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PROCEEDINGS SERIES

Food Safety and Control

Safe Food for a Better Life

PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM
Vienna, Austria, 27–31 May 2024

Joint FAO/IAEA Centre
Nuclear Techniques in Food and Agriculture

FOOD SAFETY AND CONTROL

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ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE
AND HELD IN VIENNA, 27–31 MAY 2024

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FOREWORD

Food safety is fundamental to people's well-being and affects the entire food supply chain, from production to consumption, involving multiple stakeholders. The demand for safe, high quality food is growing with the rise in the prevalence of foodborne diseases and a broad range of chemical hazards — such as antimicrobial residues, pesticide residues, naturally occurring substances and other contaminants — as well as known and emerging microbial hazards and food fraud. This has led to greater demand for regular and improved testing, monitoring and other control measures to safeguard consumers and facilitate trade.

Various measures have been implemented by IAEA Member States to enhance food safety and quality; however, food control systems need to be continually improved. These systems increasingly face new pressures, including pandemics, climate related effects, political instability and emerging zoonotic diseases. To address these challenges, concerted and diverse interventions are needed, such as targeted research and development, appropriate application of relevant technologies, and the building or strengthening of laboratory testing and regulatory capabilities. For laboratories involved in routine testing or research and development, a variety of nuclear, isotopic and complementary techniques offer solutions to challenges affecting food production and supply or food safety and quality. Food treatment science and technologies such as food irradiation play a critical role in ensuring food safety and quality. Ionizing radiation is predominantly used in post-harvest applications to prevent pest infestation for phytosanitary purposes; extend the shelf life of foods; reduce food spoilage, loss and waste; and control foodborne pathogens, among other applications. Food irradiation involves the application of gamma rays, electron beams or X rays at controlled doses.

With growing food safety and trade concerns, increasingly stringent regulatory requirements and the threat of emerging pests and diseases, as well as advances in technology, there is a need for scientists and other food safety stakeholders to periodically review the status of food safety and associated trends, share knowledge and identify or report new research areas and priorities. To help address this need, the Food and Agriculture Organization of the United Nations (FAO) and the IAEA organized the International Symposium on Food Safety and Control under the theme 'Safe Food for a Better Life', held on 27–31 May 2024 in Vienna. At least 533 participants from Member States, international organizations, the private sector, financial institutions and other stakeholder groups attended in person, with more than double that number participating virtually.

The symposium covered seven main topics: food authenticity and fighting food fraud; food and phytosanitary irradiation; chemical residues and contaminants in food and feed; preparing for and responding to emergencies and incidents affecting the food supply; detection and characterization of pathogens in food; standard setting, regulations, metrology and risk assessment; and the One Health approach. The programme consisted of a high level opening session and panel discussions, oral presentations and poster sessions, and resource mobilization activities. This publication presents the proceedings of the symposium.

The IAEA officers responsible for this publication were J.J. Sasanya, C.M. Blackburn and C. Vlachou of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture.

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1. SUMMMARY

1.1. INRODUCTION

Food control systems underpin food safety and quality, which are vital components of global food security. These systems also contribute to safeguarding public health and enhancing local and international trade in food commodities. In many countries, however, these systems are either weak or underdeveloped. Increasing pressures including the disruptive effects of pandemics and higher prevalence of foodborne diseases, and conflicts affecting food production, distribution, and supply chains further hamper the systems. Extreme weather and other climate-related effects also influence patterns of food chemical contamination and microbes, further threatening safety of the food supply. These conditions strain testing laboratories and food handling/processing facilities which are a major part of the food control system.

Various solutions, including effective analytical technologies, are needed. For instance, nuclear techniques play an important role in enhancing performance and delivery of laboratory services and in the generating and collecting scientific data required for establishing regulatory standards and guidelines. Nuclear techniques are often used in conjunction with complementary methods to provide tangible solutions to food safety and control challenges associated with a wide range of chemical, microbiological and physical hazards. Nuclear technologies also play an important role in food processing. For example, pre-packaged fresh fruits or vegetables can be exposed to a controlled dose of ionizing radiation as a phytosanitary measure to prevent the introduction and spread of regulated pests through trade in fresh produce. Irradiation is also used to destroy pathogens in food, maintain food quality, prevent foodborne illness, reduce food losses, and extend the shelf life of food products. Nuclear technologies can also contribute to the implementation of global initiatives such as the One Health approach, the fight against antimicrobial resistance and the mitigation of the effects of climate change on food supply.

The integration of nuclear techniques into food control systems is therefore essential but requires increased awareness of the comparative advantages of these techniques. Encouraging dialogue and ensuring good communication among relevant actors, including research and academia, regulatory bodies and industry, among others, is one mechanism for creating such awareness. The symposium therefore provided an appropriate forum for information-sharing on relevant cutting-edge research and developments; networking between the public and private sectors and defining future research needs and directions, as well as promoting the peaceful use of nuclear technologies.

The INTERNATIONAL SYMPOSIUM ON FOOD SAFETY AND CONTROL aimed at converging global experts and stakeholders in food safety and control systems to deliberate on the protection of the food supply chain and on measures to improve its resilience to food security-challenges such as the impacts of climate change, foodborne diseases and conditions, food fraud, and antimicrobial resistance, among others. The symposium also considered methods to address external factors that may disrupt food control systems such as, but not limited to, those caused by pandemics, conflicts or other catastrophic events. Contemporary and novel applications of nuclear and complementary techniques and technologies were presented, and future perspectives and opportunities discussed.

1.2. SUMMARY OF THE CONFERENCE

As detailed elsewhere[1] the symposium was attended by nearly 533 participants (in-person). The opening session was attended by the Directors General of the IAEA and FAO Rafael Mariano Grossi and Dongyu QU, respectively as well as the Deputy Director General and Head of the Department of Nuclear Sciences and Applications Najat Mokhtar. Other dignitaries included the President OPEC Fund for International Development Abdulhamid Alkhalifa; the Permanent Secretary, Ministry of Lands and Water Affairs, Botswana, Kekgonne Baipoledi—who also delivered a scene-setting key note address; the Director, Cabinet of the Ministry of Agriculture, Livestock and Fisheries, Republic of Benin, Dossa Aguemon; the Managing Director of the United Nations Industrial Development Organizations (UNIDO) Gunther Beger and Pleiner Duxneuner, Managing Director, Austrian Agency for Health and Food Safety.

Two technical keynote addresses on ‘Irradiation, Microbiology, Public Health, and Trade—the Interconnectedness’, as well as on ‘Diet Vs Food—Safeguarding Consumer Health’ were delivered after the official opening ceremony. These presentations were immediately proceeded by a session on chemical residues and contaminants in food and feed covering a broad range of chemical and biological hazards. A session on

[1] IAEA. Food Safety and Control Newsletter. 3 (2) July 2024. https://www.iaea.org/publications/15798/food-safety-and-control-newsletter-vol-03-no-2-july-2024

public-private partnerships, funding agencies including international financial institutions, the food industry and policy-making then followed. During the week, sessions took place on food irradiation, food authenticity and fighting food fraud and standards setting, metrology and risk assessment. An additional session on the detection and characterization of pathogens in food and antimicrobial resistance (AMR) addressed opportunities and challenges in pathogen-detection and AMR. This session transitioned into another on One Health, a concept that approaches health challenges from different perspectives and disciples such as human, animal and environmental health, as well as food safety. Each of these sessions and the symposium in general identified critical areas of research and data-generation for international food safety standards-setting and decision-making, to consider. A detailed programme of the symposium is included in this publication.

1.3. OBJECTIVE AND STRUCTURE OF THE PROCEEDINGS

This publication, an output of the symposium, focuses on the scientific and technical aspects delivered as oral or poster presentations and later submitted for internal peer-review. Findings reported on food irradiation, chemical and microbiological hazards, food fraud and authenticity, food safety standards and One Health are presented. The document is a source of information for food safety stakeholders including (but not limited to) testing laboratories, regulators and inspectors. Other potential beneficiaries are research and academia, risk managers, assessors and communicators as well as food business operators.

1.4. A SUMMARY OF PAPERS PRESENTED

The papers presented in the following section start with findings and reports on food irradiation followed by food fraud and authenticity and finally on chemical and microbiological hazards (especially their detection) efforts to address gaps in food safety standards and then aspects of One Health. On irradiation, researchers in Argentina reported the use of gamma-radiation technology to enhance food safety by eliminating pathogenic microorganisms in Fine Cut Salamín, a traditional Argentinian dry-fermented sausage (with pork, beef, bacon, salt, sugar, spices, wine and lactic-acid bacteria). They reported the effects of ionizing radiation (at doses of 0 kGy, 4 kGy and 6 kGy) on physicochemical properties of vacuum-packaged Fine Cut Salamín in 120 days in refrigerated (4.0 ± 0.5ºC) conditions. The product was dehydrated across all treatment-doses. Researchers in Mongolia reported the irradiation of potatoes using an X ray irradiator designed as a compact, self-containing cell irradiation research system operating between 40 kV and 160 kV. The locally grown potatoes were irradiated by at a range of low doses (up to 200 Gy).

Indonesian researchers reported use of cobalt gamma irradiation, with chitosan. They noted that the effect of cobalt gamma irradiation extended the shelf-life of pempek. They also noted that chitosan irradiated at doses of 10 kGy to 50 kGy had a lower molecular weight than non-irradiated chitosan. Other researchers in Indonesia reported the effect of gamma irradiation and vacuum-packaging on the shelf-life of gudeg, an ethnic cuisine. Gudeg samples were packed in laminated aluminium foil and vacuum-sealed before irradiating them at room temperature at doses of 0 kGy and 19.56 kGy. Researchers in the Russian Federation reported a study on volatile organic compounds whose concentrations can serve as indicators of bacteria suppression in a product after irradiation. They also established optimal dose range by tracing the efficiency of suppressing microorganism as well as functional and structural changes in proteins. Further on irradiation, Indonesian researchers synthesized and characterized a chitosan-edible film which was used on pempek as a wet food preservation method, protecting the food from oxidation. Activated chitosan solution was prepared by exposure it to ^{60}Co gamma in the range 10 kGy to 50 kGy. The activated chitosan edible film was then characterized using fourier transform infrared spectroscopy.

Scientists in Poland investigated the detection of irradiation in dietary supplements (effervescent tablet) following irradiation at doses of 1 kGy and 5 kGy. A thermoluminescence method was successfully used to identify irradiation. In Jordan work was done to investigate gamma-irradiation doses required to completely eliminate pathogenic bacteria and to reduce mould and yeast counts on five herbal products. In Argentina scientists investigated radiotolerance for certain pests, including the sugarcane borer. The effect (or potential) of ^{60}Co gamma radiation on both larval and pupal development stages was studied. In Sri Lanka, a study was conducted on the development of a high-energy granola bar using local raw materials. The effect of ^{60}Co gamma radiation on compositional and nutritional quality of sweet and spicy granola bars was investigated.

The effect of low energy electron beam (LEEB) on citrus fruits compared to gamma irradiation sources was reported by researchers in Türkiye. They noted that LEEB is suitable for the eradication of microorganisms on or near food surfaces or for phytosanitary purposes and could be sustainable. A team of researchers in the Russian Federation presented work on a comprehensive approach to food irradiation-planning, determining the optimal dose range for a biological object and calculating irradiation-source parameters using a mathematical model.

On food fraud, authenticity and determination of geographical origin, investigators in Myanmar conducted research to verify the authenticity of Shwebo Pawsan rice. Authentic Pawsan rice samples produced in Shwebo, Ayeyarwady, and different rice varieties were collected and elemental composition such as arsenic, cadmium, cobalt (Co) chromium (Cr) copper (Cu) iron, manganese, nickel, lead (Pb) and zinc, determined by inductively coupled plasma optical emission spectrometry. Elsewhere, scientists in Brazil reported the development of a multispectral microscope (and method) for detection of fraud in arabica and robusta coffee. The partial least squares discriminant analysis model was used to distinguish coffee types. Work was also reported by a team in the United Arab Emirates on the challenges of milk-adulteration in the Sharjah City Municipality. An innovative solution known as the Dumas method, employing fast, green, and fully automated technology to combat fraud was adopted.

Still on fraud and authenticity, in Greece, work was reported on screening of the volatilome of raw beef, pork and horse meats under different storage conditions, and determining the ratios of adulteration. Tools such as the headspace solid phase microextraction coupled with gas chromatography mass spectrometry were used, and the results showed that metabolomics with a multivariate data analysis is a potential primary tool for assessing the authenticity of raw meats. A group in Jamaica investigated the presence of toxic metals in selected spices such as turmeric and turmeric-containing products in the Jamaican market. Neutron activation analysis (NAA) and total reflection X ray fluorescence (TXRF) were used with some indications that lead (Pb) levels may exceed 1 mg/kg in some cases. Researchers in Indonesia also investigated the geographical origin and discrimination of rice in the West Java region. Work involved use of isotope ratio mass spectrometry and NAA. Four stable isotope-variables: $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$ and $\delta^{15}N$ and the 17 elements were analysed.

On chemical and microbial hazards as well as One Health, scientists in Thailand reported the testing of antimicrobials and antimicrobial resistance in fish and prawns using a One Health approach in tackling antimicrobial resistance (AMR). Samples were analysed by relevant institutions under different Ministries. Respective data-sets were then integrated and analysed in an attempt to identify correlations. A group of scientists in the Syrian Arab Republic reported the analysis of tetracycline drug residues in chicken meat, skin and liver using high performance liquid chromatography. All residue-levels in meat, skin and liver samples were within set tolerance limits.

A study in South Africa estimated the risk associated with the consumption of lettuce contaminated with shigatoxin-producing *Escherichia coli* in the country. The study identified possible mitigation measures to reduce the risk. Research was also conducted in Indonesia to investigate potentially toxic elements in breast milk and the complementary food Tangerang. NAA and TXRF were used to measure metals such as Cr, Co, Cu, and Pb. Elsewhere, scientists in Cote d'Ivoire investigated farming practices in the Western, Northwestern and Northern regions of the country between 2016 and 2022. The focus was on establishing the effects of potential exposure to drug residues in food as well as contact with chemicals used in production, and how this may also affect the farmers and performance of the chemicals in disease treatment and control.

In Morocco a study was conducted on the antimicrobial susceptibility of *Salmonella spp*., in various poultry samples (caecum-content and meat) from slaughterhouses and traditional markets in the Rabat-Sale-Kenitra region. A team of scientists in Sudan investigated the presence of oxytetracycline residues in muscle, milk, liver and kidney samples of camels, cattle, goats and sheep. Samples were obtained from slaughterhouses and farms in three states in 2021. The same scientists also conducted a study to gather basic information on food animal management practices, including rearing and treatment by animal owners in parts of Sudan.

Researchers in Pakistan reported work done to enhance radioreceptor assay measurement of chemical residues and contaminants in food through the production of in-house receptors (IHRs) for use with tracers such as ^{14}C- and ^{3}H-labelled drugs/toxins. The IHRs were isolated from non-immunized poultry liver and found to be suitable for measuring analytes such as chloramphenicol and aflatoxin B1, B2, M1. Scientists in Zimbabwe investigated contamination-sources, biofilm-forming ability and biocide resistance of *Listeria monocytogenes* recovered from a Nile crocodile meat-processing facility. Samples of processing water, meat products, and swabs from personal protective clothing, food-contact surfaces, and the processing environment were collected three times a year and tested.

Scientists in Chile reported work done on depletion of the antimicrobial amoxicillin in broiler chicken integuments following a controlled study. They found drug concentrations in the ranges of 28.63 µg/kg to 5.39 µg/kg in skin and fat and established a 5-day withdrawal period. In Egypt, work was reported on the presence/absence of sulfonamide and penicillin G residues in raw milk by radioreceptor assay testing and evaluating the presence of *Salmonella* spp. and *Staphylococcus aureus* as well as associated resistance. The possibility of degradation of these antimicrobials by gamma irradiation was also investigated.

The development of certified reference material (CRM) for food safety and quality testing was discussed along with measurement tools such as nuclear magnetic resonance and inductively coupled plasma mass

spectrometry, among others used to verify CRMs. In Mauritania, a study investigated the resistance of *Salmonella* to certain antimicrobials. This study involved 200 samples analysed between May 2022 and March 2023 with 9% of the samples containing *Salmonella* spp. resistant (100%) to ampicillin as well as amoxicillin combined with clavulamic acid. Another report discusses the detection of the pesticide dithiocarbamates in vegetables including tomatoes, lettuce, celery, chili peppers, cilantro, and cucumbers, for local consumption in Cosa Rica. Out of the 117 samples analysed, 21% exceeded set maximum residue levels (MRLs) particularly in celery. In a separate study, scientists in the Kingdom of Bahrain determined levels of several pesticide residues in local and imported plant products. This involved, among others, fruits, vegetables and spices delivered to the Public Health laboratory between 2015 and 2019.

The risk of exposure to the pathogen *E. coli* O157H7 in meat in Mozambique was reported while Sanad et al., also investigated an outbreak of staphylococcal food poisoning in the Kingdom of Bahrain. The retrospective study considering samples received by the Public Health Laboratories between 2013 and 2021 found *Staphylococcal* enterotoxin in chicken shawarma. Elsewhere, the process of establishing a risk-based pesticide residue monitoring programme for crops in Panama was reported while researchers in Bangladesh reported the screening of selected antimicrobials in chicken muscles using a radioreceptor assay method. Residues of tetracyclines, sulfonamides, beta-lactams, and macrolides/lincosamides were detected.

Related work was done in Pakistan, where scientists investigated the presence or absence of mycotoxins, including total aflatoxins, deoxynivalenol, ochratoxin and patulin, as well as the antimicrobials chloramphenicol, nitrofurans, tetracycline, penicillin, quinolones and beta-lactams in selected food and feed samples using the radioreceptor and enzyme linked immunosorbent assays. Thein et al., in Myanmar, investigated the screening of drug residues and aflatoxins in milk and animal feed. The analytes including the aflatoxins M1and B1, tetracyclines, beta-lactams, and macrolides were measured using the Charm II radioreceptor assay technique. Finally, scientists in Kuwait reported work done on estimating radioactivity in plant-based milk in Kuwait markets. They used gamma and alpha spectrometers. A study was also conducted in Zimbabwe to investigate levels of radionuclides in maize and soil from a coal mining region in the country.

PAPERS

GAMMA RADIATION EFFECTS ON THE PHYSICOCHEMICAL CHARACTERISTICS OF FINE CUT *SALAMÍN* (ARGENTINIAN DRY FERMENTED SAUSAGE)

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Abstract

The use of gamma radiation technology enhances food safety by eliminating pathogenic microorganisms in foods such as Fine Cut *Salamín*, a traditional Argentinian dry fermented sausage. The effects of ionizing radiation (0 kGy, 4 kGy and 6 kGy) on the physicochemical properties of vacuum-packaged Fine Cut *Salamín* in 120 days at refrigerated storage (4.0 ± 0.5ºC) were analysed. This study found that Fine Cut *Salamín* dehydrated across all treatments with a significant ($p<0.05$) decrease in a_w and increase in hardness and chewiness. The pH and lightness (CIE L*) decreased while springiness increased significantly ($p<0.05$) over 120 days for all treatments. There was no significant change ($p>0.05$) in adhesiveness at 0 kGy and 6 kGy but with a slight increase at 4 kGy. Redness (CIE a*) increased significantly ($p <0.05$) in nonirradiated and 6 kGy-irradiated samples but remained stable ($p>0.05$) at 4 kGy. Yellowness (CIE b*) increased for all treatments after 120 days, with significant difference ($p<0.05$) among samples at 0 kGy. In conclusion, refrigerated storage time (0–120 days) significantly impacted on physicochemical properties of vacuum-packaged Fine Cut *Salamín*, while gamma irradiation did not result in significant differences between treated and control samples.

1. INTRODUCTION

Food irradiation is a non-thermal process that applies ionizing radiation to accomplish pasteurization without a significant temperature increase, hence its characterization as 'cold pasteurization' [1, 2]. The process involves exposing food to controlled amounts of ionizing radiation from sources such as γ rays, X rays and accelerated electrons [3]. This technology helps to reduce foodborne pathogens, spoilage microorganisms as well as parasites, and extends shelf-life among other benefits [3]. Regardless of the method used, the effectiveness of the irradiation depends on the absorbed dose [4]. The dose depends on several variables including the specific objective, type and level of microbial contamination, the resistance of microorganism to radiation, and the product type being treated, while maintaining sensory properties of food products [4, 5]. The optimal radiation dose is thus a balance between the desired effect and what a product tolerates [6]. Food irradiation has been extensively studied since the 1960s and it is widely considered safe [7]. Research indicates that doses up to 10 kGy neither present toxicological hazard nor induce significant nutritional or biological alterations (World Health Organization, 1981).

The Argentine Food Code (CAA) addresses food irradiation in Chapter III, Article 174. From 1988 until October 2017, the legislation covered the general aspect of food irradiation and authorized its use for a limited range of products (such as potatoes, onions, garlic, strawberries, mushrooms, asparagus, spices, dehydrated fruits and vegetables). On 6 October 2017, the National Atomic Energy Commission (CNEA) of Argentina requested modification and update of Article 174. This resulted in a change that categorized food irradiation according to specific classes, intended objective and maximum dose. Class 7 of the regulation covers poultry, beef, pork, goat meat, other meats and their products (fresh and frozen) while Class 8 encompasses dried foods of animal origin [9].

Food irradiation has been used to reduce or eliminate various pathogens in a range of animal and plant products [10]. The technology compliments good manufacturing practices and management systems such as the hazard analysis and critical control point [1, 11]. Argentinian salami products ,have their origins in Italian immigration between 1880 and 1930. Immigrants brought recipes and techniques for dry fermented sausage (DFS)-production, including fermentation and starter cultures. The items are combined with local meat, spices and flavours. Over the past 150 years, salami became an integral part of Argentine culture and custom promoting tourism, the economy, developments in science and technology. Additionally, there is a dedicated tourist circuit

known as 'The Salamín Route'. Some studies suggest that approximately 50% of the Argentinians consume salami frequently [12, 13].

Fine Cut *Salamín* (FCS) can be a mixture of minced pork and beef up to one millimetre in size, bacon, salt, sugar, spices, wine, lactic acid bacteria (LAB) and food additives. The product is often stuffed into a natural casing and undergoes fermentation and drying processes. The commercial shelf-life is 60 days to 90 days. Due to its relatively high fat content, diverse ingredients and absence of heat treatment in the processing, the fermented sausage is highly susceptible to deterioration, including lipid oxidation and bacterial growth [14]. The presence of pathogens such as *Listeria monocytogenes (Lm) Salmonella* and *Escherichia coli* and associated outbreaks have been reported (Van Ba, y otros, 2017).

In Argentina, there have been no documented listeriosis outbreaks associated with contaminated food to date, as listeriosis was not classified as a notable disease in the National Health Surveillance System until 2022 [16] although, the Argentinian National Reference Laboratory recorded 310 cases of invasive listeriosis between 1985–2016 [17]. Recent studies on *Lm* show a potential epidemiological linkage between *Lm* isolates from humans and food in Argentina [18] with a 27 % positive rate in Argentinian processed sausages [19]. *L. monocytogenes* is one of the most frequently detected pathogens in DFS and is a document challenge to control in food manufacturing environment [20]. This is a concern to Argentina due to a zero-tolerance policy which requires intervention of much better technology such as gamma irradiation to protect public health from potential outbreak associated with this widely consumed product, as reported elsewhere [21]. A minimum absorbed dose (Dmin) of 4 kGy is required to achieve a 5 logarithmic reduction of *Lm* in vacuum-packed FCS stored at 4ºC (Raad, y otros, 22-28 May 2022). Caution is required since *Lm* is the most radiation-resistant among pathogenic vegetative bacteria [22, 23]. A Dmin necessary to eliminate *Lm* can also be effective against other vegetative pathogens.

The effect of gamma irradiation and vacuum-packing combined, on the physicochemical characteristics of DFS has been investigated. Previous research showed that pH, CIE L* and CIE b*-value of DFS were not significantly influenced by irradiation up to 4 kGy after 90 days of storage at 4ºC [24]. Other researchers found that colour parameters of vacuum-packed DFS were affected by irradiation treatment (at 1 kGy, 2 kGy and 3 kGy). Product stored at 4ºC for 0 days to 18 days were not affected [25]. Also, the Texture Profile Analysis (TPA) parameters of vacuum-packed DFS did not show significant differences between irradiated (1 kGy, 2 kGy and 3 kGy) and non-irradiated samples after 18 days storage [25].

To the best of the researchers' knowledge, no other studies have assessed the effects of gamma irradiation on any type of Argentinian DFS. Hence, the aim of the present study was to evaluate the effect of gamma irradiation (Dmin, 4 kGy or higher) on the physicochemical properties of vacuum-packed FCS stored at 4.0 ± 0.5ºC for 120 days.

2. MATERIALS AND METHOD

2.1. Experimental design

A completely randomized design with independent measures was applied to evaluate the effects of different gamma irradiation doses (0 kGy, 4 kGy and 6 kGy) and storage time (0 days, 30 days, 60 days, 90 days and 120 days) at 4.0 ± 0.5ºC on the physicochemical properties of vacuum-packed FCS. In the present study, irradiation doses and storage time were the independent variables and pH, water activity (aw) colour parameters (CIE L*, a*, b*) and TPA parameters including hardness, adhesiveness, springiness and chewiness were the dependent variables. A total of 15 combinations, consisting of 3 doses and 5 storage times, were assessed for all dependent variables. Additionally, 18 independent whole FCS samples were used for each treatment and date of analysis.

2.2. Sample preparation

Samples of FCS were produced in Santa Fe Province, Argentina, and transported to the laboratory under refrigeration conditions (5.5 ± 1.0ºC). The ingredients include a mixture of pork (50–60%) and beef (10–20%) minced in fine ground size (1–4 mm) bacon (20–30 %) salt (2.5%) nitrates and nitrites (< 0.003%) soy protein isolate (< 2%) sugar (dextrose and sucrose, 0.5 %) starch (1.5%) spices (0.5 %) wine (0.5–1%) coagulase negative cocci, lactic acid bacteria (LAB) (0.5%) and food additives (< 0.05 %) such as sodium erythorbate and phosphates. The meat paste was stuffed into natural casing (cleaned, dried, salted and refrigerated). The FCS were fermented for 18–20 h [23ºC to 25ºC, 90 % relative humidity (RH)], to attain a pH between 5.1 and 5.3. Drying was performed in three stages: 0.5 day at 14–16°C/80 % RH; 2 days at 12–14 °C/60–70 % RH and 7.5

days at 12–14°C/78–83 % RH; to achieve $a_w < 0.91$. The FSC was completely ripened (fermentation and drying stages) in 10 days, resulting in a product at a pH of 5 (20.0 ± 0.1ºC) a weight of 140–160 g, a diameter of 38 mm–42 mm and shelf-life of 60 days at 5.0 ± 0.5 ºC.

The natural casing was removed at the laboratory, and the samples were vacuum-packed at 20 hPa in high barrier bags of 100 µm made of polyamide/evoh/polyethylene (3 cc O_2/m^2/24 h and 7 g Steam/m^2/24 h, CRYOVAC, P7340B). All samples were stored in the dark at 4.0 ± 0.5ºC for 24 h before irradiation.

2.3. Irradiation, dosimetry system and storage conditions

Gamma irradiation was performed at the semi-industrial irradiation facility at the Ezeiza Atomic Centre of CNEA with a ^{60}Co source (activity of 700 kCi). The products were irradiated at 0 kGy (control) 4 kGy and 6 kGy under refrigerated conditions. The temperature after irradiation was 6.3ºC and 10ºC for 4 kGy and 6 kGy, respectively. A reference-standard dosimetry system was used according to ISO/ASTM51607-13 Standard Practice for Use of the Alanine EPR Dosimetry System in the high-doses dosimetry laboratory. Dose mapping was performed to determinate the location of Dmax and Dmin for the sample box and ensure a dose uniformity ratio (DUR, Dmax/Dmin ratio). To enhance dose uniformity, an insulated small box (37 × 20 × 21 cm) made of Tergopol for a shallow depth. Double-side irradiation was performed with the sample-boxes rotating at the half time of irradiation. The configuration of samples inside the boxes was arranged to minimize the sample depth relative to the source.

The average dose rate was 10.9 kGy/h (DUR of 1.03) and 11.3 kGy/h (DUR of 1.04) for the treatments at 4 kGy and 6 kGy, respectively. The alanine/EPR dosimetry system was used. The alanine dosimeter pellets had a calibration traceable to the National Physical Laboratory. The mean absorbed dose was within ±3 % of the target dose of 4 kGy and ±11.6% of 6 kGy. Refrigerated control samples (4 ± 0.5ºC) were located outside the irradiation room in small box of Tergopol (37 × 20 × 21 cm) to maintain the same room temperature conditions as the irradiated ones. After irradiation, control and irradiated samples were immediately stored in the dark at 4.0 ± 0.5ºC for 0 day, 30 days, 60 days, 90 days and 120 days.

2.4. Physicochemical analysis

2.4.1. pH measurement

Half of each FCS were hand-chopped into 5 mm cubes and then processed using a mini blender for 30 s until a paste was formed. A 5 g aliquot of the paste with 25 ml of distilled water at pH 7 (adjusted with a diluted solution of NaOH and H_2SO_4) was homogenized in a stomacher for 30 s. The slurries were collected in a falcon tube (50 ml) and the pH measured. The electrode was calibrated immediately before measurement using two buffer solutions at pH 4.0 and 7.0. Four independent samples, each analysed in duplicates were used.

2.4.4. Water activity (a_w) measurement

Samples were trimmed into 1 cm thick slices and first introduced in the manufacturer's capsules at room temperature for 30 min and then measure using a water activity meter. Four individual samples, each analysed in triplicate were used.

2.4.5. Chromatic parameters

Determination of the chromatic parameters were as reported elsewhere (Kim, y otros, 2012) with modifications. Samples were cut with a knife into 2 cm thick slices, placed in a refrigerator at 4.0 ± 0.5ºC for 10 min and then quickly determined at room temperature using a spectrophotometer ($\underline{\lambda}$= 360–740 nm, $\Delta\lambda$=10 nm, D65, 10º) with a port measuring area of Ø = 8 mm, calibrated with a white plate provided by the manufacturer. The surface chromatic parameters were measured at 5 different points (one in the centre and one in each quadrant) with 3 scans performed at each point. The values of the three chromatic parameters were expressed according to the CIE L*a*b* system, L* (black-white component, lightness) a* (redness/greenness) and b* (yellowness/blueness) (Commision International de l'Éclairage (CIE), 1976). Four independent samples in three replicates each were included.

2.4.6. Texture Profile Analysis (TPA)

The TPA was implemented using a Stable Micro Systems Texture Analyser, equipped with a cylindrical probe of Ø =35 mm and a 5 kg load cell, with an Exponent 32 software. The FCS cylinders (1.2 cm diameter

and 2 cm height) were used as samples cut in a room at 15ºC and stored at 4.0 ± 0.5ºC for 30 min before analysis. The assay was set up to a compression of 42% of the original portion height and probe height was calibrated to 20 cm. The test was performed with two consecutive compressions on the samples at a speed of 2 mm/s for pretest and 1 mm/s for the test. For the recovery time between compressions, the test was set up at a speed of 5 mm/s. At the end of the assay, the probe was returned to the initial position at a speed of 10 mm/s. Parameters including hardness, adhesiveness, springiness and chewiness were measured using 8 standalone samples, with twelve replicates each.

2.5. Statistical analysis

Non-parametric ANOVA (Kruskall Wallis) was performed at a significant level of $p<0.05$, using InfoStat version 2020 and the results were normalized.

3. RESULTS AND DISCUSSION

3.1. Statistical analysis

The results presented in Tables 1 and 2 show that the interaction between dose and storage time was a significant ($p<0.05$) factor for all parameters except for springiness. The impact of storage time on the response variables seem to depend on the absorbed doses. Also, the relationship (line slope) of dependent variables with irradiation doses at storage were not constant. However, the individual effect of radiation was not found to be significantly different ($p>0.05$) at any of the samples. Furthermore, the results indicate that storage time was the determining factor, as the values of the irradiated samples were insignificantly different ($p>0.05$) from those of the control samples for each storage date analysed.

3.2. Evaluation of pH, a_w and chromatic parameters

The results for pH, water activity (a_w) L*, a* and b* were set out in the Table 1 for the control and irradiated FCS samples stored under refrigeration at 4.0 ± 0.5ºC for 0 days and 120 days. Changes were observed during all storage periods (0 days, 30 days, 60 days, 90 days and 120 days) at 4.0 ± 0.5ºC for vacuum-packed FCS, with the results normalized to pH, water activity, L*, a* and b*.

TABLE 1. pH, a_w AND CHROMATIC PARAMETERS OF GAMMA-IRRADIATED (4 KGY AND 6 KGY) AND CONTROL (0 KGY) FINE CUT *SALAMÍN* (FCS) VACUUM-PACKAGED AND STORED AT 4 ± 0.5ºC FOR 0 days AND 120 DAYS.

Dose	pH		Water activity, aw (20ºC)		Chromatic parameter					
					CIE *L**		CIE *a**		CIE *b**	
	0 day	120 days	0 day	120 days	0 day	120 days	0 day	120 days	0 day	120 days
0 kGy	5.10 ± 0.03^{b}	4.98 ± 0.04^{a}	0.93 ± 0.01^{b}	0.89 ± 0.01^{a}	54.50 ± 1.44^{b}	49.26 ± 0.94^{a}	13.20 ± 0.69^{a}	16.08 ± 0.58^{b}	10.44 ± 0.66^{a}	12.31 ± 0.58^{b}
4 kGy	5.10 ± 0.03^{b}	5.00 ± 0.01^{a}	0.93 ± 0.01^{b}	0.89 ± 0.01^{a}	54.80 ± 1.94^{b}	51.72 ± 1.19^{ab}	13.40 ± 1.26^{a}	14.88 ± 0.90^{ab}	10.66 ± 0.47^{a}	11.05 ± 0.60^{ab}
6 kGy	5.11 ± 0.02^{b}	5.02 ± 0.02^{a}	0.93 ± 0.01^{b}	0.88 ± 0.01^{a}	54.07 ± 0.84^{b}	50.14 ± 1.01^{a}	13.10 ± 0.64^{a}	15.60 ± 0.58^{b}	10.29 ± 0.65^{a}	11.22 ± 0.55^{ab}

3.2.1. pH evaluation

Fermented sausages are characterized by low acidity, with the final pH in the range of 4.8–6.2 (Kim, y otros, 2012). Despite gamma-irradiation treatments, the pH range during refrigerated storage was 4.98 (at 120 days) and 5.22 (at 30 days). No significant difference ($p>0.05$) in pH values was found among treatments (0 kGy, 4 kGy and 6 kGy). This concurs with Kim et al., (Kim, y otros, 2012), who reported irradiation at 0 kGy, 0.5 kGy, 1 kGy, 2 kGy and 3 kGy did not have any effect on pH over 90 days of storage at 4ºC for vacuum-packed DFS. Their values were slightly lower nevertheless in a lower range (4.88 to 4.93). Ameer et al., (Ameer, Seleshe, & Kang, 2022) noted a significant difference ($p<0.05$) among treatments (0 kGy, 1 kGy, 2 kGy, 3 kGy and 4 kGy) for refrigerated (4ºC) vacuum-packed DFS during 60 days of storage. Their study found that the pH significantly decreased ($p<0.05$) with the decreasing irradiation doses at 15 days, 30 days and 60 days of storage, except at 30 days, where 3 kGy showed a higher value than 4 kGy (Ameer, Seleshe, & Kang, 2022).

These researchers noted that on the first day of analysis, all treated samples had significantly lower pH ($p<0.05$) compared to the control, in the following order: 1 kGy and 2 kGy < 3 kGy < 4 kGy < control (Ameer, Seleshe, & Kang, 2022). The pH range of 5.09–5.47 reported by Ameer et al., (Ameer, Seleshe, & Kang, 2022) was similar to the present study. There was a significant decline ($p<0.05$) in pH values throughout storage period for both irradiated and control samples, which also agree with Ameer et al., (Ameer, Seleshe, & Kang, 2022) and not Kim et al., (Kim, y otros, 2012), where no significant difference ($p<0.05$) was observed. These differences among studies, could be due to the formulation of DFS and the conditions for ripening. In the present study, both control and irradiated samples scored the highest pH value at 30 days of storage.

According to previous findings, the decrease in pH values as observed in the present assay, correlated with an increase in LAB throughout the storage period (Raad, y otros, 2024). Specifically, a reduction in pH levels correlated with a rise in LAB counts (at 120 days) of 1.9 log cfu/g for 0 kGy and 0.6 log cfu/g for 4 kGy and 6 kGy (Raad, y otros, 2024). The LAB counts were notable higher in control samples, (8.2±0.25 log cfu/g at 120 days) compared to irradiated FC: 3.97±0.22 log cfu/g at 30 days and 3.61±0.09 log cfu/g at 90 days, for 4 kGy and 6 kGy, respectively (Raad, y otros, 2024).

The resultant decrease in pH could be explained as reported by Demeyer et al., [29] who suggested that pH is counterbalanced by the salt solubilized and hydrolysed muscle proteins and by the ammonia produced (Demeyer, D; Toldrá, F; Leroy, F;, 2014). The growth of LAB seems to have had the greater effect on pH reduction. Fermentation and aging also seem to continue during storage of vacuum-packed FCS perhaps due to gradual growth of LAB. Ripening is also enhanced in such acidic FSC environments (Coccocelli & Fontana, 2010). Gamma irradiation appeared to limit the LAB growth (approximately 4 log cfu/g) suggesting therefore that ripening was slower in irradiated samples, which could explain the extended FCS shelf-life.

3.2.2. Water activity (aw) evaluation

Ameer et al., (Ameer, Seleshe, & Kang, 2022) saw no difference ($p>0.05$) in a_w values among irradiated and control samples for refrigerated (4ºC) vacuum-packed DFS where irradiation doses of 0 kGy, 1 kGy, 2 kGy, 3 kGy and 4 kGy were used. However, in the present investigation, significant decreases ($p<0.05$) in a_w values were observed during refrigerated storage. This discrepancy may be attributed to the longer ripening and fermentation period of DFS (35 days) in the study by Abeer et al., where dehydration took place inside a fermentation chamber unlike in the present study where it occurred throughout refrigeration. As was noted, fermentation was ongoing, as LAB increased during storage, and dehydration was the result of a continuous ripening process over the holding time. During fermentation and aging, both of which continued during storage, the rate of water loss is influenced by the drop in pH (Toldrá, Principles of dry-fermented sausage-making, 2002).

The decrease in the pH between 4.6 to 5.9 during fermentation of DFS has been reported by others [32, 30]. It is also reported that as pH reduced and approached 5.1–5.3 [30, 31], the number of negative charges decreased [31]; muscle proteins were denatured reducing their water-holding capacity [30, 31, 33, 34], thus resulting in the release of bound water [31, 33]. Salt added at grinding and mincing promoted the solubilization and extraction of proteins, primarily myosin and actin, from the myofibrils [33, 31]. Further, tissue proteases (especially cathepsin D) promote proteolysis during fermentation under optimal condition (pH of 4.8–5.2 and 15–20ºC) leading to the coagulation of solubilized proteins, releasing water [31, 32].

The reduction in pH resulted in increased water loss, which lowered a_w [34, 35] and improved the drying process [35]. In general, sausages dry faster when pH drops rapidly (Toldrá, Principles of dry-fermented sausage-making, 2002) during the drying process, but in vacuum–packaging with high barrier bags of 100 µm, the drying rate was limited by the low vapor diffusion. The present study found higher a_w values than those reported by other authors (0.84) [36], 0.74–0.75 [27] and 0.83–0.84 [37] at the end of fermentation and aging. The a_w decreased gradually during storage, regardless of the low pH values and LAB growth. This suggests that vacuum-packed FCS dried slowly preventing excessive dehydration.

As shown in Table 1, no significant effect ($p>0.05$) was observed among irradiation doses (0 kGy, 4 kGy and 6 kGy) in refrigerated vacuum-packed FCS for the three chromic parameters. A notable decrease ($p<0.05$) in lightness (CIE L*) and a significant increase ($p<0.05$) in redness (CIE a*) during refrigerated storage time for the control and 6 kGy treatments were noted. In contrast, the CIE L* and CIE a* values for the FCS samples treated at 4 kGy were found to be similar ($p>0.05$) at 0 days and 120 days. The storage period had a significant impact ($p<0.05$) on yellowness (CIE b*) for the control samples, while the CIE b* values remained stable ($p>0.05$) for the irradiated samples (4 kGy and 6 kGy). The increase in CIE b* value for the controls may indicate a higher degree of lipid oxidation (Table 1) compared to irradiated FCS.

Ji et al., (Ji, Shankar, Salmieri, & Lacroix, 2022) reported similar results where no significant effect of γ irradiation dose at 1.5 kGy was observed on chromatic parameter values of vacuum-packed DFS at room temperature (20–21ºC) compared to the control. An exception was in the values of redness, which decreased after irradiation and storage time (20 weeks). Kim et al., (Kim, y otros, 2012) observed the same trend for CIE L* and vacuum-packed DFS stored at 4ºC for up to 90 days as in this study. They also found a significant decrease in CIE L* for doses of 0 kGy, 0.5 kGy, 1 kGy and 2 kGy, with a tendency to greater decrease at 4 kGy during storage (Kim, y otros, 2012).

Unlike this study, Kim et al., [24] reported that CIE L* decreased while CIE a* and CIE b* increased with irradiation doses at day 1 of storage; but, at 90 days of refrigerated storage, the irradiated samples had lower CIE a* and CIE b* values compared to the control samples. Conversely, Gámez et al., (Gámez, García, Selgas, & Calvo, 2011) observed no changes in CIE L*, CIE a* and CIE b* values at refrigeration (4ºC) for 0 months, 1 months, 2 months and 3 months or irradiation treatment at 0 kGy, 2 kGy and 4 kGy) for vacuum-packed DFS. However, Cabeza et al., (Cabeza, De la Hoz, Velasco, Cambero, & Ordoñez, 2009) found no effect (for the three chromatic parameters) in vacuum-packed DFS during storage for 0 days and 18 days at 4ºC. These researchers also observed that CIE L* and CIE a* values increased with increasing irradiation doses, while the CIE b* value was not affected by irradiation. Ameer et al., [27] identified differences in CIE L* and CIE a* values in refrigerated (4ºC) vacuum-packed DFS irradiated at 0 kGy, 1 kGy, 2 kGy, 3 kGy and 4 kGy on the days 15 and 60 of storage, but not for days 1 and 30. The CIE b* value was affected by irradiation for all storage days. They also found that during storage, CIE L* decreased in control, 1 kGy and 3 kGy samples, but increased in those treated at 2 kGy and 4 kGy. The CIE a* decreased in irradiated samples but increased for controls. The researchers also found that CIE b* increased in control samples during storage, with a slight decrease in irradiated samples. Galán et al., [37] found that at 0 day after irradiation increasing the dose (from 0 kGy onwards led to a rise in CIE L* and, a decrease in CIE a* and CIE b*. The above findings differ from the current study's.

The colour of irradiated meat products varies with radiation source, radiation dose, animal species, muscle type, packaging atmosphere and myoglobin concentration [31–43]. For DFS, the colour is also influenced by other factors during the manufacturing process, such as raw material; additives; mixing/filling and drying/repining. Others are whether whole, sliced or packaged sausage and retail display [44]. Nitrosomyoglobin ($MbFe^{II}NO$) is largely responsible for redness in meat products containing nitrite [31, 41, 44–46]. Fermented sausages containing both nitrate/nitrite and with erythorbate up to 80% pigment conversion into $MbFe^{II}NO$ after 8 days of dehydration is possible [44]. Results from sausage extracts have shown that irradiation causes degradation of the nitrosohaeme complex (NO-Mb) due to the presence of oxygen (Badr, 2012). Ahn et al., (Ahn, y otros, 2004) demonstrated that irradiation combined with vacuum-packaging was associated with the loss of the red colour in sausages. This finding suggests that the nitrosohaeme pigment and nitrosomyoglobin are not destroyed by irradiation in vacuum-packaged products. Moreover, phenolic compounds can interrupt the oxidation reaction of irradiated meat by providing hydrogen atoms or quenching free radicals, thereby preventing the colour and texture changes caused by irradiation (Ji, Allahdad, Sarmast, Salmieri, & Lacroix, 2022).

Dehydration during the storage, the associated reduction in a_w and a subsequent loss of moisture among vacuum-packaged FCS, resulted in concentration of the fat, protein, and carbohydrate content (Santchurn, Arnaud, Zakhia-Rozis, & Collignan, 2012). Furthermore, both control and irradiated samples showed a decrease in L* due to the decreasing a_w throughout storage period. Consequently, lightness decreased while redness increased during the refrigerated-storage. Results of the present study on vacuum-packed FCS has shown that the changes in lightness (CIE L*) and redness (CIE a*) are likely due to dehydration, resulting from a continuous ripening throughout refrigeration.

3.4. Texture profile analysis (TPA)

Table 2 presents the results for the hardness, adhesiveness, springiness and chewiness test for both control and irradiated vacuum-packed FCS samples, stored for 0 days and 120 days under refrigerated conditions (4 ± 0.5ºC). The study showed varying changes across the storage period (0 days, 30 days, 60 days, 90 days and 120 days) at 4 ± 0.5ºC for both control and irradiated vacuum-packed FCS samples. This is based on the normalized TPA results for hardness, adhesiveness, springiness and chewiness.

TABLE 2. TEXTURE PROFILE ANALYSIS (TPA) MEASURED DURING STORAGE OF GAMMA IRRADIATED (4 KGY AND 6 KGY) AND CONTROL (0 KGY) VACUUM-PACKAGED FINE CUT SALAMÍN STORED AT 4 ± 0.5ºC FOR 0 DAYS AND 120 DAYS.

Dose	Hardness (*N*)		Adhesiveness (*N x s*)		Springiness		Chewiness (*N*)	
	0 day	120 days	0 day	120 days	0 day	120 days	0 day	120 days

Dose	Hardness (*N*)		Adhesiveness (*N x s*)		Springiness		Chewiness (*N*)	
0 kGy	7.98 ± 0.82[a]	12.49 ± 1.12[b]	-0.38 ± 0.09[a]	-0.41 ± 0.05[a]	0.76 ± 0.02[A]	0.79 ± 0.02[B]	4.12 ± 0.40[a]	6.93 ± 0.60[b]
4 kGy	7.78 ± 0.37[a]	10.83 ± 0.62[b]	-0.41 ± 0.07[a]	-0.37 ± 0.07[a]	0.75± 0.02[A]	0.78 ± 0.02[B]	4.04 ± 0.23[a]	5.85 ± 0.40[b]
6 kGy	7.90 ± 0.46[a]	11.76 ± 0.91[b]	-0.45 ± 0.10[a]	-0.46± 0.06[a]	0.75 ± 0.02[A]	0.78 ± 0.02[B]	4.04 ± 0.30[a]	6.29 ± 0.44[b]

Note: *Numbers are expressed as means ± standard deviations. a-b denoted means with different letters within a parameter (row and column) are significantly different ($p<0.05$). A-B means with different letters within a row for each parameter are significantly different ($p<0.05$).*

Texture parameters (hardness, adhesiveness, springiness and chewiness) of the vacuum-packed FCS stored at 4.0±0.5ºC were not significantly affected ($p>0.05$) by γ irradiation. This is consistent with earlier investigations on vacuum-packed DFS stored at 4ºC, by Cabeza et al., [25] and Ameer et al., [27]. Research on processed meat products has also reported that irradiation did not have an effect on texture-characteristics [41]. However, other studies on vacuum-packed DFS refrigerated at 4ºC found slight changes in springiness due to irradiation [38], and a decrease in hardness with increasing radiation dose [37].

The results from the present study showed that hardness, springiness and chewiness increased considerably ($p<0.05$) with storage, with the exception of adhesiveness, which did not change. Hardness increased by 57% (0 kGy) 39% (4 kGy) and 48% (6 kGy) after 120 days of storage, while chewiness increased by 68% (0 kGy) 45% (4 kGy) and 56% (6 kGy). The most notable changes were on hardness, directly impacting chewiness. The 60-day curve showed fewer peaks and less variations in the spider web plot for all texture parameters.

Results at 60 days showed the least variability compared to the other storage dates. Also, irradiation doses showed reduced effect (indirect) on all texture parameters of refrigerated (4ºC) vacuum-packed FCS at 60 days. In contrast, several studies showed no texture-modifications due to storage time [27]; minor changes on springiness only [38], or a decline in hardness up to 8 weeks [3, 36], followed by a rise at 12 weeks and another decline at 16 weeks, with a final increase at 20 weeks. These findings agree with previous observations at 1 week of storage as reported elsewhere (Ji, Shankar, Salmieri, & Lacroix, 2022). The changes in physicochemical parameters depended on the irradiation dose, sample temperature as well as pH and water content as well as the packaging and storage period as observed elsewhere [49].

The sensitivity of proteins and thus meat texture to irradiation varies [49]. This study on TPA for vacuum-packed FCS in refrigerated storage of up to 120 days showed that the primary cause of changes in texture was dehydration. Changes in texture resulted the denaturation and coagulation of meat protein during the acidification process, forming a strong gel that binds the fat and meat closely [31, 33]. Continuous dehydration also led to a significant increase ($p<0.05$) in hardness during storage [31, 33].

4. CONCLUSIONS

A study was undertaken to determine the potential effect of irradiation on FCS. The physicochemical properties of vacuum-packed FCS showed no statistically significant differences due to γ irradiation treatment at different doses up to 6 kGy. This dose can thus be considered the maximum applicable when irradiating the product. The physicochemical and microbiological properties of vacuum-packed FCS were significantly influenced by refrigeration storage time, with a decrease in pH and reduction in a_w. It was evident that FCS undergoes continuous ripening and dehydration until 120 days. Dehydration influences the development of hardness and chewiness. The control samples showed the largest reduction in pH, and the most significant increase in hardness and chewiness (0 kGy days and 120 days). Gamma radiation is shown as a viable preservation-method for Argentine dry fermented sausages without altering the physicochemical quality. Nevertheless, further studies and sensory analyses are needed to compare the acceptability of irradiated versus control FCS and ascertain the shelf-life.

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SHELF-LIFE EXTENSION OF POTATOES BY LOW ENERGY X RAY SUPPRESSION OF SPROUTING

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Abstract

This study reports the use of an X ray cell irradiator designed as a compact, self-containing cell irradiation research system and with voltages between 40 and 160 kV. Dose mapping was conducted using an electrometer and ionization chamber and showed good agreement with the reference value provided by the manufacturer. Locally grown potatoes were irradiated by low-energy X rays at various doses (up to 200 Gy). Single-side irradiation was carried out and uniformity within potatoes was not considered. No sprouting of potatoes stored at room temperature for 2 months was observed after irradiation with 150 Gy and 200 Gy.

1. INTRODUCTION

Mongolia is a landlocked country located in north-central Asia, between the Russia Federation to the north and China to the south. The country has a marked continental climate, with long cold winters (-30°C) and short cool-to-hot summers (+25°C). About three quarters of Mongolia consists of pasturelands for livestock and the remaining area [1] is about equally divided between forests and barren deserts. The human population is about 3.4 million, but the country's large territory supports approximately 70 million livestock. The climate and weather conditions support all aspects of agricultural production, and, for crops such as grains, potatoes, garlic, onions, and cabbages, there is one annual harvest season due to large temperature changes and dry weather. Once gathered, crops are stored until the next harvest. Up to 30% post-harvest loss is expected. Thus, shelf-life extension of various vegetables is critical, and the country is promoting the application of irradiation to extend the shelf-life of various foods and for phytosanitary purposes to promote exports [2].

2. MATERIALS AND METHODS

Well-shaped, damage-free, firm, and relatively clean potatoes *(Solanum tuberosum'* L. (Family: *Solanaceae*) cv. '*gala'*) used in this study were divided into 4 groups, where 3 groups were irradiated with 100 Gy, 150 Gy, and 200 Gy doses using a RS1800 Q4 X ray irradiator. Irradiated samples were incubated at room temperature for 60 days and changes in weight, length, width, sprouts, green and browning area of each potato measured according to the Mongolian National Standard MNS 0258:2021. Total sugar, dissolved solids and pH were measured and compared in each group. The X ray cell irradiator used is designed to be a compact, self-contained cell irradiation research system and has operating voltages between 40 and 160 kV [3]. Dose mapping was conducted using an electrometer and ionization chamber and showed good agreement with the reference value provided by the manufacturer [4]. Figures 1 and 2 provide more information.

Two types of texture analyses were conducted to compare the mechanical properties of irradiated and unirradiated potatoes after storage. The tests included a puncture test on the whole tuber, and a double compression test on the cylindrical potato samples. All irradiated and control groups were approximate in weight (91.92±11.83 grams) and shape. In the puncture test, the whole potato was pierced at 5 different points using a cylindrical probe of 5 mm diameter at a speed of 0.25 mm/s on a texture analyser. The probe was pushed until the potato was punctured. From the force-stroke graph of the test, the parameters such as force at puncture, work done and distance to puncture, and the elasticity modulus at puncture were determined.

In the Texture Profile Analysis (TPA)-Double Compression test, when performing the test, 2 cylindrical samples (diameter 2.7 cm × height 1 cm) were taken from the inner central part of one potato and compressed twice by 25% using the analyser at a speed of 0.25 mm/s with a 30 mm diameter flat disc on a non-lubricated

platform. Cohesiveness, gumminess and hardness of the samples were determined from the force-time graph. Hardness is the maximum force applied when the sample is compressed (N); cohesiveness is the area of the graph obtained during second compression (N m) divided by the area of the graph obtained during the first compression (N m); Gumminess (N) is a product of hardness and cohesiveness.

FIG. 1. Potato samples in the irradiator.

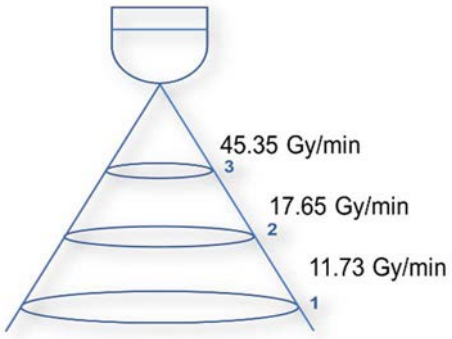

FIG.2. Absorbed-dose rate in the irradiator depending on distance from X ray tube.

FIG. 3. Appearance of potatoes after 60 days at room temperature, left is the control (unirradiated) group and right is the X ray (100 Gy)-irradiated group.

3. RESULTS AND DISCUSSIONS

Irradiation suppressed sprouting even at the lowest dose of 100 Gy (Fig. 3). There were notable differences in the sprouting of potatoes even when exposed to similar levels of irradiation (Fig. 4). In the control group, the average number of sprouts per potato was 7.25±1.25 after storage at room temperature for 60 days, whereas in the 150 Gy irradiated potatoes, the average was 4.17±1.01 (Fig. 4b). The most significant difference in sprouting was observed in the unirradiated control group, where the average number of sprouts that were longer than 4 mm was 2.88±0.88, unlike in the irradiated potatoes. When a potato sprouts more than 5%, it is not acceptable according to the Mongolian standard MNS 0258:2021 [5]. The pH of both irradiated and non-irradiated groups was not significantly different and within the normal range of 5.1–5.9 after storage at room temperature for 60 days.

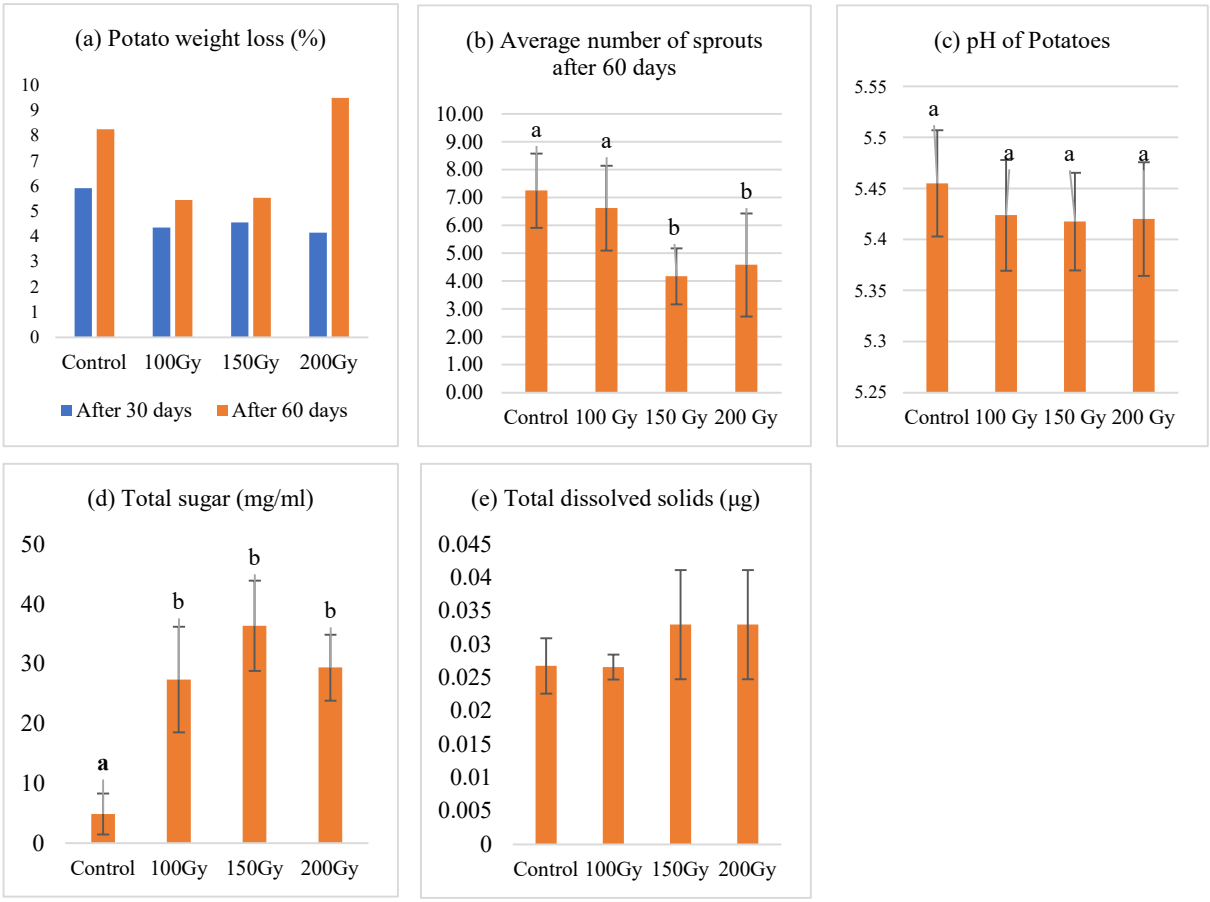

FIG. 4. Percentage of potato weight loss (a) average number of sprouts per potato (b) biochemical parameters such as pH (c) total sugar (d) and total dissolved solids (e) compared between groups.

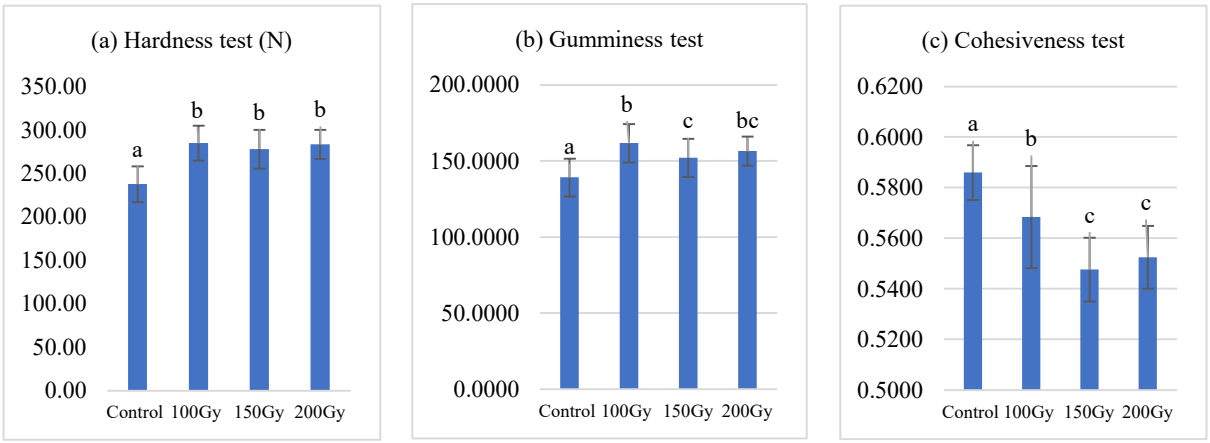

FIG. 5. Texture Profile Analysis for hardness (a) gumminess (b) and cohesiveness (c).

Based on the TPA results (Figs. 5 and 6) after 60 days of storage, hardness of the control group of potatoes was lower by 40–50 N compared to the irradiated groups (Fig. 5a) and their gumminess was also 10–20 N less (Fig. 5b). The puncture force was similar for all groups (Fig. 6a) but the Young's modulus values, work done to puncture, and puncture stroke (Figs. 6b, c and d, respectively) were significantly different between the control and irradiated groups. For instance, the 'work done' parameter values of the control group were 20–50 mJ higher than other groups. The puncture stroke was also 2 mm more for the control group, while the elasticity modulus of the control group was 1000–2000 Pa lower. Consequently, it can be concluded that the properties of potatoes stored at room temperature for 60 days after irradiation at different doses differ from non-irradiated ones.

Peng at al., [6] investigated the effect of high energy beams on potato-sprouting using doses in the range of 300 Gy to 500 Gy. They concluded that the 300 Gy dose was more suitable for inhibiting sprouting and maintaining storage quality of the potatoes. Sarkar and Mahato [7] also investigated control of sprouting in the Kufri Jyoti potato variety using gamma radiation at dose of 100 Gy and 200 Gy. The 100 Gy dose applied after 30 days of harvest and storage at 15°C showed the best results.

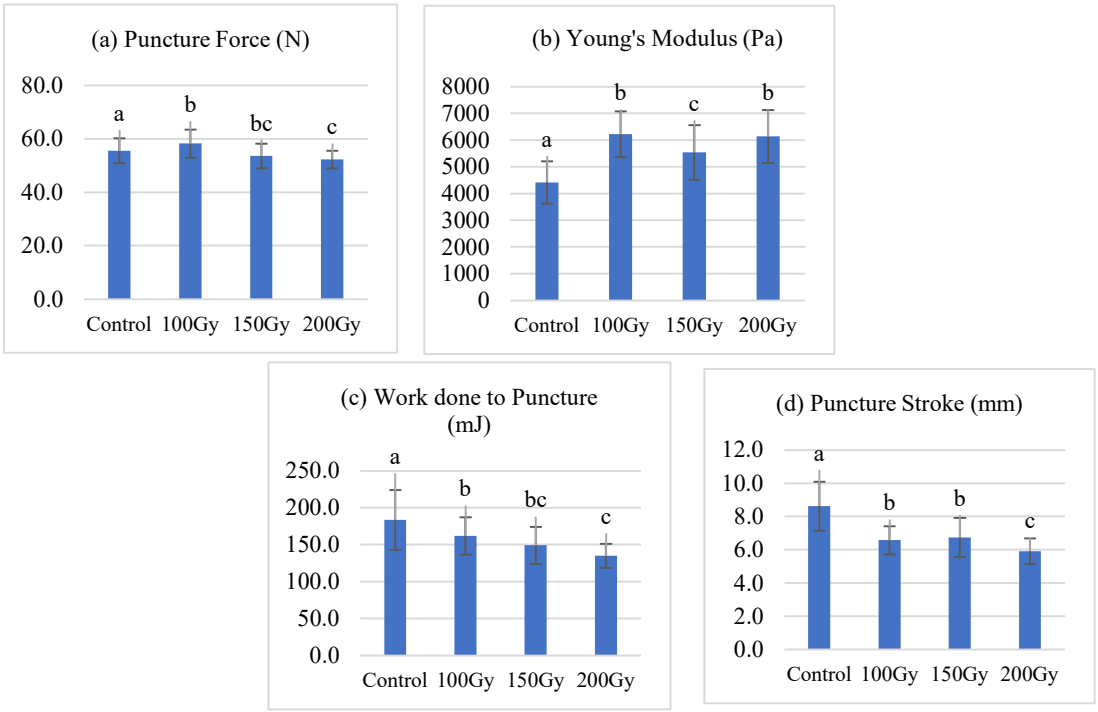

FIG. 6. Test results were expressed as puncture test (a) Young's modulus (b) work done (c) and puncture stroke (d).

4. CONCLUSIONS

Locally grown potatoes were irradiated with low energy X rays at various low doses (up to 200 Gy). Single-side irradiation was carried out and dose uniformity within potatoes was not considered in this study. Some studies have indicated that low doses of radiation can prevent sprouting and preserve potatoes. Other studies have conversely suggested that lower doses of irradiation may result in potatoes that are more susceptible to rotting, even at low storage temperature. To test this further, both irradiated and non-irradiated potatoes were incubated at room temperature (~22°C). The researchers observed significant differences including measurable physicochemical and biochemical changes between the two groups. For storage at room temperature, 150 Gy was determined as the most suitable X ray irradiation dose especially for the cv. gala variety of potato.

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EFFECT OF COBALT-60 GAMMA IRRADIATION ON SHELF-LIFE EXTENSION OF PEMPEK PRESERVATION IN INDONESIA

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Abstract

Indonesia has many islands that stretch from Sabang to Merauke. Therefore, it could take several days to transfer food or packages from one area to another. Ways of preserving traditional foods like pempek are therefore important. This study investigated edible coating for pempek with chitosan and the integration of ^{60}Co gamma irradiation (coatings of chitosan or irradiated chitosan). Cobalt gamma irradiation is known to enhance the bioactivity of chitosan solutions; chitosan is known for its potential as an edible coating material with antimicrobial properties. Control and treated pempek samples one day after treatment did not show significant differences (pempek without coating, pempek with non-irradiated chitosan coating, and pempek with chitosan coating irradiated at 10 kGy to 50 kGy). On the second day, mould was present in pempek without chitosan coating. The untreated samples also had a duller colour compared to pempek coated with non-irradiated chitosan and pempek coated in chitosan irradiated at 10 kGy to 50 kGy. Eight days post-treatment, the differences between the samples were more pronounced. Research and observations were carried out until the fourteenth day. Pempek coated in chitosan irradiated at 10 kGy to 50 kGy did not show any mouldiness, although the colour was brownish and the texture hard. The study found that chitosan irradiated at 10 kGy to 50 kGy had a lower molecular weight than non-irradiated chitosan. Gamma irradiation lowered the viscosity and decreased chitosan's surface tension significantly [($F_{calculation}$ (9.77) > $F_{criteria}$ (4.96)].

1. INTRODUCTION

Indonesia is a largely agricultural country with a very large territory consisting of 34 widely scattered provinces. The vast territory is also rich in diversity, for example in food. Every region in Indonesia has its own special food, such as pempek with its origin in Palembang, South Sumatra. Pempek (empek-empek) is made from a mixture of mashed or ground fish meat , and wheat flour. In the past, the fish meat most commonly fish meat used in making pempek was ground mackerel, because of its white flesh, aroma, texture and taste compared to other types of fish [1].

The nutritional content of pempek also varies depending on the raw materials used. Some producers add 70% of fish meat to increase the nutritional value, while others add egg fillings. Pempeks rich nutritional content makes it an excellent substrate for microbial growth, so that fish-based pempek is prone to quick spoilage [2]. This and long distances to markets (at least 2–3 days of travel/delivery from the islands to Java, and 4–5 days of Java) increase the risk of exposure to contaminated pempek. Preservation is therefore critical.

Chitosan is a natural preservative often used as a food coating as reported in previous studies such as Puspa Ayu Puswiati et al., [2] where pempek was soaked in a chitosan solution as an edible coating and affecting shelf-life. Serano et al.,[3] reported the use of chitosan active films to extend the shelf-life of a chicken product. Hussein et al., [4] also reported the use of chitosan and essential oils for the preservation of fish and fisheries products. These coatings can be improved. Gamma irradiation can degrade or reduce the molecular weight of chitosan, making it more biologically active as reported elsewhere [5].

2. MATERIALS AND METHODOLOGY

2.1. Materials

The materials and tools used included pempek mentah, chitosan, glacial acetic acid, NaOH, ethanol and aquadest. Other equipment included an oven , beaker glass , analytical balances , volumetric glass , volume pipettes, pasteur pipettes, volumetric flasks, universal pH meter, viscometer, filter paper, magnetic stirrer and stir bar.

2.2. Preparation of chitosan solution, coating, viscosity and data analysis

Four chitosan solutions were made, each consisting of 2 grams of chitosan dissolved in 200 ml of 2% acetic acid. The solution was then irradiated with a dose of 10 kGy, 20 kGy, 30 kGy, 40 kGy and 50 kGy. Pempek was coated with 100 ml of the irradiated chitosan solution and checks including odour, colour and texture were conducted every day. Visual inspection was also used to determine microbial spoilage on the surface of pempek samples. The molecular weight of the chitosan solution was measured using an Oswaltd viscometer and statistical analysis involved F-ANOVA One Way test to determine whether the ^{60}Co gamma irradiation dose significantly affected the preservation of pempek food ingredients.

3. RESULT AND DISCUSSION

The research began with the coating process of chitosan solution modified by gamma ^{60}C irradiation at doses of 10 kGy, 20 kGy, 30 kGy, 40 kGy and 50 kGy on 6 units of pempek. One pempek was not coated with chitosan and used as a control. The coating was carried out for 1 night to ensure adequate coating (Fig. 1).

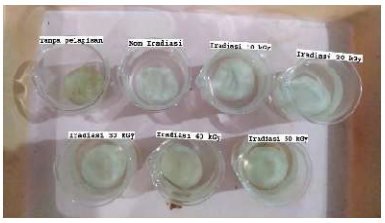

FIG. 1. Coating process for pempek.

The next day, the pempek was removed from the chitosan coating solution, left in open air and changes observed. Figures 2–11 show effects of the exposing coated pempek from days 2 to 14, for both irradiated and non-irradiated cases. Tables 1–4 summarize the appearance of pempek on days 1, 2, 8 and 14 of the experiments.

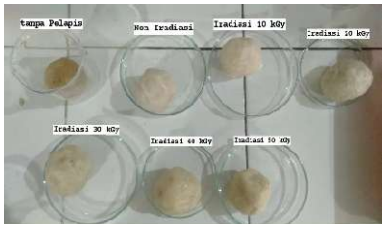

FIG. 2. First day of pempek exposure after coating.

FIG. 3. Second day of pempek exposure after coating.

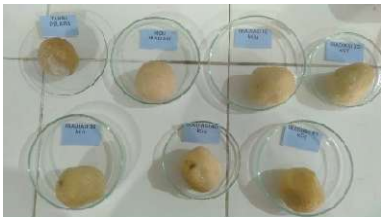

FIG. 4. Third day of pempek exposure after coating.

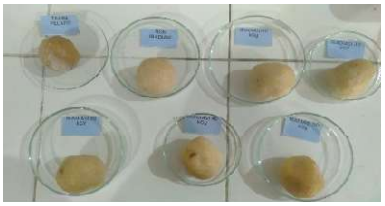

FIG. 5. Fourth day of pempek exposure after coating.

FIG. 6. Seventh day of pempek exposure after coating.

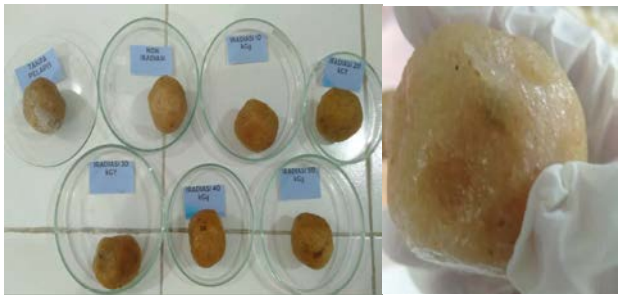

FIG. 7. Eighth day of pempek exposure after coating.

On the eighth day, pempek with non-irradiated chitosan was mouldy and the colour getting duller.

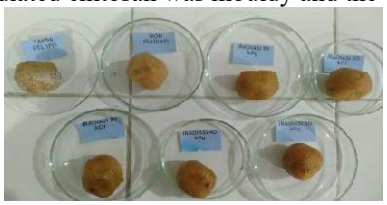

FIG. 8. Ninth day of pempek exposure after coating.

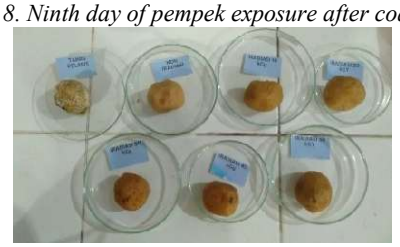

FIG. 9. Tenth day of pempek exposure after coating.

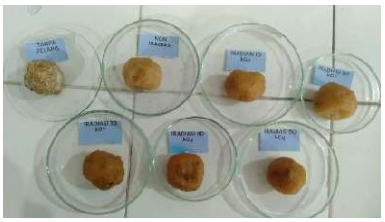

FIG. 10. Eleventh day of pempek exposure after coating.

FIG. 11. Mouldy appearance of pempek with non-irradiated chitosan on day 14.

TABLE 1. PEMPEKS' PHYSICAL APPEARANCE ON THE FIRST DAY

Sample	Texture	Colour	Odor	Bacteria/Mould
Pempek with non-irradiated chitosan coating	Still soft	White	Tasty	Not visible
Pempek with 10 kGy irradiated chitosan coating	Still soft	White	Tasty	Not visible
Pempek with 20 kGy irradiated chitosan coating	Still soft	White	Tasty	Not visible
Pempek with 30 kGy irradiated chitosan coating	Still soft	White	Tasty	Not visible
Pempek with 40 kGy irradiated chitosan coating	Still soft	White	Tasty	Not visible
Pempek with 50 kGy irradiated chitosan coating	Still soft	White	Tasty	Not visible

TABLE 2. PEMPEK'S PHYSICAL APPEARANCE ON THE SECOND DAY

Sample	Texture	Colour	Odor	Bacteria/Mould
Pempek with non-irradiated chitosan coating	Still soft	Beginning to darken	Tasty	Visible
Pempek with 10 kGy irradiated chitosan coating	Still soft	White	Tasty	Not visible
Pempek with 20 kGy irradiated chitosan coating	Still Soft	White	Tasty	Not visible
Pempek with 30 kGy irradiated chitosan coating	Still Soft	White	Tasty	Not visible
Pempek with 40 kGy irradiated chitosan coating	Still Soft	White	Tasty	Not visible
Pempek with 50 kGy irradiated chitosan coating	Still Soft	White	Tasty	Not visible

TABLE 3. PEMPEK'S PHYSICAL APPEARANCE ON THE EIGTH DAY

Information	Texture	Colour	Odor	Bacteria/Mould
Pempek with non-irradiated chitosan coating	Hard	Brownish yellow, pale	Odourless	Visible, almost on any surface
Pempek with 10 kGy irradiated chitosan coating	Start to get hard	Brownish yellow	Odourless	Not visible
Pempek with 20 kGy irradiated chitosan coating	Start to get hard	Brownish yellow	Odourless	Not visible
Pempek with 30 kGy irradiated chitosan coating	Start to get hard	Brownish yellow	Odourless	Not visible
Pempek with 40 kGy irradiated chitosan coating	Start to get hard	Brownish yellow	Odourless	Not visible
Pempek with 50 kGy irradiated chitosan coating	Start to get hard	Brownish yellow	Odourless	Not visible

TABLE 4. PEMPEK'S PHYSICAL APPEARANCE OF ON THE FOURTEENTH DAY

Information	Texture	Colour	Odor	Bacteria/Mould
Pempek with non-irradiated chitosan coating	Harder	Brownish yellow, pale	Odourless	Visible, almost on any surface
Pempek with 10 kGy irradiated chitosan coating	Hard	Brownish yellow	Odourless	Not visible
Pempek with 20 kGy irradiated chitosan coating	Hard	Brownish yellow	Odourless	Not visible
Pempek with 30 kGy irradiated chitosan coating	Hard	Brownish yellow	Odourless	Not visible
Pempek with 40 kGy irradiated chitosan coating	Hard	Brownish yellow	Odourless	Not visible
Pempek with 50 kGy irradiated chitosan coating	Hard	Brownish yellow	Odourless	Not visible

Figure 1 shows no change in each pempek, both coated with ^{60}Co gamma irradiated chitosan and those coated in non-irradiated chitosan. In Fig. 2 the pempek sample without coating started to mould and the colour began to darken 3 days after treatment. Table 1 also shows physical test-results of pempek without coating or non-irradiated, where the colour started darkening and the growth of bacteria or fungi shows on one side of the surface. Meanwhile, pempek coated with the gamma ^{60}Co irradiated chitosan solution with doses of 10 kGy, 20 kGy, 30 kGy, 40 kGy and 50 kGy did not show any significant changes different from the first day. Figs. 4–6 show that overall pempek showed no significant changes from the second day. However, in Figs. 7 and 8 the pempek without chitosan coating shows signs of spoilage with microbes observed and, pempek coated with non-irradiated chitosan started to show growth of mould on one side. The colour at this point is brownish-yellow, though pale, whereas the pempek with ^{60}Co gamma irradiated chitosan coating at doses of 10 kGy, 20 kGy, 30 kGy, 40 kGy and 50 kGy showed no visible signs of microbial growth on the entire surface, although the colour started turning brownish-yellow.

On the tenth and eleventh days (Figs 9 and 10 respectively) the pempek without chitosan coating had visible mould or bacteria growth almost all over the surface; the colour was completely brownish-yellow and pale; the texture very hard and no smell was recorded. The pempek with ^{60}Co gamma irradiated chitosan coating, still had no visible mould or bacteria growing, but the colour was dark, brownish yellow, the texture hard with no smell detected. According to Fig. 11, on the 14th day, uncoated pempek and those coated with non-irradiated chitosan showed microbial growth on the entire surface; the colour was already brownish-yellow but pale, and the texture harder. In the pempek with chitosan coating irradiated at all doses of 10 kGy, 20 kGy, 30 kGy, 40 kGy and 50 kGy, there was still no visible mould-growth on the entire surface, but the colour was brownish-yellow and there was no smell. It is therefore clear that pempek with gamma-irradiated chitosan coating would last longer on the shelf and be more resistant to microbial growth compared to the others studied. This could be due to ionizing radiation-induced modification, that can enhance chitosan's solubility and antimicrobial activity.

The gamma irradiation, degrades and breaks molecular chains. Some of the amine and amide groups in chitosan were possibly cleaved and produced ammonia gas after reacting with hydrogen radicals from irradiation. It is possible that gamma rays trigger breakage of the chitosan molecular chain into shorter length molecular chains and a reduced number of coils. The reduction in molecular chain-size is associated with the molecular weight. Typically, the greater the molecular weight, the greater the mechanical strength [5]. This may

explain the findings on viscosity as shown in Table 5 and Fig. 12 where viscosity reduces with increasing gamma irradiation doses.

The reactive amino groups and hydroxyl groups in chitosan likely play an important role in its application as a flocculant [6]. The active group in chitosan is associated with the N atom from the amine group (NH_2) and the O atom from the hydroxyl group (-OH); both have free electrons which can bind protons or metal ions to form a complex. The presence of this hydrogen interaction causes chitosan to dissolve and have binding potential. Irradiated chitosan with a lower molecular weight, would thus be more soluble and easily coat pempek.

TABLE 5. VISCOSITY OF CHITOSAN SOLUTIONS (CHITOSAN IN ACETIC ACID) TREATED WITH INCREASING DOSAGES OF IONIZING RADIATION

Sample	Viscosity (Poise)
Chitosan, non-irradiated	0.7500
Chitosan 10 kGy	0.1358
Chitosan 20 kGy	0.1293
Chitosan 30 kGy	0.0905
Chitosan 40 kGy	0.0841
Chitosan 50 kGy	0.0663

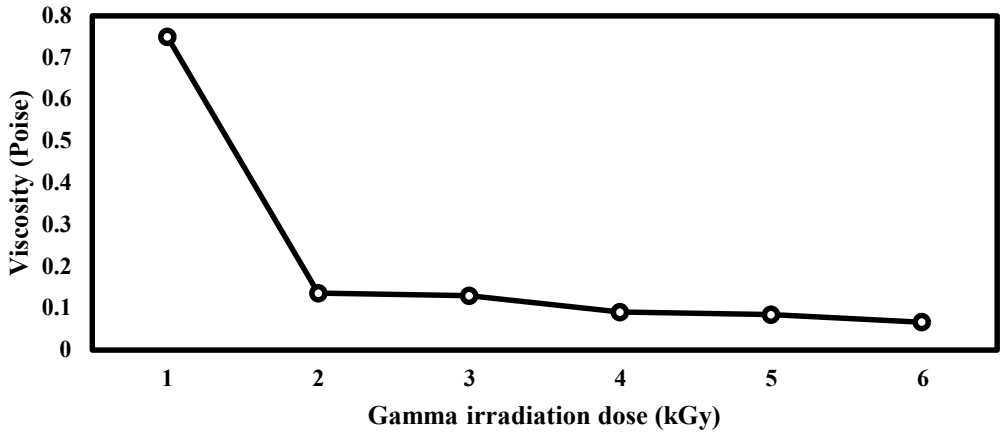

FIG. 12. Effect of various gamma irradiation doses on the viscosity of chitosan solutions used to dip and create the edible film coating on pempek.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Dose	6	150	25	350
viscosity	6	5.796	0.966	4.488

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1732.9	1	1732.9	9.77691	0.01075	4.9646
Within Groups	1772.44	10	177.244			
Total	3505.34	11				

FIG. 13. Result of ANOVA Single Way Analysis.

The findings show the importance of using irradiation and packaging as reported in other studies [7–9]. The ANOVA Single Factor (one way ANOVA) statistical analysis was performed to assess potential significant impact of gamma irradiation on the viscosity of chitosan solutions. In Table 6, the $F_{calculation}$ was 9,77691 and

$F_{criteria}$ or F_{table} was 4,9646. The $F_{criteria} > F_{calculation}$ finding shows that gamma irradiation-exposure resulted in significantly lower viscosity and decreased chitosan-surface tension, the latter improving product-coating.

4. CONCLUSIONS

A study was undertaken to investigate coating of pempek with irradiated (^{60}Co) and non-irradiated chitosan. Irradiation doses ranged from 10 kGy to 50 kGy. Pempek coated in chitosan irradiated in this range did not show any growth of moulds and bacteria while the colour was brownish and the texture hard. The irradiated chitosan had a lower molecular weight than non-irradiated chitosan, resulting in lower viscosity and a significant decrease in chitosan's surface tension both of which facilitated coating of pempek.

ACKNOWLEDGEMENTS

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EFFECT OF GAMMA IRRADIATION AND VACUUM-PACKAGING ON SHELF LIFE-EXTENSION OF GUDEG, AN ETHNIC FOOD

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Abstract

The effect of gamma irradiation and vacuum-packaging on the shelf-life of the ethnic food gudeg was investigated. Gudeg samples were packed in laminated aluminium foil and vacuum-sealed. The radiation process was carried out at room temperature, with gamma rays at doses of 0 kGy and 19.56 kGy and followed the Indonesian National Standard (SNI) for high-dose food irradiation. After irradiation, samples were held at room temperature (28±2°C) and a relative humidity of 60–70%. Chemical and microbiological changes were then observed for 16 weeks. The biochemical results revealed that both samples irradiated with large doses had no significant effect before and after irradiation based on the proximate test, water activity (a_w) pH and thiobarbituric acid (TBA) values. The TBA value for irradiation treatment remained under 1 mg of malondialdehyde (MDA)/kg for the duration of storage. The study also demonstrated that irradiation could reduce total microbial content by 2 log cycles. This study showed that gudeg irradiated at high doses could be kept for longer periods, but this could be enhanced through an optimised combination of gamma irradiation and vacuum-packaging.

1. INTRODUCTION

Indonesia is an archipelagic country with a tropical climate and awide variety of ethnic foods. The cuisine is diverse due to abundant natural resources, a sea-dominated geography, and a wealth of spices. Each region has its own distinct product. One of the famous Indonesian specialties is gudeg, typical to Yogyakarta, a region located on Java Island. This dish is made from young jackfruit cooked with coconut milk and spices [1]. Gudeg has a sweet and savoury taste and is often served with rice and a variety of toppings, including chicken, red beans, tofu, tempeh, *areh* (thick coconut milk cooked with spices) and *sambal krecek* (spicy fried cow skin). Each gudeg producer has its own cooking technique that characterizes its uniqueness.

Gudeg has a high water content and therefore cannot be stored for a long time. Traditional gudeg sellers extend the product's shelf-life by repeated heating over a certain period of time. Recently, research about extending the shelf-life of some Indonesian ethnic foods such as *empal gentong* [2] *kapurung* [3] and *gudeg* [1, 4] by canning has been successfully conducted. Canned gudeg is currently available commercially. While canning is useful for extending shelf-life, it has significant limitations. The thermal process involves excessive heating which can alter sensory features and nutritional value [5] and overcooking in the absence of close supervision altering the texture, taste, and mouthfeel. It is therefore preferable to use the best process conditions to control pathogens and enzymes while reducing food quality degradation [5, 6]. Cans are also bulky and not user-friendly.

An alternative to canning is the use of a nonthermal technique, that could significantly decontaminate foods of pathogens at low temperatures, ensuring food safety while maintaining nutritional and sensory properties [7–9]. Irradiation is one of the nonthermal processes that can be used to enhance the shelf-life of thermally sensitive food. It involves the controlled application of ionizing radiation such as gamma rays, beta rays, and X rays. Food irradiation is often performed with gamma rays from radioactive isotopes such as ^{60}Co and ^{137}Cs [10]. Previous research has shown that gamma irradiation at doses below 10 kGy could significantly reduce the number of microorganisms in dried chilies and seaweed flour [11, 12]. Ready-to-eat (RTE) foods sterilized at a high dose irradiation (45 kGy) could be stored for 1.5 years at room temperature without changing the overall quality [13]. Irradiation could increase antioxidants, and thus the nutritional status as well as the immune status of immunocompromised patients [14]. Irradiated RTE-foods also have the potential to improve nutritional status in school aged children and people in emergency situations [15, 16]. This study aimed to

examine the effect of irradiation and vacuum-packaging on nutritional and microbial quality and prolonging the shelf-life of gudeg.

2. MATERIALS AND METHODS

2.1. Sample preparation

Gudeg samples (original gudeg-GA and smoked gudeg-GB) were obtained from an RTE-food industry in Bogor city, Indonesia. Samples were primarily packed (100 g; aluminium foil contact material) under vacuum conditions (15 mbar/2.5s) and then frozen (-20°C, 24 h). Frozen samples were secondarily packed in a Styrofoam box 25×30×45cm with dry ice (1:2 w/w sample: ice) to maintain frozen conditions during irradiation.

2.2. Irradiation

Irradiation was conducted according to the Indonesian national standard (SNI) regarding food irradiation for high dose RTE food (SNI-8352-2017) [17]. The process was performed at a dose of 19.56 kGy at room temperature in the IRKA Irradiator located in South Jakarta, Indonesia (^{60}Co gamma radiation source; dose rate 1.26 kGy/h; DUR 1.08). Irradiated samples were then stored for further analysis (Fig. 1).

2.3. Proximate analysis; pH and aw value; TBA

Analysis of the water and ash content was carried out using the gravimetric method and fat content-analysis using the soxhlet extraction method. Protein analysis was carried out using the kjeldahl method and pH using a pH meter. The sample was dissolved in distilled water (1:3 w/v) then measured using a pH meter. The a_w value was measured with an a_w meter while the TBA value was measured using a spectroscopic method. The sample (10 g) was placed in a distillation flask, and 97.5 ml of distilled water; 2.5 ml of 4M HCl were added. Distillation was carried out until 50 ml of distillate was obtained, then 5 ml of the distillate was placed in a tube with a lid, 5 ml of TBA solution was added, the mixture heated in a water bath until a reddish colour formed and then cooled. The solution was then measured with a UV-Visible spectrometer at a wavelength of 528 nm. The TBA value was expressed as mg MDA/kg sample.

2.4. Total plate count (TPC)

The TPC was carried out according to Pratami et al., [18], with some changes. The samples were diluted three times using a 1% bacto peptone solution and each diluted sample (1.0 ml) was placed in the petri dish. The plate count agar liquid was 10 ml Tryptic Soy Agar (50°C, pH 7) combined with the diluted samples; the petri dish was spined. Colonies were counted after 48 hours of incubation at 35°C.

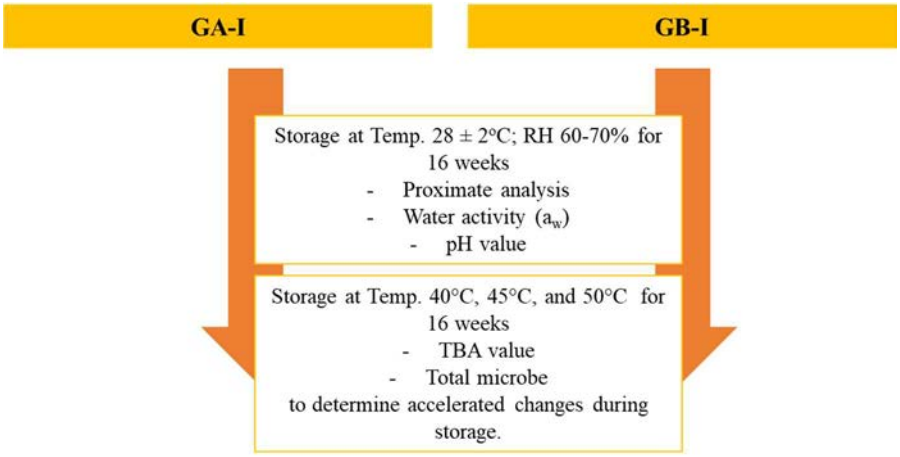

FIG 1. Storage treatment and analysis. GA-I: Irradiated gudeg A; GB-I: Irradiated gudeg B.

3. RESULTS AND DISCUSSION

3.1. pH value, water activity (aw) proximate properties

Table 1 shows the pH and water activity (a_w) values of irradiated gudeg during storage at room temperature. The table show that irradiation does not affect the pH and a_w values.

TABLE 1. pH AND WATER ACTIVITY VALUE OF IRRADIATED GUDEG AT ROOM TEMPERATURE STORAGE COMPARED TO CONTROL SAMPLES

Treatment	pH		a_w	
	0 week	16 weeks	0 week	16 weeks
GA-C	4.98 ± 0.00	—	0.91 ± 0.00	—
GA-I	4.97 ± 0.06	4.96 ± 0.08	0.91 ± 0.00	0.91 ± 0.01
GB-C	4.56 ± 0.03	—	0.89 ± 0.01	—
GB-I	4.53 ± 0.01	4.51 ± 0.02	0.89 ± 0.01	0.89 ± 0.00

***Note**: GA-C: non irradiated gudeg A; GA-I: Irradiated gudeg A; GB-C: non irradiated gudeg C; GB-I: Irradiated gudeg B; —: not available.*

TABLE 2. PROXIMATE PROPERTIES OF IRRADIATED GUDEG AT ROOM TEMPERATURE STORAGE COMPARED TO CONTROL SAMPLES

Treatment	Water (%wb)		Ash (%wb)		Protein (%wb)		Fat (%wb)	
	0 week	16 weeks	0 week	16 weeks	0 week	16 weeks	0 week	16 weeks
GA-C	78.37±0.49	—	1.40±0.23	—	2.26±0.14	—	0.01±0.00	—
GA-I	78.28±0.18	78.57±0.00	1.97±0.09	1.81±0.13	1.81±0.25	2.61±0.13	0.02±0.01	0.01±0.00
GB-C	66.74±0.82	—	1.27±0.08	—	2.03±0.09	—	0.01±0.01	—
GB-I	66.44±0.31	68.26±0.01	1.45±0.07	0.86±0.24	2.01±0.26	2.22±0.26	0.01±0.01	0.01±0.00

***Note:** GA-C: non-irradiated gudeg A; GA-I: Irradiated gudeg A; GB-C: non-irradiated gudeg C; GB-I: Irradiated gudeg B; —: not available.*

Table 2 shows no significant change in proximate properties of irradiated gudeg compared to the non-irradiated product after 16 weeks of storage. The finding indicates that the irradiation process combined with vacuum-packaging can maintain the nutritional value, a_w, and pH value of food at room temperature for 16 weeks. These findings also showed that the nutritional value of gudeg irradiated at a high dose was maintained. The irradiation process can maintain the nutritional value, freshness, and sensory quality of food products such as texture, colour, taste, and aroma as reported elsewhere [19, 20].

3.2. Lipid oxidation and the TBA value

Differences in the effect of irradiation on lipid oxidation, measured as TBA values (indicator of sample oxidation) associated with rancidity in different samples were demonstrated. The TBA values in both gudeg samples were still below permitted threshold (SNI 7764.1-2012). Generally, the maximum permitted TBA value is 3–5 mg malonaldehyde/kg. Figure 2 shows that high-dose irradiation did not significantly affect the changes in rancidity. The measurement of the TBA value of irradiated gudeg showed that both gudeg A and gudeg B samples, which were irradiated with high doses, had a TBA value below 3–5 mg malonaldehyde/kg. This result is typical of irradiation and not thermal processes that cause oxidation at high temperatures. Irradiation is thus able to maintain the nutritional quality, freshness, and organoleptic quality of food products [19].

Figure 3 shows an increase in TBA value with storage time although the value is still below the permitted threshold of 3 mg MDA/kg according to SNI 7764.1-2012. The findings show that the high-dose irradiation process combined with vacuum packaging can maintain freshness of products such as gudeg for up to 16 weeks of storage at temperatures of 40–50°C.

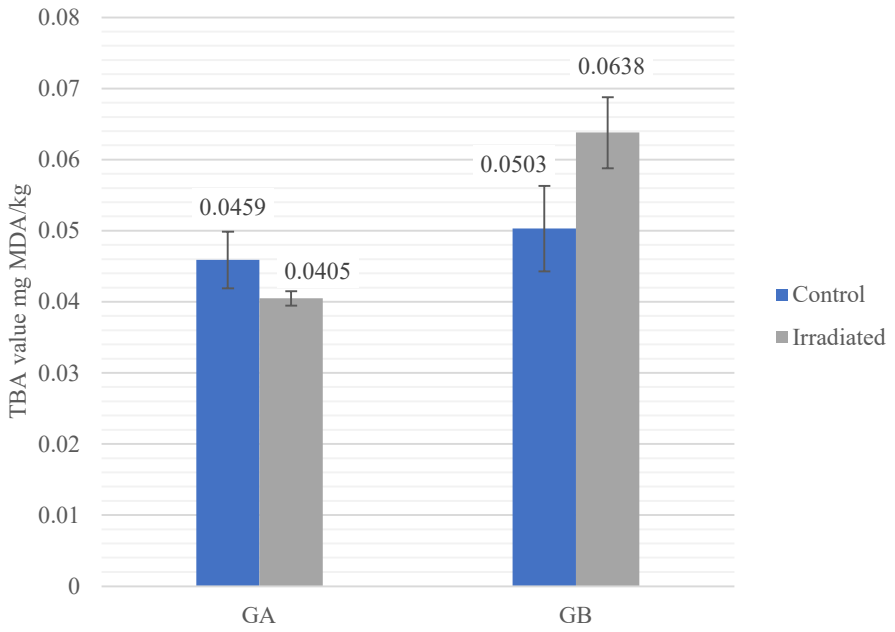

FIG 2. TBA value for control (non-irradiated) and irradiated samples. GA: gudeg A GB: gudeg B.

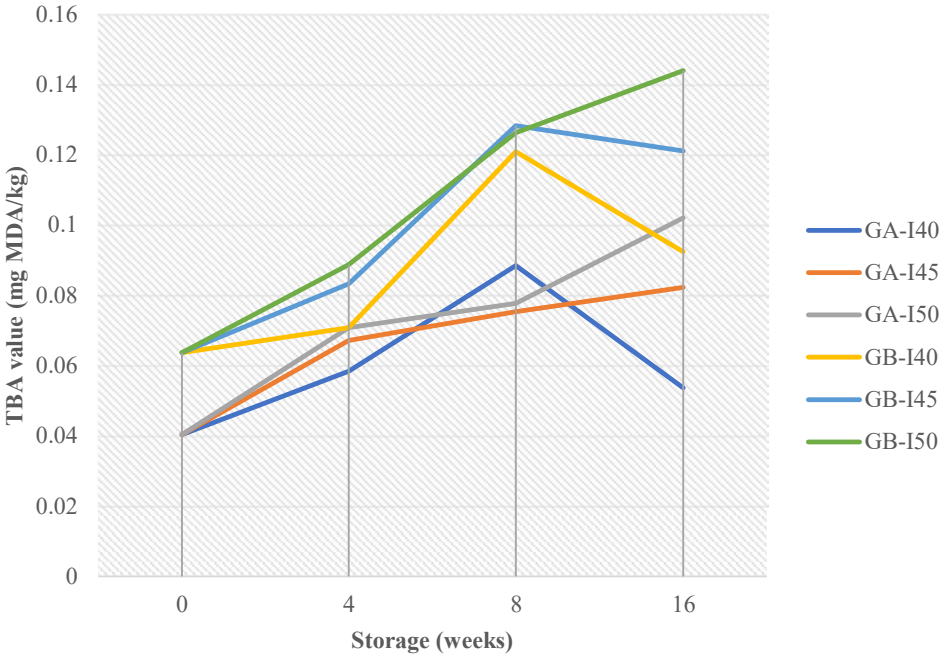

FIG 3. TBA value during storage at 40°C, 45°C and 50°C. GA-I40: Irradiated gudeg A storage at 40°C; GA-I45: Irradiated gudeg A storage at 45°C; GA-I50: Irradiated gudeg A storage at 50°C; GB-I40: Irradiated gudeg B storage at 40°C; GB-I45: Irradiated gudeg B storage at 45°C; GB-I50: Irradiated gudeg B storage at 50°C.

3.3. Microbial properties

Table 3 shows the total plate count in non-irradiated and irradiated gudeg. Irradiation is shown to be effective in killing microorganisms in vacuum-packaged gudeg. Irradiation disrupts DNA synthesis in living organisms which can ultimately inhibit the growth of pathogenic bacteria subsequently extending the shelf-life of foods [21].

TABLE 3. TOTAL PLATE COUNT (COLONIES/G) NON-IRRADIATED AND IRRADIATED GUDEG

Treatment	Non-irradiated	Irradiated
GA	2.6×10^2	—
GB	4.9×10^2	—

Note: *GA: gudeg A; GB: gudeg B; —: not detected.*

Table 4 shows that, in storage at 40–50°C for 16 weeks, the total microbes was still below the maximum allowed, which is generally 1×10^2 colonies/g the maximum limit for total microbes in sterile vegetables [22]. These results indicate that irradiated gudeg can still be stored for a longer time. Ionizing radiation can disrupt chemical bonds in DNA and other biological molecules affecting both microorganisms and insects that impact foodstuffs [21, 23].

TABLE 4. TOTAL PLATE COUNT (COLONIES/G) DURING STORAGE AT 40°C, 45°C AND 50°C

Treatment	0 week	4 weeks (°C)			8 weeks (°C)			16 weeks (°C)		
		40	45	50	40	45	50	40	45	50
GA-I	—	—	3.0×10^0	2.0×10^1	—	6.0×10^1	3.0×10^1	—	—	$3.0\text{x}10^1$
GB-I	—	—	—	1.3×10^1	—	—	1.0×10^1	—	—	—

Note*: GA-I: Irradiated gudeg A; GB-I: Irradiated gudeg B; —: not detected.*

4. CONCLUSIONS

Vacuum-packaged gudeg irradiated at a dose of 19.56 kGy shows a significantly reduced microbial load during storage for up to 16 weeks, without change in quality. At temperatures as high as 40–50°C the microbial load was still below recommended threshold. The TBA values, which indicate sample oxidation and therefore rancidity was below the set level of 3–5 mg malonaldehyde/kg, typical of irradiation compared to thermal treatment processes. Irradiation and vacuum packaging can maintain the nutritional value, aw, and pH value of food at room temperature for 16 weeks. Further studies on storage are needed to validate findings in this study on optimum storage period for irradiated gudeg. This could include investigating conventional storage for a minimum of 6–12 months.

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VOLATILE ORGANIC COMPOUNDS AND PROTEINS AS FOOD IRRADIATION MARKERS

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Abstract

The search for reliable dose-criteria for radiation processing of food products has established that oxidation products of lipids can be used as irradiation markers since lipids are easily oxidized compared to other biomacromolecules. Another effective irradiation marker is the presence of functional and structural changes in proteins since such irreversible and steady changes can show immediately after irradiation and during storage. Since the composition of volatile organic compounds (VOCs) is readily influenced by physical and chemical impacts on food products, this study aimed to identify VOCs whose concentrations can serve as indicators of bacteria-suppression in the product after irradiation. The study also aimed to establish optimal dose range by tracing microorganism suppression efficiency as well as functional and structural changes in proteins. The study compared the dose ranges in different approaches.

1. INTRODUCTION

Due to global food crises caused in part by climatic, economic, political and demographic factors that contribute to scarcity of foods, and because of constantly increasing food quality standards, food irradiation has attracted attention around the world [1]. While food irradiation can solve a wide range of problems, such as inhibiting ripening and suppressing pathogenic microflora [2, 3] it can negatively impact lipids, proteins, enzymes, and other nutrients [4–6]. Since food may be infested with various microorganisms with different radiobiological sensitivity, determining optimal dose range based on product-category is required. Food substances with low and high molecular weight whose concentrations have clear time and dose dependencies can serve as indicators of oxidation or damage to biomacromolecules and thus act as biomarkers of inappropriate/high radiation doses. Investigators including scientists and researchers from the Physics Department and Chemistry Department of Lomonosov Moscow State University in collaboration with Skobeltsyn Institute of Nuclear Physics undertook this study aimed at establishing biomarkers, whose concentrations can be used as a basis for selecting criteria for the optimal irradiation dose-range for different biological objects/substrates.

2. MATERIALS AND METHODS

2.1. Methodology

The study used real bio-objects, such as beef, turkey, chicken, salmon and trout, as well as bacterial suspensions, volatile organic compound-suspensions and bovine serum albumin suspension. The methodology of the study included irradiation of the bio-objects with 1 MeV electron accelerator. The doses absorbed by real food products and suspensions estimated using ferrous sulphate dosimetry solution ranged from 250 Gy to 10000 Gy. The dose uniformity monitored with GEANT 4 toolkit ranged from 0.6 to 0.7 depending on irradiation technique and sample size [7].

2.2. GC-MS analysis

A gas chromatography mass spectrometry method involving a gas chromatographer equipped with a headspace autosampler was used to establish volatile compound concentrations in the foods. The qualitative and quantitative analysis of chromatograms was performed using a mass spectral library and GC-MS software [8].

2.3. HPLC-MS analysis

Tests were performed to trace the structural changes in the native structure of proteins caused by irradiation. Trypsin hydrolysis of bovine serum albumin peptide bonds in 0.9% saline solution and the concentrations of three peptides (m/z=417, 461, 571) were estimated. Potential damage to the albumin structure was estimated by measuring the three peptides using an LC-MS/MS. The liquid chromatograph connected to a high resolution mass selective tandem analyser was used to measure the peptides [9].

2.4. Spectrophotometric analysis

To estimate the functional changes in proteins after irradiation, myoglobin derivatives were studied as the transformation of oxymyoglobin into metmyoglobin is a clear sign of any physical and chemical impact on a product with a high myoglobin content, such as beef. To trace the metmyoglobin level in foods after irradiation, beef samples were placed in 0.01 M phosphate buffer with 0.137 mol/l NaCl, and the wavelengths determined in the range 190–1100 nm using a UV spectrophotometer. The metmyoglobin level was estimated using mathematical processing of solution spectra [10].

3. RESULTS AND DISCUSSION

The monitoring of VOCs in meat and fish after irradiation revealed clear dose and time dependencies of aldehydes (resulting from lipid oxidation) and ethyl alcohol due to glycogen destruction as a result of bacterial fermentation. At irradiation doses of 250–5000 Gy, the concentration of lipid oxidation aldehydes peaked on day 1 and day 2 after irradiation of the meat and fish samples stored at 5°C. The ethanol content decreased in meat and fish products stored for 4 days after application of high irradiation doses. Figure 1 shows the lipid oxidation aldehyde level (a) and the ethanol content (b) in beef samples stored at 5°C during 4 days after irradiation. More high aldehyde peaks were observed at higher irradiation doses (Fig. 1a). Thus, the concentration of aldehydes can serve as a marker of lipid peroxidation, and the concentration of ethanol can serve as a marker of irradiation's bacterial suppression efficiency. The study suggests that the lower end of the optimal dose for meat and fish irradiation is determined based on the content of ethanol as a result of bacterial activity. The upper limit on the other hand is associated with the level of aldehydes. Thus, for example, when beef tenderloin is irradiated with 1 MeV electrons the lower limit is 250–350 Gy while the upper limit is 500–1000 Gy.

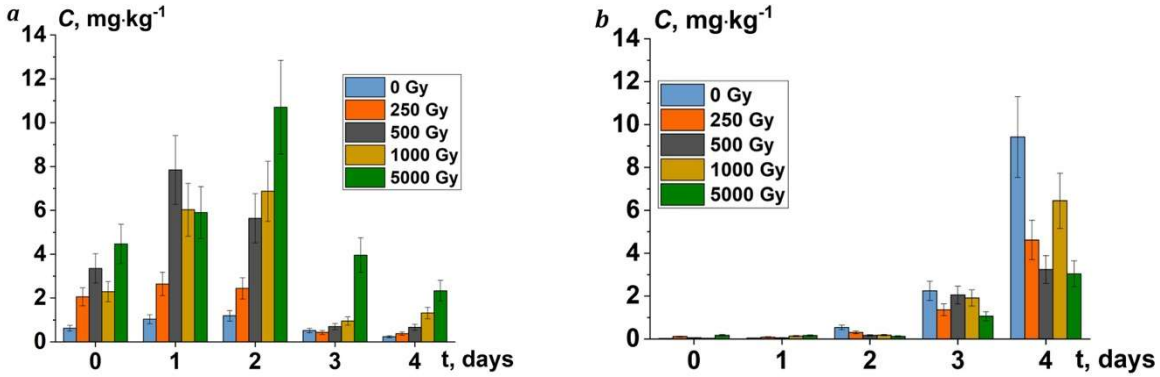

FIG. 1. Concentration of lipid oxidation aldehydes (a) and ethanol alcohol (b) in the beef samples stored at 5°C during four days after irradiation with 250 Gy–5000 Gy.

Another approach for establishing the optimal dose range by tracing the microorganism suppression efficiency as well as functional and structural changes in proteins involved the calculation of optimization function H(D) with maximum suppression of microorganisms but minimal damage to proteins. The study shows the exponential dose-dependencies of microorganism suppression in meat and fish $\varepsilon^{M}(D)$ after irradiation as well as the dose-dependencies of the extent of protein damage $\varepsilon^{P}(D)$. These can be expressed in Eq. (1) and Eq. (2)

$$\varepsilon^{M}(D)=K_1(D)\cdot(1-e^{-\alpha_M D}), \quad (1)$$

$$\varepsilon^{P}(D)=K_1(D)\cdot(1-e^{-\alpha_P D}), \quad (2)$$

Where $K_1(D)$ is a combination of the dose-uniformity, which is the ratio of the minimum absorbed dose D_{min} to the maximum absorbed dose D_{max} over the volume of the food product, and the critical dose which suppresses microorganisms or damages proteins to the required level; $\alpha^{M,P}(Gy^{-1})$ is determined by the physical parameters of irradiation, radiosensitivity and linear dimension of the microorganisms or proteins. The optimization function which can be expressed as $H(D) = e^{M}(D)\cdot(1 - e^{P}(D))$ facilitates determination of the optimal dose D_{opt}. According to the mathematical model, the maximum H_{max} of the optimization function H(D) occurs when the dose reaches the optimal level D_{opt}. The findings show that the D_{min}, D_{max} has to correspond to the H(D) or $0.9\cdot H_{max}$–H_{max}.

Figure 2a represents the experimental dose-dependency of the quantity of viable cells in beef after irradiation at 250 Gy to 2000 Gy and the function $\varepsilon^{M}(D)$ calculated using formula/Eq. (1). Figure 2a also shows the experimental dose-dependency of metmyoglobin level in beef after irradiation and the function $\varepsilon^{P}(D)$ calculated by formula/Eq. (2). According to the calculated optimization function H(D) the limits of the optimal range of beef irradiation with electrons are 220 Gy–854 Gy. Figure 2b represents the experimental dose-dependency of the rate of potential damage to native structure of bovine serum albumin in the suspension after irradiation with accelerated electrons and the function $\varepsilon^{P}(D)$ calculated by formula (2). According to the calculated optimization function H(D) the limits of the optimal range of beef irradiation with electrons are 204 Gy–755 Gy. Thus, the ranges determined by the direct measurements of microorganism suppression-efficiency and the rate of protein damage agree well with the ranges obtained by measurements of the VOC concentrations. Therefore, the suggested approaches can be used independently or complement each other.

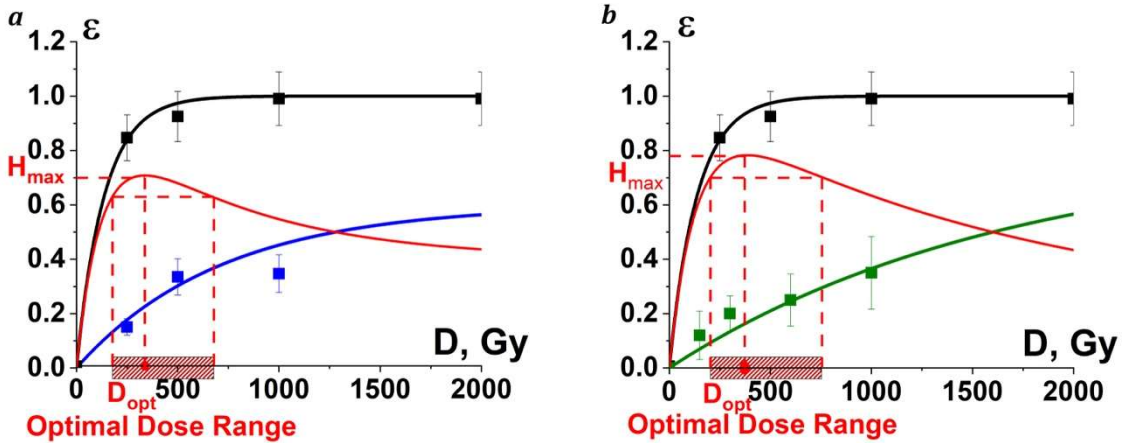

FIG. 2. Experimental dose dependency of the quantity of viable cells in beef (black squares) after irradiation and the calculated function ε^{M}(D) (black line) (a, b); a - experimental dose dependency of metmyoglobin level in beef (blue squares) after irradiation and the calculated function ε^{P}(D) (blue line); b - experimental dose dependency of the rate of potential damage to bovine serum albumin (green squares) in the suspension after irradiation and the calculated function ε^{P}(D) (green line); optimization function H(D*) (red line) (a, b).*

4. CONCLUSIONS

This research demonstrated that determining the optimal radiation dose depends on factors such as type of irradiation, energy spectrum, the dose uniformity, physicochemical food properties and storage temperature. The dose range can be influenced by the presence of biomacromolecules surrounding microorganisms and the microorganisms themselves in the food products. The analytical method used to detect microorganisms also plays a role in estimating the microbial load (including contamination) and therefore dose required.

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CHARACTERIZATION OF ACTIVATED CHITOSAN EDIBLE COATING FILM USING COBALT GAMMA IRRADIATION EXPOSURE AND ITS EFFECT ON THE PRESERVATION OF PEMPEK (A TRADITIONAL SUMATRAN FOOD)

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Abstract

Pempek is a traditional food consumed in all regions in Indonesia, but it is susceptible to microbial spoilage and contamination. Without preservation, the product has a very short shelf-life. The purpose of this research was to synthesize and characterize a chitosan edible film coating as a preservation method which is easily applied by dipping pempek into a solution and leaving it to dry. Edible films can protect food from microbial contamination, slow microbial growth and oxidation. This preliminary research involved the preparation of activated chitosan solutions using ionizing radiation (^{60}Co gamma irradiation at various doses (10 kGy to 50 kGy). Characterization of the activated chitosan edible film was carried out using Fourier Transform Infrared (FTIR) density, and weight-loss analysis. The preservation effect of activated chitosan edible film applied on pempek was observed after ten days of storage at room temperature. When characterizing the chitosan coating, the results showed that non-irradiated and irradiated chitosan films had different characteristic peaks. The 10 kGy irradiated chitosan film had a broad peak in the range 700–1300/cm, indicating skeletal C-C vibrations and cross-linking phenomenon. Irradiation significantly affected chitosan's density according to the single ways Anova analysis [$F_{calculation}$ (9.89) > $F_{criteria}$ (4.96)]. Meanwhile, irradiating chitosan did not have an effect on its percent weight loss. The visualization data shows that coating of pempek with activated chitosan edible film had significant effect on its performance after ten days of storage at room temperature. Chitosan edible film could prevent pempek from becoming mouldy. The coating could help preserve this traditional food often shipped from one part of Indonesia to the other, journeys that may take several days.

1. INTRODUCTION

In Indonesia pempek is a famous traditional food from Sumatera [1]. It is a savoury delicacy, widely consumed in all regions as a combination of fish, such as mackerel, and starch, such as sago or tapioca. It is easily perishable, especially when made with fresh fish and kept at ambient temperatures. Warm temperatures accelerate microbial growth, predisposing pempek to spoilage and microbial contamination. It is difficult to ship pempek from one region to another because of long journeys and its relatively short shelf-life of just a few days. To extend its shelf-life, traditional and modern preservation methods are employed to prevent and slow microbial spoilage.

Edible film, a thin layer of food coating, can be eaten and applied as food packaging, and its use is rising. Using edible films will not totally replace conventional food packaging, but it is an environmentally friendly preservation method as it is degradable while safe to consume. There is a recent increase in developmental studies and usage of edible films using different raw materials and ingredients based on carbohydrates, proteins, and fat. Some are also being developed with components that have antimicrobial and antioxidant properties. Active substances added to increase edible-film preservation properties include among others, plant extracts, essential oils, chlorophyll, carrageenan [2].

Active packaging is an innovative strategy for preventing lipid oxidation and protecting food from microbes. Many active substances have been investigated for antioxidant activity, preventing the formation of radicals and absorbing ultraviolet (UV) light. For example, fish fillets of rainbow trout (*Oncorhynchus mykiss,* sp.) have antimicrobial characteristics, delaying bacterial growth for 15 days [3]. Bio-composite film containing oleoresin of black pepper is the most effective packaging material in maintaining quality of bread [4]. Chitosan can prevent growth of psychotropic microorganisms and development of coliforms in cheese. The development of active edible film can extend the shelf-life of cheese and contribute to its quality and safety [5, 6].

Gelatine film from skate fish skin (*Raja kenojei* sp.) with thyme essential oil can be useful as an active packaging to extend the shelf-life of chicken tenderloin [7]. Edible film from gelatine and chitosan with essential oil (*Ferulago angulate.* sp.) inhibits the activity of *Listeria monocytogenes* and has antioxidant activity. Therefore, it can be used to inhibit microbial growth and slow lipid oxidation in cheese [8] and elsewhere. Edible film coating as active packaging mainly enhances antimicrobial and antioxidant activity. For example, the addition of orange peel extract to edible film food coatings is capable of improving physicochemical, mechanical

and antioxidant properties of a product. There are also essential oils that can be used as active substances in edible films due to their antioxidant properties and antimicrobial activity. Oregano essential oil has carvacrol and thymol as main components and it can extend the shelf-life of food when used in combination with edible films.

Chitosan is a natural biopolymer composed of β-1, 4-linked 2-acetamido-d-glucose and β-1, 4-linked 2-amino-d-glucose. It is the deacetylated derivative of chitin, a naturally abundant polymer. Chitosan is biologically active and can be used to control the growth of bacteria, fungi and yeast. Ionising radiation can be used to enhance its bioactivity by changing the physicochemical properties of chitosan as it yields simpler oligomers. An enhancement in antimicrobial activity results from the increased amounts of lower molecular weight polymer units [9]. The effect of irradiation on the antibacterial efficiency of chitosan using starch/chitosan blends irradiated at room temperature by electron beam was studied by Zhai et al., [10] who noted that chitosan's antimicrobial activity can also be enhanced by pre-treatment with ionising radiation. Chitosan films prepared using several kGys of irradiation chitosan solution exhibit significantly more antimicrobial activity in comparison to films prepared from non-irradiated chitosan solutions [11].

2. MATERIALS AND METHOD

The materials used in this study were chitosan, glacial acetate acid, sodium hydroxide, ethanol, and pempek. Chitosan solutions were prepared by dissolving 2 grams in 200 ml of 2% acetic acid. The chitosan solution was then irradiated at 10 kGy, 20 kGy, 30 kGy, 40 kGy and 50 kGy using ^{60}Co irradiation. Freshly prepared, raw pempek was dipped in 100 ml of irradiated chitosan solution. Visual checks were carried out every day, including odour, shape, and texture as well as recording of pempek's mass per day. Characteristics were monitored by FTIR and a weight-loss test.

3. RESULT AND DISCUSSION

Figure 1 shows a graph presentation of infrared spectra of activated chitosan solutions used to coat pempek and form an edible film. The solutions were activated using cobalt gamma irradiation exposure at various doses.

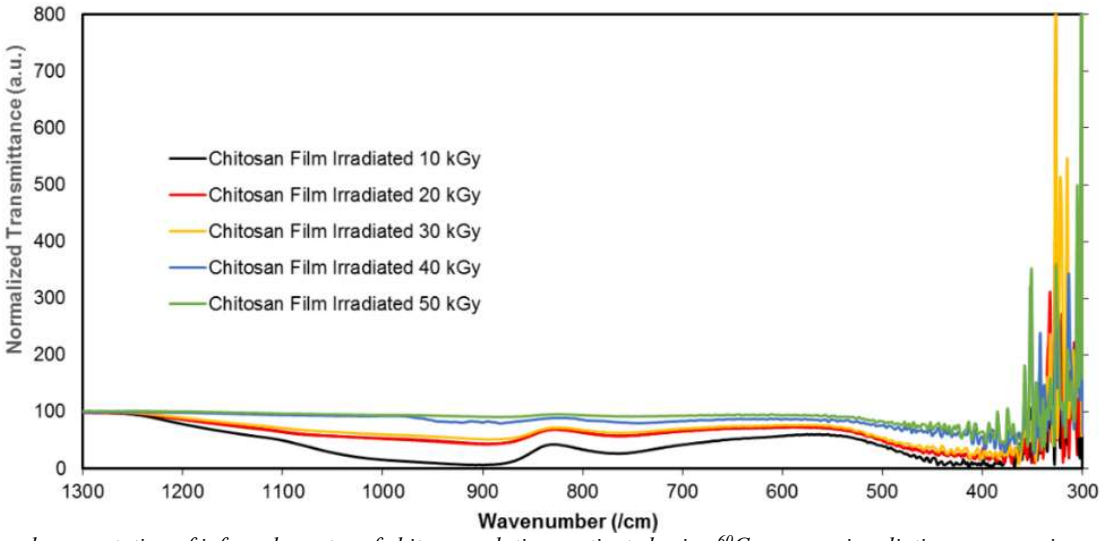
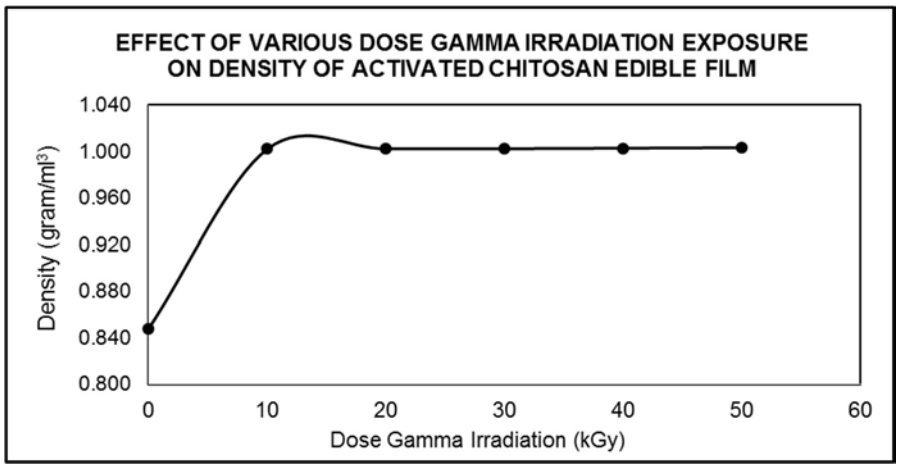


FIG. 1. Graph presentation of infrared spectra of chitosan solutions activated using ^{60}Co gamma irradiation exposure in various doses.

At 10 kGy, the peak in the range 700–1300/cm was broader than others, suggesting skeletal C-C vibrations and a cross-linking phenomenon. Irradiation resulted in free radical production and interaction with chitosan. Because of free radical-production, the surface of chitosan is rich in oxygen. Gamma radiation produces free radicals such as hydroxyl and hydrogen radicals [9]. Interaction with these radicals in solution can break chitosan's chemical bonds, modifying the chitosan and properties of the solution possibly resulting in reduced surface tension which in turn improves the pempek's coating.

Figure 2 shows the effect of various gamma irradiation dose-exposures on density of activated chitosan edible film. $F_{calculation}$ (9.89) is higher than F_{table} (4.96) suggesting that the irradiation dose on chitosan solution significantly affected its density.



Anova: Single Factor

SUMMARY

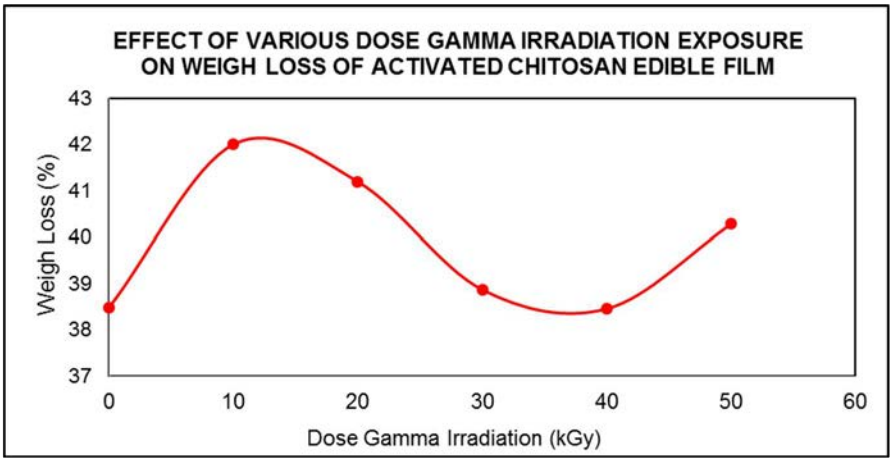
Groups	*Count*	*Sum*	*Average*	*Variance*
Dose	6	150	25	350
Densitas	6	5.8604	0.976733	0.003978

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1731.352	1	1731.352	9.893328	0.010415	4.964603
Within Groups	1750.02	10	175.002			
Total	3481.372	11				

FIG. 2. Effect of various dose gamma irradiation doses on the density of chitosan solutions used to dip and coat the pempek.

Figure 3 shows the effect of various gamma irradiation dose-exposures on weigh loss of activated chitosan edible film. $F_{calculation}$ (3.77) is lower than F_{table} (4.96).



Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Dose	6	150	25	350
%weigh loss	6	239.3	39.88333498	2.27377

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	664.541	1	664.5409807	3.77287	0.08076	4.9646
Within Groups	1761.37	10	176.1368828			
Total	2425.91	11				

FIG. 3. Effect of various dose gamma irradiation exposure on weight loss Pempek.

Pempek's performance after ten days of storage at room temperature is shown in Fig. 4 showing that various gamma irradiation doses on chitosan edible film did not significantly affect weight loss. On day 10 of storage, the pempek coated in chitosan in a non-irradiated solution had mould unlike in the irradiated coated pempek.

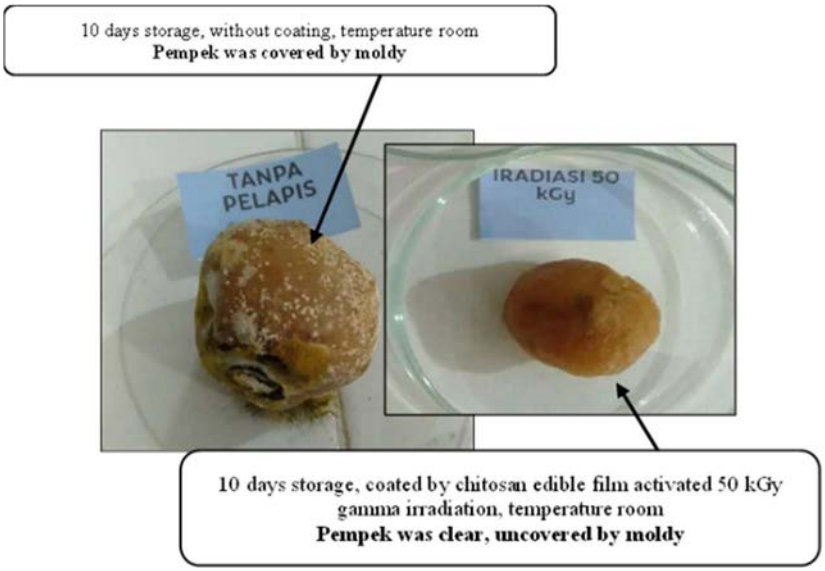


FIG. 4. Pempek's performance after ten days storage on temperature room.

4. CONCLUSIONS

Irradiated chitosan solutions have different characteristic peaks. The 10 kGy irradiated chitosan solutions had a broad peak in range 700–1300/cm suggesting skeletal C-C vibrations and cross-linking. Irradiation had a significant effect on the chitosan solution's density. Meanwhile, irradiated chitosan had no effect on weight loss of coated pempek. A coating of activated chitosan edible film on pempek significantly improved its preservation performance after ten days of storage at room temperature. Chitosan edible film can prevent microbial growth and could be a solution to challenges in shipping this food product across Indonesia over extended periods of time.

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DETECTION OF IRRADIATION IN DIETARY SUPPLEMENTS (EFFERVESCENT TABLETS) BY THERMOLUMINESCENCE

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Abstract

The aim of the research was to investigate evidence of irradiating dietary supplements in the form of effervescent tablets containing small amounts of plant ingredients using the thermoluminescence (TL) method. Selected effervescent tablets were irradiated with doses of 1 kGy and 5 kGy. The TL method was successfully used to detect irradiation. Both the high intensity of TL Glow 1 and TL Glow 2 and the registration of well-developed and defined maxima on the TL intensity curves demonstrated test reliability. This method is suitable for other irradiated products containing herbal ingredients.

1. INTRODUCTION

The human body is often exposed to harmful external factors, moreover the increasing pace of life, stress and sedentary work have their effects such as contributing to vitamin and mineral deficiencies in the body. More and more consumers now find it easier to use dietary supplements than to change their eating habits. Popular forms of supplementing vitamins and minerals are easy-to-access and tasty effervescent tablets that dissolve in water, as well as other supplements sold as tablets. The growing interest in these products [1] is due to manufacturers' claims (benefits) of excellent physical and mental condition, and disease prevention as well as increased general body performance, the latter of great interest to athletes. Dietary supplements require proper regulation. While a dietary supplement containing fewer active ingredients than claimed by the manufacturer may not affect human health, microbiologically-contaminated supplements are a concern. In order to eliminate the unintentional presence of pathogenic microorganisms in tablets or effervescent powders of dietary supplements, various disinfection methods are used, including irradiation (such as gamma ^{60}Co radiation) [2], which though safe to consumers, still arouses public suspicion. For this reason, some food manufacturers and distributors do not declare if a product is irradiated.

Supply of unlabelled irradiated food products is discouraged by the State Sanitary Inspectorate in accordance with EU Directives [3, 4] and national regulations [5]. The popular effervescent tablets are not among dietary supplements whose irradiation is effectively detected by the TL method. The European Committee for Standardisation has published standard EN 1788:2001 on the detection of radiation by TL in foods [6]. Therefore, the development of a method to detect irradiation of this product was investigated.

2. MATERIALS AND METHODS (EXPERIMENTAL)

2.1. Mode of proceedings

Nine types of dietary supplements packaged as effervescent tablets containing plant ingredients were purchased from retail stores. Their composition, labelling and the dose of gamma radiation used are presented in Table 1. Test samples (n=20 tablets) each were divided into three groups: non-irradiated as well as those irradiated at doses of 1 kGy and 5 kGy. Irradiation was performed at the Institute of Nuclear Chemistry and Technology (INCT) in the gamma source. The absorbed dose was controlled with an alanine dosimeter established guidelines [7].

TABLE 1. IDENTIFICATION OF THE SAMPLES

The composition of the sample	Labelling of the sample	Dose [kGy]
Manufacturer 1 Vitamin C=200 mg, Cranberry fruit extract DER 20:1 100 mg	T1/1/0/23	0
	T1/2/1/23	1
	T1/3/5/23	5
Manufacturer 2 Vitamin B6=1.4 mg, Vitamin B12=2.5 µg, Magnesium=56.25 mg, Caffeine=200 mg,	T2/1/0/23	0
	T2/2/1/23	1
	T2/3/5/23	5

The composition of the sample	Labelling of the sample	Dose [kGy]
Guarana extract=20 mg, Bacopa extract=20 mg, Yerba mate extract=20 mg		
Manufacturer 3	T3/1/0/23	0
Ginseng root extract=120 mg, Niacin=16 mg, Vitamin E=12 mg, Pantothenic acid=6 mg, Vitamin B2=1.4 mg, Vitamin B6=1.4 mg, Vitamin B1=1.1 mg, Vitamin A=800 µg, Folic acid=200 µg, Biotin=50 µg, Vitamin D=5 µg, Vitamin B12=2.5 µg, Iodine=150 µg	T3/2/1/23	1
	T3/3/5/23	5
Manufacturer 4	T4/1/0/23	0
Ginseng extract=40 mg, Vitamin C=80 mg, Niacin=16 mg, Vitamin E=12 mg, Pantothenic acid=6 mg, Vitamin B2=1.4 mg, Vitamin B6=1.4 mg, Vitamin B1=1.1 mg, Folic acid=200 µg, Biotin=50 µg, Vitamin B12=2.5 µg	T4/2/1/23	1
	T4/3/5/23	5
Manufacturer 5	T5/1/0/23	0
Vitamin C=1000 mg, Zinc=10 mg, Rutin=25 mg	T5/2/1/23	1
	T5/3/5/23	5
Manufacturer 6	T6/1/0/23	0
Vitamin C=1000 mg, Zinc=15 mg, Vitamin D=50 µg, Elderberry extract=150 mg, Echinacea purpurea extract=100 mg	T6/2/1/23	1
	T6/3/5/23	5
Manufacturer 7	T/27/1/23	0
Vitamin C=200 mg, Ginkgo biloba leaf extract=45 mg, Giant orange fruit extract=46 mg, Guarana seed extract=20 mg, Vitamin B6=0.67 mg, Vitamin B12=0.75 µg, Niacin=6 mg, Pantothenic acid=2 mg, Taurine=400 mg, Caffeine=120 mg, L-carnitine=5 mg, Inositol=20 mg	T/27/2/23	1
	T/27/3/23	5
Manufacturer 8	T/28/1/23	0
Lemon balm extract=25 mg, Vitamin B6=2 mg, Magnesium=75 mg	T/28/2/23	1
	T/28/3/23	5
Manufacturer 9	T/29/1/23	0
Bitter orange extract=333 mg, Black pepper extract=2.1 mg, Sodium=792 mg, Potassium=300 mg, Vitamin C=180 mg, Pantothenic acid=6 mg, Chromium=100 µg	T/29/2/23	1
	T/29/3/23	5

2.2. Procedure for isolation of silicate minerals

The basic problem to solve in detecting radiation in effervescent tablets using the TL method is the isolation of the silicate minerals from the tablets. The procedure for the separation of silicate minerals in the EN 1788:2001 [6] is based on the density method, in which the addition of sodium polytungstate increases the density of the solution where a mixture of light plant components and heavier minerals is suspended for ease of separation. Effervescent tablet solutions contain a relatively small amount of plant constituents, and the use of the density method is not appropriate and may actually complicate isolation of minerals. Thus, a modified mineral separation procedure, described below, was used in this study.

To each of nine 1000 ml glass beakers containing 200 ml of demineralized water and used in this study, 20 effervescent tablets were gradually added one by one to prevent rapid foaming. After dissolving, the beakers were filled with water to approximately 800 ml, ultrasonicated twice for about 30 mins and then left to settle overnight for the separated minerals to settle. The solutions were then carefully decanted, leaving the mineral fraction intact at the bottom. Hydrochloric acid (3M HCl) was carefully added to the beakers with heavy foaming observed due to the decomposition of carbonates in the sediment. The suspension obtained was diluted with demineralized water and left to stand for about 30 mins also for the silicate minerals to settle. The excess solution was slowly poured off and the supernatant mineral sediment carefully transferred to a centrifuge. The minerals were washed with water and then twice with acetone, and then applied to the TL measuring disc with silicone spray used to fix the mineral deposit on the disc. The discs with the silicate minerals were stored overnight in a laboratory oven at 50°C ± 5°C, drying off.

2.3. TL measurements and recording of the TL glow curves

The thermoluminescence measurement was performed using a TL/OSL thermoluminescence reader as recommended elsewhere [6]. Operation of these units is presented elsewhere [8].

3. RESULTS AND DISCUSSION

Results of the TL measurements are presented in Table 2. A reliable proof of food irradiation in the TL method is the analysis of TL glow curves. The TL glow ratio (Glow 1/Glow 2) measured in the temperature range of 150–250°C has to be higher than 0.1 (Table 2, column 4). Also the TL Glow 1 curve has to show a clear maximum at 150–250°C Table 2, column 5).

TABLE 2. TEST RESULTS

Sample reference	TL intensity		Glow 1/Glow 2, k_{TL}	TL max. Glow 1 [°C]	TL max. Glow 2 [°C]	Sample classification of
	Glow 1 (150–250°C) [counts/s]	Glow 2 (150–250°C) [counts/s]				
T1/1/0/23	861	1110340	0.0008	> 300	206	non irradiated
T1/2/1/23	549151	1082508	0.5073	189	189	irradiated
T1/3/5/23	970723	958206	1.0131	193	185	irradiated
T2/1/0/23	7427	5759528	0.0013	> 300	200	non irradiated
T2/2/1/23	238482	3079533	0.6619	208	204	irradiated
T2/3/5/23	12351335	545615	2.2644	193	194	irradiated
T3/1/0/23	10985	175859	0.0625	196	193	irradiated
T3/2/1/23	45281	69726	0.6494	181	210	irradiated
T3/3/5/23	133844	206958	1.2514	180	197	irradiated
T4/1/0/23	870	767056	0.0011	—	206	non irradiated
T4/2/1/23	440098	1533908	0.2869	232	217	irradiated
T4/3/5/23	525633	732023	0.7181	215	204	irradiated
T5/1/0/23	169	463105	0.0004	—	202	non irradiated
T5/2/1/23	2865	70897	0.0404	238	227	irradiated
T5/3/5/23	4088	25386	0.1610	208	211	irradiated
T6/1/0/23	300	456526	0.0007	> 300	166	non irradiated
T6/2/1/23	160820	189974	0.8465	202	201	irradiated
T6/3/5/23	646831	266460	2.4275	202	191	irradiated
T27/1/0/23	410	254751	0.0016	> 300	185	non irradiated
T27/2/1/23	370679	1491471	0.2485	191	172	irradiated
T27/3/5/23	239223	265825	0.8999	214	200	irradiated
T28/1/0/23	1745	14385980	0.0001	> 300	193	non irradiated
T28/2/1/23	2253475	3795038	0.5938	204	187	irradiated
T28/3/5/23	6365517	4533201	1.4042	212	193	irradiated
T29/1/0/23	7923	2408351	0.0033	> 300	197	non irradiated
T29/2/1/23	141819	947426	0.1497	212	183	irradiated
T29/3/5/23	689354	1752034	0.3935	212	197	irradiated

Silicate minerals were isolated from samples of effervescent tablets using the modified mineral separation method. Those irradiated at doses of 1 kGy and 5 kGy were clearly identified as irradiated (Table 2, column 7).

The TL glow curves at these doses showed characteristic peaks at 180–238°C (Table 2, column 5). Ideally at 150 to 250°C where, according to the standard, only the glow of irradiated samples is observed. The TL Glow 1/Glow 2 ratios (k_{TL}) of the samples irradiated at these doses were also higher than the critical value of 0.1, except for one sample. For most samples irradiated at a dose of 1 kGy, the ratios were in the range of 0.1497–0.8465, and for samples irradiated at a dose of 5 kGy, the range was 0.1610–2.2644 (Table 2, column 4).

A less clear result was obtained for a sample irradiated at a dose of 1 kGy (T5/2/1/23). The TL Glow 1/ Glow 2 ratio for this sample was slightly less than 0.1 and equal to 0.0404 (Table 2, column 4). On the other hand, in the TL glow curve of this sample, a peak at a temperature of 238°C could be distinguished (Table 2, column 5 and Figs 1–10) specifically within the range of 150–250°C typical for irradiated samples. The EN 1788:2001 standard states that if the Glow 1/Glow 2 ratio is lower than 0.1 (samples marked:T3/1/0/23 and T5/2/1/23) as in the case of the tested sample, and if a clearly outlined Glow 1 peak is observed in the range of 150–250°C, this is sufficiently reliable evidence that the sample was irradiated [6].

The method used for the separation of silicate minerals facilitated a clear assessment of all tested sets of effervescent tablets purchased in retail shops. A sample from manufacturer 3 (T3/1/0/23) was determined to be irradiated (Table 2, column 4). The label on the packaging of this sample did not provide the required information. Below are examples of Glow 1 and Glow 2 curves for silicate minerals isolated from effervescent tablets during the test: Manufacturer 3 (Sample T3)–Fig. 1 (Glow 1) and Fig. 2 (Glow 2) and Manufacturer 7 (Sample T27)–Fig. 3 (Glow 1) and Fig. 4 (Glow 2) recorded in a non-irradiated sample (0 kGy) and in samples irradiated at doses of 1 kGy and 5 kGy.

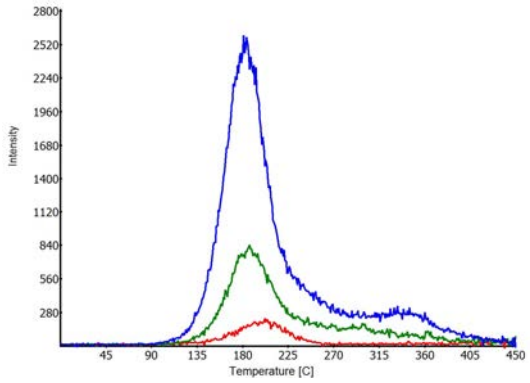

FIG. 1. Glow 1 curves of minerals isolated from Manufacturer 3 effervescent tablets of non-irradiated, 0 kGy (red curve) and irradiated at 1 kGy (green curve) and 5 kGy (blue curve).

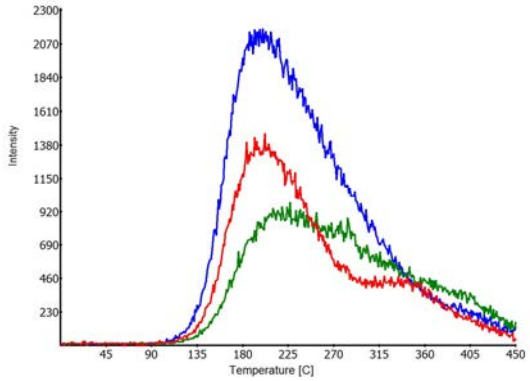

FIG. 2. Glow 2 curves of minerals isolated from Manufacturer 3 effervescent tablets of non-irradiated, 0 kGy (red curve) and irradiated at 1 kGy (green curve) and 5 kGy (blue curve).

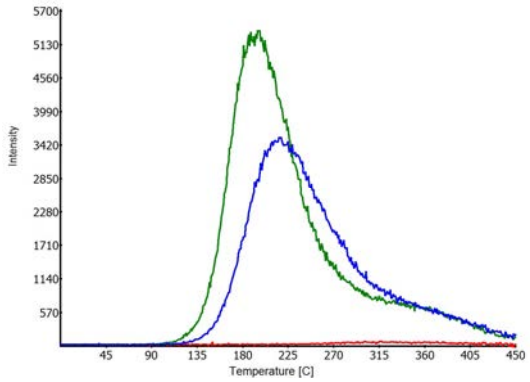

FIG. 3. Glow 1 curves of minerals isolated from Manufacturer 7 effervescent tablets of non-irradiated, 0 kGy (red curve) and irradiated at 1 kGy (green curve) and 5 kGy (blue curve).

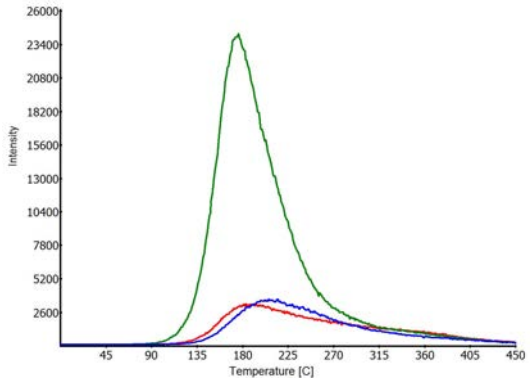

FIG. 4. Glow 2 curves of minerals isolated from Manufacturer 7 effervescent tablets of non-irradiated, 0 kGy (red curve) and irradiated at 1 kGy (green curve) and 5 kGy (blue curve).

4. CONCLUSIONS

The suitability of the thermoluminescence (TL) method in identifying irradiation is demonstrated following tests on effervescent tablets. The high intensity of TL Glow 1 and TL Glow 2 as well as the well-developed and defined maxima on the TL intensity curves confirm the reliability of test results obtained using the modified method. This method could also be used to test other irradiated products such as those containing herbal ingredients from which it is not possible to isolate a sufficient amount of silicate minerals according to European standard EN 1788:2001.

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THE STATUS OF FOOD IRRADIATION IN JORDAN

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Abstract

A little over 5 years ago, irradiation was only used in the medical industry in Jordan. Recently, the Jordan Food and Drug Administration (JFDA) and the Energy and Minerals Regulatory Commission approved gamma irradiation for herbs, spices, and nuts only. This approval has encouraged the food industry to utilize gamma irradiation for their raw materials. This trend is reflected in the growth of the industry, with products now being distributed worldwide and needing to meet various regulations. The herbs and spices industry typically irradiates raw materials to eliminate pathogenic bacteria and significantly reduce total bacteria, mould, and yeast counts. However, contamination can occur during processing and packing in less-than-optimal conditions. The paper reports the investigation of gamma irradiation dose required to eliminate all pathogenic bacteria, and to reduce the total bacteria, mould, and yeast counts. It also aimed to address contamination that may occur during processing and packing. Specimens of final herbal products were tested at three different selected doses: 3 kGy, 5 kGy, and 7 kGy. The study found that the optimum dose needed to meet the standards is 7 kGy. Five different herbs were investigated.

1. INTRODUCTION

After the JFDA and EMRC approved gamma irradiation for food, specifically herbs, spices, and nuts, the country's food industry has increasingly embraced this technology. This growth is depicted in Table 1 which illustrates the quantities of irradiated herbs, spices, and nuts over the past five years. A range of standards such those issued by the Gulf Cooperation Council [1] are followed.

TABLE 1. GROWTH IN QUANTITIES OF IRRADIATED HERBS, SPICES, AND NUTS OVER FIVE YEARS

Year	Herbs for tea Tons	Spices Tons	Medical Herbs Tons	Nuts Tons	Total Tons
2023	116.44	35.53	9.84	19.5	181.31
2022	130	25.3	5.3	17.4	177.7
2021	83	12.1	1.2	8.9	105.2
2020	28.9	5.6	0.5	8.4	43.4
2019	26.9	4.3	0.4	5.6	37.2

The herbal tea industry processes and packs its final products under minimum clean conditions, which can lead to contamination. Moreover, raw herbs pose a challenge due to high contamination levels, as they are typically grown in hot and humid climates where irrigation can partially or fully cover the herb plants. However, drying these herbs for tea purposes can significantly reduce contamination levels, although they may still exceed standard limits.

2. MATERIALS AND METHODS

The company Alattar, one of Jordan's leading producers of herbal teas, sought to irradiate its raw herbs to a specific dose. Their objectives were twofold: firstly, to eliminate all pathogenic bacteria and reduce the total bacterial, mould, and yeast counts, and secondly, to mitigate contamination occurring during processing and packing to remain below specified limits. A ^{60}Co irradiator was used.

3. RESULTS AND DISCUSSIONS

The results for the total aerobic count are presented in Table 2. As shown, the effect of the 3 kGy dose is sufficient to meet the limits (except for mountain anise) while the 5 kGy dose is more suitable. The 7 kGy dose

exceeds the requirements by at least one digit. For *E. coli*, all three selected doses are sufficient to meet the limits Table 3 and *Salmonella* was not detected at any of these doses Table 4.

TABLE 2. TOTAL AEROBIC PLATE COUNT IN DIFFERENT SUBSTRATES FOLLOWING IRRADIATION

Total aerobic count <1.0×10^3 cfu/g [2]			
Herb name	Dose 3 kGy	Dose 5 kGy	Dose 7 kGy
Mint	2.0×10^2 cfu/g	1.1×10^2 cfu/g	1.0×10^2 cfu/g
Cumin	1.4×10^2 cfu/g	1.3×10^2 cfu/g	1.2×10^2 cfu/g
Melissa	2.6×10^2 cfu/g	1.8×102 cfu/g	40 cfu/g
Wild thyme	2.5×10^2 cfu/g	90 cfu/g	<10 cfu/g
Mountain anise	1.4×10^3 cfu/g	2.3×10^2 cfu/g	<10 cfu/g

TABLE 3. *E. COLI* LEVELS IN DIFFERENT SUBSTRATES FOLLOWING IRRADIATION

E. coli <10 cfu/g [3]			
Herb name	Dose 3 kGy	Dose 5 kGy	Dose 7 kGy
Mint	<10 cfu/g	<10 cfu/g	<10 cfu/g
Cumin	<10 cfu/g	<10 cfu/g	<10 cfu/g
Melissa	<10 cfu/g	<10 cfu/g	<10 cfu/g
Wild thyme	<10 cfu/g	<10 cfu/g	<10 cfu/g
Mountain anise	<10 cfu/g	<10 cfu/g	<10 cfu/g

TABLE 4. *SALMONELLA* LEVELS IN DIFFERENT SUBSTRATES FOLLOWING IRRADIATION

Salmonella detection absent/25g [4]			
Herb name	Dose 3 kGy	Dose 5 kGy	Dose 7 kGy
Mint	absent/25g	absent/25g	absent/25g
Cumin	absent/25g	absent/25g	absent/25g
Melissa	absent/25g	absent/25g	absent/25g
Wild thyme	absent/25g	absent/25g	absent/25g
Mountain anise	absent/25g	absent/25g	absent/25g

The 7 kGy dose was chosen due to operational limitations; reaching 5 kGy was not feasible. For one cycle of operation, more than 3 kGy was achieved, and for two cycles of operation, 7 kGy was achieved. After selecting the 7 kGy dose, the next step was to test processed and packed final products. According to GSO-1016-2015-E–Microbiological Criteria for Foodstuffs, issued by the Gulf Cooperation Council Standardization Organization (GSO) [1], the required total coliform count is < 1.0×10^2 cfu/g. Additionally, other requirements such as total aerobic count, anaerobic count, and total mould and yeast count are mandated in countries such as Saudi Arabia [8].

As illustrated in Table 5, the total aerobic count is one digit below the maximum value. Both the anaerobic count and total coliform count are within acceptable limits. The total mould and yeast count also falls one digit below the maximum value. These results support the adoption of the 7 kGy dose as necessary for irradiating the raw herbs.

TABLE 5. MICROBIAL LEVELS IN THE IRRADIATED FINAL PRODUCT

Herb name	Total aerobic count [2] <1.0×10^3 cfu/g	Anaerobic count [5] <10 cfu/g	Total coliform [6] 1.0×10^2 cfu/g	Total mould and yeast count [7] <1.0×10^2cfu/g
Mint	2.1×10^2	<10 cfu/g	<10 cfu/g	40 cfu/g
Cumin	3.3×10^2	<10 cfu/g	<10 cfu/g	90 cfu/g
Melissa	2.6×10^2	<10 cfu/g	<10 cfu/g	60 cfu/g
Wild Thyme	1.9×10^2	<10 cfu/g	<10 cfu/g	90 cfu/g
Mountain Anise	8.3×10^2	<10 cfu/g	<10 cfu/g	60 cfu/g

4. CONCLUSIONS

The use and effectiveness of ionizing radiation in ensuring the safety of several foods such as spices and herbs while meeting regulatory and market standards is demonstrated in Jordan. Three doses of 3 kGy, 5 kGy and 7 kGy were investigated providing a basis for selecting the lowest dose. Only five years prior, irradiation was used solely in the medical industry in Jordan, but growth in the food industry has been reported. Companies such as Alattar, a major producer of herbal tea, embrace the technology.

ACKNOWELDGMENTS

The authors thank the management of the Jordan Atomic Energy Commission for supporting this study

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ANALYSIS OF RADIOTOLERANCE IN THE DEVELOPMENTAL STAGE OF *DIATRAEA SACCHARALIS*

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Abstract

The study aimed at comparing various pest species for radiotolerance. The investigation started with the sugarcane borer *Diatraea saccharalis* Fabricius (*Lepidoptera*: Crambidae) a significant agricultural pest in South America due to its destructive impact on sugarcane, corn, rice, and wheat. To assess the impact of gamma radiation (^{60}Co) on both larval and pupal development stages, the pests were reared on an artificial diet under controlled conditions. Larvae were subjected to doses ranging from 100 Gy to 200 Gy, while pupae were exposed to doses in the range 150–350 Gy at a rate of 14 Gy/h. The percentage inhibition of emergence of normal adults was measured in both cases. Findings indicate that pupae exhibit greater radiation tolerance; larvae demonstrated complete inhibition above 100 Gy, whereas pupae showed 48–57% inhibition within the range of 150–350 Gy. Further investigations are warranted to determine an effective dose resulting in complete inhibition for the pupa-development stage. Larvae were more radiosensitive than pupae.

1. INTRODUCTION

The work done in this report is part of a project 'Quarantine treatment of food products and market promotion' under the IAEA coordinated research project D61026. The goal is to compare different economically important agricultural pests and determine the most radiation-resistant. The research started with *Diatraea saccharalis*, a pest present in all regions where sugar cane is grown. It is an American specie distributed from the south of the United States of America to Argentina, reaching 40° south latitude in the province of Buenos Aires. It attacks sugar cane, corn, sorghum, wheat and rice, as well as forage grains such as oats, rye and millet [1].

In Argentina, the province of Tucumán, the highest producer of sugar cane (66% of national production) is the most affected. The pest form galleries in the stems, which reduces the weight and number of stems produced per hectare [2]. It is also responsible for the death of the main shoot or guide shoot, if the damage is caused during the first months of the cane's age, and for the proliferation of lateral shoots when the crop is mature. Furthermore, these perforations produced are gateways to other fungal and bacterial diseases, causing damage such as red rot due to the fermentation of the sugars [3]. This pest also causes the greatest losses in corn crops, as well as damage to sorghum, although its impacts have not been studied and quantified as exhaustively as in the case of sugarcane and corn [4].

The objective of this study was to investigate the effects of gamma radiation on the developmental stages of both the larva and pupa of *D. saccharalis* to determine at which stage it shows greater radiotolerance.

2. MATERIALS AND METHODS

2.1. Rearing of *D. saccharalis*

D. saccharalis was reared in the Ezeiza Atomic Center´s entomology laboratory, Argentina. It fed on artificial diet as reported elsewhere [5], in a climate chamber with controlled temperature of 26 ± 2°C, relative humidity 70 ± 10% and a 12 h photoperiod.

2.2. Radiosensitivity test on *D. sacharalis* larvae

The tests were carried out in March and May 2023. Polypropylene boxes 25 cm long, 15 cm wide and 10 cm high were used. Stage V larvae were placed in polypropylene vials of 8 cm high and 2.5 cm in diameter and covered with cotton plugs (Fig. 1). The dose range used was 100–200 Gy and a control was included. The number of individuals per dose used was 100. Once irradiated, the larvae were checked once a week and the trial ended after 90 days. Three replicates of this trial were carried out and the absence of normal adults emerging analysed, Fig. 2.

FIG.1: Stacked boxes containing polypropylene tubes. Each vial contains a last instar larva and a portion of diet, and a cotton plug is placed to prevent the larva from escaping.

FIG. 2: Images of abnormal adults that have emerged. They cannot fully emerge from the cocoon, are unable to fly and leave offspring.

2.3. Radiosensitivity test on pupae of *D. sacharalis*

The tests were carried out from October to December 2023. The same boxes were used as for the radiosensitivity assay in larvae state as previously described (Fig. 1). The scheme and the distribution of the vials was the same as shown in Figs 3A and 3B. The polypropylene vials used (5 cm high and 4 cm in diameter) contained a single pupa and were capped with a plastic lid (Fig. 3C). The dose range used was 150–350 Gy and the number of pupae per dose was around 200. Once irradiated, the pupae were observed once a week and the trial ended after 30 days. Two replicates of this trial were carried out and the absence of normal adults analysed (Fig. 2).

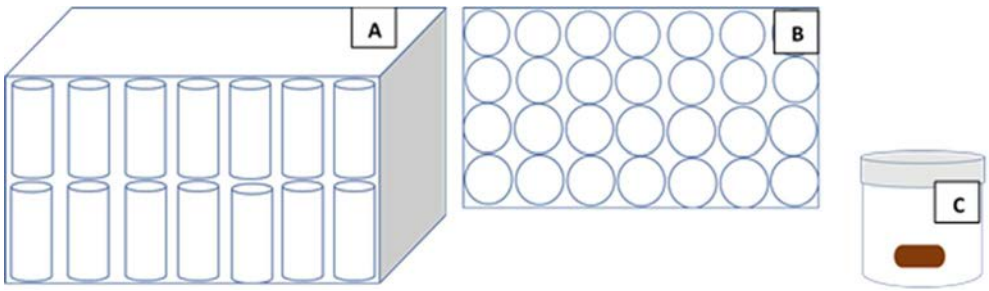

FIG. 3: Scheme A, front view of boxes with vials of D. sacharalis pupae at the time of irradiation. Scheme B, top view of the box with vials. Scheme C, A vial containing a pupa.

2.4. Irradiation and radiosensitivity tests

Samples were irradiated at the Semi-Industrial Plant of the Ezeiza Atomic Centre, in a ^{60}Co source with an activity of 600000 Ci (Fig. 4). Absorbed dose was determined with alanine dosimeters on each box; an alanine-EPR system was used. Two dosimeters were placed in the boxes, one in the area of maximum and the other in the area of minimum absorbed dose. For dose uniformity (DU) the boxes were rotated 180° at half the irradiation time. After irradiation the dosimeters were analysed in the High Dose Dosimetry Laboratory of the National Atomic Energy Commission. The DU was calculated for each irradiation.

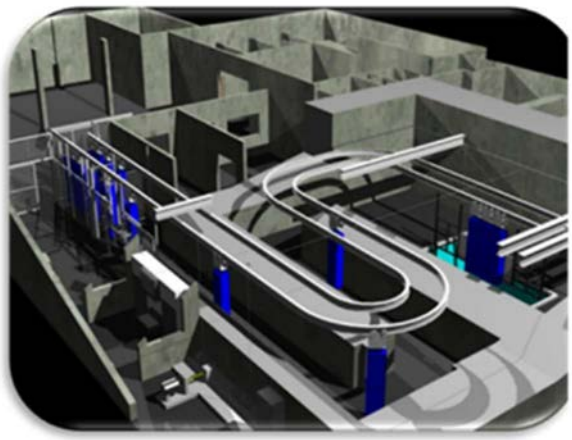

FIG 4: Diagram of the interior of the semi-industrial irradiation plant, National Atomic Energy Commission.

3. RESULTS AND DISCUSSION

3.1. Rearing of *D. saccharalis*

The eggs have a diameter ranging from 0.9 mm to 1.3 mm; laid eggs are creamy white in colour, turning orange as the embryo develops (Fig. 5 A). The larvae hatch around 6 days after oviposition. They are 1.5 mm to 2 mm long, with a brown head and prothoracic shield, the rest of the body is yellowish white, covered by setae. At its full development a larva reaches a length of 22 mm to 35 mm. The larvae usually undergo 5 molts, lasting between 20 days and 30 days (Figs 5 C and D). The pupa measures 12 mm to 22 mm in length, has a light brown colour which turns dark brown close to emergence. The pupal stage lasts from 7 days to 13 days, depending on the environmental temperature. (Fig. 5 E). Adults are straw-coloured moths, 1.5 cm–1.7 cm in length. They have the palps extended forwards, a typical characteristic. They also have two oblique dark lines and a central point on the forewings. The females are bigger than the males (Figs 5 F and G).

Diatraea saccharalis is a major concern to the sugarcane industry and calls for integrated countermeasures. One of the potential measures is the use of hyphomycetes fungi that hibernate in the pest [6]. Use of pesticides is another option. However, due to challenges in managing the pest with pesticides alone, the use of Bt corn MON810, transformed with a gene from *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) has also been explored [7]. The larvae of *D. saccharalis* (Fabricius) which are not only a problem to the sugarcane industry but to maize and sorghum as well, have been investigated under laboratory controlled conditions to facilitate strategies for the management and control of the pest's populations [8]. The biological control of the pest with mass rearing of relevant parasitoid and optimum diet have also been investigated as an additional control measure [9].

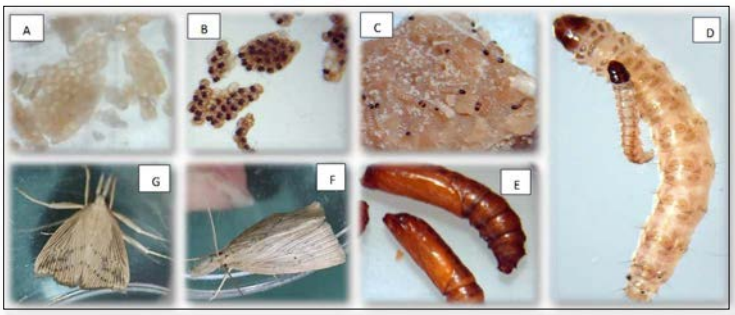

FIG. 5: A: Newly laid eggs.; B: eggs with larvae to emerge.; C: newly emerged larvae on artificial diet.; D: third and the last larval development stages with a brown head and prothoracic shield, the rest of the body is yellowish White covered by setae.; E: pupa stage with dark brown coloration.; F: adult female.; G: adult male.

3.2. Radiosensitivity test on *D. sacharalis* larvae

Larvae were subjected to 5 different doses of 100 Gy, 125 Gy, 150 Gy, 175 Gy and 200 Gy. About 200 individuals were exposed at each dose and a control without irradiation was included. Table 1 shows the absorbed dose received for each target dose, the minimum, maximum and average absorbed dose, the dose rate and the DU. The DU was in a range of 1.01–1.08. The dose rate for all was 14 Gy/min.

TABLE 1. RADIOSENSITIVITY TEST IN LARVAE *D. SACCHARALIS* ANALYSIS OF THE DOSE ABSORBED BY THE SAMPLES

Dose targeted (Gy)	Minimum dose, D_{min} (Gy)	Maximum dose, D_{max} (Gy)	Dose Uniformity ratio D_{max}/D_{min}	Dose rate (Gy/min)	Absorbed dose (X)
100	86.4	102.4	1.08	14	94.4
125	121.9	123.3	1.01	14	122.6
150	140.0	147.0	1.05	14	143.5
175	165.0	178.4	1.08	14	171.7
200	188.5	205.0	1.08	14	196.7

4. CONCLUSIONS

A 99.9% inhibition of the emergence of normal *D. saccharalis* adults was demonstrated at a dose of 155 Gy during radiosensitivity tests on larvae of the development stages. The inhibition rate for the pupal stage was 60% at doses between 150 Gy and 350 Gy. Based on these findings, a decision was taken to continue with additional tests for the pupal stage of development, using the same dose range (150–400 Gy). Adult-sterility rather than adult-emergence would be evaluated. Literature shows that the pupal stage of *D. saccharalis* is present in the host agricultural commodity. Continued research and testing to determine the dose-efficacy for quarantine purposes during the pupal stage of development is therefore encouraged.

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DEVELOPMENT OF GRANOLA BARS USING LOCAL RAW MATERIALS AS AN EMERGENCY FOOD AND EVALUATING THE EFFECT OF GAMMA IRRADIATION ON NUTRITIONAL QUALITY AND SENSORY ATTRIBUTES

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Abstract

High energy granola bars have been progressively introduced to the modern day market as a convenient alternative to conventional breakfast cereals. Development of energy-rich cereal bars with improved storage quality has become a timely requirement to fulfil the daily nutrient requirements of adolescents, military and victims of calamity among others. The aim of this study was to develop a high energy bar using locally available raw materials and to evaluate the effect of gamma irradiation (^{60}Co) as a preservation technique on the compositional and nutritional quality of granola bars. Two types (sweet and spicy) of granola bars with three different formulas (F_1, F_2 and F_3) were initially tested for sensory properties to select the optimum formula. Bars with best selected formulas were irradiated (^{60}Co) at 0 kGy, 1 kGy, 3 kGy, 5 kGy, and tested for the changes in physicochemical, microbiological and sensorial properties. Results showed that the moisture content and the peroxidase value of each bar significantly increased ($p<0.05$) while microbial counts significantly decreased ($p<0.05$) with increasing dose. The highest sensory score was achieved with the bars irradiated at 1 kGy compared to 5 kGy. Therefore, 1 kGy gamma irradiation treatment of granola bars was appropriate for optimum nutritional quality and sensory attributes.

1. INTRODUCTION

In the busy modern day schedule, many people tend to skip or miss breakfast [1]. As such, food manufacturers are increasingly developing cereal bars as a compact nutritious meal in a tiny bar for the convenience of consumers [2]. The granola bar is an alternative to commonly available breakfast cereal bars and is becoming popular among people as a high energy food source in small serving size [3]. These bars usually consist of grains (millet, rice) legumes (green gram, soybean) nuts, dried fruits, honey or other sweeteners (sugar syrup or kithul treacle) with a crispy, toasted, golden brown appearance. Cereal bars boost energy required for highly intensive workouts and replenish energy [4].

Several granola bar variations are known with different ingredients and several flavours, colours and textures [5]. More importantly such energy-dense foods could be effectively use as an 'Emergency Food' to meet the nutritional needs of all age groups during situations such as severe floods, droughts, landslides, and for security forces. However, quality deterioration (sogginess and rancidity) with shorter shelf-life has become one of the major issues in granola bars and this remains challenging even with several preservation techniques.

Gamma irradiation has been widely used as a non-thermal processing technique in the manufacturing process of cereal bars to minimize nutritional losses while preserving its wholesomeness [6]. Some studies on quality assessment of cereal bars due to gamma irradiation have been reported [7]. Safety and efficiency of food irradiation have been approved by several regulatory authorities and widely used in many countries to-date. It is nevertheless critical to regulate the radiation dose and the exposure time to avoid negative health consequences when threshold limits are exceeded [8].

2. MATERIALS AND METHODS

2.1. Sample preparation

Two types of granola bars (spicy and sweet) were prepared using locally available raw materials such as pearl millet powder, green gram powder, puffed rice, soy powder, kithul treacle, roasted groundnut, spices, plums among others. Dry ingredients were weighed first according to the predetermined recipes in Tables 1 and 2. Sugar syrup, kithul treacle, water and soy lecithin were measured by weight and heated altogether at 103°C for 10 min before adding other dry ingredients. The dry ingredients were then added into the heated content and mixed well. The mixture was flattened on an aluminium tray and baked at 150°C for 10 min, allowed to cool to room temperature (25°C) and cut into identical rectangular pieces (6 cm × 2 cm × 1 cm) weighing approximately 30–35 g. Finally, the prepared granola bars were packed in nylon LDPE pouches and vacuum packaged separately using a vacuum packaging machine with a 15s of exposure time.

TABLE 1. TREATMENT COMBINATIONS USED FOR THE SWEET BAR

Ingredients	Formulations		
	Formula 1 (% w/w)	Formula 2 (% w/w)	Formula 3 (% w/w)
Green gram powder	12	12	12
Millet powder	10	10	10
Soya powder	9.8	9.8	9.8
Glucose syrup	22	22	32
Kithul treacle	10	0.0	0.0
Honey	0.0	10	0.0
Water	8.0	8.0	8.0
Ground nut	6.0	6.0	6.0
Puffed rice	5.8	5.8	5.8
Soy lecithin	1.0	1.0	1.0
Salt	0.4	0.4	0.4
Dates	6.8	6.8	6.8
Plums	5.0	5.0	5.0
Non-fat milk powder	3.0	3.0	3.0
Cinnamon powder	0.1	0.1	0.1

TABLE 2. TREATMENT COMBINATIONS USED FOR SPICY BAR

Ingredients	Formulations		
	Formula 1 (% w/w)	Formula 2 (% w/w)	Formula 3 (% w/w)
Green gram powder	20	20	30
Millet powder	15	15	15
Soya powder	10.02	10.02	10.02
Glucose syrup	20	20	20
Kithul treacle	10.00	0.00	0.00
Honey	0.0	10	0.0
Water	8.0	8.0	8.0
Ground nut	8.0	8.0	8.0
Puffed rice	5.0	5.0	5.0
Soy lecithin	1.0	1.0	1.0
Salt	0.4	0.4	0.4
Spices	0.5	0.5	0.5
Ginger	2.0	2.0	2.0
Curry leaves powder	0.08	0.08	0.08

2.2. Irradiation

Granola bars packed in the nylon LDPE package were treated with ^{60}Co gamma rays at the Sri Lanka Gamma Centre at Biyagama, Sri Lanka at different doses (0 kGy, 1 kGy, 3 kGy and 5 kGy) at a dose rate of about 5.8 Gy/min. Physicochemical, microbiological and sensorial qualities of the developed granola bars were tested based on the different doses.

2.3. Sensory analysis

A preliminary sensory evaluation was carried out using 10 trained panellists in the Food Technology Section (FTS) Industrial Technology Institute (ITI) to select the best formula for each sweet and spicy granola bars. Sweet and spicy granola bars which were developed with best selected formula were then subjected to different irradiation doses and tested for sensory properties by 20 panellists at the sensorial laboratory ITI. The appearance/colour, taste, smell, texture and acceptability of the samples were evaluated using a preference test based on a 9 point hedonic scale.

2.4. Proximate analysis, physicochemical parameters and microbiological analysis

Proximate composition of the granola bars was determined according to an Association of Official Analytical Chemists (AOAC)' method [9]. Colour and firmness of the samples were measured using a digital chroma meter and texture analyser , respectively. Free fatty acid, acid value and peroxide values of granola bars were determined according to an American Association of Cereal Chemists Official methods of analysis [10]. Water activity was measured using the water activity meter while individual sugars (sucrose, glucose, and fructose) were measured as specified elsewhere [11] using a HPLC system equipped with an organic Acid H^+ analytical column and refractive index detectors. Peaks were identified as retention time and quantified as area under the curve using a software . Sweet and spicy granola bars treated with different doses of gamma irradiation were tested for total plate count, yeast and moulds as well as coliform counts according to the standard methods [12–14].

2.5. Statistical analysis

Physicochemical and microbiological data were analysed using one way ANOVA followed by Tukey's error rate test while the sensory data were analysed using Kruskal wallis test at a significant level of ($p<0.05$) using the SPSS statistical software. All experiments were run as triplicates.

3. RESULTS AND DISCUSSION

3.1. Selection of best formulation for the development of sweet and spicy granola bars

Sweet and spicy granola bars prepared with sweet (plums, dates, milk powder) and spicy (spices, ginger) ingredients had a golden brown colour with crispy toasted texture. Bars were prepared in rectangular shape with a uniform size of 6 cm × 2cm × 1 cm to maintain the product consistency. A preliminary study with three different product formulations (F_1, F_2, F_3) for sweet (Table 1) and spicy granola bars (Table 2) were tested separately for basic organoleptic properties to select the best formulation and proceed with irradiation. Different combinations of kithul treacle and honey were used as the major variations in three tested formulations for both sweet and spicy bars. According to sensory analysis (Fig. 1) significantly higher ($p<0.05$) mean ranks in all tested parameters were shown by F_1 and F_2 compared to F_3 without honey or kithul treacle. Texture was the most significantly affected attribute by the level of kithul treacle or honey. However, there was no significant difference among all F_1 and F_2 tested quality parameters.

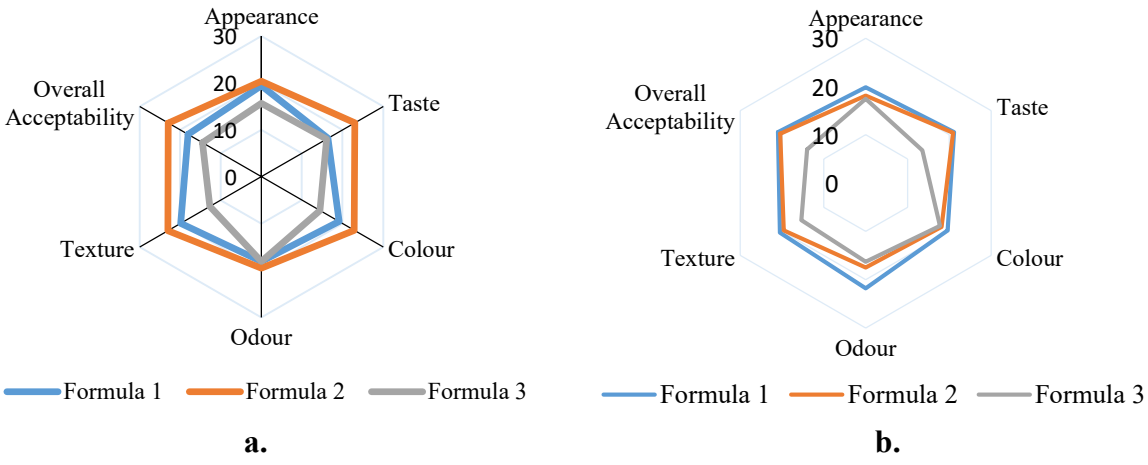

FIG. 1. Sensory attributes of granola bars at preliminary test (a) Sweet granola bar. (b) Spicy granola bar.

F_1 was selected as the best formulation considering the cost-effectiveness and the health effects of each ingredient (Honey; 475 LKR/L; Kithul Treacle; 330 LKR/L). Kithul containing sweet and spicy granola bars with energy values of 451.1kcal/bar and 464.7kcal/bar, respectively, were selected for the gamma irradiation and further physicochemical, microbiological and sensory analysis. Another study [15] highlighted the importance of cost-benefit analysis prior to commercialization of a granola bar, to better understand market flow and consumer purchasing behaviour. Kithul treacle is well known as a healthy natural functional sweetener with claims of antidiabetic and antioxidant properties as well as a better remedy for arthritis, skin complexion, blood sugar levels [16]. Researchers have reported that consumer acceptance along with the price and health claims influence product-purchase [17].

3.2 Compositional changes of gamma irradiated granola bars

3.2.1 Moisture content

As shown in Tables 3 and 4, the moisture content significantly varied ($p<0.05$) in both sweet and spicy granola bars based on the dose of irradiation. The moisture content in the irradiated granola bars significantly increased ($p<0.05$) compared to the control samples at the room temperature. According to literature, this may be due to induced changes in cellular integrity through the depolymerization of polysaccharides, which leads softening and easier water release in foods at ambient temperature [18]. This observation is supported by several research studies conducted on pigeon pea flour [19] and wheat flour [20]. Another study reported Increased moisture content as relatively lower in irradiated food samples (with time) compared to control samples and that this effectively extended shelf-life [21].

TABLE 3. PROXIMATE COMPOSITION OF THE GAMMA-IRRADIATED SWEET GRANOLA BAR

Irradiation Dose	Moisture (% wb)	Ash (% wb)	Crude Fat (% wb)	Crude Fiber (% wb)	Crude Protein (% wb)	Carbohydrate (% wb)	Total Energy (% wb)
0 kGy	$8.97^{a}\pm0.1$	$2.20^{a}\pm0.1$	$10.78^{a}\pm0.1$	$2.66^{a}\pm0.0$	$16.80^{b}\pm0.1$	$58.58^{a}\pm0.1$	$398.59^{b}\pm0.4$
1 kGy	$9.78^{b}\pm0.2$	$2.12^{a}\pm0.0$	$10.62^{a}\pm0.1$	$2.51^{a}\pm0.1$	$8.91^{a}\pm0.5$	$65.99^{b}\pm0.4$	$395.18^{ab}\pm1.7$
3 kGy	$9.95^{b}\pm0.1$	$2.13^{a}\pm0.0$	$10.28^{a}\pm0.1$	$2.56^{a}\pm0.0$	$8.74^{a}\pm0.1$	$66.29^{b}\pm0.3$	$392.64^{a}\pm0.6$
5 kGy	$9.73^{b}\pm0.1$	$2.04^{a}\pm0.1$	$10.28^{a}\pm0.2$	$2.48^{a}\pm0.1$	$8.61^{a}\pm0.2$	$66.14^{b}\pm0.2$	$394.33^{ab}\pm0.6$

Note: *Data is expressed as mean ± SD, n=3, Significant difference ($p<0.05$) within a column for each tested nutritional parameter was denoted as superscripts (values with the same letters A and b are not significantly different).*

TABLE 4. PROXIMATE COMPOSITION OF THE GAMMA IRRADIATED SPICY GRANOLA BAR

Irradiation Dose	Moisture (% wb)	Ash (% wb)	Crude Fat (% wb)	Crude Fiber (% wb)	Crude Protein (% wb)	Carbohydrate (% wb)	Total Energy (% wb)
0 kGy	$8.79^{a}\pm0.0$	$2.15^{a}\pm0.1$	$7.81^{a}\pm0.0$	$2.67^{a}\pm0.0$	$14.69^{a}\pm0.3$	$64.42^{a}\pm0.2$	$386.69^{a}\pm0.5$
1 kGy	$10.00^{b}\pm0.1$	$2.12^{a}\pm0.1$	$7.27^{a}\pm0.3$	$2.58^{a}\pm0.1$	$14.55^{a}\pm0.1$	$63.85^{a}\pm0.4$	$379.07^{a}\pm0.9$
3 kGy	$9.49^{ab}\pm0.2$	$2.13^{a}\pm0.0$	$7.69^{a}\pm0.2$	$2.60^{a}\pm0.0$	$14.28^{a}\pm0.0$	$64.04^{a}\pm0.0$	$382.49^{a}\pm2.1$
5 kGy	$9.70^{b}\pm0.2$	$2.13^{a}\pm0.0$	$7.44^{a}\pm0.4$	$2.50^{a}\pm0.1$	$14.20^{a}\pm0.2$	$64.45^{a}\pm0.3$	$381.55^{a}\pm2.7$

Note: *Data is expressed as mean ± SD, n=3, Significant difference ($p<0.05$) within a column for each tested nutritional parameter was denoted as superscripts (values with the same letters a and b are not significantly different).*

International standards [22] of cereal based snacks/bars provide that moisture content has to be below 10%. All samples developed in this study fulfilled this requirement. The findings also meet specifications for processed grain based food product/multigrain products recently established [23]. Literature [24] shows that the moisture content of granola bars prepared with whole flour varies from 7.19%–8.24% and is attributed to the fruit content in the mixture. Therefore, it is important to improve the water-holding capacity of the product for longer storage [25].

3.2.2 Protein content

The protein content significantly reduced ($p<0.05$) with irradiation doses compared to control samples in spicy granola bars (Table 4) while no change was observed for sweet bars (Table 3). Gamma irradiation induces structural transformations in proteins and medium doses of irradiation (3–5 kGy) affect secondary and tertiary protein structure [26]. The protein content correlates with the grain concentration in each granola bar. The irradiated spicy bars may contain higher grain content (and thus protein content) than the sweet granola bars, and this could also explain the significant changes.

3.2.3 Energy values

Energy values of spicy granola bars differed significantly ($p<0.05$) from control samples while there was no significant change observed in sweet granola bars. Since granola bars are considered an energy rich diet [27] the energy content of cereal-based energy bars is more than 300 kcal and could vary with the ingredients added [25]. Energy values of all samples developed in this study ranged between 375–392 kCal and no significant losses were observed after irradiation.

3.2.4 Fat, fibre, ash and carbohydrate content

Tables 3 and 4, show significantly higher ($p<0.05$) carbohydrate content in the sweet granola bars compared to spicy bars. In contrast, fat, fibre and mineral content (ash %) were not significantly different regardless of the dose or flavour and this agrees with a previous study [28]. Higher quantities of puffed rice and milk powder in sweet bars may account for higher carbohydrate content compared to spicy bars.

3.3 Physicochemical changes in irradiated granola bars

3.3.1 Acid value, free fatty acids, peroxide value

In Table 5, acid and free fatty acid contents decreased significantly ($p<0.05$) with increasing dose of gamma radiation while peroxide values increased significantly ($p<0.05$). The acid value and free fatty acid content in sweet bars were higher compared to spicy bars probably due to the absence of ingredients with antioxidant properties. Though the spicy bars are high in fat content (10.78% wb) compared to sweet bars (7.81% wb) the presence of spices and curry leaves may help in preventing lipid oxidation. The acid value, peroxide value and free fatty acids are key indicators of food quality often manifesting as rancidity [29].

TABLE 5. VARIATIONS IN ACIDITY OF GAMMA IRRADIATED SWEET AND SPICY GRANOLA BAR

Irradiation dose	Acidity					
	Free fatty acids (%)		Peroxide value (meq/kg)		Acid value (%)	
	Sweet bar	Spicy bar	Sweet bar	Spicy bar	Sweet bar	Spicy bar
0 kGy	$3.23^{a} \pm 0.04$	$2.35^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	$5.36^{a} \pm 0.22$	$7.47^{a} \pm 1.02$	$4.46^{b} \pm 0.09$
1 kGy	$2.93^{a} \pm 0.06$	$1.89^{ab} \pm 0.01$	$29.98^{b} \pm 0.68$	$22.06^{b} \pm 0.25$	$5.82^{a} \pm 0.13$	$2.12^{a} \pm 0.01$
3 kGy	$2.77^{a} \pm 0.09$	$1.84^{a} \pm 0.16$	$31.29^{b} \pm 1.27$	$24.57^{b} \pm 0.72$	$5.52^{a} \pm 0.18$	$2.13^{a} \pm 0.00$
5 kGy	$2.71^{a} \pm 0.15$	$1.82^{a} \pm 0.07$	$31.52^{b} \pm 0.33$	$24.79^{b} \pm 0.75$	$5.38^{a} \pm 0.29$	$2.04^{a} \pm 0.10$

Note: *Data is expressed as mean ± SD, n=3, Significant difference ($p<0.05$) within a column for each tested nutritional parameter was denoted as superscripts (values with the same letters ^{a} and ^{b} are not significantly different).*

This study can claim that development of rancidity is significantly controlled by gamma irradiation. Radiation could result in free radicals followed by the formation of carbonyl groups, and it is therefore advisable to consider the dose of irradiation [30]. Also, irradiation could induce oxidation of unsaturated fatty acids compared to saturated fatty acid [31]. Other researchers [32] have shown that the acid values have to be lower than 25% for better quality at ambient temperature. Therefore, it is vital to develop products within safe acid-value limits. Peroxide values are less than 10–20 meq/kg with rancidity noticeable at 20–40 meq/kg [33].

3.3.2 Sugar (sucrose, glucose, fructose) content

In Table 6, the sugar content in the sweet bar was significantly higher ($p<0.05$) compared to the spicy bar. The glucose concentration is generally enriched by adding glucose syrup and sugar plays a vital role in keeping quality during long term storages and is considered as a key quality indicator microbial spoilage [34]. Another study [35] reported that gamma irradiation increases the reducing sugar content of starches present in foods and that the effect of radiation on sugar contents is negligible even at higher doses (>10 kGy). Other researchers have shown how radiation increases the contents of invert sugars due to breakage of the glycosidic linkage of sucrose molecule, without changing the total sugar content [36].

TABLE 6. VARIATIONS IN SUGAR COMPOSITION OF GAMMA IRRADIATED SWEET AND SPICY GRANOLA BAR

Irradiation dose	Sugar content							
	Glucose (mg/100g)		Fructose (mg/100 g)		Sucrose (mg/100g)		Total sugars (mg/100 g)	
	Sweet bar	Spicy bar	Sweet bar	Spicy bar	Sweet bar	Spicy bar	Sweet bar	Spicy bar
0kGy	6700 [a]	3705 [a]	5800 [a]	2215 [a]	3730[a]	3985[a]	16230[a]	9905[a]
1kGy	7520 [a]	3470 [a]	6620 [a]	2160 [a]	3935 [a]	3390[a]	18075[a]	9020[a]
3kGy	6525 [a]	3065 [a]	5815 [a]	1885 [a]	3980 [a]	3350 [a]	16320[a]	8300[a]
5kGy	7370 [a]	3395 [a]	6350 [a]	2145 [a]	4060 [a]	3185 [a]	17780[a]	8725[a]

Note: *Data is expressed as mean, n=3, Significant difference ($p<0.05$) within a column for each tested nutritional parameter was denoted as superscripts (values with the same letters a and b are not significantly different).*

3.3.3 Vitamin profile

The sweet granola bars developed in this study were rich in niacin while spicy bar was a good source of riboflavin. However, no significant changes were observed in both types of granola bars. Vitamins B, C, and E are highly sensitive to irradiation (1–4.5 kGy) and may result in approximately 11%–95% loss in Vitamin B complexes [37]. Thiamine is the most radiolabelled vitamin in the group of Vitamin B complex, and a significant loss could be experienced even at a lowest radiation dose (0.5 kGy) in maize and chickpea. In contrast, riboflavin is highly stable against irradiation and the effect of irradiation on niacin was negligible. Researchers [38] reported that significant decrease in riboflavin occurs only at higher doses of irradiation (2.5–5kGy) while pyridoxine (B6) is not affected until doses reach 1 kGy [39].

3.3.4 Colour, firmness and water activity

Major changes in physical parameters of irradiated granola bars are shown in the Table 7. Colour did not significantly change with irradiation dose, but firmness of the irradiated granola bars changed significantly ($p<0.05$) with increasing irradiation dose. The firmness of sweet bars decreased significantly ($p<0.05$) while that of spicy bars increased significantly ($p<0.05$) with increasing irradiation doses. Researchers reported that irradiation causes loss of firmness in granola bars, and softening occurs due to several biochemical and physiological reactions [40]. Water activity is a significant property to note as it influences microbial growth in food. No significant change was observed with increasing irradiation doses in this study compared to a previous study that reported a decrease in water activity due to irradiation [25]. Others [32] reported water activity between 0.330 and 0.730 as optimum for ensuring quality.

TABLE 7: VARIATIONS IN PHYSICAL PARAMETERS OF GAMMA IRRADIATED SWEET AND SPICY GRANOLA BAR

Irradiation dose	Colour (L)		Firmness (kPa)		Water activity (a_w)	
	Sweet bar	Spicy bar	Sweet bar	Spicy bar	Sweet bar	Spicy bar
0 kGy	$41.24^{a}\pm1.73$	$49.5^{a}\pm1.21$	$5.28^{b}\pm0.13$	$3.45^{b}\pm0.27$	$0.67^{a}\pm0.01$	$0.65^{a}\pm0.01$
1 kGy	$39.82^{a}\pm1.02$	$48.28^{a}\pm0.15$	$4.92^{ab}\pm 0.18$	$1.98^{a}\pm0.25$	$0.67^{a}\pm0.01$	$0.67^{a}\pm0.00$
3 kGy	$43.86^{a}\pm1.23$	$47.87^{a}\pm1.19$	$7.36^{c}\pm0.48$	$2.53^{a}\pm0.18$	$0.65^{a}\pm0.00$	$0.67^{a}\pm0.01$
5 kGy	$44.78^{a}\pm1.48$	$48.15^{a}\pm0.61$	$3.97^{a}\pm0.33$	$5.18^{c}\pm0.22$	$0.63^{a}\pm0.00$	$0.67^{a}\pm0.01$

Note: *Data is expressed as mean ± SD, n=3, Significant difference ($p<0.05$) within a column for each tested nutritional parameter was denoted as superscripts (values with the same letters a and b are not significantly different).*

3.4 Sensory properties of sweet and spicy bars

Based on the consumer perception on sweet and spicy granola bars, the highest rank on overall acceptability was for granola bars irradiated at 1 kGy dose (Fig. 2). The sensory attributes deteriorated with increasing irradiation dose. A study [41] reported that the textural alteration and development of off-flavours are minimized at irradiation doses lower than 2 kGy. Observations in this study are supported by others [42] where lower consumer acceptance of cereals bars at 3 kGy and 5 kGy irradiation doses was reported. Changes in aroma and taste are mainly caused by cleavage of sulfhydryl groups in sulfur-based amino acids and lipid oxidation [43].

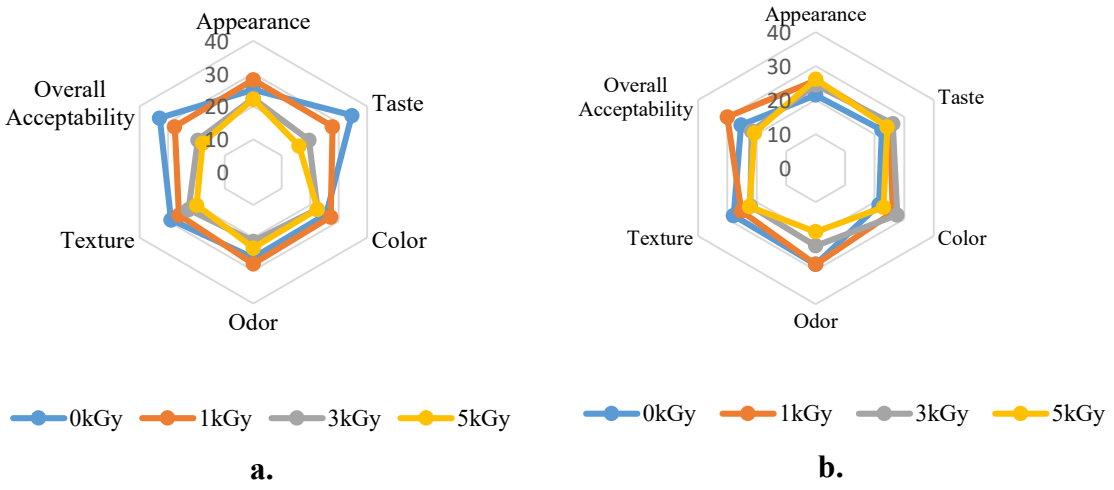

FIG. 2. Sensory attributes of Gamma irradiated granola bars (left) Sweet granola bar (right) Spicy granola bar.

3.5 Microbiological analysis

Table 8 shows that aerobic plate counts, yeast and mould as well as coliform counts reduced significantly ($p<0.05$) with increasing irradiation doses. The observed levels in each microbiological assessment of both sweet and spicy bars did not exceed the maximum limits stated in the SLSI 516 standards. The irradiation dose of 1 kGy was selected as the best treatment for sweet and spicy granola bars for safety and quality purposes. Radiation energy is absorbed by the radiolytic compounds, and some chemical bonds may break and produce free radicals which are highly reactive and unstable [44]. Therefore, it is important to select the safest dose of irradiation along with optimum quality characteristics.

TABLE 8. MICROBIOLOGICAL PROPERTIES OF SWEET AND SPICY GRANOLA BAR

Irradiation dose	Aerobic plate count		Yeast and Moulds		Total Coliforms	
	(cfu/g)		(cfu/g)		(MPN/ml)	
	Sweet bar	Spicy bar	Sweet bar	Spicy bar	Sweet bar	Spicy bar
0 kGy	2.3×10^3	2.2×10^3	—	—	—	—
1 kGy	2.1×10^2	8.6×10^2	—	—	—	—
3 kGy	1.8×10^2	3.6×10^2	—	—	—	—
5 kGy	4.0×10^1	1.8×10^2	—	—	—	—

Note: *— Denoted that microbes not detected or microbial counts were below the lowest limit of detection.*

The DNA in microorganisms is highly sensitive to irradiation which causes severe damages in the tertiary structure of both DNA and the proteins [45]. Furthermore, irradiation of DNA at approved level (>10 kGy) causes damages in nucleotide-bases, breakage of DNA strands and cross linkages. Ultimately, damages caused in the microbial DNA will result in growth-inhibition and reproducibility. The dose and the exposure time are critical for both quality and safety attributes of food commodities. A safer irradiation dose is also required to ensure safer human consumption while building consumer confidence [27]. Thus, while the technology has several benefits, special labelling to indicate that a food is irradiated is required to inform consumers [46].

4. CONCLUSIONS

A study has determined that 1 kGy irradiation dose was the best in preserving all physicochemical, nutritional, microbiological and sensory properties of both sweet and spicy granola bars developed. The developed sweet (451.1 kcal) and spicy (464.7 kcal) granola bars with high energy values fulfil standard nutritional requirements as a breakfast cereal. The moisture content was the most affected nutritional property by gamma radiation. In addition, protein and carbohydrates in both sweet and spicy granola bars changed significantly ($p<0.05$) although there were no significant changes in crude fat, fibre ash and energy values. The peroxide value of sweet and spicy bars increased significantly ($p<0.05$) with increasing irradiation doses while acid and free fatty acid values in spicy granola bars decreased significantly ($p<0.05$) with increasing ionization doses. There was a significant difference ($p>0.05$) in vitamin B2, B3 and B6 in the vitamin profiles of both sweet and spicy bars and aerobic plate counts were reduced significantly ($p<0.05$) after gamma irradiation. This study concludes that, the physicochemical, microbiological and sensorial properties of sweet and spicy granola bars varied significantly with effect of irradiation dose.

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CONCEPTUAL EVALUATION OF LOW-ENERGY ELECTRON BEAM AND COMPARISON WITH GAMMA IRRADIATION — A CASE OF CITRUS FRUITS

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Abstract

In Türkiye, for the agriculture sector to develop linearly, risk assessment and management has to satisfy the requirements by food importing countries. The sector also requires that trade-related biosecurity risks are appropriately managed. Citrus fruits account for a significant portion of Türkiye's exports. Conventional approaches are used to battle the Mediterranean fruit fly, a major phytosanitary concern, in addition to microbiological-related losses in the export of agricultural products. It is widely accepted that risk factors associated with fruits can be minimized through the application of irradiation. Low-energy electron beam (LEEB) applications for fresh fruit phytosanitary purposes have gained significance. It has emerged that using LEEB for the eradication of microorganisms on or near food surfaces or for phytosanitary purposes could be both a sustainable and effective option for the food industry. This study provides a review of LEEB and gamma irradiation applications on citrus fruits based on previous research. A comparison is needed between both methods in terms of phytosanitary procedures, microbiological decontamination, and food quality. Potential routine use of LEEB is discussed.

1. INTRODUCTION

Fruits of the genus citrus are from flowering trees and shrubs of the *Rutaceae* family and include important cash crops such as oranges, mandarins, lemons, and grapefruits, among others [1]. Addressing the nutritional needs of the rapidly growing global population necessitates not only enhancing the yield of crops grown on limited agricultural lands but also ensuring the preservation and maintenance of the harvested produce. Post-harvest losses of fresh fruits and vegetables range from 20%–60%, principally due to inadequate control of abiotic and biotic factors that lead to the losses and cultivation flaws within the producer-to-consumer chain [2]. Another critical factor contributing to the loss of fruits and vegetables is damage by pests. Fruit flies (*Diptera*: *Tephritidae*) are recognized as the insect group causing the most severe damage to commercially valuable agricultural products [3]. The Mediterranean fruit fly (*Ceratitis capitata;* Medfly) inflicts damage by larvae feeding on the fruit. Intensive pesticide applications against this pest not only escalate the product's cost but may also adversely affect human health and the environment [4].

Quarantine is one of the major interventions to prevent the spread of pests found in plants/crops or foods from one region to another [5]. The application of irradiation technology to fresh fruits for quarantine purposes is practiced in many countries [6]. The Codex Alimentarius Commission (CAC) and the International Plant Protection Convention (IPPC) provide international standards, a framework, and guidelines for the use of irradiation as a food treatment and a phytosanitary measure [7–9].

This study evaluated the applications of LEEB and gamma irradiation on citrus fruits, based on previous studies. In addition, these methods were assessed for phytosanitary processes, microbiological decontamination, and food quality. Conceptual and practical suggestions for future application of the LEEB approach are offered.

1.1. Sources of citrus fruits-loss postharvest

1.1.1. Mediterranean fruit fly Ceratitis capitata (Wiedemann)

The Medfly is a major insect pest that infests over 300 fruit species, including various citrus fruits. The fly causes significant post-harvest losses in citrus fruits. Its larvae feed on the pulp of the fruit, rendering it unmarketable and facilitating fungal and bacterial decay. Infestations can lead to fruit market-drop and trade restrictions due to quarantine regulations. Fruit infestation rates may vary depending on local environmental conditions, season, citrus species, and cultivars within the species [10]. The pest overwinters either as pupae in the soil or as larvae within fruits remaining on the trees.

Upon feeding, adult flies oviposit their eggs into fruits by creating punctures once the fruits reach an appropriate stage of ripeness. The emerging larvae feed on the fruit's mesocarp, progressing through three larval instars. The larval development duration is highly temperature-dependent, ranging from 9 days to 18 days. The pupal stage lasts approximately 10 days–12 days during the summer. After emerging, adults attain sexual maturity after a feeding period of 4 days–7 days. For oviposition to occur, ambient temperatures have to exceed 16°C, and a single female can lay approximately 200–300 eggs over her lifespan. The average lifespan of an adult under natural conditions ranges from 30 days to 50 days [11]. The larvae, by consuming the fruit pulp, inflict physical damage that hinders the fruit's marketability [12]. The life cycle of the Medfly has been reported elsewhere [13, 14] and is depicted in Fig. 1.

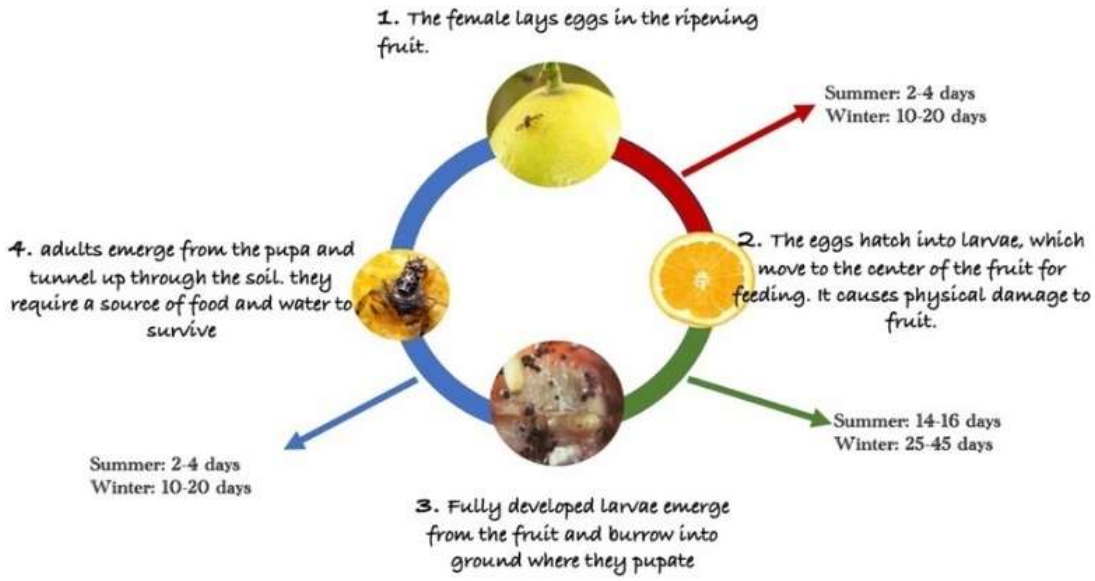

FIG. 1. Life cycle of the Medfly.

The citrus peel serves as the primary barrier to *Tephritidae* eggs and larvae. For survival and development, newly hatched larvae inevitably breach the peel to access the more nutrient-rich fruit pulp. The penetration of the citrus peel is associated with high larval mortality rates, primarily due to the toxic effects of the essential oils within the peel [7]. A female Medfly typically deposits 1–10 eggs in a cavity approximately 1 mm deep; she can lay up to 22 eggs per day and may lay up to 800 eggs (commonly around 300) throughout her lifespan [15].

Phytosanitary measures are imperative for export of citrus fruits from infested to non-infested regions. Various treatment options are available to meet this requirement. Traditionally, quarantine treatments include cold storage (1.1–2.2°C for 14–18 days) methyl bromide fumigation, and hot water treatment (immersion in water at 46.1°C for 65–110 mins) for different fruits. Ionizing radiation, a non-chemical and innovative method, is also utilized [16]. Irradiation stands out as the most broadly applicable commercial treatment method for managing *Tephritidae* fruit flies at effective dosages [17]. A generic dose of 150 Gray (Gy) is recognized as effective against all *Tephritidae* species, and studies support the application of a general dose of 400 Gy for controlling all insect pests except the pupae and adults of lepidoptera [18].

1.1.2. Microbial contamination on citrus fruits

Microbial contamination is another critical factor causing post-harvest product loss. The predominant post-harvest physical losses in citrus fruits are attributable to green and blue moulds, caused by *Penicillium digitatum Sacc*. and *P. italicum*, respectively [19]. Spores produced *en masse* by contaminated or decayed fruits are disseminated through the air and can infect adjacent fruits [20]. *P. digitatum* can induce decay in 60–80% of fruits under ambient conditions, whereas *P. italicum* is predominant in fruits stored under cold conditions [21]. Other notable post-harvest pathogens include *Galactomyces citri-aurantii* and *Botrytis cinerea*, while *Penicillium ulaiense*, *Trichoderma viride*, *Aspergillus niger* and *Rhizopus stolonifer* are less frequently encountered [20].

To mitigate post-harvest spoilage and preserve the quality of citrus fruits, synthetic chemical fungicides have been traditionally employed. For example, Imazalil is predominantly used as a post-harvest treatment for citrus fruits [22]. However, the emergence of resistant pathogen populations, increasing regulatory restrictions on chemical residues, and the environmental impact of these chemicals have amplified demand for residue-free fruits. Consequently, novel, safer, and more environmentally friendly strategies have been developed [23]. The nonthermal, residue-free, irradiation treatment for foods including fruits and vegetables is recognized as a safe technology by the WHO, FAO, IAEA, as well as the CAC [24]. Recently, the electron beam (E-beam) technology has been explored as an alternative to thermal post-harvest treatments for phytosanitary purposes [25]. Similar to gamma irradiation, E beam treatment causes double strand breaks in DNA, inactivating microorganisms in produce. Dosages of 50–750 Gy are necessary for insect pest control, whilst dosages of typically 1–1.75 kGy are required for post-harvest disease control [26].

1.1.3. Irradiation regulations of fruits

An FAO/IAEA/WHO expert committee in the 1980's deemed the maximum absorbed dose of 1 kGy for irradiation of fresh fruits and vegetables suitable for quarantine purposes [27]. This figure is a technological limit and not a food safety limit; most fruits and vegetables can tolerate up to 1 kGy of ionizing radiation without a significantly change to their organoleptic properties. Although some fruits and vegetable can tolerate much higher dosages, the 1 kGy limit has become widely accepted and is mandated in the regulations of many countries as the maximum dose for fresh fruits and vegetables. Therefore, maintaining the irradiation dose within the 1 kGy limit is considered a prerequisite for facilitating the international trade of irradiated fresh agricultural products. An absorbed treatment dose of at least 0.4 kGy is generally considered to be one of the highest phytosanitary treatment doses because it is more than sufficient to meet quarantine regulations for most pest species (except adult lepidoptera).

However, many commercial irradiation units typically deliver dose within a dose uniformity ratio (maximum imparted dose/minimum imparted dose) of 3.0. A treatment dose of 0.4 kGy could result in part of a batch of fruit receiving up to three times that dosage and 1.2 kGy would exceed the legal limit. Hence, careful optimization of the process may be necessary to reduce the dose uniformity ratio and therefore deliver a commercial treatment of at least 0.4 kGy to all parts of the batch of fruit being processed without any part of that batch receiving a dosage higher than the 1 kGy limit and possibly adversely affecting the physicochemical and nutritional properties of some fruits and vegetables [28]. Additionally, the recommended 1.0 kGy maximum dose is also applicable to inhibit growth and maturation [29]. The literature indicates that irradiation reduces spoilage-microorganisms in foods such a fresh fruits and vegetables enhancing shelf-life [30]. Gamma irradiation is internationally recognized as an effective method for preserving the quality of foods over extended periods [31].

A growing ionizing radiation approach in food and agriculture is the use of electrons with energies below 300 keV (low-energy electrons) [32]. This method applies ionizing radiation to food and is advantageous due to the shorter exposure times and easier control of radiation doses compared to other methods [33]. Although far less penetrating than conventional electron beam irradiation, low energy electrons can still be effective treatments. Since microorganisms are predominantly found on food surfaces, surface-irradiation effectively eliminates foodborne microorganisms [32]. Electron beam irradiation also controls insects in stored products [34].

2. MATERIALS AND METHODS

2.1. Food irradiation technology

Food irradiation fundamentally relies on ionizing radiation. It is a non-chemical food treatment that significantly reduces microbial spoilage and/or contamination [35]. The objectives of food irradiation include reducing the risk of foodborne illnesses, slowing spoilage, controlling sprouting or ripening, and preventing infestation. Irradiation may involve gamma photons from ^{60}Co, electron beams (E-beams) or X rays [36].

2.2. Principle of E-beam and comparison with gamma irradiation

Industrial electron beam applications utilize E-beam energies ranging from 75 keV to 10 MeV. Electron beam usage falls into three main categories based on the accelerator electron energy: high energy E-beam of 5.0 MeV to 10 MeV; medium energy E-beam of 400 keV to 5.0 MeV; low energy E-beam of 80 keV to 300 keV. The application of E-beams with energies up to 10 MeV ensures the irradiation of all food substances, whereas electron energies below 300 keV reduce the penetration depth of electrons. Electron beams at these lower energy

levels are referred to as LEEB [37] and is recommended as a method for processing fresh fruits and vegetables [38] among others. The LEEB systems preserve quality by preventing microorganisms on dried spices and edible insect surfaces; LEEB also effectively prevents insect infestation and plant disease on grains (Table 1).

TABLE 1. REVIEWING LEEB FOR PHYTOSANITARY AND MICROBIOLOGICAL USE

Material	Purpose	Observed effect	Ref.
Dry spices	Microbial decontamination	Reduced microorganisms on the spice surface.	[39]
Dry pepper	Microbial decontamination	Reduced microorganisms on the spice surface.	[40]
Seeds	Microbial decontamination	Preserves its chemical and sensory properties.	[41]
Grain	Pest control	Larvae and pupae of insects were neutralized in vitro. Disinfected grain and beans.	[42,43]
Grain crops	Insect pests and plant disease prevention	Irradiation of seeds before planting reduced the disease incidence. It prevented adult insects.	[44]
Dried insect products	Microbial decontamination	Microbial inhibition has been achieved in edible insects. There has been no change in quality.	[45]

Gamma irradiation requires non-renewable radioactive sources and presents more technical, financial, and operational challenges compared to alternative irradiation technologies. Although gamma irradiation penetrates deeper than E-beams as reported elsewhere (Table 2) it can lead to certain quality losses in foods. For instance, scientists [46] irradiated soybeans using both gamma and LEEB methods to achieve microbial decontamination but found that gamma radiation altered some properties of the soybeans, while LEEB had less impact.

TABLE 2. COMPARISON OF GAMMA RAYS AND ELECTRON BEAM

	Electron beam	Gamma rays
Power Source	Electricity	Radioactive isotope
Power Activity	Electrical on-off	5.27-year half-life
Properties	Electrons mass=9.1×10^{-31} kg (10 MeV)	Photons (1.25 MeV) $\lambda=1.0\times10^{-3}$ nm
Charge	1.60×10^{-19} coulombs	None
Emission	Unidirectional	Isotropic
Penetration	Finite range	Exponential attenuation

Existing literature does not report studies specifically examining the application of LEEB on citrus fruits; most studies reported high-energy electron beam (Table 3). The findings from these studies suggest that high-energy electron beam application effectively achieves microbial decontamination without adversely affecting the quality of the fruit. Furthermore, some citrus fruits have been shown to be highly sensitive to gamma irradiation [47, 48].

TABLE 3. EFFECT OF ELECTRON BEAM AND GAMMA IRRADIATION ON CITRUS FRUITS

Material	Treatment	Dose (kGy)	Observed effects	Ref.
Mandarin	Gamma	0, 0.15, 0.4 and 1	Irradiation of 0.4 and 1 kGy caused a decrease in fruit firmness, vitamin E and carotenoids. Fruit is highly sensitive to gamma irradiation.	[48]
Mandarin	E-beam 5 MeV	0, 0.4 and 1.0	Dose-dependent microbial inhibition was seen for up to 15 days. 0.4 kGy provided microbial decontamination. It did not affect its quality.	[49]
Grapefruit Lemon	E-beam 10 MeV	0, 0.4 and 1.0	It affected the product quality. Minimized quality deterioration during storage.	[50]
Orange	E-beam 10 MeV	0, 0.2, 0.4, 0.6, 0.8 and 1.0	Up to 0.6 kGy is the optimum dose to minimize quality changes and for pesticide treatment.	[51]
Orange	E-beam 10 MeV	0, 0.2, 0.4, 0.6, 0.8 and 1.0	E-beam irradiation at dosages lower than 1 kGy does not affect antioxidant compound levels and antioxidant activities.	[52]
Grapefruit	E-beam 10 MeV	0, 1.0, 2.5, 5.0 and 10	Vitamin C content did not affect at 1 kGy. Doses above 1 kGy significantly reduced vitamin C content. Naringin flavonoid showed a significant increase compared to the control at 10 kGy. Low dose E-beam radiation has little effect on bioactive compounds and can provide a safe solution.	[53]
Grapefruit Orange Mandarin	Gamma	0, 0.5, 1.0 and 1.5	A gamma radiation irradiation dose of 0.1 kGy was sufficient against the Mediterranean fruit fly. There was no negative effect of low dose (<1.0 kGy) application on fruit quality in these fruits, except for mandarin.	[54]

Material	Treatment	Dose (kGy)	Observed effects	Ref.
Orange	Gamma	0, 0.3, 0.6 and 0.9	It significantly affected the microbial load. An increase in respiration rate was observed in irradiated fruits with the irradiation dose during the storage period. During early shelf life, the decay rate of irradiated fruits is higher than control in a dose-dependent manner. Irradiation showed no significant effect on titratable acidity and total organic acids during storage. To maintain shelf life quality, 0.6 kGy may be an appropriate radiation dose.	[55]
Orange	Gamma	0, 0.25 and 0.5	With gamma irradiation, the weight loss of the fruit decreased throughout its shelf life. 0.5 kGy irradiation is at an acceptable level in terms of sensory and chemical properties of the fruit.	[56]

2.3. New approaches for LEEB

Recent years have seen an increasing focus on the application of LEEB to food products. The limited penetration depth of LEEB enables effective surface sterilization without compromising the quality of the internal food matrix. Another LEEB advantage is that it can be integrated into existing processing lines. The LEEB systems, often referred to as electronic pasteurization [57] do not involve radioactive isotopes rather, generate ionizing beams using electricity. Despite the limited number of studies on LEEB, recent investigations have garnered attention. A prominent example is the Laatu system developed by Bühler AG, an industrial LEEB system with a continuous capacity of 1000 kg per hour. The process flow of the Laatu system involves the product entering through an upper feed hopper and passing through two shaking beds (feeders) which facilitate the even distribution of the product as it enters the treatment zone.

The treatment zone features two E-beam lamps, positioned on either side, through which the product passes and undergoes irradiation processing [58]. The Laatu system has been used on spices [59] and edible insects [55]. An analysis of these studies indicates that no quality degradation occurred in the products and effective microbial decontamination was achieved. The mechanization developed for industrial applications serves as a precursor for future research. Ongoing research also involves seed sterilization using LEEB systems known as E-VITA with capacity to irradiate 25 metric tons of grain per hour and is designed for use as a mobile unit [60]. Thus, LEEB represents an advancing non-thermal and chemical-free irradiation technology.

Considering its potential future applications, several critical considerations have been highlighted in recent studies [58]. Given the variability in size and shape of food products, it is essential to conduct further research on dose depth specific to each product. Investigations into dose distribution in three-dimensional applications are necessary.

3. RESULTS AND DISCUSSIONS

3.1. Conceptual considerations on the LEEB application of citrus fruits

Selecting the right and effective technology in food irradiation applications depends on many factors. The characteristic properties of the food to be irradiated and the determination of quality parameters are points that need to be established at the initial step. Additionally, clarity on the purpose of irradiating food is needed.

3.2. Process of citrus fruits in facilities

The species of orange, mandarin, lemon, and grapefruit belong to the *Citrus* genus. It is estimated that global production in the 2022/23 period will be 36.9 million tons for mandarins, 47.8 million tons for oranges, 6.8 million tons for grapefruits, and 9.1 million tons for lemons [61]. The countries with the highest citrus exports globally are China, South Africa, Türkiye and Egypt [61, 62]. The packaging of fresh fruits and vegetables constitutes one of the most critical post-harvest steps in the export process. Effective packaging safeguards the produce from all forms of deterioration and damage before, during, and after transportation, thereby enhancing its shelf-life. The facilities where fruits and vegetables are packaged and made ready for the market postharvest are referred to as packing houses [63]. The key processes and their critical points undertaken in packing houses include pre-sorting, washing, waxing, grading, packing, and storage (Fig. 3).

A study examining the fungal and bacterial species and their densities in the microbiota of fruits processed in different packaging facilities and the ambient air of the facility [64] found that fungi such as *Penicillium, Cladosporium, Alternaria, Botrytis, Fusarium, Stemphylium, Aspergillus, Rhizopus* and *Mucor* can be transported by air currents and remain suspended in the air. These airborne fungi can cause symptoms like tissue softening and decay in fruits. Fruits exposed to these fungal spores can suffer significant losses by the time they reach export destinations. Since the most substantial losses in fruits are due to fungal decay, these losses often reach levels of 30%–40%. The identified fungal species can contaminate packaging equipment, ripening, and storage rooms. Spores produced by infected fruits can contaminate the surface of healthy fruits, perpetuating this cycle within the packing houses and storage facilities [64].

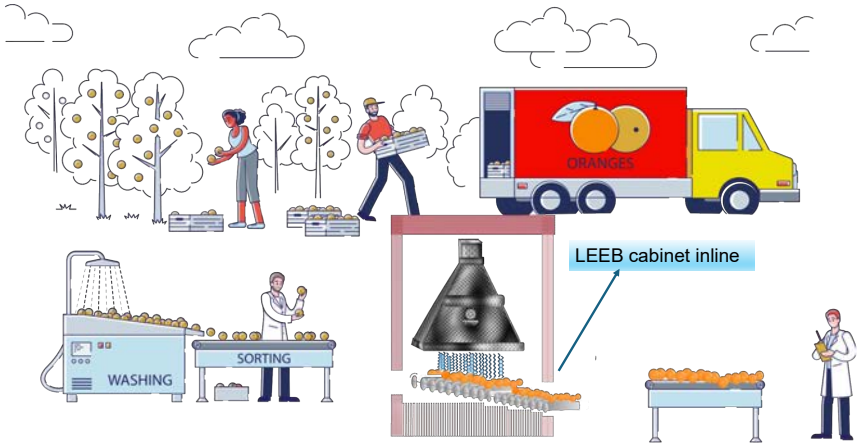

FIG. 3 Conceptual evaluations for citrus processing facility flow chart.

Various methods are employed to inhibit pathogenic microorganisms before the product-packaging stage. For instance, in a certain study [65] chemicals such as thiabendazole, guazatine, prochloraz and imazalil were used in washing water or waxing processes in packing houses to prevent disease transmission. These are often coupled with other interventions such as disinfecting all tools and equipment within the system [66]. *Penicillium italicum* and *Penicillium digitatum* have been reported as the most significant microorganisms affecting the shelf-life of citrus fruits [67]. To mitigate microbial contamination, prevent product loss, and ensure food safety, irradiation technology is considered crucial, especially for fresh fruits and vegetables. Studies have shown that irradiation significantly inhibits *Penicillium italicum* and *Penicillium digitatum* in a dose-dependent manner [68, 69].

It is known that citrus losses are attributed to microbial contamination, and without adequate measures in packing houses, these losses are likely to increase. Therefore, it is considered that irradiation applied in the final processing stage in packing houses can reduce and mitigate contamination in fruits. However, Medfly larvae cause physical damage to the fruit pulp as they feed on it. Damaged fruits are separated at the pre-sorting stage. Female Medflies lay their eggs on the surface of the fruit skin [15]. Therefore, eggs that cannot be separated at the pre-sorting stage pose the greatest risk factor. Surface irradiation can offer solutions. The integration of LEEB applications into the process stands out compared to other irradiation methods due to its ease of integration and ability to perform surface sterilization without causing quality loss in the food matrix [47]. It is anticipated that following irradiation treatment and appropriate packaging, the shelf-life of packaged fruits will increase, product loss will decrease, infestation will be prevented, and food safety ensured (Fig. 3).

3.3. Strategies and design considerations of LEEB in citrus processing facilities

The integration of radiation technologies into food processing facilities has emerged as a significant topic in recent years. The meticulous selection of the appropriate irradiation application, including equipment and applied dosage levels, along with precise process conditions, directly influences the quality and safety of the product. Fig. 4 delineates the procedural steps essential for selecting the optimal irradiation process as indicated elsewhere [70].

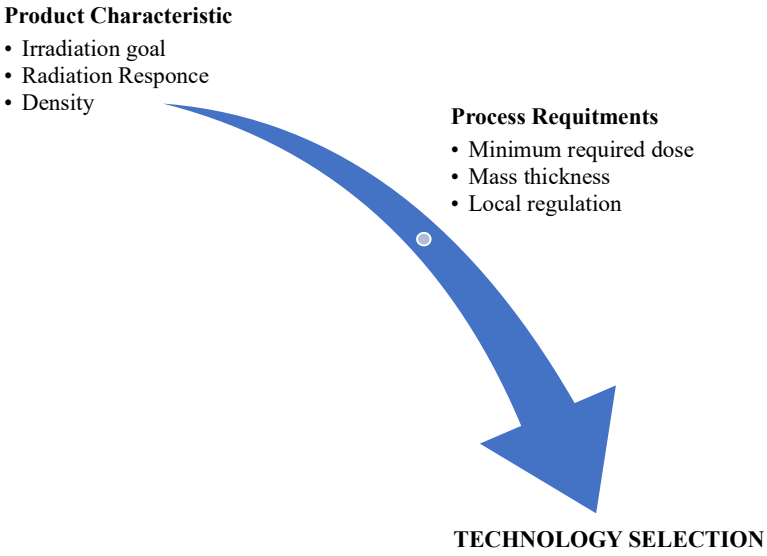

FIG. 4. Steps for choosing the proper irradiation technique.

The LEEB applications represent irradiation units with energy levels ranging from 80 keV to 300 keV. Studies have been conducted in food irradiation using LEEB. Due to the low electron energies in LEEB systems, their penetration into the product is limited. This capability often allows for surface sterilization of food without compromising its organoleptic characteristics [49]. Table 4 discusses terminology related to LEEB systems as detailed elsewhere [71] for a better understanding.

TABLE 4. TERMINOLOGY OF THE LEEB SYSTEM

Terminology	Description
Beam energy	It expresses the kinetic energy of the radiation. Higher energy provides deeper penetration.
Average energy	It describes how deeply the radiation will penetrate the material. It is used in calculating the average beam power of the accelerator.

Terminology	Description
Beam width	It is the size of the radiation field perpendicular to the direction of movement of the product at a specified distance from the accelerator window.
Beam length	It ensures the minimum dose capacity and dose uniformity for low doses.
Product thickness (Mass-thickness)	It represents the weight of the material interacted with per unit area to determine the performance of the electron beam system.
Depth-dose distribution curve	It is a graph that shows the variation of radiation dose deeper into the object from its surface.
Product handling system	It ensures the orientation of the products as they are transferred from the loading area to the radiation treatment area.

The depth-dose distribution curve is a graph of the energy deposited per electron per unit area versus depth. The energy distribution is proportional to the dose absorbed. The penetration depth of ionizing radiation is defined as the depth where the tail of the dose-depth curve extrapolates to intersect the x-axis. The absorbed dose increases with the depth within the product, peaking around the midpoint of the electron penetration range and then decreases rapidly [71]. Due to the variability in electron energy deposition, there exists a location within the product where the minimum dose is received and another where the maximum dose is attained. Another important parameter is the ratio of maximum absorbed dose to the minimum dose (dose uniformity ratio, DUR). A ratio close to 1 signifies uniformity, though in commercial applications, DUR ratios of 2 or 3 are often deemed acceptable [70]. Food irradiation can be carried out either in packaged or bulk form.

A range of considerations are essential for ensuring the integration of LEEB systems into these facilities. As seen in Fig. 3, in citrus processing facilities, after the classification process, fruits are packed and transferred to storage areas. Throughout these processes, citrus fruits are conveyed using roller conveyor systems to prevent physical damage by maintaining certain speeds without coming into contact with each other. Citrus fruits have high mass-to-volume ratios due to their small volumes and high masses, resulting in high fruit density per unit area. This high density can lead to increased product weight per unit area and subsequently lower radiation penetration. Therefore, radiation may not penetrate the fruit, facilitating surface sterilization without causing quality loss. However, eggs deposited by female Medflies on the fruit peel pose the greatest risk of fruit and economic losses. Since they have not yet developed into larvae, visible damage may not occur, potentially leading to infestation during storage if infected fruits are not separated and stored during the pre-sorting stage. The primary objectives of irradiating the products are to target eggs deposited by female Medflies on fruit peels and to address microbial contaminations.

Microbial contaminations can originate from the fruit itself as well as from the air within the facility. Therefore, it is better that irradiation is performed before the products are packaged and sent to storage. When determining the mass thickness of citrus fruits, average values for each product are calculated. Mass thickness represents the weight of the material interacted with per unit area for determining the performance of the E-beam system, with fruit thickness always defined in the direction of electron movement. Therefore, a spherical model needs to be used when considering the penetration depth of citrus fruits. To ensure dose uniformity, if roller conveyor systems are selected for product transfer, the fruit can rotate around its axis during irradiation to achieve a high uniform dose distribution. It is necessary to model the dose distribution for each individual product.

4. CONCLUSIONS

Preserving and maintaining the supply of fruits in the genus *Citrus* such as oranges, mandarins, lemons, and grapefruits is essential in meeting global consumer needs. Post-harvest losses of fresh fruits and vegetables can be very high especially in developing countries, with averages of 20% and 60%. These losses are primarily due to inadequacies in controlling abiotic and biotic factors that cause deterioration of produce after harvest. Fruit flies also contribute significantly to these losses. Ionizing radiation is effective in reducing the microbial load, preventing infestation, and thereby extending the shelf-life of foods such as, but not limited to fruits and vegetables. Gamma irradiation, recognized internationally as an effective method for preserving food quality over extended periods, has recently been supplemented by LEEB. Despite its efficacy, gamma irradiation relies

on non-renewable radioactive sources and presents technical, financial, and operational challenges. Due to its shallow penetration ability, LEEB can be used for microbial decontamination without damaging the food matrix. Integrating LEEB systems into processing lines is expected to ensure microbial decontamination, restrict contamination by eggs deposited by the Medfly, reduce product losses, and contribute to the economic growth while delivering safe food to consumers.

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COMPREHENSIVE TWO-PHASE APPROACH TO FOOD IRRADIATION

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Abstract

This paper presents a comprehensive approach to food irradiation-planning, which involves experimental determination of the optimal dose range for a given biological object and calculation of irradiation source parameters. To determine the optimal dose range, a mathematical model was developed based on experimental data that relates the efficiency of radiation treatment to the uniformity of the absorbed dose distribution and damage to targeted (related to microorganisms or phytopathogens) and non-targeted (such as proteins, lipids, carbohydrates, enzymes) molecules. Empirical dependencies relating the electron beam energy and the uniformity of the absorbed dose distribution were obtained to calculate the irradiation source parameters. The DosePreview and Demetra software tools make it possible to expedite and improve the accuracy of dose distribution calculations, as well as to optimize the process of calculating the accelerator operation parameters to deliver the planned dose to the target.

1. INTRODUCTION

The food industry has diverse products with different physical and biochemical properties. Effective use of irradiation therefore requires optimizing doses across the range of products [1]. It is suggested that specific criteria for choosing the optimal irradiation dose ranges are established for each category of food products considering fat, protein, carbohydrate, and water content, and other factors such as packaging, the background microbial levels and the type of the microorganisms [2–5]. After finding the optimal irradiation dose range for the food category it is necessary to determine the irradiation parameters which would ensure irradiation within the optimal dose range for the product from the category having specific physical properties, such as linear dimensions, shape and density. This study proposes a comprehensive approach consisting of two phases. The first involves the determination of the optimal dose range that would maximize microorganism suppression whilst minimizing the damage to the surrounding molecules. The second phase involves the calculation of the irradiation energy spectrum for electrons or photons and the irradiation parameters.

2. MATERIALS AND METHODS

2.1. Determining optimal dose

The basis of the approach suggested in this study is to represent food as a combination of targeted microorganisms or phytopathogens, and nontargeted molecules, such as proteins, lipids, carbohydrates, enzymes. For the purpose of the study the irradiation efficiency for targeted microorganisms and nontargeted surrounding molecules, such as proteins, is measured as the ratio of the damaged microorganisms or proteins to all microorganisms or proteins present in the food product. In the study the irradiation efficiency is determined by irradiation parameters as well as physical and biological properties of food product. The dependency of irradiation efficiency on the dose D can be represented as in Eq. (1)

$$\varepsilon(D)=F(K\_1\ (D),K\_2\ (D),K\_3\ (D)), \quad (1)$$

Where K1 is a combination of the absorbed dose uniformity U throughout the food product and the dose required to damage biotargets at the established level; K2 determines the share of homogeneous bio-targets that receive an estimated amount of ionization events required to damage bio-targets; K3 is a radiosensitivity heterogeneity

of the bio-targets which form a statistical ensemble. According to the experimental data, the dependency of irradiation efficiency on the dose for different bio-targets can be represented as two different scenarios, exponential dependency, and sigmoidal dependency. If all biotargets have the same radiosensitivity, in other words, the factor K3=f3(D) tends towards 1, then the irradiation efficiency is represented in Eqs (2 and 3)

$$\varepsilon(D) = K_1(D) \cdot K_2(D). \quad (2)$$

The function K_2 depends on the dose in the following manner:

$$K_2(D)=1-e^{-\alpha D} \quad (3)$$

Where a (Gy-1) is determined by both physical parameters of irradiation as well as radiosensitivity and linear dimension of bio-target. The results of this study's experiment show that the dependency expressed by formula/Eq. (2) is typical for microorganisms and for the extent of the damage to the native structure of proteins. If the probability of irradiation hitting the bio-targets K2-f2 (D) tends towards 1, then the irradiation efficiency is represented in Eqs (4 and 5)

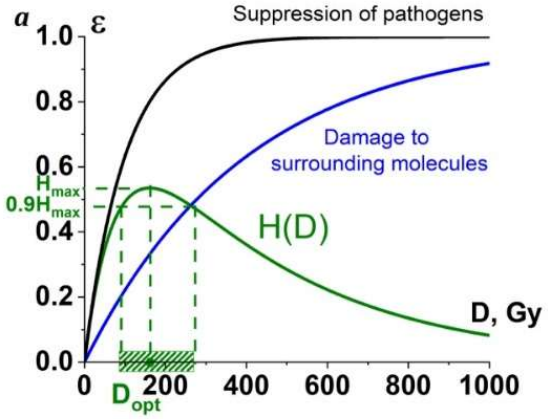
$$\varepsilon(D)=K_1(D)\cdot K_3(D). \quad (4)$$

The function K3 is described by the sigmoidal function:

$$K_3(N)=\frac{1}{1+e^{-\frac{(N-\bar{N})}{\delta}}} \quad (5)$$

where $\bar{N}$ is the number of ionization events that result in damage to 50% of biotargets, and δ is the width of the transition region of the function. Such dependency is typical for bio-targets found on the surface of the food product, for instance, phytopathogens on seed potato tubers or potato sprouts. The study suggests the optimization function H(D)=ε^targeted·(1-ε^(non-targeted)) where εtargeted is the irradiation efficiency of microorganism suppression and εnon-targeted is the irradiation efficiency of damage to surrounding molecules. The function H(D) has the maximum H_{max} which corresponds to the optimal dose Dopt at which the suppression of targeted microorganisms is maximized while minimizing the damage to nontargeted surrounding molecules. This study proposes that the optimal dose range (D1, D2) corresponds to the values at which the optimization function H(D) runs from $0.9 \cdot H_{max}$ to H_{max}.

Figure 1 presents model exponential dependencies of irradiation efficiency, Fig. 1(a) represents targeted microorganisms (targeted, black curve) and nontargeted proteins (non-targeted, blue curve). Fig. 1(b) represents sigmoidal dependencies of irradiation efficiency for the suppression of pathogens (targeted phytopathogens) and nontargeted biological structures found on the surface of seed material. Although the dependencies are different, with an exponential curve for microorganisms in food and a sigmoidal dependency for phytopathogens on seed surfaces, they both have a common feature at high doses in that the damage to surrounding molecules (damage to nontargeted molecules at higher doses) compared to targeted microorganisms is quite similar. This similarity prompted application of the same approach to calculating the optimization function H(D).



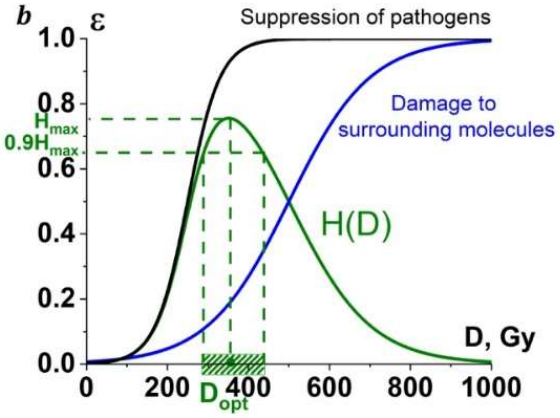


FIG. 1. Model exponential dependencies of irradiation efficiency for targeted microorganisms and non-targeted molecules (a) and sigmoidal dependencies of irradiation efficiency for targeted pathogens and non-targeted biological structures (b).

3. RESULTS/DISCUSSION—CALCULATION OF OPTIMAL PARAMETERS

The minimum dose uniformity U throughout the food product to comply with the optimal dose range (D1, D2) is estimated as the ratio of D1 to D2. The second phase of the comprehensive approach to food irradiation involves the calculation of the irradiation parameters including the effective energy of electrons or photons in order to ensure that dose-uniformity in food product with specific physical parameters, such as linear dimensions, density and shape, is not below U. The calculation of the optimal irradiation parameters for irradiation of food products was performed using Geant4 toolkit. Since the effective atomic number for biological tissue is 7.2, which is close to 7.43, the effective atomic number of water, computer simulation modelled food products as water parallelepipeds, spheres, ellipsoids, cylinders with linear dimensions ranging from 1 cm to 10 cm with the densities ranging from 0.6 g/cm^3 to 1.3 g/cm^3.

To ensure a high accuracy 2D and 3D dose distributions were obtained through a series of computer simulation sessions involving modelling the irradiation with 109 electrons with the energy ranging from 0.1 MeV to 10 MeV in each simulation session. X ray processing was modelled as the irradiation with bremsstrahlung photons with the maximum energy ranging from 0.1 MeV to 5 MeV obtained as 109 electrons passed through a 2 mm thick tantalum plate. The study suggests that empirical dependencies which allow to calculate the electron beam energy Ee (MeV) required to achieve the specified dose uniformity U in the water parallelepiped with the density ρ ranging from 0.6 g/cm^3 to 1.3 g/cm^3 and the thickness L ranging from 0.1 cm to 5 cm can be expressed in Eqs (6 and 7)

$$E_e[\text{МэВ}]=\frac{L[\text{cm}]\cdot\rho[\text{g}\cdot\text{cm}^{-3}]}{4[\text{cm}^{-1.88}\cdot\text{g}^{0.96}\cdot\text{MeV}^{-1}]}+\frac{159}{400}[\text{MeV}\cdot\text{g}^{-0.5}\cdot\text{cm}^{-1.5}]\sqrt{\rho[\text{g}\cdot\text{cm}^{-3}]}, \quad (6)$$

$$U=0.01\ [\text{МэВ}^{-1}]\times E_e[\text{MeV}]+0.57. \quad (7)$$

According to the calculations, the energy Ee (MeV) of electrons which generate bremsstrahlung photons as they hit the 2 mm thick tantalum plate would not be below the value calculated as in Eq. (8)

$$E_e[\text{МэВ}]\geq\frac{\left[-L[\text{cm}]\frac{\rho[\text{g}\cdot\text{cm}^{-3}]}{\log(U)\rho_0[\text{g}\cdot\text{cm}^{-3}]}-10[\text{cm}]\right]}{0.6[\text{cm}\cdot\text{MeV}^{-1}]} \quad (8)$$

Where L is the thickness of water parallelepiped with the thickness ranging from 0.1 cm to 50 cm, $\rho 0$ = 1 g/cm^3 is the density of water.

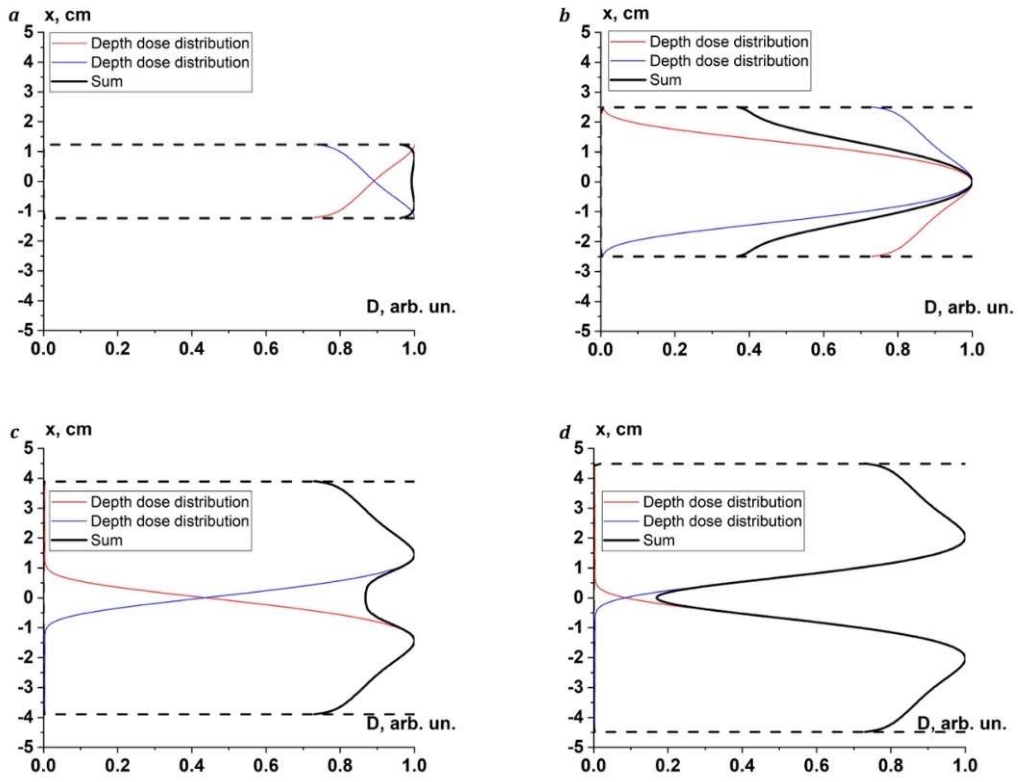


FIG. 2. Depth dose distributions in 2.5 cm (a) 5 cm (b) 8 cm (c) and 9 cm (d) water parallelepipeds irradiated with 10 MeV electrons from above (blue curve) below (red curve) and from two opposite sides (black curve).

Whilst one-side irradiation causes the dose uniformity (U) to increase with a higher energy of electrons or photons, an increase in the irradiation energy with two-side irradiation can lead to both increase and decrease in dose uniformity depending on the combination of irradiation energy and the thickness of the food product. Figure 2 shows the depth dose distributions in water parallelepipeds with the thickness ranging from 2 cm to 9 cm irradiated with 10 MeV electrons from two opposite sides. As it can be seen, whereas the dose uniformity for a 2 cm thick parallelepiped is 0.97 (Fig. 2a) with an increase in the phantom thickness up to 5 cm the dose uniformity drops to 0.4 (Fig. 2b). A further increase in the phantom thickness up to 8 cm causes the dose uniformity to increase to 0.75. However, as the thickness of the phantom exceeds 8 cm the dose uniformity tends to decrease to eventually drop to zero.

Since the dose uniformity demonstrates a non-linear dependency on the thickness of the phantom (simulated food product) and the energy of electrons, the study has revealed that the empirical dependencies of the phantom thickness on the electron energy are linear and differ depending on the empirical thickness. For the phantom thickness ranging from 0.1 cm to 10 cm irradiated with electrons with the energy ranging from 0.1 MeV to 10 MeV the dose uniformity varies from 0.2 to 0.99. The dependencies of the phantom thickness ranging from 0.1 cm to 10 cm on the electron energy and dose uniformity ranges are represented as in Eqs (9–11)

$$L[\text{cm}]=0.273\left[\text{cm}\cdot\text{MeV}^{-1}\right]*E_e[\text{MeV}],\ L\leq 2.5\ \text{cm},\ U\geq 0.9, \quad (9)$$

$$L[\text{cm}]=0.551\left[\text{cm}\cdot\text{MeV}^{-1}\right]*E_e[\text{MeV}],\ 2\ \text{cm}\leq L\leq 4\ \text{cm},\ U\leq 0.4, \quad (10)$$

$$L[\text{cm}]=0.847\left[\text{cm}\cdot\text{MeV}^{-1}\right]*E_e[\text{MeV}],\ 4\ \text{cm}\leq L\leq 6\ \text{cm},\ 0{,}6\leq U\leq 0.8. \quad (11)$$

Dependences (9–11) are obtained with the maximum interpolation error of 2%. Now that industrial irradiation facilities predominantly use electron sources since they achieve a higher processing rate compared to

radionuclides, there is a growing demand in high performance and easy-to-use simulation software. In response to this demand a DosePreview by IRT was developed [6, 7] to facilitate and accelerate calculations of dose distribution in biological objects, such as food products, irradiated with accelerated electrons with a specified energy spectrum. The DosePreview software contains an extensive database of depth dose distributions in water phantoms irradiated with electrons with the energies from 0.1 MeV to 20 MeV with a step of 0.1 MeV obtained using Geant4 toolkit.

To demonstrate how DosePreview software works Fig. 3a shows one of possible energy modes of the industrial electron accelerator. The DosePreview software modifies the original energy spectrum data and calculate the depth dose distribution in the water phantom considering the specified electron beam energy spectrum (Fig. 3b). In comparison, Fig. 3b shows the depth dose distribution obtained using Geant4 computer simulation without the use of the DosePreview software. The relative deviation between the depth dose distribution calculated using the DosePreview software and the distribution simulated using Geant4 toolkit does not exceed 4%. It is worth noting that the time it takes to calculate the depth dose distribution using DosePreview is less than one second, while the same procedure using Geant4 takes 35 mins on average.

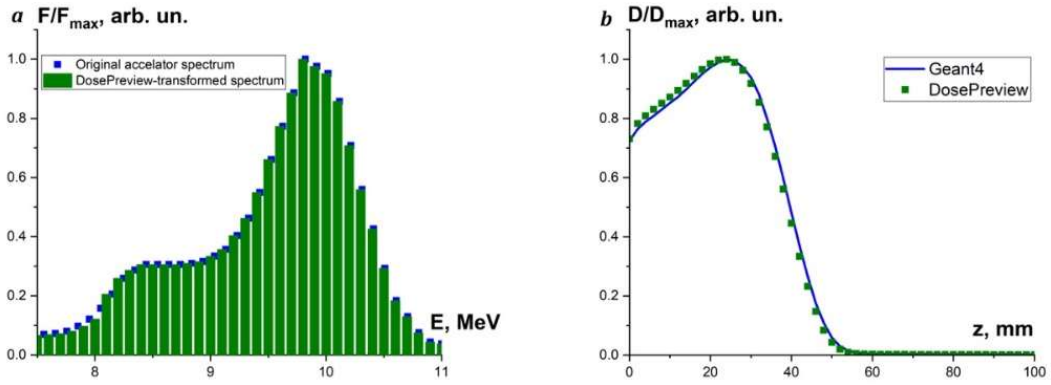


FIG. 3. (a) The blue squares denote the specified electron beam energy spectrum; the green columns show the spectrum transformed to DosePreview format; (b) the blue line shows the depth dose distribution obtained using Geant 4 computer simulation; the green points show the depth dose distribution calculated with the use of the DosePreview software.

After running a series of tests which proved the DosePreview software to be reliable for performing calculation of depth dose distributions in biological objects irradiated with accelerated electrons DEMETRA by IRT software was developed [8] to calculate optimal irradiation parameters for an industrial electron accelerator so that the irradiated biological object receives the specified absorbed dose and the required dose uniformity is achieved throughout the objects. The essence of DEMETRA by IRT software is that the operator of the accelerator inputs length, width and mass of the object, the required absorbed dose and the dose uniformity, and the interface shows the parameters of accelerator, that is accelerator mode, beam sweep width, sweep period, beam sweep frequency, beam scan frequency, conveyor belt speed, beam current, and irradiation method, either one side or two side irradiation.

4. CONCLUSIONS

The two-phase approach suggested in this paper which involves the experimental determination of the optimal dose range for the specified biological object and the calculation of irradiation source parameters is applicable to a wide range of matrices, such as food transplantations and medical items. The algorithm used to determine the optimal dose range considers the individual composition of targeted microorganisms and biochemical properties of food products. The empirical dependencies, which encompass, on the one hand, the individual physical parameters of food products, such as linear dimensions and density, on the other hand, irradiation energy of electrons or photons, allow to estimate the dose uniformity throughout the food product and ensure that irradiation is performed precisely within the optimal dose range.

To increase the precision of depth dose distribution and dose uniformity during irradiation with electron beams, the Dose Preview software can facilitate faster and more precise calculation of dose distribution. Considering that each food product calls for a specific dose range which is determined by individual physical

and biochemical properties of the food product, it is required to use a special algorithm to find optimal irradiation parameters for each food category. To meet the demand of industrial irradiation facilities equipped with electron accelerators with variable energy spectrum use of the DEMETRA by IRT software which calculates the optimal irradiation parameters to ensure that irradiation is performed within the optimal dose range was proposed.

ACKNOWLEDGEMENTS

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DETERMINATION OF THE AUTHENTICITY AND SAFETY OF SHWEBO PAWSAN RICE BY ICP-OES ANALYSIS AND CAFE SOFTWARE

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Abstract

Myanmar has an agri-based economy, and rice is the primary agricultural product. Local high quality Pawsan rice is popular among Myanmar consumers, and there is great potential for premium export markets. The price of Pawsan rice from Shwebo region is two to three times higher than 'ordinary' rice and this leads to an economic incentive for substitution of the high-value product in the domestic market. Consequently, mislabelling and adulteration of Shwebo Pawsan has become a major problem for traders and consumers. This research aims to verify authenticity and ensure the food safety and quality of Shwebo Pawsan rice. In 2022, authentic Pawsan rice samples (n=100) produced in Shwebo, Ayeyarwady, and a few different rice varieties were collected, and an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was used to measure arsenic (As) cadmium (Cd) cobalt (Co) chromium (Cr) copper (Cu) Iron (Fe) lead (Pb) manganese (Mn) nickel (Ni) and zinc (Zn). Authenticity-verification was conducted using Soft Independent Modelling by Class Analogy (SIMCA) method of the Chemometrics Add-in for Excel (CAFE) software and results showed 100% sensitivity and 84 % specificity in discriminating between Shwebo Pawsan and other rice. A comparison of the elemental concentration levels with Codex standards for contaminants and toxins in food, recommended by the Codex Alimentarius Commission, was performed.

1. INTRODUCTION

Historically, rice is the primary agricultural product of Myanmar not only as a staple food but also as a major agriculture export commodity. Although Myanmar was the number one global rice exporter before the second world war, at present Myanmar ranks just among the top ten countries in world's rice production and export. According to data of the United States Department of Agriculture, it was found that annual average production of milled rice was around 12.53 million metric tons, and average global rice exportation was around 2.3 million metric tons produced from 6.93 million-hectare farms in Myanmar during the five years (2018/19-2022/23) [1–2].

The global rice market is extremely competitive, and higher-quality rice demanded by consumers has been constantly rising in the global rice market. Myanmar has predominantly focused on the low-quality rice export market. In recent years, the local high-quality Pawsan rice, mostly preferred by consumers in Myanmar, has gained great potential for higher-quality rice export markets. Pawsan or Pawsan Hmwe rice is also known as Myanmar Pearl rice. It has a short or medium grain, unique fragrance, soft and smooth texture, good tasting quality and two to three times grain-elongation when cooking. It has been considered as a market driven export rice after receiving the award of World's Best Tasting Rice at the World Rice Conference held in Ho Chi Minh city, Vietnam in 2011.

Rice production holds a very important place in global food security and economy since over half of the world population consumes it as a staple food. In Myanmar, about 12.8 million of 67.6 million hectares of total land area are for agriculture, with rice production covering 7.53 million hectares [1–4]. Pawsan rice can be grown across the country depending on rainfed, irrigation and weather conditions. The rice's quality differs with geographical location and growing *terroir* [3]. The country has three agroecological zones: the Delta Zone, Central Dry Zone (CDZ) and the Hilly Zone. Farmers in the Delta Zone known as the Ayeyarwady region are primarily engaged in rice production during the monsoon season. Farmers in the CDZ such as the Sagaing region produce rice, oilseeds, beans and pulses depending on the rainfed and irrigated water. In the Hilly Zones such as Shan state, farmers cultivate rice, maize and pulses along with rain-fed tree crops and horticulture products [4].

The most productive area of Pawsan rice is Ayeyarwady delta followed by Shwebo District in the Sagaing region. Pawsan rice from the Shwebo District dominates the domestic market with the highest price due to its popularity among the consumers and it is well known as Shwebo Pawsan. According to Myanmar's Shwebo Pawsan Farming, Milling and Trading Association, Shwebo Pawsan rice has prospects of expanding to an international market as Myanmar's first geographical indication (GI) product, which will greatly benefit

farmers, local manufacturers/traders and consumers [5]. Due to occasional low yields and high demand, the rice's price rises to 2–3 times more than the 'ordinary' rice. Among the aromatic rice traded in the world market, the two most prominent are the Basmati rice grown in India and Pakistan, and Jasmine rice grown in Thailand. Myanmar's own, often referred to as Myanmar Jasmine rice, also contributes to a small share of the high-value global rice market. The current market share for Paw San rice in total rice exports is less than 1%. However, its export potential is estimated as progressing due to increasing global demand of high quality fragrance rice.

Production of safe and high-quality food has become essential in Myanmar as the global demand for safe and quality food for export rises. In addition, the ability to demonstrate the authenticity and traceability of food products has become a major concern to food regulators and trading partners as corrupt tendencies grow. Mislabelling and adulteration of Shwebo Pawsan rice has been a burning issue for consumers and reputable traders. Existing research in Myanmar has focused on how to improve the yield and quality of Pawsan rice with analyses performed using tools such as atomic absorption spectroscopy (AAS) and ICP-OES.

Food traceability and authenticity are required by all stakeholders in the food supply chain. In recent years, several successful applications including isotope ratio mass spectrometry (IRMS) ICP-MS near-infrared spectroscopy (NIRS) coupled with advanced data analysis methods have been reported for the authenticity verification of rice [6–9]. Gaps in specific verification methods for authentication of Myanmar's unique rice variety and increasing consumer-demand for authentication of high-quality rice, have necessitated establishing a robust analytical method that can effectively verify the Shwebo Pawsan rice.

A recent study has demonstrated that the fourier transform NIRS (FT-NIRS) and/or headspace gas chromatography ion mobility spectrometry (HS-GC-IMS) or fusion of FT-NIRS and HS-GC-IMS combined with orthogonal partial least squares discriminant analysis (OPLS-DA) or data-driven soft independent modelling of class analogy (DD-SIMCA) can be successfully used for rapid determination of the geographical origin of Paw San rice [10]. The aim of the study was to determine the authenticity, safety and quality of Shwebo Pawsan rice by ICP-OES combined with the CAFE software.

2. MATERIALS AND METHODS

This section includes sample collection, instrumentation, sample preparation and measurement as well as data analysis for food authenticity and food safety.

2.1. Sample collection

One hundred rice samples (~2 kg each) comprising of 68 authentic Pawsan rice samples from Shwe Bo District, 25 Pawsan rice samples from Ayeyarwady (AYA) Division, and another 7 rice varieties from different areas were collected between January and May 2022. The main sample areas were Yangon Region (Lower Myanmar) Nay Pyi Taw Region (Middle of Myanmar) and Mandalay Region (Upper Myanmar). The rice was grown in the monsoon season (Jun–Oct) of 2021, and collected from reliable sources, available retail markets and supermarket areas. All samples were dried, vacuum-sealed, coded by their respective names, and kept in clean plastic boxes until measurement.

2.2. Instrumentation, sample preparation and measurement

An ICP-OES was used to determine the concentrations of trace elements such as As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in each rice sample. Every purified rice sample was ground on a multi-functional grinder and passed through a 50-mesh sieve size to obtain a homogeneous fine powder. Each rice powder sample was weighed (200 mg) using an analytical balance and 200 mg of rice powder placed coded closed vessel; 4 ml of concentrated HNO_3 (~70% w/w) 1 ml of H_2O_2 (~ 30% w/w) and 3 ml H_2O were added and the solution digested for ICP-OES analysis at a temperature of 180°C for 40 min in an advanced microwave digestion system. Standard solutions of 0 µg/kg, 3 µg/kg, 30 µg/kg, 60 µg/kg and 150 µg/kg, for the elements reported, were also prepared for calibration and all measurements were repeated three times for precision and accuracy.

2.4. Data analysis

Data was analysed using a spread sheet and the Soft Independent Modelling by Class Analogy (SIMCA) model, of the CAFE software to verify authenticity and ensure the food safety and quality of Shwebo Pawsan rice. The estimated daily intakes (EDI) of the heavy metals were calculated to investigate for potential health risks based on Myanmar's daily rice consumption rate of 60 kg body weight for an adult person and average heavy metal content.

3. RESULTS AND DISCUSSION

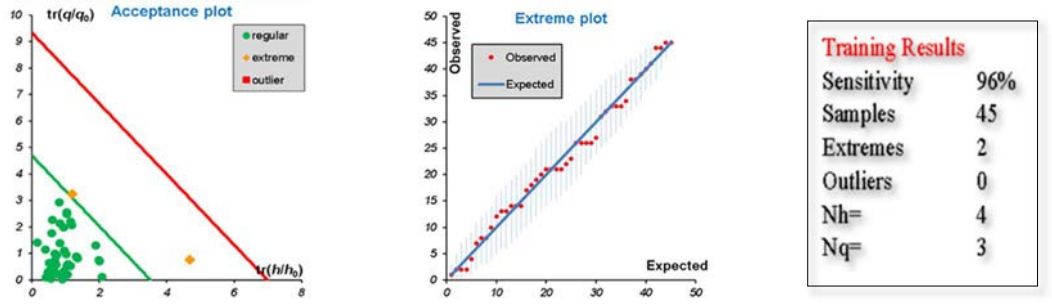
Results for authenticity verification by chemometric add-ins with concentrations of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn obtained by ICP-OES method are described and discussed in this section. Comparison of results of elemental concentration levels with Codex maximum limits (MLs) is also included.

3.1. Authenticity verification of Shwebo Pawsan rice by ICP-OES data and CAFE

The one class targeted SIMCA model of CAFE was used, in conjunction with the multi-element data from ICP-OES, to identify the authentic Shwebo Pawsan rice and other Pawsan rice or different rice varieties. Fifteen samples were randomly selected from the 68 authentic Shwebo Pawsan samples and used as a test set; 45 authentic samples were included in the training set, and 8 samples in test set. The alternative set contained 25 AYA samples, and 7 other rice (ALT) samples. As shown in Fig. 1A, the samples could be identified at a 95% confidence limit with 96% sensitivity for the training set. Fig. 1B shows 100% sensitivity for test set; and 84% specificity for the alternative set is shown in Fig. 1C.

Figure 1C shows that five samples were misclassified as Shwebo Pawsan, and 27 samples were correctly classified as these were truly AYA samples and ALT varieties. Data for only ten elements were obtained and used in the CAFE software. If more elemental data than the current observed data were obtained, the applied authenticity verification method could have provided more powerful and better SIMCA performance results. Nevertheless, the ICP-OES technique coupled with SIMCA model of the CAFE software can be considered as a reliable tool for determination of authenticity of Shwebo Pawsan rice. The ICP-OES generated information on multi-elemental concentration levels; it is therefore of food quality and safety significance.

A



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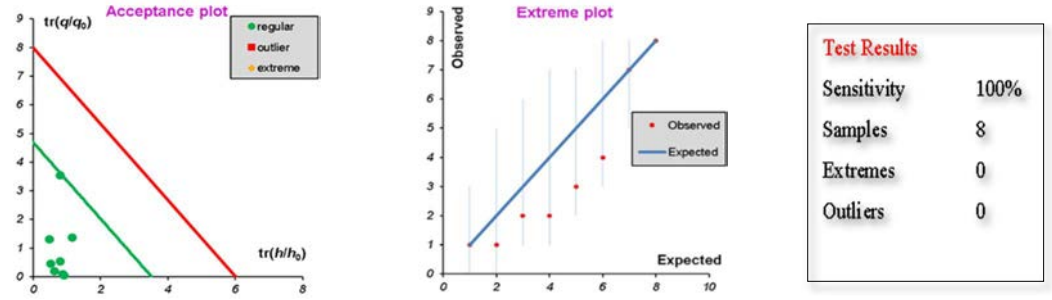

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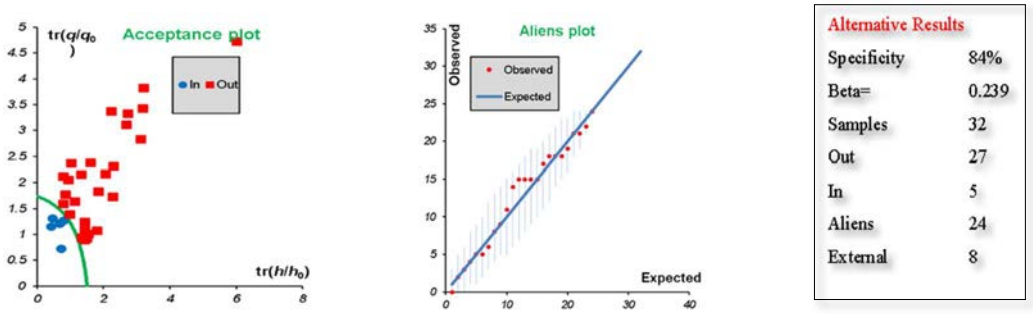


FIG.1. Acceptance plots of the applied SIMCA Model for verification of Shwebo Pawsan Rice samples using ICP-OES data (A) Training Results (B) Test Results (C) Alternative Results (The red squares are correctly classified as the Pawsan rice samples from Ayeyarwady region and others, and blue circles are the samples misclassified as Shwebo Pawsan).

3.2. Elemental concentration level against codex standards

A comparison of the elemental concentration levels (especially for the toxic heavy metals As, Cd, and Pb) with Codex MLs was performed. Table 1 shows the average, minimum, and maximum concentrations in 68 Shwebo Pawsan rice (SBPS) collected from respective coded areas along with the MLs. Table 2 describes the results of 25 AYAPS collected from the coded areas, and Table 3 shows the results of seven other rice varieties or ALT collected from different locations in Myanmar.

Arsenic levels in nine out of one hundred samples (six from SBPS and three from A YAPS collected from the NK and MD coded area) were a slightly higher than Codex ML (0.2 ppm or mg/kg). All concentration levels of Cd and Zn in all samples were under the MLs (0.4 and 50 ppm respectively). Although previous studies in 2019–2021 reported Pb under the ML (0.2 ppm) the maximum concentration in the current study were higher than the ML in 44 out of 100 samples (35/68 SBPS, 6/25 AYAPS and 3/7 ALT). Table 1 shows all products from Shwebo region, with different concentrations depending on the sampling areas. Similar trends are shown in Table 2.

TABLE 1. ELEMENTAL CONCENTRATIONS IN SHWEBO PAWSAN RICE FROM THE RESPECTIVE CODED AREAS

Location (Shwebo)	Element	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
	ML (ppm)	0.2	0.4	0.01	1	10	5	5	1.5	0.2	50
NPT (18)	AVG	—	0.0023	0.0059	0.0355	2.2075	30.0033	7.8697	0.5742	0.0205	10.4910
	MIN	—	—	—	—	—	16.9426	5.5641	—	—	8.3348
	MAX	—	0.0160	0.1065	0.5613	32.6310	57.4769	15.8223	4.5509	0.3694	16.3428
Brand (10)	AVG	0.0392	0.0119	0.0272	0.2184	0.0807	9.6802	7.3064	0.1400	0.1667	11.1523
	MIN	—	—	—	—	—	—	5.5908	—	—	8.5734
	MAX	0.1511	0.1186	0.0945	0.7456	0.4921	28.2634	8.9290	0.6840	0.6379	23.9131
KS (12)	AVG	0.0144	0.0291	0.0221	0.2356	2.2177	15.2970	7.1684	0.6253	0.5242	9.3118
	MIN	—	—	—	—	—	2.6974	5.1982	—	0.0918	5.9960
	MAX	0.1616	0.2251	0.0748	1.0692	4.3595	31.9623	9.5933	2.6700	1.1746	11.5823
HD (5)	AVG	0.0090	0.0004	0.0071	0.9415	4.7232	19.3620	8.2826	0.9046	0.3390	11.2025
	MIN	—	—	—	0.8251	2.9163	17.6060	7.8678	0.0648	—	10.2542
	MAX	0.0239	0.0020	0.0248	1.2053	7.7556	21.7047	8.9817	1.8360	1.0440	12.2793
SPK (5)	AVG	—	0.0206	—	0.3934	—	7.1603	8.6220	0.0387	—	10.7364
	MIN	—	—	—	0.1835	—	6.2275	8.0742	—	—	9.9166
	MAX	—	0.0626	—	0.9530	—	7.8548	9.2868	0.1020	—	11.6140
NK (10)	AVG	0.1783	0.0189	0.1327	3.4987	10.0216	39.8026	8.3999	2.0003	0.8209	11.8654
	MIN	0.0910	—	0.0888	0.7339	6.9693	34.2276	6.9426	—	0.5186	10.2459
	MAX	0.3029	0.1472	0.1661	4.2215	14.5282	49.5270	9.6638	4.2574	1.0668	12.9798
MD (8)	AVG	0.2041	—	0.1079	0.8590	6.9788	32.9491	8.2342	0.2218	0.7426	10.6420
	MIN	0.1349	—	0.0662	0.3220	4.5547	29.5112	6.9598	—	0.3925	9.6408
	MAX	0.3067	—	0.1523	2.2893	12.3587	38.0440	9.2947	0.8239	0.8787	11.5424

Note: *NPT=Nay Pyi Taw; KS=Kyaukse; HD=Hledan; SPK=Shwe Pauk Kan; Nk=North Okkalapa; MD=Mandalay*

The levels of As, Co, Cu and Pb were under the detection limits in all samples collected from Shwe Pauk Kan as shown in Tables 1 and 2. The levels of Pb were higher in the samples (especially) from the NK, HD, MD, and KS coded areas than others. This may be due to environmental pollution caused by industrial developments and other anthropogenic activities in those areas during that period. Daily consumption of rice contaminated with high concentration levels of Pb can negatively impact the renal, reproductive, immune and nervous systems [11]. The global risk assessor, Joint FAO/WHO Expert Committee on Food Additives recommends withdrawal of existing provisional tolerable weekly intake (PTWI) of 25 μg/kg body weight per week (~3.57 μg/kg bw/d) after a review of new scientific literature [12]. Furthermore, toxic metals such as lead can accumulate in the body, yet are unbiodegradable or not readily excreted. Thus, appropriate control measures such as routine monitoring in the food chain are needed to safeguard human health.

TABLE 2. ELEMENTAL CONCENTRATIONS IN PAWSAN RICE (AYEYARWADY)

Location (AYA)	Element	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
	ML (ppm)	0.2	0.4	0.01	1	10	5	5	1.5	0.2	50
NPT (7)	AVG	—	0.0121	—	0.1193	0.2551	24.1919	7.7015	—	0.0131	12.4188
	MIN	—	ND	—	—	—	12.5110	5.9059	—	ND	10.2057
	MAX	—	0.0207	—	0.8126	1.2625	36.7481	8.6538	—	0.0920	14.0913
Brand (2)	AVG	0.0208	0.0181	0.0232	0.1203	0.0521	5.9969	6.3282	—	0.3256	12.4291
	MIN	0.0162	—	—	0.0074	—	3.5689	6.2830	—	0.3205	12.4274
	MAX	0.0253	0.0362	0.0463	0.2331	0.1042	8.4248	6.3734	—	0.3307	12.4307
HD (5)	AVG	0.0063	0.0012	—	0.1328	0.5304	7.7867	7.1246	0.2475	0.1292	13.8994
	MIN	—	—	—	—	—	—	5.5689	—	—	10.5988
	MAX	0.0313	0.0060	—	0.2738	2.4031	14.2359	10.2290	1.2375	0.5007	18.0677
SPK (8)	AVG	—	0.0462	—	0.6734	—	11.8285	6.3562	0.2043	—	15.5208
	MIN	—	0.0238	—	0.2252	—	3.6663	3.7924	—	—	11.8751
	MAX	—	0.0644	—	2.0543	—	20.2723	9.3196	0.4831	—	19.6452
NK (2)	AVG	0.2486	—	0.1172	0.7051	12.2324	35.2269	9.0886	0.1323	0.7405	18.3153
	MIN	0.2311	—	0.1093	0.5231	12.0427	33.1222	8.9327	—	0.6591	17.3390
	MAX	0.2661	—	0.1251	0.8871	12.4222	37.3316	9.2445	0.2645	0.8220	19.2916
MD-9	AVG	0.2016	—	0.0633	0.5139	11.7572	37.2482	9.1203	—	0.7448	14.6635

TABLE 3. ELEMENTAL CONCENTRATIONS IN DIFFERENT RICE VARIETIES

ID (ALT)	Element	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
	ML (ppm)	0.2	0.4	0.01	1	10	5	5	1.5	0.2	50
JP-1	AVG	—	—	0.0012	1.3542	3.5853	31.1531	6.9162	0.3416	1.0807	10.0388
NS-2	AVG	—	—	—	1.0065	7.2496	34.0309	5.2441	0.7748	0.1124	11.8685
BMS-3	AVG	—	—	—	0.8998	7.3031	23.0808	4.4242	0.5907	—	9.0578
AYSZ-4	AVG	0.0194	—	—	1.2562	9.4091	53.5253	6.4424	0.5362	0.3006	12.8947
B-13	AVG	0.1102	—	0.0435	0.2368	0.17	7.7598	5.7688	—	0.1194	8.7697
B-14	AVG	—	0.2588	0.04	1.0311	—	4.7895	3.4616	0.7618	0.2769	6.2922
KS-13	AVG	0.0064	0.0198	0.0128	—	—	—	5.4298	0.0667	0.1952	4.0267

Note: *JP=Japanese Rice; NS=Naung Shae Rice; BMS=Beautiful Myanmar Shan Rice; AYSZ=Ayeyar Special Zeya Rice;B-13=Beautiful Myanmar Basmati Rice (Heho); B-14=India Gate Basmati Rice; KS-13= Kyaukse Magyantaw Rice.*

TABLE 4. AVERAGE CONCENTRATIONS OF METALS IN SBPS, AYAPS AND ALT

ID/ ML (ppm)	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
	0.2	0.4	0.01	1	10	5	5	1.5	0.2	50
SB	0.064	0.012	0.043	0.883	3.747	22.036	7.983	0.644	0.373	10.772
AYA	0.080	0.013	0.034	0.377	4.138	20.380	7.620	0.097	0.326	14.541
ALT	0.019	0.040	0.014	0.826	3.960	22.048	5.384	0.439	0.298	8.993

Table 4 shows the comparison of average levels of metals found in Shwebo Pawsan (SB) AYA, and ALT with the MLs. Cobalt (Co) Cr, Mn, Ni, and Pb content in SB samples were higher than the AYA and ALT samples. The average levels of As, Cu, Zn content were higher in AYA samples than others. The average levels of Cd, and Fe of ALT samples were higher than the rest. Generally, the average concentration levels of As and

Cd were below the MLs and only the average level of Pb was higher than the codex ML for the three sample types. The levels of Cr, Cu, Ni and Zn were below the MLs, while Co, Fe and Mn levels were above the MLs as described elsewhere [13].

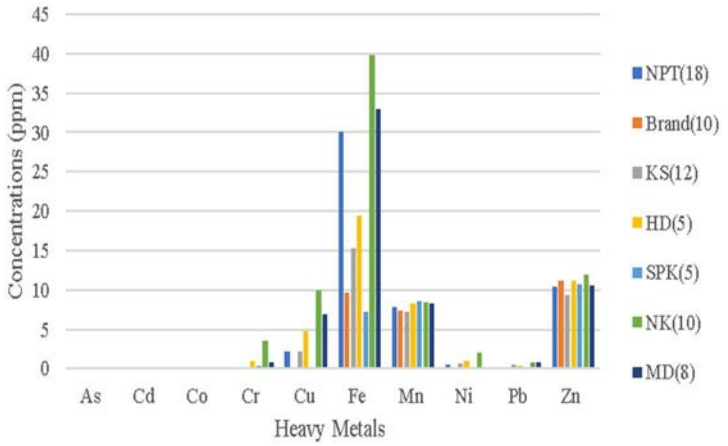
The WHO in 1996 noted that intake levels of Cr in adults has to be within the 250 mg/d upper limit while VKM [14] observed that the upper intake level for an adult may not exceed 1 mg/day. If the estimated daily intake values were calculated based on the Cr content and average daily consumption rate for Myanmar (~0.425 kg) [15] the maximum limits detected for SB, AYA, and ALT samples would be 375 mg/d, 160 mg/d, 351 mg/d, respectively. While Cu is not carcinogenic in humans or animals, JECFA in 1982 reaffirmed a provisional value for a maximum tolerable intake of 0.05–0.5 mg/kg bw per day (PMTDI) for Cu from all sources [16]. The European Food Safety Authority (EFSA) Scientific Committee established an acceptable daily intake (safe level) of 0.07 mg/kg.bw Cu for the adult population as no retention is expected for intake of up to 5 mg per day [17].

The calculated EDI values of Cu were 1.59 mg/d, 1.76 mg/d, and 1.68 mg/d for SB, AYA and ALT, respectively and all were well within the safe intake value of 5 mg/d. The EFSA established a Tolerable Daily Intake (TDI) of 13 µg/kg.bw for Ni in 2020 [17] and the calculated EDI values for SB, AYA, and ALT were well below the TDI value at 4.56 µg/kg.bw, 0.69 µg/kg.bw, and 3.11 µg/kg.bw, respectively. The daily intake for Zn according to the WHO is 15 mg/day. The EDI values determined in the study for SB AYA, and ALT were 0.076 mg/kg bw/d, 0.103 mg/kg bw/d, and 0.064 mg/kgbw/d, respectively, which were below the PMTDI for Zn of 0.3–1.0 mg/kg bw/d. The related Zn intake values per day were 4.58 mg, 6.18 mg and 3.82 mg equivalent to 30%, 41%, and 25 % respectively required daily intake of SB, AYA, and ALT samples.

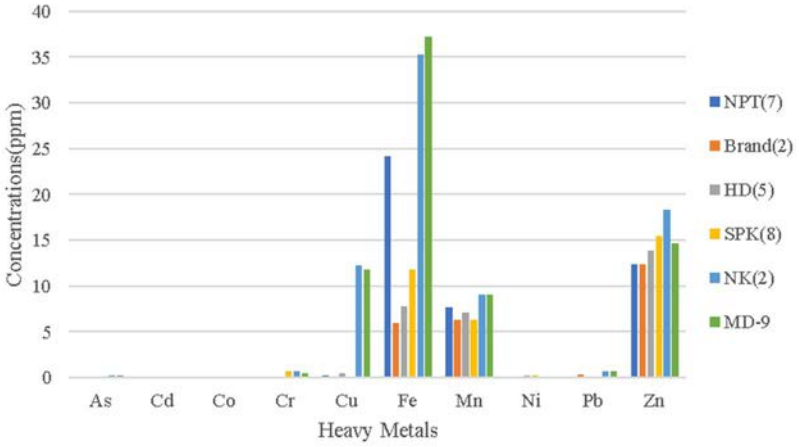
Although low levels of Co are essential for humans as a part of vitamin B12 for instance, high levels can adversely affect respiratory and haematological systems. According to International Agency for Research on Cancer (IARC) the daily cobalt intake for the general population ranges between 1.7–100 µg diet being the main source. The minimum recommended daily intake for an adult is 3 µg, corresponding to 0.012 µg of cobalt. The IARC evaluated Co and associated compounds as possible human carcinogen (group 2B) [18]. Estimated population average intake of Co have been reported at 0.12 mg/day in the UK, 0.005–0.04 mg Co/day in the US, 0.011 mg Co/day in Canada, and 0.029 mg Co/day in France [14].

The observed Co EDI values for SB, AYA, and ALT samples were 0.018 mg/d, 0.014 mg/d and 0.006 mg/d respectively, and these values were within the daily intake range from diet, and well below the oral threshold of 600 µg per capita [14]. Food and Nutrition Board of Institute of Medicine of the National Academies has described the estimated average requirements as Dietary Reference Intakes (DRIs). The DRI for iron (Fe) was 8–18 mg/d and Mn was 1.8–2.3 mg/d [19]. The EDI values for Fe were 9.36 mg/d, 8.66 mg/d and 9.37 mg/d, respectively, and 3.39 mg/d. 3.24 mg/d, and 2.29 mg/d, for Mn. While Fe plays a vital role in the human body [20] and the values are within acceptable range, it may have negative side effects. Since the Tolerable Upper Intake Level (UL) for adults is 45 mg/day for Fe, the current findings results were well below the UL [19]. Manganese another essential nutrient was found below an established level of 11 mg/day set for adults and presents no health risk [19].

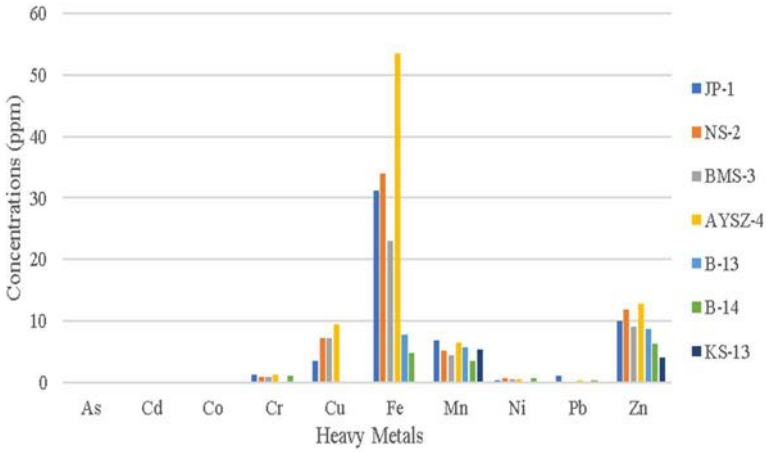
Figures 2. A, B and C show the average concentration levels (ppm) of heavy metals in SBPS, AYAPS, and ALT collected from different areas. The average levels of Cu, Fe, Mn and Zn were higher than the other metals in all sample types. Depending on production and collection areas, the different levels of heavy metals can be seen in the figures below. Figure 2D shows the comparison of average As, Cd and Pb levels in SBPS, AYAPS and ALT samples. The average levels for As and Cd were below the MPLs, unlike Pb which was above the ML. Lead is in the environment, in industrial effluents or a result of human activities [11]. This could explain the high values in the 2022 samples.


45
40
35
30
25
20
15
10
5
0
Concentrations (ppm)
As
Cd
Co
Cr
Cu
Fe
Mn
Ni
Pb
Zn
Heavy Metals
NPT(18)
Brand(10)
KS(12)
HD(5)
SPK(5)
NK(10)
MD(8)

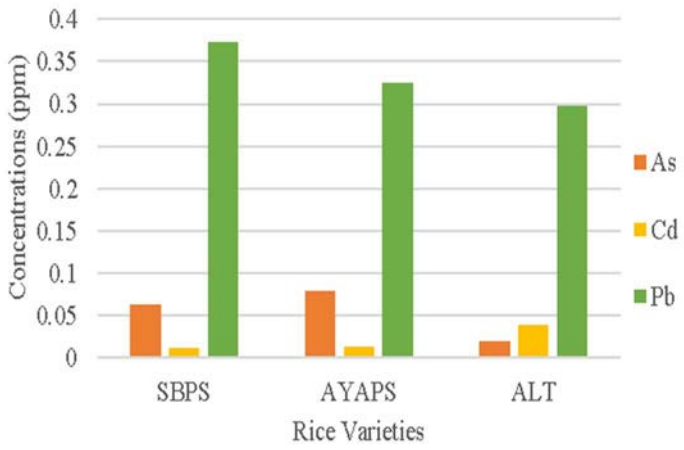
(A)


40
35
30
25
20
15
10
5
0
Concentrations(ppm)
As
Cd
Co
Cr
Cu
Fe
Mn
Ni
Pb
Zn
Heavy Metals
NPT(7)
Brand(2)
HD(5)
SPK(8)
NK(2)
MD-9

(B)


60
50
40
30
20
10
0
Concentrations (ppm)
As
Cd
Co
Cr
Cu
Fe
Mn
Ni
Pb
Zn
Heavy Metals
JP-1
NS-2
BMS-3
AYSZ-4
B-13
B-14
KS-13

(C)



(D)

FIG.2. Concentrations (ppm) of heavy metals in (A) SSBPS, (B) AAYAPS) (C) Different Rice Varieties (ALT) (D) SBPS, AYAPS and ALT.

4. CONCLUSIONS

The metals/elements including As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were determined by ICP-OES to verify authenticity of Pawsan rice samples produced in Shwebo, Ayeyarwady, and other rice varieties. The SIMCA model of the CAFE software was used and a comparison of the elemental concentration levels with Codex standards was carried out. Authenticity-verification results showed 100 % sensitivity and 84% specificity in discriminating between Shwebo Pawsan and others. Authenticity verification for high-quality rice such as Shwebo Pawsan by its geographical origin is essential to protect consumers, producers, reputable distributors and in expanding the global rice export market. It is speculated that the specificity could exceed 85% if further investigation on authenticity verification included stable isotopes for hydrogen (H) carbon (C) nitrogen (N) oxygen (O) sulphur (S) etc and trace elements: rubidium (Rb) molybdenum (Mo) samarium (Sm) mercury (Hg) strontium (Sr) all in combination with chemometrics software. Lead levels were above Codex MLs, and overall, the heavy metal contents were higher in the Shwebo Pawsan rice samples than those in others although still within the acceptable intake and quite well below the UL.

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ENHANCING COFFEE-SECTOR INTEGRITY THROUGH MULTISPECTRAL MICROSCOPY: AN ACCURATE, PORTABLE AND LOW-COST SOLUTION

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Abstract

The roasted and ground coffee sector faces a regulatory challenge with a lack of a specific method for detecting adulteration fraud, highlighting the need for more robust approaches. In collaboration with Brazil's Ministry of Agriculture, Livestock, and Supply (MAPA) OpenScience developed a multispectral microscope for scientific-fraud detection in coffee. Samples (n=142) were provided by MAPA for analysis and included arabica and robusta coffee with common adulterants. Spectra from images underwent Principal Component Analysis (PCA) flagging impurities. For distinguishing coffee types, a Partial Least Squares-Discriminant Analysis (PLS-DA) model was applied, offering a swift fraud control solution. This innovation not only addresses regulatory gaps but also establishes a robust industry-standard, ensuring coffee quality. These technologies enhance transparency, providing a tool for authorities and producers. Efficient fraud detection is crucial for preserving the sector's integrity and global economic significance, making it vital to pursue more effective methods. The project's promising results showcase its potential in maintaining the coffee sector's reputation and authenticity.

1. INTRODUCTION

Coffee is one of the most emblematic and economically significant products in Brazil, playing a crucial role in the country's economy and generating millions of jobs [1]. With a history dating back to the 18th century, Brazil has established itself as the largest producer and second largest consumer of coffee in the world, accounting for approximately 34% of global production [2]. In this context, it is important to ensure the safety of coffee. Adulterations frequently occur during roasting and grinding, where materials such as husks, corn and barley are added either accidentally or with the aim of increasing volume and reducing costs, compromising the quality and safety of coffee [3, 4]. Identifying this type of adulteration is difficult since coffee particles and impurities are almost indistinguishable to the naked eye [5].

The global demand for high-quality coffee has been growing, with consumers seeking guarantees of origin and sustainable practices. Importing countries, especially in Europe and North America, have established stringent quality standards, requiring certification and traceability. This pressures Brazilian producers to maintain high standards of quality to compete in the global market. Brazilian legislation stipulates that coffee with more than 1% foreign material is considered unfit for consumption [6]. Historically, the detection of adulterants in coffee has been carried out using traditional methods, such as sensory, physicochemical, and microscopic analysis [7]. Sensory analysis involves evaluating the flavour, aroma, colour, and texture of coffee by trained experts to identify atypical characteristics that may indicate adulteration [8]. While effective in some cases, this method is subjective and heavily depends on the evaluator's experience, besides being impractical for large sample volumes.

Physicochemical methods, such as liquid chromatography and nuclear magnetic resonance, are more precise and objectively quantify the chemical components present in coffee. However, these methods can be expensive, time consuming, and require sophisticated equipment and highly qualified personnel. Additionally, many of these techniques are destructive, meaning the coffee sample cannot be reused after analysis [9, 10]. Traditional microscopy is also used to identify visible adulterants in ground coffee. Although it is a relatively cheap and easy-to-implement technique, it requires a specialist and demands time and sample preparation [11]. Furthermore, manual identification of microscopic adulterants can be extremely labour-intensive and prone to human error [12].

The detection of adulterant has gained prominence, driven by the growing demand for faster, more accurate, and non-destructive methods. Technologies such as near-infrared spectroscopy (NIRS) raman spectroscopy and mass spectrometry have shown great potential in coffee analysis [9]. The NIRS quickly

identifies chemical compounds without destruction but may be insufficient for very low concentrations of adulterants. Raman spectroscopy is highly sensitive and detailed but can be limited by fluorescence interferences and high costs. Mass spectrometry offers precise and quantitative identification, but its cost and complexity can restrict its large-scale use [13].

Multispectral microscopy is an advanced technique that combines optical microscopy with spectral analysis, allowing the acquisition of detailed spectral information at each pixel of a microscopic image. This can be achieved by capturing multiple images at different light wavelengths, which are then combined to create a three-dimensional hyperspectral cube. Each layer of this cube corresponds to an image acquired at a specific wavelength, providing a complete spectrum for each point of the sample [14].

Spectral images have been widely used in various food industries to ensure product authenticity and quality [15]. They have already been employed to identify adulterations in grains such as wheat, rice, and corn [16–18] in fruits and vegetables, assess product maturity and quality, and detect internal defects not visible to the naked eye [19, 20] and in detecting fraud in animal products such as meat and dairy [21, 22]. In this context, to address the mentioned challenges, a multispectral microscope was developed for a project in collaboration with MAPA. This technology allows for detailed sample analysis, accurately and quickly identifying adulterants. By incorporating multivariate data analysis techniques such as PCA and PLS-DA, the system offers a solution for inspection and quality control in the coffee sector.

2. MATERIALS AND METHODS

2.1. Multispectral microscope

To develop a low-cost screening methodology for identifying adulterations in roasted and ground coffee, the study presents the construction of a multispectral microscope. The development of the device is based on capturing 24 images of the same sample, illuminated by LEDs (light emitting diodes) of different wavelengths. These images are concatenated to form a multispectral data cube. The components used for the construction of the microscope are detailed in Table 1 and illustrated in Fig. 1.

TABLE 1. MAIN COMPONENTS USED FOR THE CONSTRUCTION OF THE MULTISPECTRAL MICROSCOPE

Materials	Functionality
Camera	High-quality Raspberry Pi camera module, adapted for capturing high-resolution images.
Telecentric Lens	Ensures uniform magnification of the sample.
Controllers	Atmega328 for CNC and LED control, and a Raspberry Pi for camera control.
Display	Interface for viewing captured images.
Batteries	Rechargeable batteries allow autonomous operation of the microscope for about a day.
LEDs	Illumination provided by high-brightness LEDs emitting light in 24 distinct wavelengths (365 to 1050 nm).
Dome	3D printed to ensure uniform illumination free from external contamination.
CNC Table	Allows automated movement of the microscope for sequential image capture.

The equipment was designed for high analytical capacity, resulting in a portable microscope that allows prolonged analyses without the need for internet connection or cables, in addition to being more cost effective. These characteristics are important for democratizing access to crucial technologies in ensuring food quality and safety. Although the microscope was initially employed to detect adulterations in roasted and ground coffee, its versatility also makes it suitable for diagnosing plant diseases and studying contamination by fungi and other microorganisms.

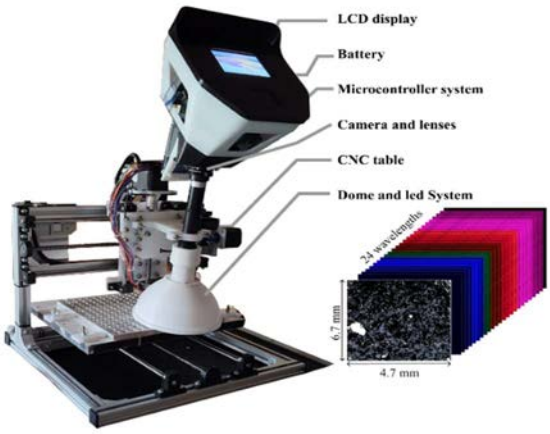

FIG. 1. Multispectral microscope along with an example of a data cube obtained through this equipment.

2.2. Samples and treatment

The Brazilian MAPA prepared 131 samples of dark roasted and ground coffee. These samples were sourced from various regions of Brazil (Bahia, Espírito Santo, Minas Gerais, Paraná, Rondônia, and São Paulo) including Arabica and Robusta varieties, as well as blends in different proportions. Regardless of origin and variety, some samples were adulterated with coffee husks, wheat, açaí seeds, barley, or corn. The coffee and the adulterants were roasted separately, then ground and blended in various proportions of coffee-to-impurity.

The samples provided by MAPA comprised 60 binary mixtures and 14 ternary mixtures of Arabica and Robusta coffees combined with the following adulterants: coffee husks, wheat, açaí seeds, barley, and corn. These samples were adulterated in varying proportions ranging from 3% to 20% (m/m) of adulterant content. The ternary mixtures featured Arabica and Robusta in a typical ratio of 70:30, along with one adulterant in the specified proportions, while binary mixtures consisted of a single coffee variety blended with one adulterant. There were 44 pure Arabica samples and 13 pure Robusta samples, used as the base for these mixtures. For each sample, the roasted and ground powder was compressed using a manual press. With the aid of the CNC table attached to the microscope, programmed in Gcode, four images were obtained from each sample, one from each quadrant, totalling 592 multispectral images (each with 24 wavelengths).

2.3. Data analysis

Data analysis was performed in MATLAB. The images were imported and converted to grayscale. To identify adulterants and locate adulterant pixels, a pixel-by-pixel analysis was performed. Each pixel had its spectrum extracted, which was processed with L1 type normalization, followed by SNV (standard normal variate) processing. To construct the PCA model, spectra from 160 pure coffee samples (100% Arabica and 100% Robusta) were used. The PCA with four principal components (PCs) achieved an accumulated variance of approximately 91%. With this PCA, the scores (T) and loadings (P) matrices were obtained. The reconstructed matrix was obtained by multiplying $T \times P'$. The residual matrix (E) was derived by subtracting the original matrix from the reconstructed matrix. The Q matrix was obtained by summing the squares of the values in each row of the E matrix, resulting in a training Q matrix used to construct a pseudo univariate control chart.

For each new image, the conversion process to spectrum described above is performed. Then, the spectra are evaluated by PCA, which returns a Q test value. This value is compared with the previously constructed control to determine if the spectrum belongs to the group modelled in the PCA (Robusta and Arabica coffee) or not. This allows differentiation between coffee and foreign material pixels. Subsequently, the pixels identified as coffee were subjected to a PLS-DA model, trained with 100% Arabica and 100% Robusta coffee samples, to differentiate between these two groups.

After these two analyses, the pixel spectra are classified as Robusta coffee, Arabica coffee, or impurity. The image is then reconstructed, showing the distribution of impurities. Additionally, the pixels of each class per sample were counted to determine the percentage of apparent impurities in the sample. This information is used to identify the presence of foreign material in the sample. Furthermore, the study evaluated another analysis methodology, considering the impurity in the entire image rather than pixel by pixel. The grey scale-converted images were transformed into pixel distribution histograms. These histograms were normalized using Pareto scaling and centred on their means. The histograms were grouped into two categories: pure and adulterated

samples, separated into calibration and validation sets in a 3:1 ratio. With these data, PLS-DA was employed using PLS Toolbox 9.0, facilitating sample differentiation based on attributes from the histograms.

3. RESULTS AND DISCUSSION

3.1. Partial least squares discriminant analysis (PLS-DA)—a sample level approach

The constructed PLS-DA models effectively differentiated adulterated and unadulterated samples, at an overall accuracy of 95%. Additionally, the models reached calibration and prediction sensitivities and specificities of 0.975, 0.970, 0.962, and 0.878, respectively. Figure 2 shows the calibration and prediction confusion matrices.

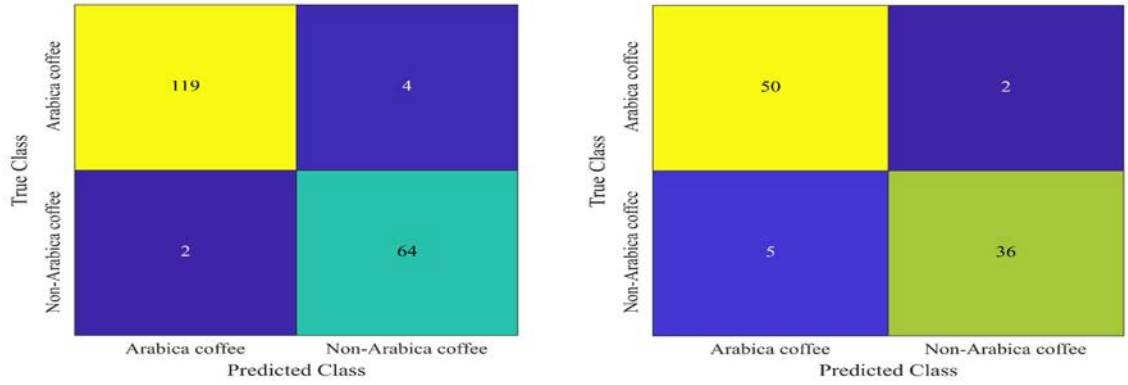

FIG. 2. Confusion matrices: a) Calibration and b) Prediction.

3.2. Integration of PCA and PLS-DA techniques-a pixel level approach

The joint application of PCA and PLS-DA techniques enabled detailed pixel-by-pixel classification, as shown in Fig. 3, 4.2% impurity was identified in a sample with 4% real impurity (wheat). In Fig. 3, the detection was 3.3% impurity, compared to a real value of 3% impurity (barley). The low residual values in these analyses highlight the potential of the developed method for identifying adulterations. Moreover, the pixel-by-pixel identification approach offers higher detectability for small impurities in samples. At the pixel level, the presence of impurities is often abundant, which improves the quantification limits of the technique. This allows spectral microscopy to be used as a combined tool with complementary local analysis methods, such as laser-assisted rapid evaporative ionization mass spectrometry.

However, some precautions have to be considered. Detecting adulterations in coffee requires a meticulous analysis of the physico-chemical characteristics of ground coffee. The LED system, which provides 24 specific wavelengths, facilitates the identification of a broad spectrum of information. Light absorption at different wavelengths can provide a chemical fingerprint of the foreign materials mixed with coffee, potentially providing an additional level of information about the source of adulteration. Despite the relatively low spectral resolution of about 28 nm, this limitation is compensated by the high spatial resolution of the system. It is worth noting that the developed method may present limitations when applied to samples with altered moisture and that precautions have to be taken to isolate the sample from external light during image capture and to consider the effects of vignetting.

In summary, the developed method for verifying the presence of 100% coffee proved sensitive to impurity detection. Developed from low-cost images obtained by multispectral microscopy, it offers a quick way to identify impurities. Furthermore, pixel-by-pixel identification is crucial as it allows visualization of the location of impurities in the image, complementing other analytical methods.

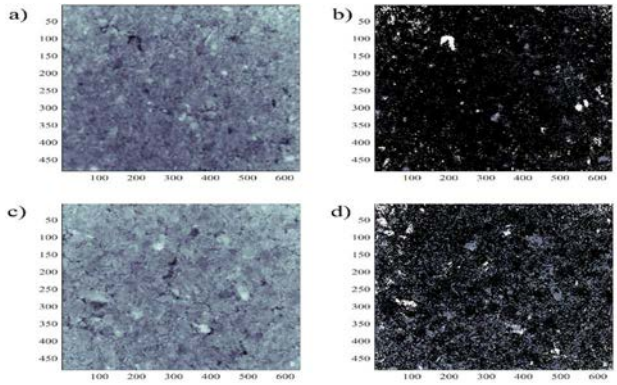

FIG. 3. Images (a) and (c) represent the average of the captured wavelengths. The images (b) and (d) are the reconstructed images using PCA and PLS-DA techniques for detailed pixel-by-pixel classification.

4. CONCLUSIONS

The study highlighted the importance of developing alternative and complementary methods to detect adulterations in coffee, a product of significant economic and cultural importance to Brazil. Since the prevalence of adulterations during roasting and grinding compromises the quality and safety of coffee, it directly affects consumer trust and competitiveness in the international market. By using a low-cost, portable multispectral microscope, the study demonstrated the effectiveness of this technology in identifying adulterants. The integration of advanced chemometric techniques, such as PCA and PLS-DA, allowed visualization of impurities in the sample, revealing points of interest for a specialist to analyse complementarity, either with a microscope or other equipment of local analysis. Similarly, results obtained from a whole image analysis with PLS-DA models achieved an overall accuracy of 95% in identifying 100% Arabica coffee.

The effectiveness of multispectral microscopy demonstrated in the study can be transferred to other fields in the food industry, where product authenticity and purity are equally important. The ability to quickly analyse large volumes of samples and provide accurate results positions this technology as a valuable tool for food regulators and producers, and it can be used in parallel with other analytical techniques. Ultimately, adopting detection technologies like multispectral microscopy is vital to maintaining the integrity of Brazilian coffee in the global market. Promoting sustainable and transparent practices that benefit the entire production chain is essential for the sector's sustainability. The study not only validates the applicability of multispectral microscopy in coffee quality control but also sets a precedent for continuous innovation in quality assurance methods across the food and beverage industry, ensuring the safety and trust of global consumers.

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EFFORTS OF SHARJAH CITY MUNICIPALITY TO UNVEIL FRAUD IN PROTEIN CONTENT OF MILK POWDER PRODUCTS

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Abstract

Consumption of milk powder promotes nutrition and enhances public health among communities. As one of the most accessible and widely embraced dietary essentials, milk powder provides an array of vital nutrients, including high-quality protein and beneficial fats. These nutrients are readily assimilated by the body, fostering optimal growth and development. However, milk powder can be subjected to adulteration, which can pose threats to public health, consumer trust, and undermine the food industry. A prevalent form of this deception involves the manipulation of protein content in milk powder. Addressing this challenge, Sharjah City Municipality has adopted an innovative solution known as the Dumas method. This method employs fast, green, and fully automated technology to combat fraud related to protein content in milk powder. This proactive approach is based on subjecting food products to a series of reactions, including combustion and reduction using helium and oxygen gases under specific conditions, to produce nitrogen gas. The resulting gas is then detected by the detector and converted into a protein percentage through mathematical operations. This result ensures the provision of genuine and nutritional products that are essential for consumer well-being, as well as enhancing consumer trust and upholding the credibility of the food industry.

1. INTRODUCTION

Milk is among the most versatile and valuable products in the food industry [1]. It is a complex, heterogeneous emulsion of proteins, lactose, fat globules, minerals, and other minor constituents [2]. Milk is the main dietary source of nitrogen in humans [3]. Moreover, milk contains an uncountable number of distinct proteins, which are categorized into caseins and whey proteins as well as non-protein nitrogen components. Milk powder can be adulterated with vegetable protein, other milk type, whey, and water. A wide range of adulterants such as acids, formalin, and melamine among others, can cause adverse health effects [4].

Evaluation of milk quality parameters are necessary for quality control, food labelling, and research and development. Common parameters to evaluate milk quality are fat percentage solid-not-fat (SNF) percentage, protein content and freezing point [4]. Several studies indicated fraud issues related to the protein content in milk powder [5–8]. Therefore, identifying and quantifying milk proteins has to be a priority in food testing laboratories to ensure its authenticity. DNA-based techniques can be used to detect adulterants in traded commodities of plant and animal origin utilizing three approaches, including polymerase chain reaction (PCR)-based techniques, sequencing, and hybridization-based techniques [9]. Moreover, different analytical methods are used for determining total protein and can be broadly categorized into three main categories: direct protein determination, indirect protein determination and total nitrogen determination. DNA-based techniques can be used to detect adulterants in traded commodities of plant and animal origin utilizing three approaches, including PCR-based techniques, sequencing, and hybridization-based techniques [9]. Moreover, different analytical methods are used for determining total protein and can be broadly categorized into three main categories: direct protein determination, indirect protein determination and total nitrogen determination.

Direct protein determination involves chromatographic methods such as size-exclusion chromatography, ion-exchange chromatography and reverse-phase chromatography and electrophoresis [10]. Immunology-based techniques such as Enzyme-linked Immunosorbent Assay (ELISA) [9] have also been used. Indirect protein determination includes spectroscopic methods such as near- and mid-infrared spectroscopy. Lastly, total nitrogen determination involves chemically digesting the sample by kjeldahl method or combusting it by Duma's method. The kjeldahl method consists of multiple time-consuming analysis steps and employs hazardous chemicals. In contrast, the Dumas method is a fully automated technique that is environmentally friendly, replacing toxic chemicals with helium and oxygen gases [11]. Therefore, this study utilizes the Dumas method to determine protein content in milk powder.

2. MATERIALS AND METHODS

2.1. Chemicals

Glycine of ≥99.7% purity, high pure oxygen and helium gases, were used for oxidation-reduction process.

2.2. Method principle

The Dumas/AOCS Ba 4e 93 method [12] is a combustion technique for quick and easy determination of nitrogen by heating food at very high temperatures (95–1100°C) in the presence of high oxygen levels. The method now validated by various professional societies such as the American Oil Chemistry Society (AOCS) under the name AOCS Ba 4e 93. The gases generated are passed through filters to remove non-nitrogen containing gases. The nitrogen-containing gases are then passed through a copper filled tube reducing the content to N_2 which is measured through a thermal-conductivity detector as detailed elsewhere [12, 13].

2.3. Sample preparation and analysis

Glycine standard and milk powder samples (200 mg) were separately weighed in different ceramic crucibles using an analytical balance, then placed into the autosampler of the instrument for analysis. Calibration was conducted once a month. According to the manufacturer, glycine standards of varying amounts — 20 mg, 40 mg, 60 mg, 100 mg, 140 mg, 180 mg and 220 mg — were also weighed using an analytical balance and subsequently placed into the autosampler [13]. Conformity assessment of the measurement results was performed by applying a binary decision with a guard band rule, in accordance with a guideline specified in ISO/IEC 17025:2017 [15] and the lower tolerance limit that was used to calculate lower acceptance limit according to Codex Alimentarius [16]. Conformity assessment of the measurement results was performed by applying a binary decision with a guard band rule, in accordance with known guidelines [15] and the lower tolerance limit that was used to calculate lower acceptance limit was obtained from the Codex Regulation [16].

3. RESULTS AND DISCUSSION

The adulteration of milk composition to mask the quality parameters is a common practice [14]. A total of 40 samples of milk powder were analysed for total nitrogen according to the manufacturer's instrument manual based on Dumas/AOCS Ba 4e-93 [12] . Table 1 shows the protein not-fat content in milk powder, which ranged from 1.73% to 36.12%.

TABLE 1. THE TOTAL PROTEIN AND PROTEIN IN MILK SOLIDS-NOT-FAT CONCENTRATION IN MILK POWDER SAMPLES

Sample ID	Origin	Total protein (%)	Protein in milk solids-not-fat (%)
S1	United Arab Emirates	14.10	20.03
S2	United Arab Emirates	13.5	18.85
S3	United Arab Emirates	11.23	15.18
S4	United Arab Emirates	13.33	19.02
S5	United Arab Emirates	14.73	20.21
S6	United Arab Emirates	2.10	2.68
S7	United Arab Emirates	12.69	18.69
S8	United Arab Emirates	14.99	19.85
S9	United Arab Emirates	13.59	19.41
S10	United Arab Emirates	14.57	20.18
S11	United Arab Emirates	5.68	6.84
S12	New Zealand	1.34	1.78
S13	United Arab Emirates	0.89	1.23
S14	New Zealand	12.10	16.64
S15	New Zealand	1.02	1.50
S16	New Zealand	0.64	0.80

Sample ID	Origin	Total protein (%)	Protein in milk solids-not-fat (%)
S17	United Arab Emirates	1.28	1.73
S18	United Arab Emirates	9.06	12.33
S19	United Arab Emirates	9.38	12.66
S20	United Arab Emirates	1.60	2.26
S21	New Zealand	23.3	34.31
S22	New Zealand	23.19	34.56
S23	United Arab Emirates	24.18	35.44
S24	United Arab Emirates	25.52	33.94
S25	United Arab Emirates	24.5	35.53
S26	United Arab Emirates	24.4	34.54
S27	United Arab Emirates	24.88	36.12
S28	United Arab Emirates	24.4	35.36
S29	United Arab Emirates	24.94	34.69
S30	United Arab Emirates	24.11	33.10
S31	United Arab Emirates	25.39	34.42
S32	United Arab Emirates	25.07	34.04
S33	United Arab Emirates	23.8	32.62
S34	New Zealand	23.7	32.24
S35	New Zealand	24.2	34.47
S36	Belarus	24.88	34.31
S37	New Zealand	23.41	34.56
S38	United Arab Emirates	23.44	35.44
S39	United Arab Emirates	24.3	33.94

The results were subjected to conformity assessment to determine the compliance status. To illustrate further, the evaluation of conformity employs a decision rule that considers measurement uncertainty when determining compliance with specification standards. There are two approaches to expressing conformity based on ISO/IEC guide 98-4:2012, each of which contains a level of risk (false positive and false negative). The first approach employs a binary decision with simple acceptance, where the acceptance threshold equals the tolerance limit. This method dictates that results below the specification limit are accepted, and those above are rejected. However, it entails a 50% risk of false acceptance and false rejection when the result is close to the tolerance limit. The second approach uses a binary decision with a guard band, which involves the usage of the uncertainty of the method to calculate the guard band [15]. Equation (1) shows the guard band calculation of the second approach [16].

$$w=rU \tag{1}$$

Where w is the guard band and r is a factor that varies according to guidelines and is chosen to ensure a minimum conformance probability for an item that is accepted. A common choice is r=1, therefore r=w and is multiplied by U which represents the expanded uncertainty [15]. That is equal to 0.055% and is obtained from the uncertainty measurement of the method. The value of the guard band is then used to calculate the acceptance limit through Eq. (2) [16].

$$w=TU-AU \tag{2}$$

Where TU is the upper tolerance limit and AU is the upper acceptance limit.

The binary decision with a guard band has a global risk level for false acceptances (global risk) ≤ 2.0, which is lower than that of the binary decision with simple acceptance [15]. Therefore, the compliance assessment of the study's results was conducted using a decision rule based on binary statements with a guard band following set guideline [15]. The Codex guideline defines a singular minimum tolerance limit for the protein in milk solids-not-fat parameter, which equals 34% [16]. Accordingly, in this study, only the lower

acceptance limit was calculated. Results exceeding this limit are considered compliant, while those below are deemed noncompliant [17]. Sixty four percent of the milk solids-not-fat in the milk powder samples were below the acceptance limit of 33.95%, calculated using Eq. (2) while 36% satisfied this criterion which is shown in Fig. 1.

Thus, screening of the protein content in milk using the Dumas method revealed that the majority of the samples failed to meet the calculated acceptance limit. As a result, it is suggested to screen more milk powder with further steps and confirm using other techniques to identify the type of adulteration. Sixty four percent of the milk solids-not-fat in the milk powder samples were below the acceptance limit of 33.95%, calculated using Eq. (2) while 36% satisfied this criterion which is shown in Fig. 1. Thus, screening of the protein content in milk using the Dumas method revealed that the majority of the samples failed to meet the calculated acceptance limit. As a result, it is suggested to screen more milk powder with further steps and confirm using other techniques to identify the type of adulteration.

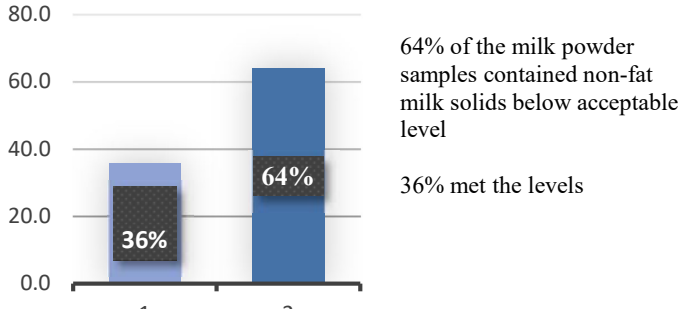

FIG. 1. Percentage levels of milk solids-not-fat in the powdered milk samples.

4. CONCLUSIONS

Milk protein is an essential macronutrient for the maintenance and building of body tissues and muscle. The current project studied the protein content using a Dumas method. Through this study, it was found that the results of the screened milk powder samples ranged from 1.73% to 36.12%. The majority of the milk powder samples did not meet the requirements of the calculated acceptance limit for protein in milk solids-not-fat, which is 33.95%. The study suggests further investigation using other techniques such as confirmatory tests, such as gel electrophoresis [18] NMR spectroscopy [19] ELISA [20] and PCR [21] to ensure the authenticity of protein in milk powder products. Additionally, other components in milk powder products, such as fat and sugars, need to be screened to prevent adulteration.

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VOLATILOMICS FOR AUTHENTICITY ASSESSMENT OF RAW RED MEATS

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Abstract

Typically, authenticity assessment of meat involves time-consuming techniques, which require trained personnel and provide retrospective results. The aim of this work was to screen the volatilome of raw red meats (beef, pork and horse) under different storage conditions and ratios of adulteration and explore it to trace back the meat's history. In total 640 HS/SPME-GC-MS chromatograms were collected from different experimental procedures. Based on the meat's volatilome and applying supervised methodologies, validated models for the preliminary screening of meat samples were developed for authenticity assessment and potential adulterant identification. High classification rates (>90%) were achieved during the prediction of meat class. Lastly, Greedy community detection on estimated partial correlation networks through the graphical LASSO (gLASSO) revealed the grouping of compounds that were not related structurally or by any known biochemical pathway. Metabolomics with multivariate data analysis can be an important tool for assessing the authenticity of raw meats.

1. INTRODUCTION

Under the umbrella of meat quality, several critical issues arise, including its physicochemical composition, microbiological condition and authenticity. Meat is vulnerable to spoilage and the development of off-odours due to its particular chemical composition (high water activity and richness in nutrients) [1]. Food authenticity is closely linked to the issue of food adulteration, which may pose risks to human and animal health, and result in billions of euros in lost revenue [2]. Analysis of rapid alert system for food and feed (RASFF) reports highlight that incidents of food fraud are increasing annually, with 17.1% of cases referring to meat and its products [3]. A notable example is the horse meat scandal of 2013, which significantly affected consumer trust.

To address the issue of meat authentication, several methods have been proposed, including DNA-based, omics approaches (proteomics, peptidomics, lipidomics, metabolomics, volatilomics) spectroscopic techniques (mid- and near-infrared spectroscopy and spectral imaging). Traditional methods for assessing spoilage and authenticity of meat are often time-consuming, providing retrospective and sometimes biased results. In response to these challenges, the era of foodomics in meat science has introduced metabolomics as an innovative tool for quality and authenticity-assessment, as well as for detecting potential adulteration. Rapid, cost-effective, accurate and reliable approaches that could provide robust results in a time-efficient manner are increasingly needed. These advanced techniques are increasingly supported by machine learning tools, enhancing their precision and efficiency. Within this context, this study aims to screen the volatilome (volatile metabolome) of raw red meats, including beef, pork and horse, and its adulterants under various microbiological conditions. By exploring the volatilome, the researchers sought to trace the history of the meat, providing a more efficient and accurate method for ensuring its quality and authenticity.

2. MATERIALS AND METHODS

2.1. Sample preparation

Domestically and/or industrially produced minced meat from different simulations were utilized in this study, in particular selected minced beef, pork, horse and its adulterants from previous studies [4–5] and all minced beef, pork and mixed samples (70%/30% beef/pork) as reported elsewhere [6]. These samples were subjected to microbiological analysis for Total Viable Counts (TVC) and then stored at -80°C prior to headspace solid phase microextraction coupled to gas chromatography mass spectrometry (HS/SPME-GC/MS).

2.2. Meat volatilomics

Sample preparation is detailed elsewhere [6]. Minced meat (2.5 g) from the whole burger (80 g) was homogenized in a 20 ml vial with using 5 ml of NaCl solution. The closed vial was agitated with a magnetic stirrer before headspace analysis on a gas chromatography mass spectrometer using well-optimized conditions [6].

2.3. Data analysis

The peak areas based on the target ion were normalized to *z*–scores and subjected to multivariate data analysis. *Z*-score transformation centres the data around a mean of 0 and scales it to have a standard deviation of 1. Different unsupervised (Principal Component Analysis, PCA) and supervised (Linear Discriminant Analysis, LDA, Partial Least Squares Analysis, PLSDA, Random Forests, RF) data analysis tools were applied to visualize, discriminate and predict meat classes. The dataset was split into 80%/20% ratio for training and testing, respectively. A LASSO regression model [7] was developed to predict the meat classes in terms of origin or based on the microbiological classes (Satisfactory: TVC < 5×10^5 cfu/g; Acceptable: $5\times10^5 \leq$ TVC < 5×10^6 cfu/g, Unacceptable TVC $\geq 5\times10^6$ cfu/g) according to the European Regulation (EC) 2073/2005. The models were tested by calculating the true positive, true negative, and Area Under the Curve (AUC) of a Receiver Operating Characteristic (ROC) curve.

3. RESULTS AND DISCUSSION

3.1. Volatilome-based authenticity assessment of red meats

In total 640 HS/SPME-GC/MS chromatograms were collected from different simulations and subjected to multivariate data analysis. To begin with, the peak areas were standardized to *z*-scores to have the data comparable across different scales and to improve the performance of prediction models. PCA showed that pure meat samples could always be distinguished from the adulterated samples, which had a distinct profile. A validated model for the preliminary screening of meat samples for authenticity assessment and potential adulterant identification was developed, based on meat's volatilome applying supervised methodologies. The developed LASSO regression model yielded an accuracy of 96%, while sensitivity ranged from 71.4% to 100% and specificity from 96.1% to 100% across meat classes. The AUC was always close to 1.0 indicating perfect discrimination, which meant that the model could perfectly distinguish between the positive and negative classes.

3.2. Volatilome-based characterisation of the microbiological condition of meat

The next question was whether volatilome could be utilized to discriminate meat according to the microbiological condition. Thus, samples were categorized as satisfactory (TVC < 5.7 log cfu/g) acceptable (5.7 log cfu/g ≤ TVC < 6.7 log cfu/g) and unacceptable (TVC ≥ 6.7 log cfu/g) according to the European Union regulation concerning the microbiological criteria for foodstuffs (EC 2073/2005). In this case, gLASSO regression model yielded 66.7% accuracy (Table 1).

TABLE 1. CLASSIFICATION RESULTS BASED ON THE MICROBIOLOGICAL CONDITION OF RAW RED MEATS

Statistics	[a]Satisfactory	[b]Acceptable	[c]Unacceptable
Sensitivity (%)	67.2	75.0	35.7
Specificity (%)	72.6	71.8	97.3
Pos predicted value	71.7	62.1	62.5
Negative predicted value	68.2	82.4	92.4
Prevalence (%)	50.8	38.1	11.1

[a]Satisfactory: TVC < 5×10^5 cfu/g; [b]Acceptable: $5\times10^5 \leq$ TVC < 5×10^6 cfu/g, [c]Unacceptable: TVC ≥ 5×10^6 cfu/g

The ROC curve is presented graphically in Fig. 1A. From the classification results it is evident that the misclassified samples were classified in neighbour classes. Further, the classes of satisfied and acceptable were merged and a new logistic regression model was developed to distinguish and discard unacceptable meat. In this case a 95.3%, 96.5% and 85.7% values were obtained for accuracy, sensitivity and specificity, respectively. An AUC of ~0.88 was determined implying that the model had a good level of separability between the positive and negative classes (Fig. 1B).

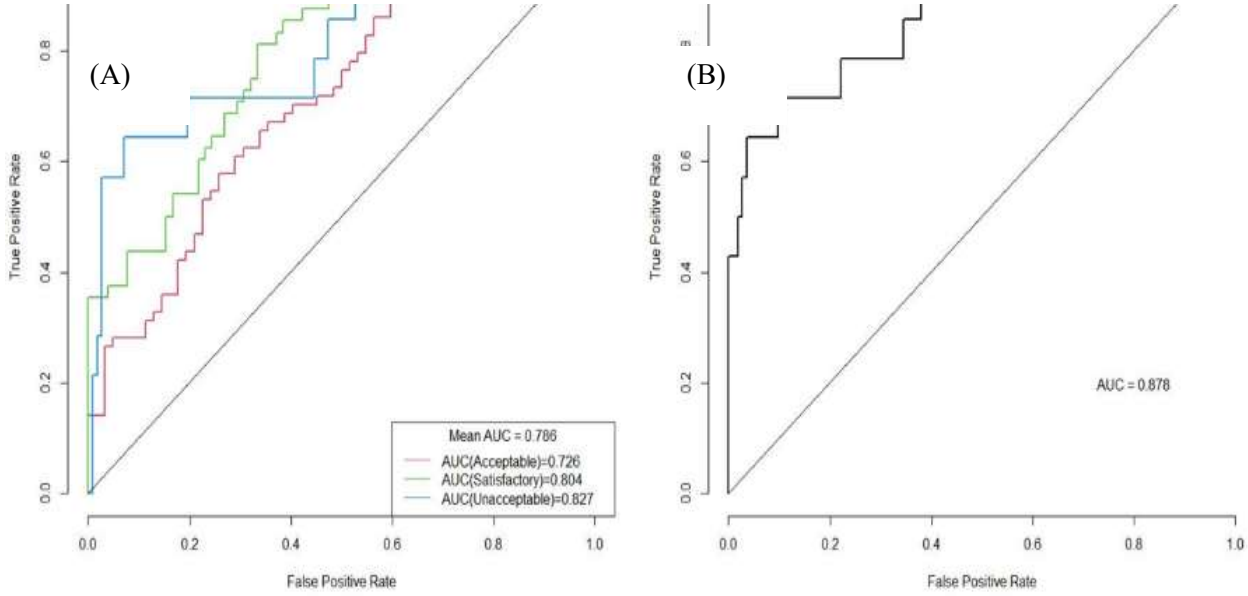

FIG. 1. ROC curve during classification according to the microbiological condition utilizing (A) 3 classes e.g., satisfactory, acceptable, and unacceptable, and (B) 2 microbiological classes (< or > 5×10^6 cfu/g).

4. CONCLUSIONS

This approach has considerable potential for further development and will aid both the meat sector and food safety regulatory bodies. The use of several batches and samples of meat is crucial in model development and validation. The results show that the metabolomics supported by multivariate data analysis could be applied as a primary tool for microbiological and authenticity assessment of raw meat. Historically, science has been moving from bulk room-size instruments to more compact benchtop applications. Nowadays, emerging tools, such as portable instruments, that can be used on-line and/or in the field could validate this approach.

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EVALUATION OF TOXIC ELEMENTS IN TURMERIC AND TURMERIC PRODUCTS ON THE JAMAICAN MARKET

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Abstract

The contamination of spices with potentially toxic elements such as lead is a global issue, and one which has raised concern in Jamaica, particularly after curry powder samples branded by Jamaican companies were recalled in the United States of America because of elevated lead concentrations. Such adulteration may be incidental, such as uptake from soil or contamination from processing equipment, as soils in Jamaica's agricultural zones contain elevated levels of some potentially toxic elements and trace elements. Adulteration of spices may also be economically motivated. A significant proportion of turmeric products on the local market, including curry powders and teas, are from local production, but some are of uncertain origin, and there are small and medium retailers that import products independently which would not necessarily be subject to surveillance monitoring as there is no local standard or technical regulation. A preliminary assessment showed some samples contained in excess of 1 mg/kg lead. This study assesses the trace element content using nuclear analytical and complementary techniques, including neutron activation analysis (NAA) and total reflection X ray fluorescence (TXRF) and the potential risk to the Jamaican population from consumption of these products.

1. INTRODUCTION

Turmeric and turmeric products regularly consumed in Jamaica and the wider Caribbean are a legacy of Indian migrants who emigrated to the region post slavery abolition. Aside from its use as a culinary spice, more recently turmeric has found medicinal use as herbal tea and dietary supplement. Agri-processors, including large multinational companies, have adopted its use as a natural food colouring agent in an effort to reduce or phase out artificial colorants. This increased usage in turn increases the potential for economically motivated adulteration and the need for increased surveillance. Few jurisdictions have established specific limits for lead in turmeric, which is a primary concern given past incidences of adulteration/contamination with lead chromate. Even for products labelled as being produced in Jamaica, the raw materials may be sourced from overseas because of insufficient domestic supply, and those imports are not necessarily tested (or retested) before being used in 'Jamaican-made' products.

Some labels state 'packed/packaged in', 'manufactured for' or 'distributed by', which does not necessarily indicate the country of origin of the product. Production statistics for turmeric are not available from the Ministry of Agriculture, Fisheries and Mining, Jamaica. The potential brand harm was underscored by the discovery of elevated levels of lead found in 'Jamaican curry powder' products sold in the USA [1, 2] although none of the products were produced in or sold in Jamaica [3]. Some of the products, however, were branded by a well-known Jamaican-based company [1]. In May 2024, a large quantity of counterfeit curry powder was seized by police from a supermarket in rural Jamaica [4]. In addition to the potential brand harm, there is also the consideration of potential adulteration of the product and threat to consumer safety.

With Jamaicans potentially consuming up to 0.59 kg of spices per capita per year [5] the possibility for dietary exposure to lead from consumption of spices cannot be overlooked. Admittedly, this is likely an overestimation but allows for a worst-case scenario of the dietary exposure estimate. The Provisional Tolerable Weekly Intake (PTWI) of 25 µg/kg bw has been replaced for not being adequately protective [6]. The PTWIs for arsenic and cadmium were also withdrawn in 2010; a provisional tolerable monthly intake of 25 µg/kg bw was established for cadmium [6]. Also, the PTWI for inorganic mercury, applicable to dietary exposure from foods other than fish and shellfish, is 4 µg/kg bw [6].

2. MATERIALS AND METHODS

Nineteen samples of dried turmeric, turmeric based teas (some containing ginger) and curry powder/seasoning were purchased on the open market and were representative of availability at the time of the study. Six (6) samples were dried ground turmeric powder; one (1) was dried whole turmeric rhizome, which was ground prior to analysis using a pulverisette automated agate mortar and pestle. Nine (9) samples were curry seasoning blends, seven (7) of which were powders; the remaining two (2) were 'wet' seasoning blends. The remaining three (3) samples were herbal teas, two of which contained ginger in addition to turmeric.

Samples were analysed for arsenic and cadmium by Instrumental Neutron Activation Analysis (INAA) using a research reactor. Short lived radioisotopes were measured using approximately 0.5 g of samples in appropriate bags and vials. Samples were irradiated for 3 min at 5 × 1011 cm^{-2} s^{-1} with a decay-time of 5 min and 60 min prior to measurement. Intermediate and long-lived radioisotopes were measured using ~1 g of the sample irradiated for 4 h at 1×1012 $cm^{-2}s^{-1}$ with a decay-time of 4 days and 14 days, respectively, prior to measurement. All samples were analysed using a high purity germanium coaxial gamma photon detector system with an efficiency of 40 % and a resolution of 2.1 keV at the ^{60}Co 1332 keV gamma line.

Samples were digested for TXRF analysis using a microwave digestion system. Approximately 0.5 g of each curried powder sample was digested in 10 ml of trace metal grade nitric acid at 200°C for 15 mins (power level 1600W, pressure 800 psi). After cooling, each digest was transferred to a vial and made up to 20 ml with deionized water. Lead was determined using a TXRF spectrometer. Calibration and internal standards were prepared from a solution of 10000 mg/l in 5% HNO_3. An aliquot of a cobalt (Co) solution was added as an internal standard (10 mg/l). The samples were then homogenized with a vortex mixer and analysed after pipetting 10 µl onto a quartz sample carrier/reflector and evaporating the solvent on a hot plate. The samples were irradiated under vacuum for 1000 s at 50 kV and 40 mA in a TXRF spectrometer. Reagent blanks were treated identically to the samples. Mercury was determined using a direct mercury analyzer. Approximately 0.1 g of sample was used for analysis. NIST 3299 (ground turmeric rhizome) was analysed using the same protocols.

3. RESULTS AND DISCUSSION

Range, mean and median for As, Cd, Hg and Pb are shown in Table 1 below. All data are expressed in mg/kg.

TABLE 1. ELEMENTAL DATA (RANGE) FOR TURMERIC AND TURMERIC PRODUCTS PURCHASED IN KINGSTON, JAMAICA

	As	Cd	Hg	Pb
range	0.018–0.407	0.033–1.44	0.0016–0.034	0.110–2.39
mean	0.117	0.515	0.009	0.648
median	0.079	0.375	0.006	0.48

Data for individual samples are shown in Table 2. All data are expressed as mg/kg.

TABLE 2. ELEMENTAL DATA FOR TURMERIC AND TURMERIC PRODUCTS BY CATEGORY

	Country of origin	As	Cd	Hg	Pb
Turmeric					
G-TURM-MFG0123	Jamaica	<0.054	0.035	0.003	0.41
IS-TURM-MFG0423	Jamaica	<0.083	0.496	0.031	0.28
ID-TURM-I1023323	Jamaica	0.078	0.369	0.006	0.44
FO-TURM-MN092022	Jamaica	<0.075	0.096	0.002	0.33
SW-TURM-KX27922IL	India	0.019	0.226	0.019	0.26
LX-TURM-03F2025	India	0.035	0.351	0.002	0.25
HM-TURM-1002022	India	<0.081	0.111	0.002	0.11
Curry powders/blends					
BP-CURRY-3J12092017	Jamaica	0.055	0.373	0.034	0.49
ISHI-CURRY-D2018	Jamaica	0.023	1.084	0.007	—

	Country of origin	As	Cd	Hg	Pb
HCI-CURRY	Trinidad and Tobago	0.044	1.018	0.003	0.39
BHI-CURRY-0517	Jamaica	<0.161	0.345	0.005	0.480
TH-CURRY-JL20186203	UK	<0.407	0.814	0.010	0.5
GL-CURRY-N0918	USA	<0.092	<0.828	0.008	2.387
BDJ-CURRY-148845	USA	<0.262	1.441	0.006	0.75
SPTW-CURRY-J162019	Jamaica	—	—	—	1.23
SHVM-06S2024 Teas	Pakistan	<0.258	0.856	0.002	0.78
CD-TURMERIC-GINGER-T5-1151	Jamaica	0.017	0.033	0.003	—
SPC-TURMERIC-S0923P19	Jamaica	0.238	0.376	0.014	1.33
CD-TURMERIC-GINGER-TEA-00727	Jamaica	0.115	0.409	0.007	0.6

***Note**: — Nothing detected*

The EU has established 1.50 mg/kg as the ML of Pb in root and rhizome spices [7]. Only one (1) sample exceeded this limit, a curry powder brand which was labelled as 'Packed in the USA'. Two (2) other samples exceeded 1.0 mg/kg but were below the ML set by the EU. These were a 'wet' curry seasoning blend (1.23 mg/kg) labelled as 'Product of Jamaica', and a sample of dried turmeric tea bags (1.33 mg/kg) also labelled as 'Product of Jamaica' and bearing the 'Jamaica Made Mark'. Use of the 'Jamaica Made Mark' requires a product to contain at least 51% of raw materials grown/produced in Jamaica. The certification is administered by the National Certification Body of Jamaica. A limit of 0.8 mg/kg Pb is also established for fresh ginger and turmeric; MLs are not specified for the other elements reported. The USA has not established limits for heavy metals; Bangladesh has a limit of 2.5 µg/g for lead, while India has a limit of 10 µg/g [8, 9].

Elevated levels of lead have been found in turmeric and turmeric products in other jurisdictions, up to 2.6 mg/kg in curry powder and as high as 74 mg/kg in turmeric in the USA [10] and more than 700 mg/kg in some samples from India and Bangladesh [9]. A limit of 0.1 mg/kg Cd is established for root and tuber vegetables, which would be exceeded by all but three (3) samples. Similarly, the Codex Alimentarius limits for both Cd and Pb is set at 0.1 mg/kg in roots and tubers. All samples analysed were above the ML for Pb. However all but one sample were dry mass, therefore these limits would not apply. FAO Food Balance Sheets for 2021 (the most recent data available) estimate the per capita supply of spices at 0.59 kg/capita/year [5]. While this is an overestimation as the figure includes other spices and spice blends, the data was used to make a 'worst case' estimate of dietary intake of potentially harmful trace elements from consumption of turmeric and turmeric products using the maximum concentrations found (See estimates in Table 3).

TABLE 3. ESTIMATED DIETARY-INTAKE OF TRACE ELEMENTS FROM IN TURMERIC AND TURMERIC PRODUCTS

Element	Estimated intake (µg/day)
As	0.66
Cd	2.33
Hg	0.05
Pb	3.86

The estimated daily intake of these elements is low, including in comparison to the PTMI for cadmium and the PTWI for inorganic mercury. It is worthwhile noting that some samples are tea or tea bags, which would be consumed as brews or infusions and not as the whole sample. This would result in lower intake than indicated from the calculated estimate. For example, a previous study at ICENS showed between 1% and 15% of Cd was

extracted from brews/infusions of different herbal teas [11]. Given the increased use of turmeric as an herbal tea this is a possible area for further investigation.

While studies have focused on lead exposure, the cadmium concentrations found also indicate that surveillance of other metals is necessary. It is also noted that while these concentrations are lower than those found in other studies, the importation of both raw materials and finished products does not preclude the need for surveillance. The concentrations found indicate incidental contamination, and not necessarily EMA. However, it cannot be ignored that some samples exceeded the EU limit for Pb, a significant consideration for trade. Mitigative actions, such as following the Codex Code of Practice for the Prevention and Reduction of Lead Contamination in Foods [12] could reduce future occurrences.

4. CONCLUSIONS

While the estimated intake of these elements from consumption of these products is low, the importance of increased surveillance is evident. This is also underscored by the recently launched JASpices project, which aims to boost production of specific spice commodities, with turmeric being one of the targeted crops for increased production to satisfy both domestic and export markets. Potential dietary exposure from tea infusions can be investigated. Other emerging products, including dietary supplements/capsules and turmeric ginger 'shots and infusions may be considered as candidates for surveillance.

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GEOGRAPHICAL IDENTIFICATION OF RICE CULTIVARS IN DIFFERENT REGIONS IN WEST JAVA USING STABLE ISOTOPES AND ELEMENTAL ANALYSIS WITH UNSUPERVISED LEARNING

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Abstract

A study about rice geographical origin discrimination in the West Java regions was undertaken to identify and map the rice characteristics of rice cultivars in these areas. Sixteen samples from 13 areas (Bogor, Purwakarta, Majalengka, Ciamis, Cirebon, Tasikmalaya, Cianjur, Indramayu, Karawang, Sumedang, Kuningan, Subang, Sukabumi) were analysed using isotope ratio mass spectrometry (IRMS) and neutron activation analysis (NAA). The four stable isotopes variables $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$ and $\delta^{15}N$ and the 17 elements aluminium (Al) arsenic (As) bromine (Br) calcium (Ca) cobalt (Co) chlorine (Cl) chromium (Cr) caesium (Cs) iron (Fe) potassium (K) magnesium (Mg) manganese (Mn) sodium (Na) rubidium (Rb) scandium (Sc) zinc (Zn) and molybdenum (Mo) were measured and analysed using descriptive statistical analyses with violin plot, correlation analysis and clustering analysis using unsupervised machine learning techniques such as Fuzzy C-Means, Hierarchical Cluster, Nearest Neighbour and Random Forest methods. This study found that altitude affects the distribution of $\delta^{2}H$ and $\delta^{18}O$ data with a positive correlation, p-value < 0.05. Different rice cultivar areas cause the distribution of mineral content in rice. Fuzzy C-Means gave the best result for clustering both isotopes and elements of rice based on the geographical origin of the cultivation area.

1. INTRODUCTION

In 2014, according to FAO data, Indonesia was the third highest consumer of rice in the world after China and India [1]. There are various varieties of rice in Indonesia, which are the primary source of carbohydrates. The largest rice production is on the island of Java. However, rice is also cultivated on islands other than Java with different characteristics and qualities due to geography, environment and climate, as well as different cultivation techniques that farmers have practiced. The provenance of rice can be difficult to identify in the market using only the label printed on the product [2]. Therefore, there are many cases of food fraud, such as mixing premium and low-priced products or dangerous chemical ingredients such as bleach and other preservatives that harm consumers [3]. Based on data from the Grocery Manufacturers' Association, at least between $10 billion and $15 billion is lost by primary producers per year due to food counterfeiting. PricewaterhouseCoopers in 2017 estimated around $30 to $40 billion annually [4].

Many attempts have been made to develop techniques to distinguish the geographical origin of food products [5]. Regarding food safety and quality, this analysis is used to detect and deter adulteration of high- and low-value food products [6]. Several methods are commonly used to identify the origin of food, including DNA testing to identify species related to the product's origin, chemical markers based on certain compounds, metabolomics, blockchain technology, and others [7]. The combination of stable isotope and elemental analysis techniques is one of the methods that has received much attention for verifying the claimed origin and authentication of food products [2, 8–10]. The isotope ration mass spectrometer (IRMS) is a method used to measure the relative abundance of stable isotopes in a particular sample. In rice tracing and authentication, the isotopes used in general are those of the 'light' bio-elements that comprise the nutrients in rice, $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$ and $\delta^{15}N$ [11]. These analyses will show different isotope abundance measurements depending on the geographical characteristics and the agricultural method of cultivating rice [8, 12].

Elemental analysis was carried out based on the expected element content found in plants, macro elements (Ca, K, Mg, Cl) microelements (Fe, Mn, Zn) and trace elements (Al, As, Br, Co, Cr, Cs, Na, Rb, Sc, Mo) [2]. Elemental analysis shows the distribution of certain minerals contained in rice and related to nutritional adequacy [13]. Deficiency or excess intake of certain minerals can adversely affect human health [14]. In addition to health functions, mineral analysis combined with chemometrics can be used to predict food origin using mineral-profiles in the production-area [15, 16]. This technique has been widely used for analysis of tea, oil, coffee among others [17–20]. This study used stable isotopes and elemental concentrations analysis, combined with unsupervised machine learning, to determine the geographical origin of the Ciherang variety of rice grown in West Java Province.

2. MATERIALS AND METHODS

2.1. Sample information

Rice used in this experiment was sampled from 13 areas in West Java, including Bogor, Purwakarta, Majalengka, Ciamis, Cirebon, Tasikmalaya, Cianjur, Indramayu, Karawang, Sumedang, Kuningan, Subang, Sukabumi. The areas have elevations from 6 m to 773 m above sea level (masl).

2.2. Sample collection and preparation

The rice samples were collected from various locations in grain form and then ground into 200 mesh flour using a grinder. The powdered samples were stored in a desiccator. Sample collection was conducted in November 2018, represent both the rain and dry seasons.

2.3. Stable isotope analysis

Stable isotope analysis was performed using IRMS and an elemental analyser (EA). The isotope composition of each sample is measured relative to international standards; $\delta^{13}C$ (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard, $\delta^{2}H$ (‰) and $\delta^{18}O$ (‰) relative to Vienna Standard Mean Ocean Water (V-SMOW) and $\delta^{15}N$ (‰) relative to atmospheric AIR-N_2. Measurements are made following Eq. (1)

$$(‰) = R\_sample/(R\_standard-1) \times 1000 \quad (1)$$

For isotopic analysis, approximately 0.3 ± 0.1 mg of the powdered rice samples was encapsulated in tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis or silver capsules for $\delta^{18}O$ and $\delta^{2}H$ analysis. The analysis was performed using a robust and sensitive EA IRMS. For $\delta^{13}C$ and $\delta^{15}N$, the samples in tin capsules were combusted in the elemental analyser at ~1020°C in the presence of excess oxygen, producing CO_2 and N_2 gases, which were separated using a gas chromatography and introduced into the IRMS for isotopic ratio determination. For $\delta^{18}O$ and $\delta^{2}H$, the samples in silver capsules were pyrolyzed at ~1450°C, releasing molecular hydrogen (H_2) and carbon monoxide (CO) which were analysed for $\delta^{2}H$ and $\delta^{18}O$, respectively. Calibration was performed using international standards such as NBS-19 for $\delta^{13}C$ and $\delta^{18}O$, IAEA-CH-7 for $\delta^{2}H$, and USGS 40 for $\delta^{15}N$.

2.4. Elemental analysis

The samples were dried in an oven at 40°C, ground and 100 mg was weighed into polyethylene vials which had been cleaned with HNO_3 5N and deionized water (1:1) and then sealed. The standard used for quantitative determination is MA-A-2/TM Fish Flesh reference material, where the standard was weighed as much as 200 mg the same with sample and irradiated in a reactor. Samples and standards put into polyethylene bags which had been cleaned with ethanol and then sealed. Samples and standard wrapped in aluminium foil and irradiated in Reactor GA Siwabessy, BATAN Serpong using flux~10^{13} for 1 hour. After exposure to radiation safety (1 week) samples were measured using a gamma spectrometer with HPGe detector which is connected by means of Multi Channel Analysis (MCA) that can measure about 20 elements at the same time. The HPGe detector had a resolution of 1.89 keV at 1332 keV ^{60}Co energy and peak to Compton ratio of 38.1:1. Qualitative calibration was conducted using multi 152 Eu gamma rays' standard.

2.5. Statistical analysis

Descriptive analysis, correlation analysis and cluster analysis were used in this experiment to map the geographic origin of rice. Data were pre-processed prior to analysis by removing entries with more than 20% missing values. Subsequently, missing values in the remaining data were filled using the imputation method. Then, the data was normalized using standard scaling. Data preprocessing and analysis were conducted using appropriate statistical tools.

3. RESULTS AND DISCUSSION

The parameters measured are summarized in Table 1.

TABLE 1. DESCRIPTIVE ANALYSIS OF STABLE ISOTOPES AND ELEMENTS

	Mean [‰]	Std. deviation	IQR	Minimum	Maximum	25th percentile	50th percentile	75th percentile
Elevation	355.438	297.441	522.750	6.000	773.000	65.000	477.000	587.750
$\delta^{13}C$	-28.151	0.649	0.532	-29.490	-26.830	-28.392	-27.965	-27.860
$\delta^{2}H$	-59.431	7.863	7.150	-82.100	-47.600	-62.825	-57.200	-55.675
$\delta^{18}O$	26.551	1.844	2.690	22.090	28.700	25.775	26.590	28.465
$\delta^{15}N$	4.612	1.717	1.650	1.990	8.060	3.415	4.580	5.065
Al	8.477	1.756	2.207	5.951	12.401	7.105	8.636	9.312
As	0.110	0.097	0.072	0.025	0.375	0.058	0.080	0.129
Br	0.240	0.096	0.063	0.110	0.508	0.191	0.220	0.253
Ca	138.987	37.915	29.737	88.760	240.742	116.383	138.829	146.120
Co	0.015	0.004	0.003	0.006	0.024	0.013	0.015	0.016
Cl	326.272	45.085	66.822	243.619	396.856	295.975	321.811	362.797
Cr	7.086	2.351	1.957	4.610	14.300	5.572	6.825	7.530
Cs	0.103	0.105	0.152	0.005	0.310	0.025	0.063	0.177
Fe	28.127	29.931	18.944	6.869	122.525	9.587	17.572	28.530
K	833.396	191.646	248.183	516.119	1199.807	719.295	879.694	967.479
Mg	564.510	150.663	223.610	363.185	812.600	462.699	527.226	686.309
Mn	14.980	5.277	6.633	7.794	27.236	11.250	13.381	17.883
Na	8.408	3.213	2.083	6.043	16.448	6.493	6.845	8.576
Rb	5.687	2.545	1.086	1.028	10.571	5.117	5.681	6.202
Sc	0.007	0.006	0.004	0.003	0.020	0.003	0.005	0.007
Zn	23.197	4.965	4.139	17.846	34.120	19.977	22.366	24.116
Mo	0.619	0.359	0.290	0.203	1.370	0.402	0.505	0.692

Data from isotopes descriptive analysis in Table 1 shows that δ^2H isotope measurement values range from -47.60 to -82.10‰, δ^{13}C from -26.83 to -29.49‰, δ^{18}O from 22.09 to 28.70‰ and δ^{15}N from 1.99 to 8.06‰. Among these isotopes, the δ^2H isotope has the highest standard deviation and widest interquartile range; the δ^{13}C isotope has the smallest standard deviation and the narrowest interquartile range. The concentration of K ranges from (516.11–1199.81) mg/kg, Mg (363.18–812.60) mg/kg, Cl (243.62–396.86) mg/kg and Ca (88.76–240.74) mg/kg, Fe (6.87–122.52) mg/kg and Na (6.04–16.45) mg/kg.

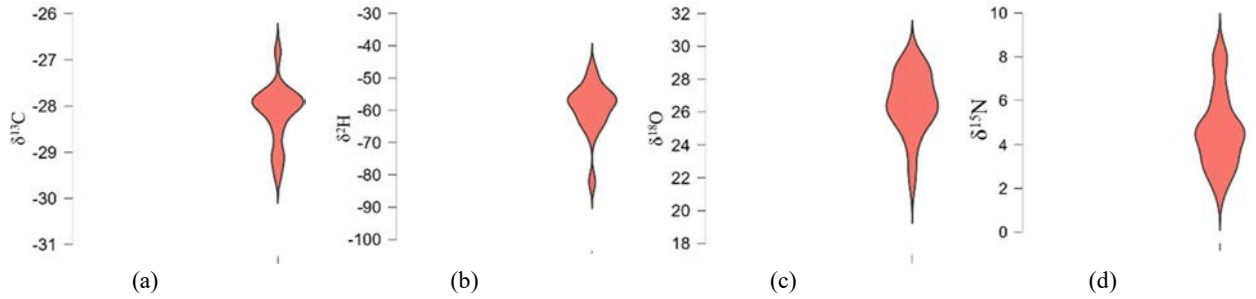


FIG. 2. Violin plot isotopes (a) δ^{13}C; (b) δ^2H (c) δ^{18}O (d) δ^{15}N.

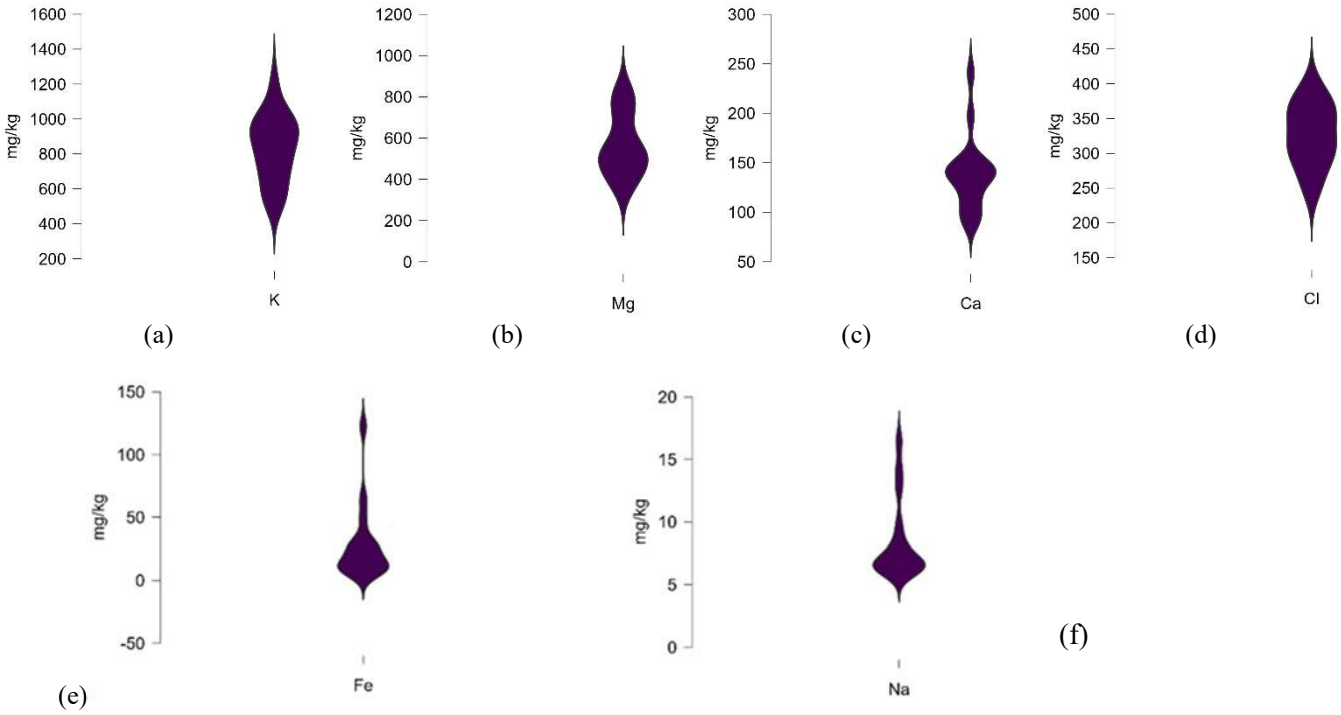


FIG. 3. Violin plot isotopes (a) K; (b) Mg (c) Ca (d) Cl (e) Fe (f) Na.

In Fig. 2, the violin plot shows the distribution of stable isotopes (a) δ^{13}C, (b) δ^2H, (c) δ^{18}O, and (d) δ^{15}N from Ciherang rice in West Java and in Fig. 3 is the distribution of elements (a) K; (b) Mg; (c) Ca; (d) Cl; (e) Fe; (f) Na.

TABLE 2. CORRELATION ANALYSIS OF STABLE ISOTOPES AND ELEVATION

Pearson's correlations				
			Pearson's r	p
δ^2H	—	δ^{13}C	0.299	0.260
δ^2H	—	δ^{15}N	0.354	0.178
δ^2H	—	δ^{18}O	0.882[c]	< .001

Pearson's correlations				
			Pearson's r	p
δ^2H	—	Elevation	0.499[a]	0.049
δ^{13}C	—	δ^{15}N	0.321	0.226
δ^{13}C	—	δ^{18}O	0.471	0.066
δ^{13}C	—	Elevation	-0.038	0.889
δ^{15}N	—	δ^{18}O	0.330	0.212
δ^{15}N	—	Elevation	0.203	0.451
δ^{18}O	—	Elevation	0.567[b]	0.022

[a]$p < 05$, [b]$p < 01$, [c]$p < 001$

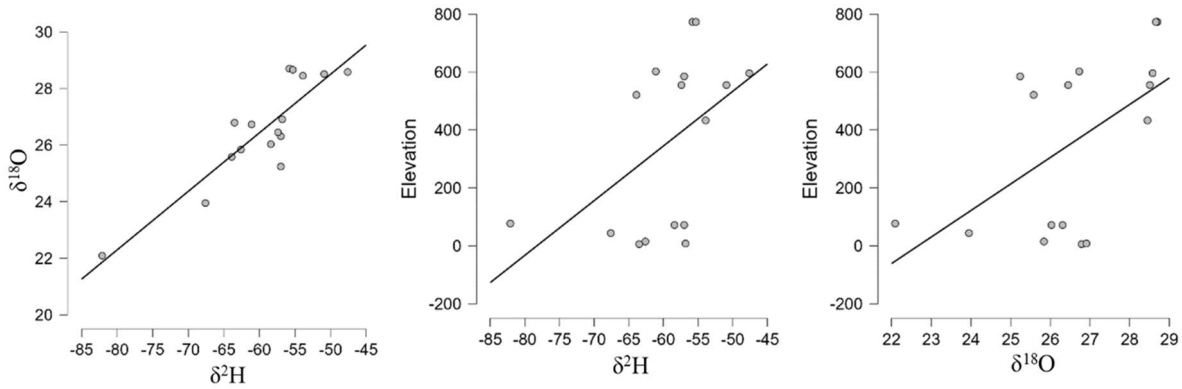
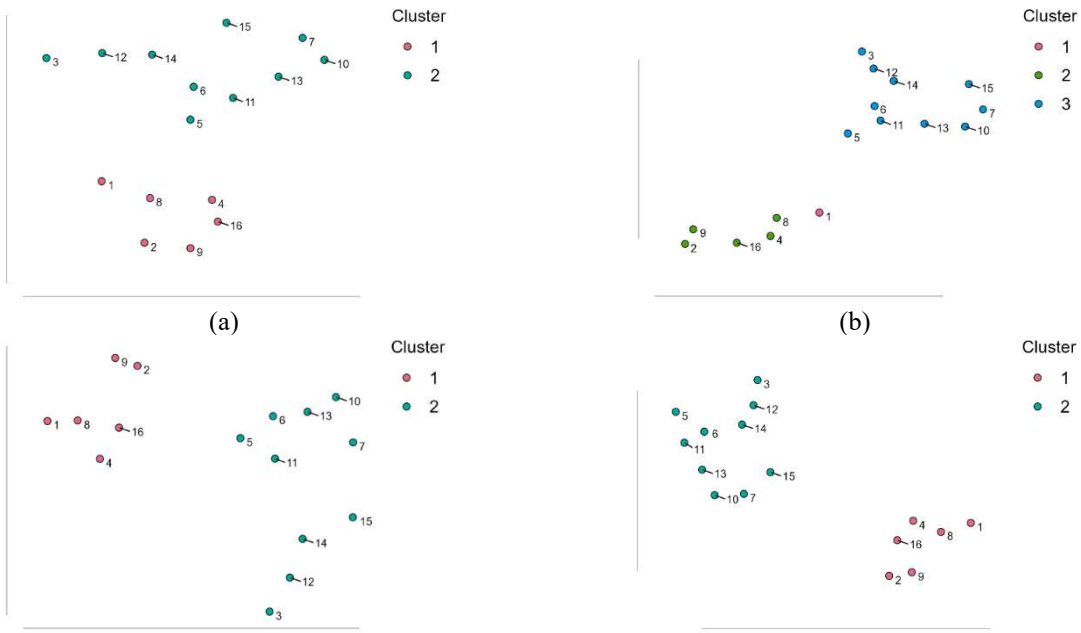


FIG. 4. Correlation analysis (a) δ^2H to δ^{18}O*; (b)* δ^2H *to Elevation (c)* δ^{18}*O to elevation.*

Correlation analysis was used to evaluate the correlation between isotopes and elevation. Pearson correlation with a 95% confidence level was chosen to conduct the analysis. It is found that the δ^2H values had a strong correlation with the δ^{18}O isotope; furthermore, both isotopes also statistically correlated with the elevation.



(c) (d)

FIG. 5. t-SNE Cluster plots of isotopes using (a) Fuzzy C-Means (FCM) Method; (b) Hierarchical Method (c) Neighbourhood Based Method; (d) Random Forest Method.

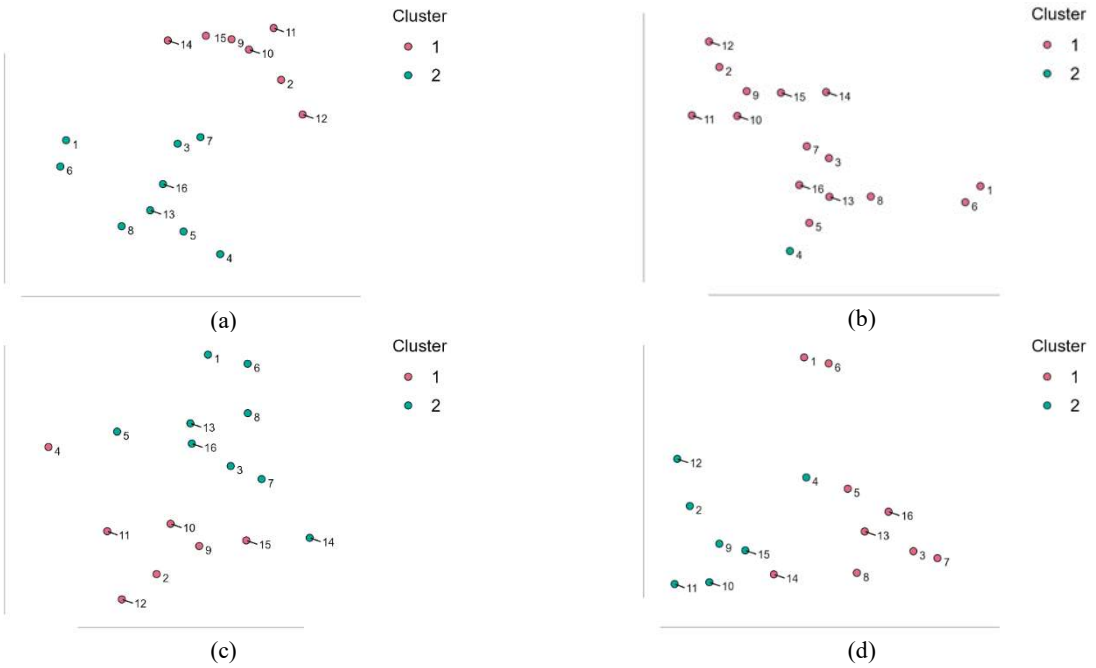


FIG. 6. t–SNE Cluster plots of elements using (a) Fuzzy C-Means Method; (b) Hierarchical Method (c) Neighbourhood Based Method; (d) Random Forest Method.

t-SNE Cluster plots visualize unsupervised machine learning analysis of isotopes and elements using FCM, Hierarchical, NB and RF models. In isotopes analysis from Fig. 5, all methods display the same results for two data clusters except for the Hierarchical method, which has three clusters. Elements analysis in Fig. 6 shows more complex results, which only NB and RF have similar results. FCM separates two clusters clearly; meanwhile, Hierarchical only has one data point for cluster 2.

TABLE 3. ISOTOPES RESULTS OF CLUSTERING USING FCM, HIERARCHICAL, NB AND RF MODEL

Method	Cluster 1	Cluster 2	Cluster 3
FCM	Bogor, Ciamis, Cirebon, Majalengka, Purwakarta, Tasikmalaya	Cianjur, Indramayu, Karawang, Kuningan, Subang 1, Subang 2, Sukabumi 1, Sukabumi 2, Sumedang 1, Sumedang 2	—
Hierarchical	Bogor	Ciamis, Cirebon, Majalengka, Purwakarta, Tasikmalaya	Cianjur, Indramayu, Karawang, Kuningan, Subang 1, Subang 2, Sukabumi 1, Sukabumi 2, Sumedang 1, Sumedang 2
Neighbourhood Based (NB)	Bogor, Ciamis, Cirebon, Majalengka, Purwakarta, Tasikmalaya	Cianjur, Indramayu, Karawang, Kuningan, Subang 1, Subang2, Sukabumi 1, Sukabumi 2, Sumedang 1, Sumedang 2	—
Random Forest (RF)	Bogor, Ciamis, Cirebon, Majalengka, Purwakarta, Tasikmalaya	Cianjur, Indramayu, Karawang, Kuningan, Subang 1, Subang 2, Sukabumi 1, Sukabumi 2, Sumedang 1, Sumedang 2	—

Table 3 shows the sampling area list from isotopes categorized as Cluster 1, Cluster 2 and Cluster 3. The hierarchical clustering method of isotopes analysis divides the data into three clusters. Cluster 1 only Bogor area, Cluster 2 consists of Ciamis, Cirebon, Majalengka, Purwakarta, and Tasikmalaya, and Cluster 3 members are Cianjur, Indramayu, Karawang, Kuningan, Subang 1, Subang 2, Sukabumi 1, Sukabumi 2, Sumedang 1, Sumedang 2. It is seen that samples taken from the same area are in the same cluster.

TABLE 4. ELEMENTS RESULTS OF CLUSTERING USING FCM, HIERARCHICAL, NB AND RF MODEL

Method	Cluster 1	Cluster 2
Fuzzy C-Means (FCM)	Ciamis, Purwakarta, Subang 1, Subang 2, Sukabumi 1, Sukabumi 2, Sumedang 2	Bogor, Cianjur, Cirebon, Indramayu, Karawang, Kuningan, Majalengka, Sumedang 1, Tasikmalaya
Hierarchical	Bogor, Ciamis, Cianjur, Indramayu, Karawang, Kuningan, Majalengka, Purwakarta, Subang 1, Subang 2, Sukabumi 1, Sukabumi 2, Sumedang 1, Sumedang 2, Tasikmalaya	Cirebon
Neighbourhood Based (NB)	Ciamis, Cirebon, Purwakarta, Subang 1, Subang 2, Sukabumi 1, Sumedang 2	Bogor, Cianjur, Indramayu, Karawang, Kuningan, Majalengka, Sukabumi 2, Sumedang 1, Tasikmalaya
Random Forest (RF)	Bogor, Cianjur, Indramayu, Karawang, Kuningan, Majalengka, Sukabumi 2, Sumedang 1, Tasikmalaya	Ciamis, Cirebon, Purwakarta, Subang 1, Subang 2, Sukabumi 1, Sumedang 2

Table 4 describes the varied results of element analysis techniques. Unlike isotopes analysis, in which samples from the same area are gathered in one cluster, elements show different results. Hierarchical method results. Almost all samples belong to one cluster except Cirebon. The NB and RF models show similar results, but samples from the same area, Sumedang, belong to two clusters. Only the FCM model represents the appropriate result.

The West Java province consists of the steep mountainous region in the south with a height of more than 1500 m above sea level, the area of sloping hillside in the middle with a height of 100–1500 m above sea level, the vast plains in the northern region with a height of 0–10 m above sea level, and River flow region [21]. Its topography gives unique characteristics for analysing stable isotopes and elements in rice that create characteristic fingerprints. It becomes the basis for authentication techniques and food traceability using stable isotopes and elemental analysis [22]. The rice samples used in this experiment are from the same variety, Ciherang, an original variety of rice from the West Java area and is cultivated by most farmers in this Province during the sampling timeline. Rice plants are included in the C_3 plant type, which bind CO_2 through the action of the enzyme ribulose bisphosphate carboxylase. This study used one variety to eliminate the effect of subvariety on isotope composition [23]. Nevertheless, the isotope and elemental analysis results forms two clusters that environmental conditions can cause.

The best results of identification show in $\delta^{13}C$ isotopes, which range from -26.83 to -29.49‰ with a mean value of -28.15‰. The violin–shaped graph in Fig. 1 shows that carbon isotope values are concentrated around the median -27.96‰. The pattern has the narrowest interquartile range, and the lowest standard deviation obtained because $\delta^{13}C$ generally influenced by the overriding effects of the common Calvin (or C_3) photosynthetic pathway used by all rice varieties. However, a range of environmental factors can still influence stomatal opening times, CO_2 conductance, and ultimately the fractionation of carbon stable isotopes [24, 25]. $\delta^{15}N$ isotope analysis results range from 1.99 to 8.06‰ and can be used for identification. However, some regions in West Java use different rice cultivation techniques so that the isotope range is wider. Figure 1(d) shows that the data is aggregated around the lower value to the median value. It means most nitrogen isotope values are below the 3rd percentile of 5.06‰. The addition of fertilizer influences nitrogen stable isotope measurements [26–30]. Further experiments are needed to conclude whether higher nitrogen isotope ratios correlate with organic rice cultivation in West Java.

Rice samples were taken from 13 regions with altitude variations ranging from 6 m to 773 m above sea level (masl). Based on statistical data of West Java, rainfall ranges from 2000–4000 mm/year with a high level of rainfall intensity [21]. It is attributed to the $\delta^{18}O$ isotope and $\delta^{2}H$ isotope measurement results [31]. Table 2 and Fig. 1(a) also supported that $\delta^{2}H$ isotopes strongly correlate with $\delta^{18}O$ with $p < 0.001$. $\delta^{18}O$ isotopes range from 22.09 to 28.70‰ with a median of 26.59‰. The violin graph in Fig. 1(c) shows the oxygen isotope distribution spread above the 1st percentile $\delta^{2}H$ isotope measurement values range from -47.60 to -82.10‰, which is the widest range among others since it was mentioned that the contours of West Java vary, affecting its altitude and groundwater depth. Figure 1(b) shows that the distribution of isotope values spread between the 1st and 3rd percentile. Correlation analysis in Table 2 and Figs. 3 (b and c) describe that the elevation positively correlates with the hydrogen and oxygen isotope values. It was also mentioned in Suzuki's research that altitude correlates with the range of hydrogen isotopes [32].

Elemental descriptive measurements exhibit more data variance than isotope values. Seventeen elements were characterized to determine their 'fingerprint' pattern based on cultivation area. Those elements are categorized as macro, micro, and trace elements [2]. Macro and microelements are essential components of human nutrition intake and trace elements provide micronutrition [33]. The elements shown in the figure represent macro (K, Mg, Ca, Cl) micro (Fe) and trace (Na) which have high deviation standards. Figure 2 delineates that element composition differs for each cultivation area, affecting the amount of macro-, micro-, and trace elements in rice samples. For K, Mg and Ca, most elements are distributed under the 3rd percentile; meanwhile, in Cl, elements are evenly distributed in the range. For Fe and Ca elements, data congregate around the median value. Figure 2 shows some outliers detected from Ca, Fe and Na elements and need further evaluation. Some areas are steep mountainous regions, which are active volcanic areas. Volcanoes store solid silicate material called magma in the earth's depths. They are composed of materials in the form of gasses (volatile) such as H_2O, CO_2 and non-gaseous materials that generally consist of Si, O, Fe, Al, Ca, Mg, Na, K and minor elements, such as V, Sr, Rb and others [34–39].

4. CONCLUSIONS

In this study, unsupervised machine-learning techniques FCM, Hierarchical, NB and RF were used to map rice stable isotope and elemental distribution. Multivariate statistical techniques demonstrated that most of the stable isotope and elemental concentration data congregated into two main clusters except for the Hierarchical method. The FCM, NB and RF are effective methods to interrogate multivariate data to establish clusters from stable isotope data. The NB and RF models show similar results in the clustering of rice elemental concentration data, but both methods classified rice from the same area, Sukabumi, into two clusters; meanwhile, FCM classified with the best performance. The FCM is an effective method for mapping the rice distribution for both isotopes and elemental analysis. Figure 7 shows that nearby cultivation areas are in the same cluster.

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SURVEILLANCE OF ANTIMICROBIAL RESIDUES IN AQUATIC ANIMALS IN CENTRAL RIVER BASIN IN THAILAND—A ONE HEALTH APPROACH

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Abstract

Thai local and national institutions have been working in silos to tackle antimicrobial resistance (AMR) specifically in terms of surveillance. Pieces of information obtained were fragmented and rarely underwent a combined analysis. As a result, they lost the ability to function as a scientific basis for Thailand to enable its national strategic plans on AMR to become more effective and relevant. This work was part of the first joint attempt among Thai government agencies in human, animal, food, and environmental sectors, to introduce a One Health approach to AMR surveillance. Aquatic sample collection across the above mentioned sectors was coordinated and carried out in a specified area, the Central River Basin, simultaneously at a specific time from January to March in 2023 and in 2024. Afterwards, further analysis of integrated data was performed to identify any correlation across One Health sectors in Thailand. In this study, as representatives of food of aquatic animal origin, 174 fish and prawn samples were collected annually and analysed for 47 antimicrobial residues by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The paper highlights recent insights from the application of the One Health approach and considers the gaps to be overcome for Thailand to have an effective AMR surveillance program.

1. INTRODUCTION

In Thailand, there are several government agencies responsible for addressing AMR. However, until recently they had been working in silos, specifically in terms of surveillance, because of excessive bureaucratic regulations and complex bureaucratic structures. Several data sets of AMR surveillance obtained over the years were available but were sometimes inaccessible, fragmented, and never collated for analysis thus compromising the potential impact on Thailand's strategic plans on AMR. To tackle AMR, six strategies had been identified in both Thailand's National Strategic Plan on Antimicrobial Resistance 2017–2021 [1] and the 2nd Thailand's National Action Plan on AMR 2023–2027 [2] of which the first strategy being 'AMR surveillance system using the One Health approach'. Working under the scope of the first strategy, stakeholders across One Health sectors had gathered and initiated a collaborative surveillance study in 2023. This work was part of the first joint attempt among relevant Thai government agencies in the animal, environmental, food and human sectors.

2. MATERIALS AND METHODS

2.1. Sampling plan

The Thai government agencies collaboratively developed a sampling plan for AMR surveillance (Table 1). Sample collection across above mentioned sectors was coordinated and carried out in a specified area, the Central River Basin, which is the most populated area in the country, and within the same time frame, in 2023 and in 2024. The collection points and collection times were appointed by the Pollution Control Department, Ministry of Natural Resources and Environment. As the Pollution Control Department was responsible for collecting water samples from the rivers, collection points, distributed along the two main rivers in the centre of Thailand: Chao Phraya River, and Tha Chin River, were chosen. In total 29 collection points were located. These collection points covered the areas of 12 provinces. Corresponding collection time for each collection point was then scheduled between January and March.

The detailed sampling plan was shared with all stakeholders and used by each stakeholder. The Bureau of Quality and Safety of Food, responsible for aquatic animal samples, collected samples from local markets nearest to the location of each designated collection point. Fish and prawn samples were purchased from three different local markets (one fish sample and one prawn sample from one local market where possible). The collection time was within the time frame of 14 days pre- and post- the scheduled time of that specific collection point. Likewise, other stakeholders collected their samples as described above in their own contexts.

TABLE 1. SAMPLING PLAN FOR AMR-SURVEILLANCE ACROSS ONE HEALTH SECTORS

Stakeholder	Collection point	Sample type	Scope of work	
			Antimicrobial resistance	Antimicrobial residues
(a) Ministry of Natural Resources and Environment. (i) Pollution Control Department.	Chao Phraya River and Tha Chin River	Water samples	Yes	No
(b) Ministry of Public Health. (i) Department of Medical Sciences; National Institute of Health of Thailand;	Hospitals	Biological samples	Yes	No
Bureau of Quality and Safety of Food.	Local markets	Aquatic animals – Fish (Nile Tilapia or *Oreochromis nilotica*) – Prawn (Giant Freshwater Prawn or *Macrobrachium rosenbergii*)	Yes	Yes
(ii) Department of Health.	Hospital sewage treatment plants	Water samples	Yes	No
Ministry of Agriculture and Cooperatives. (i) Department of Fisheries.	Freshwater animal farms	Water samples	Yes	No

2.2. Chemicals and reagents

Commercial antimicrobial standards used were: amoxicillin, ampicillin, cefalexin, cefapirin, cefazolin, chloramphenicol, chloramphenicol-d5 (Internal standard (IS) for amphenicols) chlortetracycline, ciprofloxacin, cloxacillin, danofloxacin, dapsone, dicloxacillin, difloxacin, doxycycline, enrofloxacin, erythromycin, florfenicol, flumequine, josamycin, levofloxacin, lincomycin, marbofloxacin, nafcillin, nalidixic acid, norfloxacin, norfloxacin-d5 (IS for quinolones) ofloxacin, ormetoprim, oxacillin, oxolinic acid, oxytetracycline, sarafloxacin, sulfadiazine, sulfadimethoxine, sulfadimidine/sulfamethoxine, sulfadoxine, sulfadoxine-d3 (IS for sulfonamides) sulfamerazine, sulfamethizole, sulfamethoxazone, sulfamonomethoxine, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisoxazone, tetracycline, thiamphenicol, tilmicosin, and trimethoprim, and erythromycin-d6 (IS for beta-lactams, macrolides, and tetracyclines). Other items were acetonitrile (HPLC) n-hexane (HPLC) formic acid (AR) and ultrapure water.

2.4. Analytical method

Antimicrobial residues determination was carried out by an in-house method developed as reported elsewhere [3] and was validated and accredited to ISO/IEC 17025:2017. Simultaneously 47 veterinary drug residues including amphenicols, beta-lactams, macrolides, quinolones, sulfonamides, and tetracyclines, were analysed. The characteristics of the method were as follows: the LOD and the LOQ were described in Table 2, and the linearity determined with a reasonable correlation coefficient of ≥ 0.98. Internal quality control included the analysis of procedural blank, sample blank, duplicate samples, and spiked samples (at the LOD, and mid-range).

TABLE 2. LODS AND LOQS FOR THE ANALYTICAL METHOD USED

Analytes	LOD (µg/kg)	LOQ (µg/kg)
Amphenicols		
— chloramphenicol	0.1	1.0
— florfenicol	0.3	3.0
— thiamphenicol	0.3	3.0
Beta-lactams	30	50
Macrolides	30	50
Quinolones	5.0	10
Sulfonamides	5.0	10
Tetracyclines	30	50

Note:
The beta-lactams are: amoxicillin, ampicillin, cloxacillin, dicloxacillin, cefalexin, cefapirin, cefazolin, nafcillin, and oxacillin
The macrolides are: erythromycin, josamycin, lincomycin, and tilmicosin
The quinolones are: ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, flumequine, levofloxacin, marbofloxacin, nalidixic acid, norfloxacin, ofloxacin, oxolinic acid, and sarafloxacin
The sulfonamides are: dapsone, ormetoprim, sulfadiazine, sulfadimidine/sulfamethoxine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethizole, sulfamethoxazone, sulfamonomethoxine, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisoxazole, and trimethoprim
The tetracyclines are: chlortetracycline, doxycycline, oxytetracycline, and tetracycline

2.4. Sample preparation/extraction

Fish and prawn (only muscle) weighing 300–500 g was chopped and finely homogenized using a food blender. Aliquots were stored fat -15°C until analysis. The extraction procedure involved the following steps: 2 g of sample was weighed in a polypropylene centrifuge tube (50 ml) and then spiked with the IS. Ultrapure water (0.5 ml) was added, and the mixture was vortex for 1 min. Acetonitrile (6 ml) was added, the content shaken for 10 min and spined on a centrifuge at 4000 rpm (-4°C for 10 min) the extract was collected, and the sample re-extracted with 4 ml of acetonitrile. Fat was the removed using 10 ml of n-hexane the rest evaporated and reconstituted in 1 ml of acetonitrile: water with 0.1% formic acid (50:50 v/v) before analysing an aliquot of 10 µl with the HPLC-MS/MS system.

2.5. Liquid chromatography tandem mass spectrometry instrumentation

The HPLC apparatus was equipped with a binary pump, degasser, autosampler, and column oven. The separation was performed on a C18 column (100 mm × 4.6 mm, i.d., 3.5 µm). An LC-MS/MS with corresponding software control system was used.

2.6. Exposure assessment

Probabilistic approach was employed to assess health risk to consumers. Random sampling estimate from food consumption and chemical concentration distributions were selected for the calculation [4]. The simulation was conducted in Excel version 2410 through 10^5 iterations for each scenario. Only when the food samples showed positive results, the estimated daily intake (EDI) of an antimicrobial drug was evaluated by using the Eq. (1)

$$EDI = C \times IR \times F / BW \times 1000 \quad (1)$$

Where C denotes the antimicrobial drug concentration found in a particular sample (µg/kg); IR (Ingestion rate) represents the daily consumption of the respective food item (g/person/day); F is a conversion factor to account for the difference between the forms of food for concentration data and consumption data; BW refers to the body weight of Thai population (kg). The EDI is expressed in micrograms per kilogram of body weight per day (µg/kg bw/day).

For parameter C, the actual data set (non-parametric) was used in the estimation, while for parameters IR and BW, input values for the calculation were derived from Food Consumption Data of Thailand [5] assuming normal distribution. Two exposure assessment scenarios were considered: an average intake scenario and a high intake scenario. Per capita daily consumption data and eater only daily consumption data were used to calculate average intake and high consumer intake respectively. Since antimicrobial drug residues in the study was analysed in raw food samples but the consumption data available was for samples in cooked form, conversion factors were introduced. Due to lack of data of cooking yield for the species of aquatic animals in the study, data were obtained from comparable studies. The mean cooking yield for fish fillets ranged from 72.6% to 88.9% [6] thus the cooking yield of 80% was approximated and used in the model (F_{fish} = 100/80). For prawn samples, data from the study of shrimp was used. Weight loss during cooking of shrimp was 11.0%, with a standard deviation of 2.35% [7]. Accordingly, the input values for parameter F_{prawn} were generated, assuming normal distribution. The afore mentioned variables were expressed in Table 3. For risk characterization, the EDI was compared to the ADI of the drug evaluated by JECFA.

TABLE 3. VALUES ASSIGNED AND PROBABILISTIC DISTRIBUTION IN EXPOSURE ASSESSMENT

Quantity	Description	Unit	Value	Standard uncertainty	Probability distribution	Comment
C	Concentration of antimicrobial	µg/kg				concentrations used
IR	ingestion rate					
— IR_{fish} (per capita)		g/person/day	11.35	23.36	normal	
— IR_{fish} (eater only)		g/person/day	67.34	67.68	normal	
— IR_{prawn} (per capita)		g/person/day	1.64	4.99	normal	
— IR_{prawn} (eater only)		g/person/day	59.17	71.85	normal	
F	conversion factor					
— F_{fish}			100/80			
— F_{prawn}			100/(100–x)			
x	weight loss at cooking	%	11.0	2.35	normal	
BW	body weight	kg	57.57	17.71	normal	minimum BW used (8.88)[a]

[a] *Value derived from Food Consumption Data in Thailand*

3. RESULTS AND DISCUSSION

Table 4 summarizes the number of samples (tested and positive (≥ LOD)) mean, and range of antimicrobial residues found in the samples. In 2023, of 174 samples, only 2 samples, representing 1.1%, showed concentrations above the LOD. The antimicrobial residue found was oxytetracycline at levels lower than the LOQ in prawn samples. In contrast, a higher occurrence rate of 8.0% was recorded in 2024. Oxytetracycline was detected in four prawn samples at concentrations ranging from <LOQ-589 µg/kg. Quinolones, mainly enrofloxacin, were detected in ten samples, both in fish and prawn. There was only one sample (fish) containing multiple antimicrobial residues, including enrofloxacin at 268 µg/kg, and ciprofloxacin at 14.4 µg/kg. The presence of ciprofloxacin in this sample might possibly result from the degradation of enrofloxacin [8–9].

For quinolones, some geographical patterns were identified for the contaminated samples. Four out of 5 (80%) of the contaminated fish samples were collected from one single collection point in Nakhon Sawan province, while 60% (3 out of 5) of contaminated prawn samples were obtained from a single collection point in Nakhon Pathom province. For oxytetracycline, each of the contaminated samples over the years 2023 and 2024 was from different collection points distributed in five provinces.

Thai regulation, Notification of the Ministry of Public Health (No. 303) B.E. 2550 (2007) Re: Veterinary Drug Residues in Foods [10] has laid down MRL for veterinary drugs applied or administered to any food producing animal. Nonetheless, there is no MRL established for oxytetracycline in giant freshwater prawn (*Macrobrachium rosenbergii*) or for enrofloxacin/ciprofloxacin in fish, implying that residues of these substances are not allowed to be detected. Therefore, all the positive samples (1.1% in 2023 and 8.0% in 2024) which showed detectable amounts of the antimicrobial residues, in the study, were considered non-compliant. Although, this regulation has come into force since 2007, there are still some challenges in its implementation and enforcement. First, MRLs listed were established more than 15 years ago and have not been updated ever since.

Moreover, there are MRLs for only limited numbers of substances, in only limited species of animals. For those drugs not listed or for animals not listed in the regulation, drug residues are not allowed to be detected. However, lack of harmonisation on analytical performance of methods and evaluation of the analytical results have caused difficulty for field officers, since the analytical result of one food sample might vary depending on the competence of the laboratory. A sample analysed in a laboratory with a lower LOD might give positive result, while it might give a non-detected result when analysed in a laboratory with a higher LOD. This discrepancy has led to disputes between authority and the private sector. Due to limitations mentioned above, the Thai Food and Drug Administration is currently in a process of reviewing the regulation, so that the implementation of the regulation can be achieved in practice.

The EU has also established levels and guidelines for veterinary drug residues in foodstuffs of animal origin [11]. The MRL for oxytetracycline in all food-producing species equals 100 μg/kg (muscle) and the MRL for enrofloxacin (sum of enrofloxacin and ciprofloxacin) in all the food-producing species other than bovine, ovine, caprine, porcine, rabbit, and poultry, equals 100 μg/kg (muscle). If the EU regulation were to be applied to the results found in this study, only two fish samples (one sample detected 109 μg/kg of enrofloxacin, the other sample detected 268 μg/kg of enrofloxacin and 14.4 μg/kg of ciprofloxacin) would exceed the MRL for enrofloxacin, and only three prawn samples (oxytetracycline concentrations detected were, 112 μg/kg, 311 μg/kg, and 589 μg/kg) would exceed the MRL for oxytetracycline. As a result, only five samples from 348 samples (2023 and 2024) representing 1.4 % of all samples, would be considered non-compliant.

Exposure assessment was performed to evaluate the health risk of antimicrobial residues via consumption of fish and prawn. The actual data set of concentrations detected in the samples was used for the calculation. The antimicrobial residue results presented more than 90% of concentration data reported to be below the LOD, resulting in a left-censored distribution of occurrence values. These left censored data were treated with the widely used substitution methods: upper bound and lower bound. The LOD and a value of zero were substituted for the censored observations in the former method and latter method, respectively. Concentration data reported to be below the LOQ were handled in a similar manner.

Given the results summarized in Table 5, the EDI for general Thai consumers (>3 years old) of the three veterinary drugs detected were well below their respective ADIs recommended by JECFA. Even for the high consumer scenario (with the upper bound substitution method) which can be considered the most conservative scenario, the exposure at the 97.5^{th} percentile of the intake distribution for enrofloxacin, ciprofloxacin and oxytetracycline were 0.07422 g/kg bw/day, 0.03147 g/kg bw/day, and 0.19496 μg/kg bw/day, respectively. The EDI of enrofloxacin and ciprofloxacin were summed up and compared to the group ADI of enrofloxacin and its metabolite, ciprofloxacin of 2 μg/kg bw/day, resulting in 5.28 %ADI. The EDI of oxytetracycline was compared to the ADI of 30 μg/kg bw/day, representing 0.65 % ADI. The % ADIs estimated in the study suggested low health risks. Figures 1–3 present the distribution of simulated daily exposure of antimicrobials from the consumption of fish and prawn.

There was no population exceeding the ADI in any scenario except for the high consumer scenario (with the upper bound substitution method) to enrofloxacin (Table 5). There were merely 9 occasions out of the 10^5 iterations simulated where the ADI of enrofloxacin was exceeded (resulting in 0.00009% population exceeding ADI) which could be considered extreme cases. The concern regarding this particular group of population is that whether they are loyal consumers who always consume foods that contain antimicrobial residues due to their preferences toward certain types of foods or specific food brands/shops/markets. Those who are loyal consumers are likely to be exposed to higher levels of health risks.

TABLE 4. MONITORING RESULTS OF ANTIMICROBIAL RESIDUES FOUND IN AQUATIC ANIMALS IN 2023 AND 2024

		tested	≥ LOD	Amphenicols	Beta-lactams	Macrolides	Quinolones	Sulfonamides	Tetracyclines
2023	Fish	96	—	—	—	—	—	—	—
	Prawn	78	2	—	—	—	—	—	Oxytetracycline 2 (25.0 / < LOQ)
	Total	174	2						
2024	Fish	87	5	—	—	—	Enrofloxacin 5 (103/< LOQ– 268) Ciprofloxacin 1 (14.4/14.4)	—	—
	Prawn	87	9	—	—	—	Enrofloxacin 5 (11.1/< LOQ– 35.6)	—	Oxytetracycline 4 (259 / < LOQ –589)
	Total	174	14						

Note: *Mean values were calculated from positive samples, assuming <LOQ as half the LOQ values; —: Not detected*

TABLE 5. ESTIMATED DIETARY EXPOSURE TO ANTIMICROBIALS

Antimicrobial	Average scenario				High consumer scenario				% population exceeding ADI[a]
	Lower bound EDI		Upper bound		Lower bound EDI		Upper bound		
	mean	97.5th % ile	mean	97.5th %ile	mean	97.5th %ile	mean	97.5th %ile	
Enrofloxacin[b]	0.00114	0	0.00338	0.01170	0.00542	0	0.02229	0.07422	0.00009
Ciprofloxacin	0.00003	0	0.00198	0.00826	0.00014	0	0.00917	0.03147	0
Sum of Enrofloxacin and Ciprofloxacin	0.00117	0	0.00536	0.01996	0.00556	0	0.03146	0.10570	
Oxytetracycline	0.00041	0	0.00235	0.01012	0.00947	0	0.05417	0.19496	0

[a]*Calculated for the high consumer scenario (upper bound method)*

[b]*Sum of the estimates from consumption of fish and prawn*

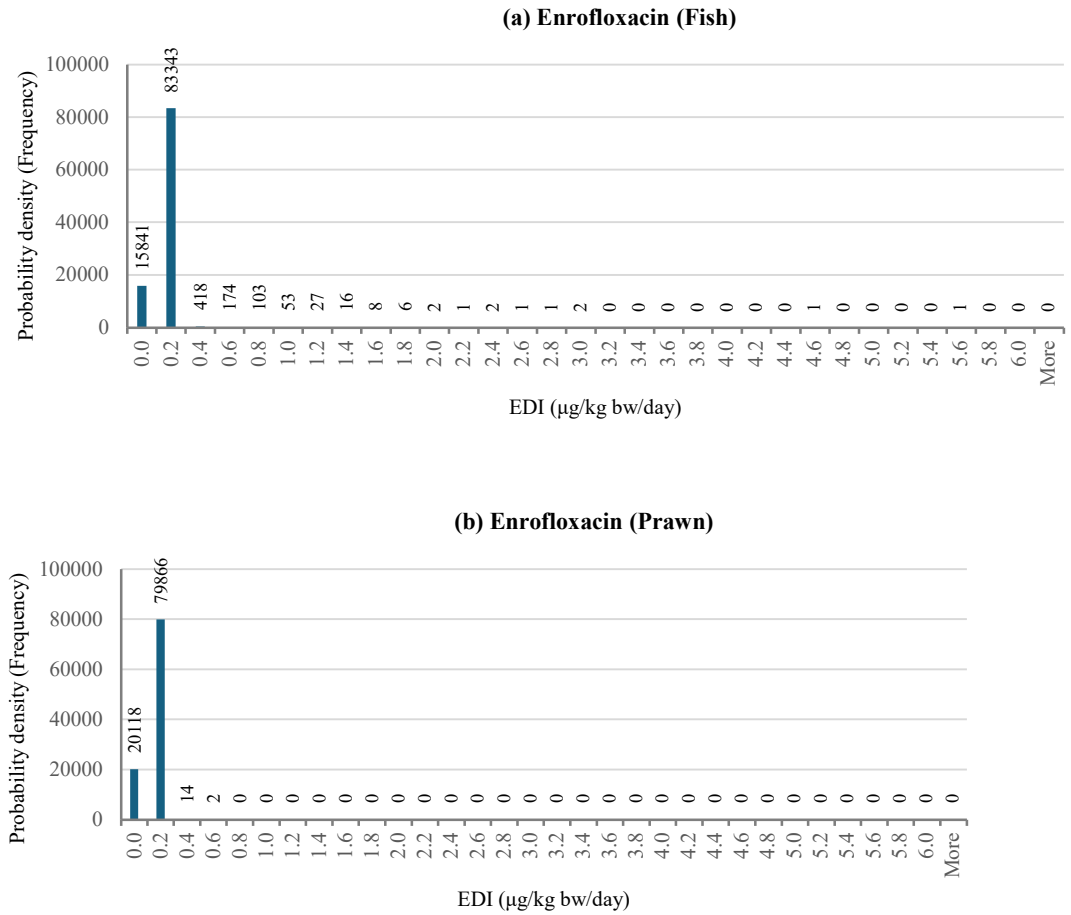

FIG. 1. Simulated distribution of estimated daily intake of enrofloxacin (a) from consumption of fish and (b) from consumption of prawn, for the high consumer scenario with the upper bound substitution method.

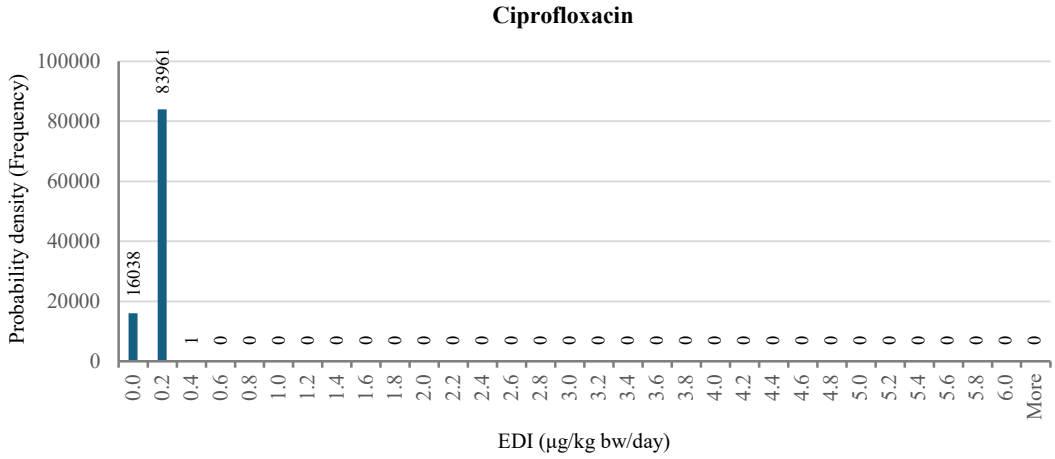

FIG. 2. Simulated distribution of estimated daily intake of ciprofloxacin from consumption of fish for the high consumer scenario with the upper bound substitution method.

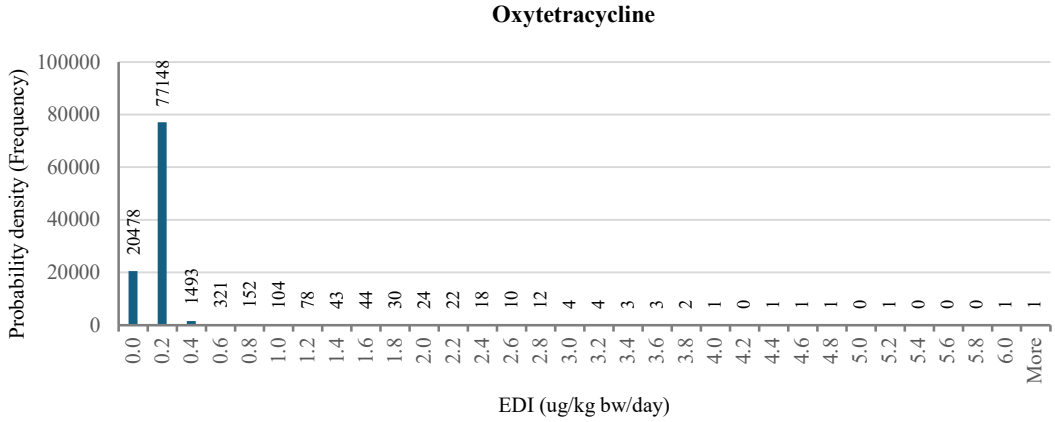

FIG. 3. Simulated distribution of estimated daily intake of oxytetracycline from consumption of prawn for the high consumer scenario with the upper bound substitution method.

The result in the study indicated that antimicrobial-use in freshwater animal production in Thailand is evident and occasionally good agricultural practices were not strictly implemented. Overuse of antimicrobials and/or ignorance of withdrawal period might have led to the MRL exceedance. Even though the exposure assessment indicated low risk levels from consumption of fish (Nile Tilapia) and prawn (*Macrobrachium rosenbergii*) the exposure from other food products was not yet considered. For example, in a survey of 60 muscle samples from bovine, swine, and chicken in Bangkok and Nonthaburi, Thailand, in 2013, oxolinic acid was found in one sample (29.5 µg/kg) and enrofloxacin was detected in two samples (16.1 µg/kg and 16.9 µg/kg) [12].

In another study of 90 samples of *Pangasius* catfish products in the country in 2014, 14 and 3 samples were contaminated with enrofloxacin and sulfamethoxazole, respectively [13]. In another survey of 225 samples of aquatic animals (sea bass, Red Tilapia, Nile Tilapia, siriped catfish, *Litopenaeus Vannamei* and *Macrobrachium rosenbergii*) in Thailand in 2020, 12 samples showed positive results for antimicrobial residues: enrofloxacin, sulfadiazine and sulfamethoxazole (Unpublished data). To better understand the overall risk, further investigation is necessary, to assess and combine the risk from exposure to drugs linked to consumption of multiple food commodities representing diets consumed by Thai population on a daily basis.

From a food safety perspective, it can be concluded that fish (Nile Tilapia) and giant freshwater prawn that were available to consumers in central river basin in Thailand were still safe for consumption. More than 90 % of the samples were free from antimicrobial residues analysed. Values that exceeded EU MRLs were found in only 1.4 % of total samples (0 % in 2023 and 2.9 % in 2024). The exposure assessment also showed that in all scenarios considered in the study, the intakes of antimicrobial residues were much lower than the ADIs, indicating no appreciable health risks.

From an AMR perspective, consumption of foods contaminated with antimicrobial residues (at levels below and above the MRLs depending on the volume consumed) exposes consumers to sub-inhibitory (non-lethal) doses of drugs. The exposure to too low a dose or too short a duration of antimicrobials can induce and accelerate antimicrobial resistance in microorganisms [14–15] in this case, in normal human flora. These antimicrobial resistant strains can sometimes become opportunistic pathogens causing infections that are difficult or impossible to cure. Technically, foods with drug levels above the MRLs are considered non-compliant. Therefore, these have to be banned and subsequently removed from the markets in which can be accomplished by enabling the strong enforcement of relevant legislation. However, for those foods containing drugs at levels below the MRLs, the control measure is not as simple. From a regulatory standpoint, these foods are considered ‘safe’ for consumption, and it is difficult for authorities to exercise any intervention in the absence of legal basis considering the risk of resistance promotion.

Currently, prudent use of antimicrobials remains the most realistic measure to minimize their release into the ecosystem. Adherence to good veterinary practices is necessary. In the meantime, the use of alternatives to antimicrobials such as vaccines, immune modulators, or probiotics, can be encouraged. It is suggested that Thailand necessitates continuous monitoring programs for antimicrobial residues in order to gain insight into types and doses of antimicrobials its population is being exposed to via dietary consumption.

Thailand has successfully initiated the AMR surveillance system using a One Health approach based on its own unique interpretation. The results from this study would be mapped against data from other stakeholders once it becomes available, to identify any correlation (antimicrobial residues-resistance and/or antimicrobial

resistance–resistance genes) within and/or across One Health sectors. New insights into AMR were anticipated and expected to be a scientific foundation for policy-making in the future. This collaboration among Thai government agencies not only helped strengthen partnerships but also helped enable data sharing across sectors. Furthermore, it was demonstrated that Thailand has a rather comprehensive antimicrobial resistance surveillance system. Nonetheless, in terms of antimicrobial residues, there are gaps to be filled.

Since 'Thailand's National Strategic Plan on Antimicrobial Resistance 2017–2021' had been endorsed by the Cabinet resolution in August 2016, AMR surveillance system was prioritized and established because it was a tool for monitoring AMR trends. The common understanding among partners of the working group under the National Strategic Plan on AMR, was that most of the time the term 'AMR surveillance' referred to works related to antimicrobial resistance microorganisms. Participating members of the working group were also mostly from microbiology laboratories. The major change that has been introduced in the 2nd Thailand's National Action Plan on AMR 2023–2027 is that establishment of surveillance system of antimicrobial residues in foods and environments has been clearly stated to be one of the objectives of the first strategy in the plan. Even though publicized data on residues is very scarce, the surveillance of antimicrobial residues in Thailand has been conducted mainly in animal and food sectors. Therefore, laboratory capacity building to enable determination of antimicrobial residues in other sectors is urgently needed.

4. CONCLUSIONS

The surveillance reported in the paper demonstrated that aquatic animals, fish (Nile Tilapia) and prawn (*Macrobrachium rosenbergii*) in Central River Basin in Thailand were safe for consumers, since most of the samples did not contain detectable antimicrobial residues. For antimicrobials detected in the study, exposure assessment was performed, and the findings suggested no appreciable health risks. Consumption of the foods with residues (even at levels below the MRLs) might contribute to the development of AMR. Although there is no regulatory system considering the risk of antimicrobial resistance development, data collection on the types and the doses of antimicrobials the population is being exposed to through dietary consumption is indispensable. Regular monitoring of antimicrobial residues in foods is of importance and required in order to ensure the safety and quality of foods in the country and to fight against AMR. Finally, as part of the comprehensive AMR surveillance system, the results from the paper needed to be further analysed with data from other sectors and translated into action to support a One Health response.

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DETECTION OF TETRACYCLINE, CHLORTETRACYCLINE, DOXYCYCLINE AND OXYTETRACYCLINE RESIDUE LEVELS IN SYRIAN CHICKEN MEAT, SKIN AND LIVER

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Abstract

Tetracycline (TC) chlortetracycline (CT) doxycycline (DC) and oxytetracycline (OT) residues in 62 chicken samples, including meat (31) skin (22) and liver (9) collected for one year were analysed using HPLC-DAD monitoring at a wavelength of 265 nm; with a C18, 4.6 × 100 mm column maintained at a temperature of 35°C with a mobile phase at a flow rate 0.6 ml/min and 10 µl injection volume). Results indicated that all residue levels in meat, skin and liver samples were under the MRL. However, there was a big difference in the levels among the samples for the same compound as follows: meat (31) TC: 7 samples (92–132 µg/kg and the remaining were not detectable: (ND) CT: all samples were ND, DC: 4 samples ranging from 125 µg/k and 162 µg/kg, while the rest were ND, and for OT: 3 sample (14–174 µg/kg) and the others were ND. For skin (22) TC: 6 samples (46–239 µg/kg and ND for the remaining) CT: all samples were ND, DC: only one sample had a level of 112 µg/kg and ND for the rest and for OT: 3 samples (10–197 µg/kg, with no residues detectable for the other samples). For liver samples (9) TC: 7 samples (55–151 µg/kg and ND for the other 2 samples) CT and DC: all samples had no residue and for OT: 2 samples had 313 µg/kg and 336 µg/kg. The overall percentage of TC, CT, DC and OT in chicken meat, skin and liver samples detected in 248 analysis was 13.7%, the highest was 32.3% for TC, followed by 14.5% for OT and 8.1% for DC, whereas all CT samples were ND. Routine testing in the country and transboundary is encouraged.

1. INTRODUCTION

A range of drugs including anabolic compounds are still widely and illegally used in animals in many countries to promote their growth. Therefore, there is an increasing concern regarding the harmful effects of these compounds on animal and human health. Groups of drugs include antimicrobials, β-agonists, growth promoters, anthelmintics and others. They could be administered through ear implants, by injections, with drinking water and/or with feedstuffs. As early as the 1990's, the effects of such substances on animals were studied in a series of experiments. In one study [1, 2] researchers assessed the effect of trenbolone acetate (TBA) on growth rate and reproductive function of beef heifers, and reported that treated heifers grew faster, but the mechanisms leading to ovulation or preovulatory development were suppressed. In another study on guinea pigs [3] TBA and testosterone phenyl propionate were administered, and it was reported that, in addition to the suppression of Follicle stimulating hormone (FSH) production, signs of abnormality in the livers of the treated animals were noticed.

Antimicrobials are used as therapeutics and in some cases as growth promoters among other applications, in animal husbandry [4]. This application can, however, contribute to the development of antimicrobial resistance [5] a global human and animal health concern [6]. Drug residues at unacceptable levels can also affect the international trade [7]. There are dozens of classes of antimicrobials used in animal production, and tetracyclines are among the important used particularly in poultry [8, 9] due to their broad spectrum of activity as well as their low cost [4].

Due to the widespread use of the tetracyclines and rising concern about the safety associated with their residues, many studies have been conducted around the world to determine the extent of the problem. Residue levels of tetracycline, chlortetracycline, doxycycline, and oxytetracycline in chicken meat and liver have thus been estimated [10]. Residues of tetracycline, chlortetracycline, methacycline, demeclocycline, chlortetracycline, and doxycycline were also investigated in chicken meat and egg yolk in Greece [11] while a related study on the residues of chlortetracycline and oxytetracycline in beef was studied in Kenya [12].

Chlortetracycline, oxytetracycline, tetracycline and doxycycline residues were also studied in beef and liver in Italy [13]. The residues of the tetracyclines were detected in chicken breast, thigh and liver using screening and quantitative techniques [7]. In a comprehensive study to demonstrate the extent of abnormal use of antimicrobials in livestock production and its repercussions on public health in Cameroon it was reported that tetracycline, chloramphenicol, and neomycin were the most commonly used in poultry, with concentrations exceeding guidance [14]. The same study also reported the presence of food borne pathogens: *Salmonella* spp., *Staphylococcus* spp., *Listeria* spp., *Clostridium* spp. and *Escherichia* spp., which proved resistant to the different antimicrobials tested. Studies in Syria Arab Republic have reported the concentration of some hormones, mainly progesterone and testosterone, in plasma or blood serum [15].

The monitoring of veterinary drug residues in animal products was not addressed, and therefore the current study aimed to measure chicken meat, skin and liver samples periodically from different regions, and to estimate the level of residues of tetracycline, chlortetracycline, doxycycline, and oxytetracycline by HPLC-DAD method.

2. MATERIALS AND METHODS

2.1. Location and samples

This study was carried out at the Veterinary Drug and Growth Promoter Laboratory, Animal Production Division, Department of Agriculture, in Deir Al-Hajar area, about 33 km southeast of Damascus. A total of 62 chicken samples (31 meat, 22, skin and 9 liver) were bought from the markets (private) from 9 different locations in Damascus city and Damascus suburbs to estimate residues of tetracycline, chlortetracycline, doxycycline, and oxytetracycline in them.

2.2. Instruments and HPLC conditions

An HPLC-DAD was used. Separation was achieved with a C18, 4.6 × 100 mm, column and the mobile phase of 13% methanol, 17% acetonitrile and 70% oxalic acid (10 mM) used at a flow rate 0.6 ml/min following injection of 10 µl of the samples. The analytes were detected at a wavelength of 265 nm with the analytical column maintained at a temperature of 35°C.

2.3. Chemical and reagents used

Acetonitrile and methanol; oxalic acid and citric acid; nitric acid; HPLC water and the standards tetracycline hydrochloride (250 mg) chlortetracycline hydrochloride (250 mg) doxycycline hyclate (100 mg) oxytetracycline hydrochloride (250 mg) were used.

2.4. Sample preparation and extraction; standard solutions

Skin was separated from meat, and bone from the meat. The meat and skin samples were cut and ground independently using an electric grinder. Then 25 g per sample for each of the meat, skin, and liver was weighed in order to estimate the residues of tetracycline, chlortetracycline and doxycycline and oxytetracycline. Tetracycline, chlortetracycline, doxycycline and oxytetracycline standard solutions (stock solutions of 1 mg/ml) were prepared with methanol and diluted to three calibration standards at concentrations of 1 µg/ml, 10 µg/ml and 20 µg/ml. Calibration curves were established using these calibration standards.

3. RESULTS AND DISCUSSION

A correlation coefficient (R^2) greater than 0.999 for all standard series of the four compounds was determined as shown in Figs 1–4.

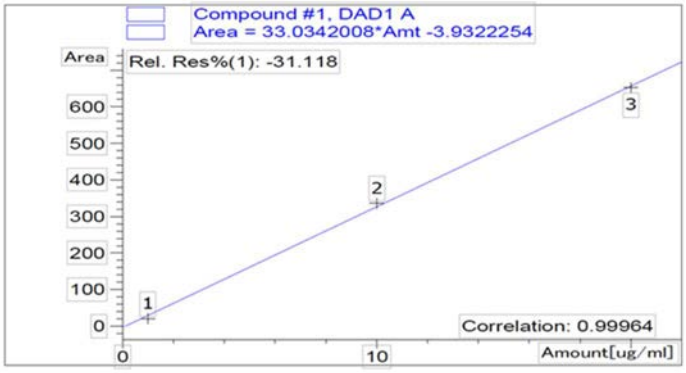

FIG.1. Standard curve for oxytetracycline.

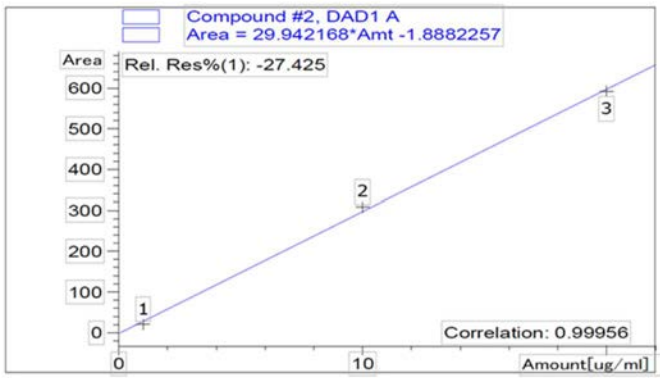

FIG. 2. Standard curve for tetracycline.

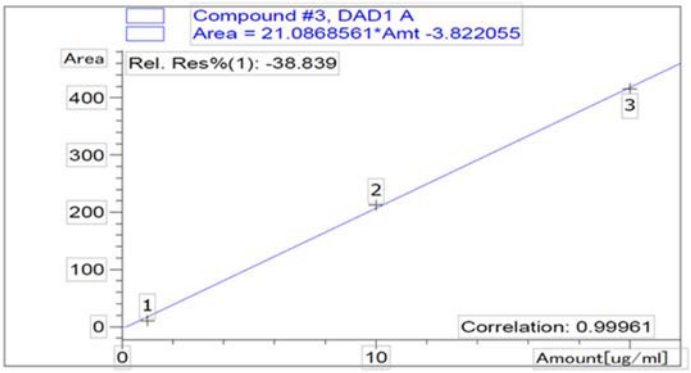

FIG. 3. Standard curve for chlortetracycline.

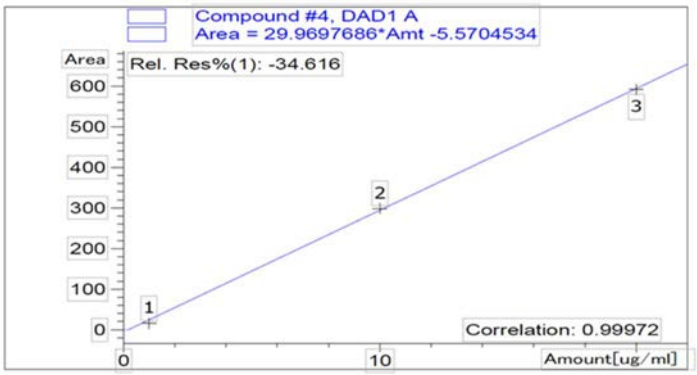

FIG. 4. Standard curve for doxycycline.

Table 1 presents the results for the 4 compounds in 9 chicken liver samples.

TABLE 1. CONCENTRATIONS OF THE DRUG RESIDUES TISSUE

Sample Nc	Sample	Tetracycline (µg/kg)	Chlortetracycline (µg/kg)	Doxycycline (µg/kg)	Oxytetracycline (µg/kg)
1	Liver	—	—	—	313
2	Liver	—	—	—	336
27	Liver	121	—	—	—
28	Liver	151	—	—	—
29	Liver	55	—	—	—
30	Liver	115	—	—	—
31	Liver	87	—	—	—

32	Liver	103	—	—	—
33	Liver	65	—	—	—

Note:*— = Not Detectable*

The results for the concentrations of the 4 compounds in 22 chicken skin samples are illustrated in Table 2.

TABLE 2. CONCENTRATIONS OF THE RESIDUES OF TETRACYCLINE, CHLORTETRACYCLINE, DOXYCYCLINE AND OXYTETRACYCLINE IN CHICKEN SKIN

Sample number.	Sample Type	Tetracycline (μg/kg)	Chlortetracycline (μg/kg)	Doxycycline (μg/kg)	Oxytetracycline (μg/kg)
11	Chicken Skin	145	—	—	—
12	Chicken Skin	239	—	—	—
13	Chicken Skin	214	—	—	—
14	Chicken Skin	197	—	—	—
15	Chicken Skin	—	—	—	—
16	Chicken Skin	—	—	—	—
17	Chicken Skin	—	—	—	—
18	Chicken Skin	—	—	—	—
21	Chicken Skin	—	—	—	197
22	Chicken Skin	—	—	—	—
23	Chicken Skin	—	—	—	10
39	Chicken Skin	46	—	—	30
41	Chicken Skin	—	—	—	—
43	Chicken Skin	—	—	—	—
45	Chicken Skin	—	—	—	—
47	Chicken Skin	—	—	—	—
49	Chicken Skin	87	—	—	—
51	Chicken Skin	—	—	—	—
53	Chicken Skin	—	—	—	—
55	Chicken Skin	—	—	—	—
57	Chicken Skin	—	—	—	—
59	Chicken Skin	—	—	112	—

Note: *— = Not detectable*

The residues of the 4 compounds in 31 chicken meat samples are presented in Table 3.

TABLE 3. CONCENTRATIONS OF THE RESIDUES OF TETRACYCLINE, CHLORTETRACYCLINE, DOXYCYCLINE AND OXYTETRACYCLINE IN CHICKEN MEAT SAMPLES

Sample number.	Sample	Tetracycline (µg/kg)	Chlortetracycline (µg/kg)	Doxycycline (µg/kg)	Oxytetracycline (µg/kg)
3	Chicken meat	—	—	—	—
4	Chicken meat	—	—	—	—
5	Chicken meat	—	—	—	—
6	Chicken meat	—	—	—	174
7	Chicken meat	—	—	—	—
8	Chicken meat	—	—	—	—
9	Chicken meat	—	—	—	—
10	Chicken meat	—	—	—	—
19	Chicken meat	—	—	—	—
20	Chicken meat	—	—	—	—
24	Chicken meat	—	—	—	—
25	Chicken meat	—	—	—	—
26	Chicken meat	—	—	—	—
34	Chicken meat	92	—	—	—
35	Chicken meat	—	—	—	—
36	Chicken meat	108	—	—	—
37	Chicken meat	124	—	—	—
38	Chicken meat	—	—	—	22
40	Chicken meat	—	—	—	—
42	Chicken meat	132	—	—	—
44	Chicken meat	92	—	—	—
46	Chicken meat	97	—	—	—
48	Chicken meat	—	—	—	14
50	Chicken meat	115	—	—	—
52	Chicken meat	—	—	—	—
54	Chicken meat	—	—	—	—
56	Chicken meat	—	—	—	—
58	Chicken meat	—	—	125	—
60	Chicken meat	—	—	162	—
61	Chicken meat	—	—	125	—
62	Chicken meat	—	—	135	—

***Note**: — = Not detectable*

The current study is the first in Syrian Arab Republic to evaluate antimicrobial residues in meat, skin and liver of the Syrian chicken. A number of these chemicals are associated with carcinogenesis among other toxic or

allergenic effects [16]. The presence of residues of antimicrobials, which are widely used in poultry, has been demonstrated elsewhere [17, 18]. This study determined residues in 62 samples consisting of 31 chicken meat, 22 skin and 9 liver samples. In each sample, the residues of 4 compounds were measured namely tetracycline, chlortetracycline, doxycycline and oxytetracycline (total of 248 analyses) and they were detected using HPLC-DAD.

The results showed that 33 (out of 248) analysis were positive although within Codex MRLs [19] in poultry meat of 600 µg/kg for tetracycline, oxytetracycline and chlortetracycline in poultry liver and 200 µg/kg for poultry meat. The levels of doxycycline residues in poultry skin were also within the EU MRL of 300 µg/kg [20]. Table 4 gives details of the number of samples in which residues were detected for each compound, and the percentages of residues present. It also shows that the overall percentage of tetracycline, chlortetracycline, doxycycline and oxytetracycline residues in chicken meat, skin and liver samples detected in 248 analysis was 13.3% and the highest percentage was in tetracycline (32.3%) then oxytetracycline (12.9%) and doxycycline (8.1%) while no chlortetracycline was detected in all samples.

TABLE 4. DETAILED DATA ON SAMPLE NUMBER AND THE CONCENTRATIONS OF THE RESIDUES OF THE TETRACYCLINES' GROUP IN SOME CHICKEN TISSUES

Compound	Tissue	Samples containing residues	(%) Total
Tetracycline	Chicken meat (M)	7	(22.6%) 31
	Chicken skin (S)	6	(27.3%) 22
	Chicken liver (L)	7	(77.8%) 9
	M + S + L	20	(32.3%) 62
Chlortetracycline	M	0	(0%) 31
	S	0	(0%) 22
	L	0	(0%) 9
	M + S + L	0	(0%) 62
Doxycycline	M	4	(12.9%) 31
	S	1	(4.5%) 22
	L	0	(0%) 9
	M + S + L	5	(8.1%) 62
Oxytetracycline	M	3	(9.7%) 31
	S	3	(13.6%) 22
	L	2	(22.2%) 9
	M + S + L	8	(12.9%) 62
	Total	33	(13.3%) 248

A study in Pakistan [21] reported that 60% of antimicrobials are used in poultry farms in Pakistan without consulting doctors, and that their misuse threatens the health of both humans and animals. Another study [22] reported that the presence of higher than permissible concentrations of doxycycline was found in 24.8% of poultry liver samples (98 samples) collected from informal markets in South Africa, and the concentrations varied significantly by regions.

A recent study conducted in Iran Republic of [23] detected the residues of tetracycline, chlortetracycline, doxycycline and oxytetracycline in meat and milk of cows, sheep, goats as well as in chicken and fish (360 samples) using ELISA and HPLC methods. They reported the presence of positive samples in meat at a rate of 21.3% and in 90 milk samples, the rate of 25.5% for tetracycline. The presence of tetracycline compounds within the permissible limits means that they have been used in poultry to prevent diseases or to promote growth, but this is done considering the appropriate withdrawal period. The results of the current study agree with others [24, 25] where the presence of tetracyclines in poultry within permissible limits was confirmed.

4. CONCLUSIONS

This study showed that some of the chicken meat, liver, and skin samples collected from shops in Damascus and suburbs of Damascus contained residues of the tetracyclines. Although the concentration levels were lower than the levels recommended by the European Union, Syrian Arab Republic's and Codex, they are indicative of the use of these compounds in animal production and disease control. Given the potential health effects proper control and regulations including the establish and effective implementation of relevant systematic testing and monitoring are required.

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QUANTITATIVE RISK ASSESSMENT OF SHIGATOXIN PRODUCING *E. COLI* (STEC) ASSOCIATED WITH CONSUMPTION OF CONTAMINATED LETTUCE: A CASE STUDY IN SOUTH AFRICA

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Abstract

There is increasing concern about the safety of South African agricultural produce, especially raw leafy vegetables. A substantial portion of this produce is consumed raw or with minimal processing, and the number of associated foodborne outbreaks has increased. This study estimated the risk of consuming STEC-contaminated lettuce under existing production and marketing conditions in South Africa. The study also identified possible mitigation measures to reduce the risk of STEC in lettuce before consumption, with the potential to significantly impact public health. The study's data came from published works in South Africa and other countries. The exposure assessment model was developed using a suitable software. A beta Poisson dose response model was employed to estimate the risk probability due to consuming STEC-contaminated lettuce. The baseline model's mean estimated chance of sickness per serving for scenario A was 0.45 (5^{th} and 95^{th} percentiles: 1.50×10^{-7}, 0.12) and 0.055 (5^{th} and 95^{th} percentiles: 1.80×10^{-5}, 0.88) for scenario B. Comparing the estimated mean number of cases to the baseline model forecast, all intervention techniques (irradiation, washing lettuce at household level, and lowering the initial concentration of *E. coli* in irrigation water to 1 log cfu/ml) considerably decreased it. Sensitivity analyses revealed the most important variables influencing the predicted risk were retail and home storage temperatures. This study was greatly assisted by the developed Quantitative Microbial Risk Assessment (QMRA) model, which served as a framework for estimating the risk of consuming STEC-contaminated lettuce and for directing the assessment and creation of intervention strategies meant to lower that risk.

1. INTRODUCTION

Consumption of fresh and minimally processed fruits and vegetables has been on the rise in the past few decades across the globe [1, 2]. The increased global demand for fresh produce has also led to the growth of its production and market reorientation in South Africa [2]. In South Africa, iceberg lettuce (*Lactuca sativa*) is among the most common free-land cultivated vegetables [3, 4]. The commodity is consumed raw or with minimal processing in South Africa, subjecting a considerable risk to public health if contaminated with pathogens. Across the globe, there have been some challenges in producing safe fresh lettuce. Outbreaks linked to the consumption of pathogen-contaminated lettuce have been well documented [5–7].

In a previous case study (case study 2) on the ranking of microbiological risks associated with the consumption of leafy vegetables in South Africa, shigatoxin-producing *E. coli* (STEC) was the most incriminated. Lettuce contaminated with STEC, particularly the O157 serotype, is a global challenge that has recently received considerable attention from governments, industry and academic research [8]. STEC can cause severe disease, with haemorrhagic diarrhoea, haemolytic uremic syndrome, and neurological symptoms [9]. No reported outbreaks have been documented to date linked to STEC-contaminated lettuce in South Africa. Nonetheless, STEC-contaminated lettuce has caused serious fatalities in Germany [10] Japan [11] USA [12] and some European countries [13].

Sources of STEC on lettuce are assumed to be environmental contamination, and the most implicated is contaminated irrigation water [1, 14, 15]. Since water comes into direct contact with the edible portion of the lettuce plant during irrigation, mainly if the process is overhead irrigation, this causes a significant risk of transfer of pathogens from the water to the plant. Most studies in South Africa which isolated STEC in irrigation water have implicated contamination from sewage and industrial effluent [4, 15, 16]. Studies have documented that lettuce can also be contaminated by STEC from a variety of sources along the farm-to-fork supply chain [17].

Equipment used during lettuce harvesting, such as coring rings and knives on farms and in processing plants, have been reported as a source of STEC [17]. Because of its inherent qualities, lettuce presents a challenge for epidemiologists and academics trying to understand the incidence and population level of STEC linked to outbreaks and the impact of different risk factors. While several intervention techniques, including bacteriophage, ultrasound, irradiation, and chlorine treatment have been developed in an effort to lower the contamination of STEC in lettuce and other fresh produce, their efficacy has to be assessed [8, 17].

The QMRA has been used more frequently in recent years as part of continuous efforts to manage risks related to food safety and is acknowledged as a useful technique for evaluating and controlling potential risks related to foodborne pathogens. This envisaged estimating the risk of consuming STEC-contaminated lettuce under existing production and marketing conditions in South Africa. The study also identified possible mitigation measures to reduce the risk of STEC in lettuce consumption.

2. MATERIALS AND METHODS

2.2. Lettuce pathway from field to consumption; exposure model

Consumers buy lettuce from retail stores in South Africa. From the farm, the commodity either passes through processing or straight to retail shops. The model was developed starting from production at the farm to consumption at the household level. Figure 1 presents the flow process of lettuce from farm to household that served as the foundation for the mathematical model used to predict consumer exposure. A 'modular process risk' framework [18] simulated lettuce (safety) from the farm to consumption. Changes in the prevalence and concentration of STEC in lettuce from farm to consumption after storage at home were also modelled. Microbial and lettuce-handling processes were identified and applied.

The model consisted of: (i) production at the field, (ii) lettuce processing, (iii) lettuce storage, (iv) consumption at home and exposure to STEC, and (v) dose response. Sequentially, each node/step generated one or more output distributions that were used as final outputs of estimating the probability and concentration per serving or as inputs to the subsequent node/step. Input data from available literature and expert opinion were used to create the model.

2.3. Exposure assessment

2.2.1. Production at the field

At the field production stage, the following were considered: prevalence and concentration of STEC in irrigation water, transfer to lettuce and concentration of STEC in lettuce at harvest (Table 1 and Fig. 1). The authors considered the national percentage of lettuce grown under irrigation and the percentage of irrigated crops that use surface water irrigation sources to calculate the initial prevalence of STEC in the water used to irrigate lettuce in South Africa. The study's analysis was based on data published in South Africa [3, 4] (Table 1). The concentration of STEC in irrigation water was determined using the distribution of *E. coli* levels in surface water used for irrigation in South Africa [3, 4] and the predicted ratio of *E. coli* O157:H7 to *E. coli* as reported by Ottoson et al., [19].

To estimate the concentration of STEC transferred from irrigation water to lettuce, an assumption was made that all the STEC would be on lettuce if the overhead irrigation method was used. After overhead irrigation, the amount of water that remained on the lettuce was described by a normal distribution truncated to a minimum limit of zero, as reported from field experiments [20, 21]. The concentration of STEC in lettuce after irrigation with contaminated water was then calculated, considering the volume transferred from irrigation water to lettuce (Table 1). The level of STEC in lettuce at harvest was computed considering holding time and solar decay between the last irrigation and harvest. The holding time followed a triangular distribution [22]. Using a Weibull survival function with parameter values from a study by Bezanson et al., [23] in their investigation of *E. coli* O157:H7 on romaine lettuce, the rate of pathogen decay/inactivation was expressed. The lettuce would then be transported to processing plants at around 5°C.

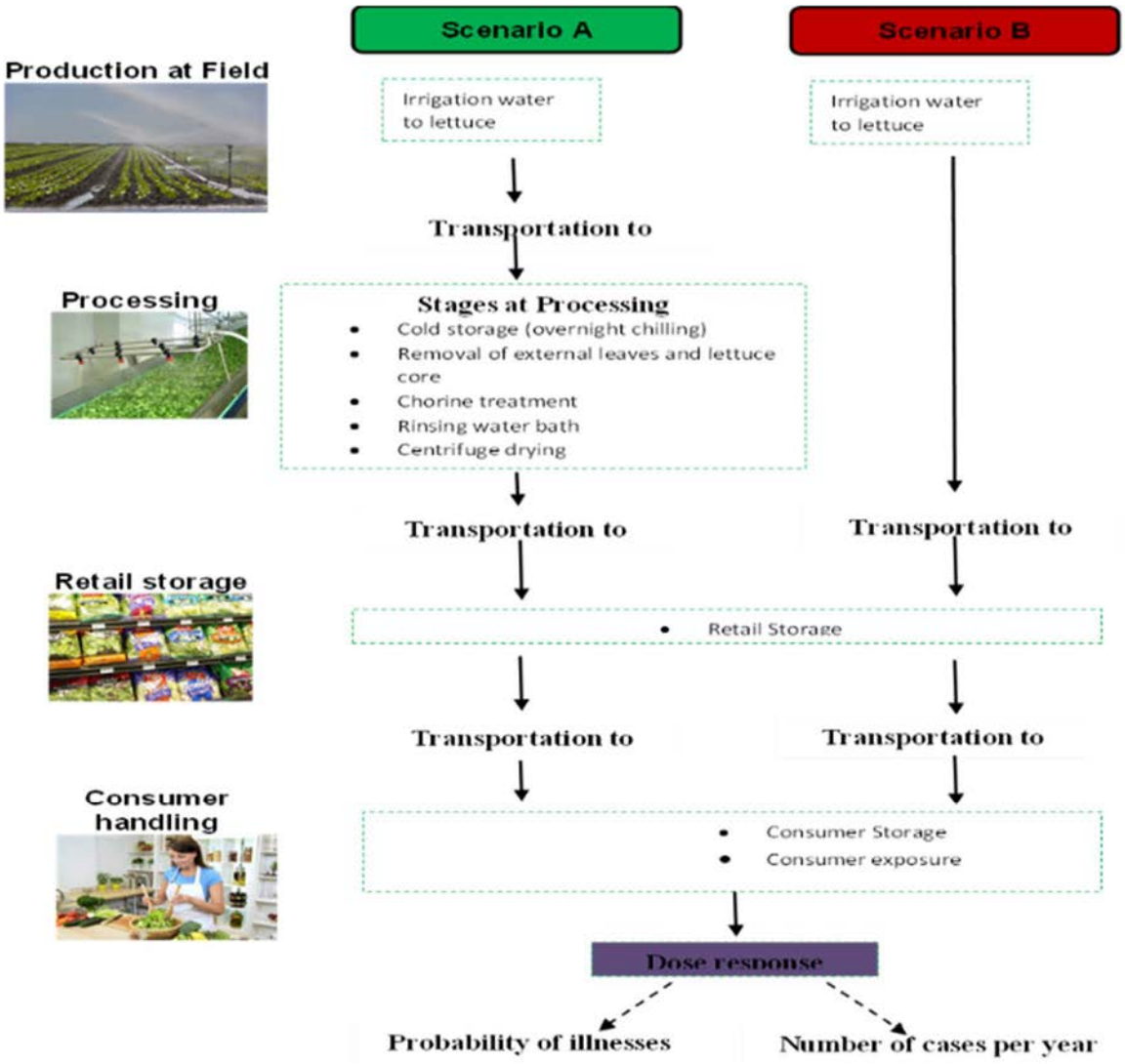

FIG. 1. Flow process of consumer exposure to STEC from irrigation water contaminated lettuce.

2.2.2. Lettuce processing

Lettuce is collected and stored at 5°C overnight; external leaves and the core are removed, and the leaves shredded followed by washing in chlorinated water at 200 ppm with a pH of 4.8 and rinsed in a clean water bath. The lettuce is then dried by centrifugation before packaging. Overnight storage temperatures, including those for transportation from farm to processing plants and during lettuce processing, ranged from 1.7°C to 6°C [24]. Therefore, it was considered that because of adequate temperature controls, microbial changes during transportation from the farm and throughout processing are minimal. There is a possibility that lettuce contaminated with STEC can transfer to shredders, conveyor belts, flume tanks, shakers, and centrifuges during washing and shredding, making it necessary to predict the prevalence and concentration of STEC in lettuce following processing.

The reverse of this contamination process may be true, and thus, the initially uncontaminated lettuce may be cross-contaminated. Data obtained from previous studies [22, 25, 26] and Jensen et al., [26] was incorporated in estimating new prevalence and concentration of STEC after cross-contamination. Assuming that every batch of lettuce delivered to the processing facility had the same weight, a batch of lettuce was used as the unit of calculation [17]. A triangular distribution was used to estimate cross-contamination according to an FDA risk assessment [22]. Calculations are shown in Table 1.

2.2.3. Lettuce storage

After processing, lettuce is transported to retail or consumer handling. In South Africa, no data has been collected on storage temperatures, both at retail and household refrigeration. A normal distribution (μ=4.44, σ=2.96°C) truncated (0°C, 20°C, 56°C) was used to estimate the uncertainty of storage temperature during storage as reported by EcoSure [27]. Although transportation of lettuce from retail to household is done at abused temperatures (above 4°C) in South Africa, growth was not calculated due to short distances and short times, as reported by Njage and Buys [28]. Storage temperatures at household levels were estimated in accordance with Pouillot et al., [29]. Time for storage at retail and household was assumed by the author (Table 1). The model for the growth of STEC on lettuce that uses temperature as a variable was developed by McKellar and Delaquis [30]. Growth of STEC is halted below and enhanced above 5°C on lettuce [30]. A 5°C threshold temperature was used to compute the growth of STEC on lettuce using primary and secondary models [31, 32].

The following primary model was used to calculate the growth of STEC at a given temperature as in Eq. (1)

$$\log Nt = \log NO + (\mu + t) \quad (1)$$

where Nt is the concentration at time *t* (cfu/g) μ is the growth rate at a specific temperature (log cfu/g/h)

N_O is the concentration at time 0 (cfu/g).

The growth rate at a specific temperature (μ) was calculated by using the secondary model as in Eq. (2)

$$\sqrt{\mu} = b\,(T - T_{min}) \quad (2)$$

Where b is the temperature coefficient, T is the temperature (°C) and T_{min} is the theoretical minimum growth temperature.

2.2.4. Consumption at home and exposure to STEC

APERT distribution of 50/80 g minimum and 100 g maximum was set as the serving size according to Nel and Steyn [33]. The number of STEC cells at time of serving was computed by multiplying concentration of STEC after storage at home and serving size (Table 1).

2.2.5. Dose response

The beta Poisson dose response with parameters described by Cassin et al., [34] for *E. coli* O157:H7 in ground beef was used in this study as in Eq. (3)

$$P = 1 - (1+D/\beta)\alpha \quad (3)$$

Where P is the probability of illness for each serving with contamination, D is the dose; α and β are model parameters.

The probability of illness for each serving was calculated as a product of prevalence of contaminated lettuce and probability of illness per contaminated serving (Table 1). The estimated per capita lettuce consumption in South Africa per year was computed from the quantity consumed per year and the proportion of lettuce consumers (in Table 1). South Africa has a population of 56 521 900 [35] and 8.9% [33] consume 34201 tons of lettuce per year [36]. Subsequently, the yearly number of illness cases in South Africa was estimated as the product of the probability of illness per serving and total number of annual servings (Table 1).

TABLE 1. PARAMETERS FOR STEC IN LETTUCE-EXPOSURE MODEL

Steps	Parameter	Description	Distribution/equation/values	Units	Data source/reference
Production in field	N_{sample}	Number of samples	200	No units	[3, 4]
					[3, 4]
	$N_{positive}$	Number of positive samples	32	No units	
	P_w	Prevalence of STEC in water	Risk Beta ($N_{positive}$ + 1, N_{sample} - $N_{positive}$ + 1)	No units	Calculated
	Pro_{crop}	Proportion irrigated crop	Risk Uniform (***60,62***)	%	[3, 4]
	$Pro_{surwater}$	Proportion irrigated from surface water	Risk Beta (***9500+1,12871-9500+1***)	No units	[3, 4]
	P_{STEC}	Prevalence of STEC in irrigation surface water	$P_w \times Pro_{crop} \times Pro_{surwater}$	No units	Calculated
	C_{irr}	Concentration of *E. coli* in irrigation water	Risk Normal (***2.26,1.50,*** Risk Truncate(0,))	Log cfu/ml	[3,4]
	$Ratio_{STEC:E.coli}$	Ratio STEC: *E. coli* in water	10^Risk Normal (***-1.9,0.6***, Risk Truncate(,0))	No units	[21, 37]
	C_{STEC}	Concentration of STEC in irrigation water	$C_{irr} \times Ratio_{STEC:E.coli}$	Log cfu/ml	Calculated
	W_{irr}	Water transferred to plant during irrigation	Risk Normal (Risk Uniform(***0.1,21.6***),***0.019***,RiskTruncate(0,))	ml/g	[20, 21]
	C_{Wirr}	Concentration of STEC in lettuce after irrigation with contaminated water	(10^ CSTEC) × Wirr	cfu/g	Calculated

Steps	Parameter	Description	Distribution/equation/values	Units	Data source/reference
	$Hold_{irr}$	Holding time after irrigation	Risk Pert (***2,4,8***)	d	[22]
	$Decay_{E.coli}$	*E. coli* decay during holding (Log reduction)	-POWER(($Hold_{irr}$/(***2.45/24)),0.3***)	Log cfu/g	[23]
	C_{Decay}	Concentration of STEC in lettuce after solar irradiation	Log_{10} (C_{Wirr})+ $Decay_{E.coli}$	Log cfu/g	Calculated
Lettuce processing	Washing	Log reduction after washing with chlorinated water	Risk Uniform (***1,3***)	Log cfu/g	[38, 39]
	C_{Chl}	Concentration after washing with chlorinated water	C_{Decay} - Washing	Log cfu/g	Calculated
	Rinsing	Log reduction after rinsing with water	Risk Pert (***0.6,1,1.4***)	Log cfu/g	[38, 39]
	C_{water}	Concentration after water-rinsing	C_{Chl} - Rinsing	Log cfu/g	Calculated
	N_{cfu}	Number of STEC cells in a unit batch after water-rinsing	(10^ C_{water}) × P_{STEC}	cfu	Calculated
	Tr_F	Transfer from lettuce to flume	Risk Triang (***0,0.02,0.02***)	%	[25]

Steps	Parameter	Description	Distribution/equation/values	Units	Data source/reference
	Tr_{SHAKER}	Transfer from lettuce to shaker	Risk Triang (***0,0.01,0.02***)	%	[25]
	$Tr_{SHREDDER}$	Transfer from lettuce to shredder	Risk Triang (***0,0.02,0.02***)	%	[27]
	Tr_{C}	Transfer from lettuce to centrifuge	Risk Triang (***0.01,0.04,0.08***)	%	[27]
	Tr_{CONV}	Transfer from lettuce to conveyor	Risk Triang (***0,0.1,0.24***)	%	[27]
	Tr_{H}	Transfer from lettuce to hands	Risk Uniform (***0.3,1***)	%	[26]
	Tr_{EQP}	Transfer from equipment to lettuce	Risk Triang (***9.9,15.33,18.83***)	%	[26]
	Tr_{HtoL}	Transfer from hands to lettuce	10	%	[26]

Steps	Parameter	Description	Distribution/equation/values	Units	Data source/reference
	N_{LtoEH}	Cells transferred from lettuce to equipment surfaces and hands in a unit batch	$N_{cfu} \times (Tr_F + Tr_{SHAKER} + Tr_{SHREDDER} + Tr_C\ Tr_{CONV} + Tr_H)$	cfu	Calculated
	N_{EHtoL}	Cells transferred from equipment-surfaces and hands to lettuce in a unit batch	$N_{LtoEH} \times (Tr_{EQP} + Tr_{HtoL})$	cfu	Calculated
	S_{cont}	Spread of contamination due to cross contamination with the organism	RiskPert (***1,1.2,2***)	No units	[22]
	Pre_{cross}	Prevalence after cross contamination	$P_{STEC} \times S_{cont}$	No units	Calculated
	C_{cross}	Concentration after cross contamination	N_{EHtoL} / Pre_{cross}	cfu/g	Calculated
Lettuce storage	S_{TR}	Storage temperature at retail	Risk Normal (***4.44,2.96***,RiskTruncate(***0,20.56***)) °C		[27]
	S_{tR}	Storage time at retail	(Risk Pert (***0.5,4,7***)) × 24	h	User input
	S_{TH}	Storage temperature at home	Risk Normal (***4.06,2.44***,RiskTruncate(***-1,20***))	°C	[29]
	S_{tH}	Storage time at home	(Risk Uniform (***1,3***)) × 24	h	User input

Steps	Parameter	Description	Distribution/equation/values	Units	Data source/reference
	b	Growth model parameter (*b*)	0.023	No units	[30]
	T_{min}	Growth model parameter (T_{min})	1.17	°C	[30]
	μ_{SR}	Growth rate at retail-storage	$(b \times (S_{TR} - T_{min})^{(2/2,303)}$	Log cfu/g/h	Calculated
	μ_{HR}	Growth rate at home-storage	$(b \times (S_{TH} - T_{min})^{(2/2,303)}$	Log cfu/g/h	Calculated
	G_{SR}	Growth during storage at retail	$IF(S_{TR}>5,1,0)$		
	C_{SR}	Concentration of STEC after storage at retail	$IF (G_{SR} =1,((\mu_{SR} \times S_{tR}) + C_{cross}), C_{cross})$	Log cfu/g	Calculated
	G_{SH}	Growth of the pathogen during home-storage	$IF (S_{TH} >5,1,0)$		
	C_{SH}	Concentration of STEC after storage at home	$IF (G_{SH} =1,((\mu_{HR} \times S_{tH}) + C_{cross}), C_{cross})$	Log cfu/g	Calculated
Consumption at home and exposure to STEC	C_{consu}	Concentration of STEC at consumption	$IF (C_{SH} <7,C_{SH},7)$	Log cfu/g	Calculated

Steps	Parameter	Description	Distribution/equation/values	Units	Data source/reference
	$Serving_{size}$	Portion size per-serving	RiskPert (***50,80,100***)	g/serving	[33]
	$D_{serving}$	Dose per-serving	$(10\text{^}C_{consu}) \times Serving_{size}$	cfu/serving	Calculated
Dose response	α	Dose response alpha parameter	0.267	No units	[40]
	β	Dose response beta parameter	229.29	No units	[40]
	P_{ill}	Probability of illnesses	$(1 - (1+ D_{serving} / \beta)\text{^}-\alpha) \times P_{STEC}$	No units	Calculated
	Pop_{consu}	Population consuming lettuce in SA	0.089 × 55 000 000	No units	[33]
	$Consu_{percapita}$	Annual consumption of lettuce per-capita in SA	$(34201 \times 10\text{^}6) / Pop_{consu}$	g/year	Calculated
	$Consu_{serving}$	Servings consumed per-person annually	$Consu_{percapita} / Serving_{size}$	No units	Calculated
	$N_{serving}$	Servings consumed in SA annually	$Pop_{consu} \times Consu_{serving}$	No units	Calculated
	N_{case}	Annual cases of illness	$N_{serving} \times P_{ill}$	No units	Calculated

2.4. Assumptions

A few assumptions had to be made in this work, primarily because of gaps in the knowledge, to finish the quantitative model. The assumptions considered were supported by literature and professional judgment. Table 2 summarizes the assumptions made in this study and the possible impacts they can have on the risk output.

TABLE 2. ASSUMPTIONS USED IN THE MODEL AND THE IMPACT THEY HAVE ON MODEL OUTPUT

Assumptions	Impact on model output
• Overhead irrigation as the only method of irrigation	• Risk overestimation
• Prevalence of STEC in lettuce is the same as the one calculated in irrigation water	• Both underestimation and overestimation of the risk
• Ratio of STEC to *E. coli*	• Both underestimation and overestimation of the risk
• All STEC cells in irrigation water would be transferred from the water and attached to lettuce using the overhead irrigation method.	• Risk overestimation
• Minimum growth temperature of *E. coli* on lettuce is 5°C	• Risk underestimation
• Storage times at retail and at households	• Risk Underestimation
• Environmental temperature has no effect on bacterial decay in the plant tissue	• Risk of overestimation

2.5. Simulation and analysis

A suitable software was used to model STEC for all scenarios based on the Monte Carlo simulation technique. Every model underwent 10000 iterations of simulation as conducted by Ottoson et al., [19] and Pang et al., [17]. Model outputs were the median risk of illness per serving. Sensitivity analysis was performed to determine the critical parameters. Spearman's regression correlation coefficient was used to estimate the impact of production and handling practices in the lettuce value chain on variability annual STEC-exposure through lettuce . On the baseline model, various alternative scenarios and their combinations were examined. These included adding radiation during the packaging stage and lowering the initial *E. coli* contamination to the WHO-recommended limits (less than 1000 cfu/100 ml irrigation water). A log reduction of 5 was achieved when lettuce contaminated with *E. coli* O157 was treated with 0.55 kGy gamma radiation [41]. These settings were applied to the data, and the model was re-simulated.

3. RESULTS AND DISCUSSION

3.1. Exposure assessment

The estimated levels of STEC per serving are depicted in Figs 1 and 2, respectively. The initial STEC concentration in irrigation water, subsequent handling and storage influence how much STEC a consumer is exposed to per serving of lettuce. A higher dose per serving of STEC was observed in scenario B than in scenario A. Results from this study highlight that decontamination of lettuce through a processing stage (scenario A) is essential in reducing the risk of STEC infections. Compared to other cooked vegetable products, lettuce has a higher risk of producing foodborne diseases because it is typically ingested raw [17, 38]. Based on the results (Figs 1 and 2) the mean doses for both scenarios A and B are very high and have the potential to cause illnesses.

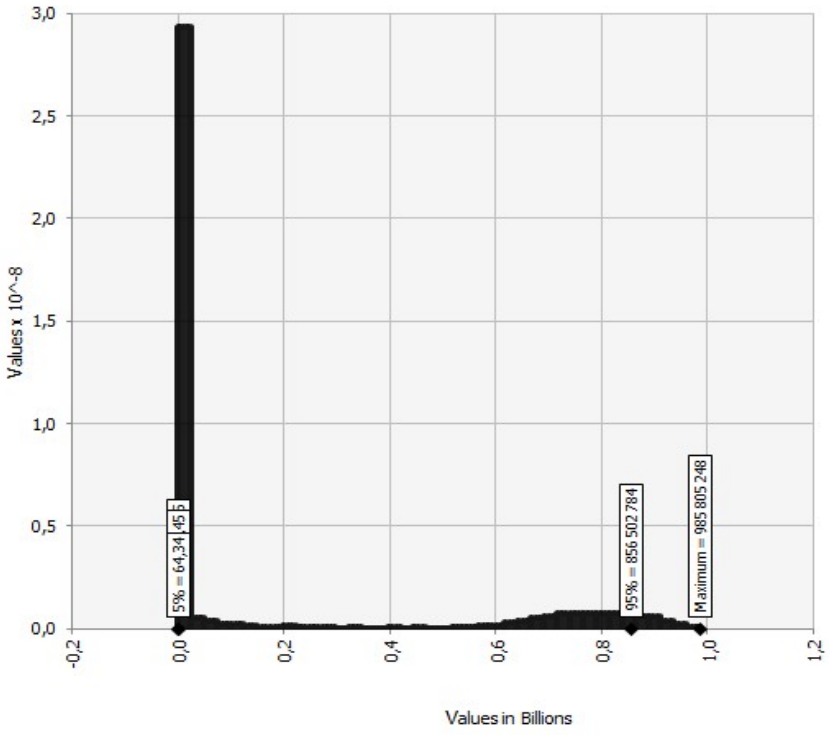

FIG. 2. Concentration of STEC at consumption (scenario A)

Values in the x-axis are in cfu per serving.

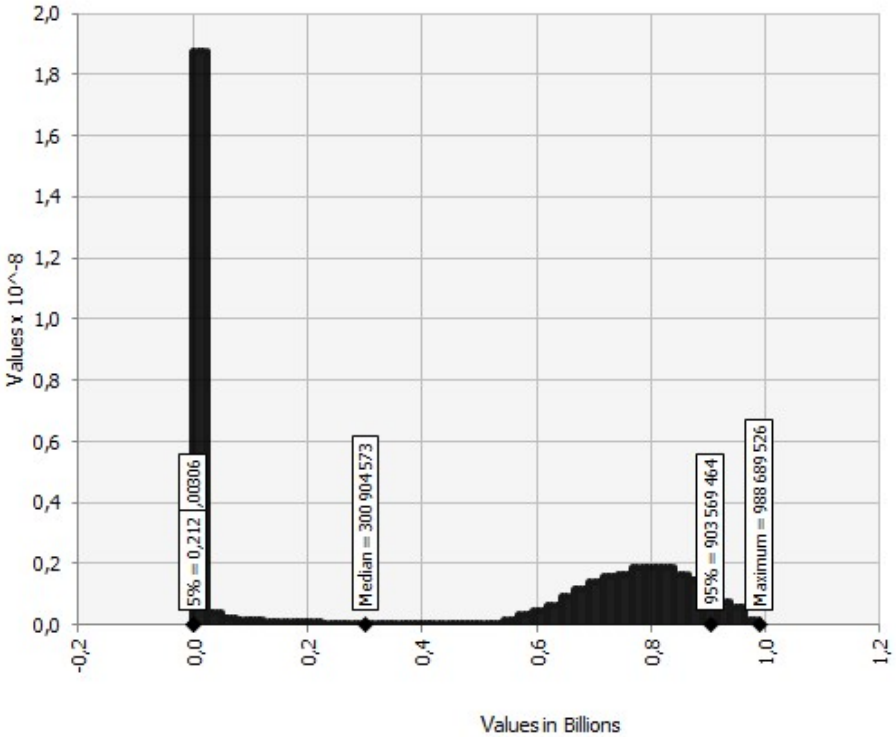

FIG. 3. Concentration of STEC at consumption (scenario B); Values in the x-axis are in cfu per serving.

3.2. Risk characterization

The average expected likelihood of illness per serving according to the baseline model for scenario A was 0.45 (5^{th} and 95^{th} percentiles: 1.50×10^{-7}, 0.12) and 0.055 (5^{th} and 95^{th} percentiles: 1.80×10^{-5}, 0.88) for scenario B. Median number of cases for scenarios A and B was 5955 and 8245, respectively (Table 3). There are no epidemiological data in South Africa to justify the model estimates. However, a study conducted by Pang et al., [17] provides model estimates in line with epidemiological statistics on the number of cases from consumption of *E. coli* O157 contaminated lettuce.

TABLE 3. PROBABILITY OF ILLNESS DUE TO STEC-CONTAMINATED LETTUCE AND ANNUAL NUMBER OF CASES

Scenario	Probability of illness per-serving Median (5^{th}, 95^{th}) percentiles	Number of cases per-year Median (5^{th}, 95^{th}) percentiles
A	0.071 (1.50×10^{-7}, 0.12)	5955 (1, 9483)
B	0.083 (1.80×10^{-5}, 0.88)	8245 (3, 10329)

3.3. Potential exposure mitigation strategies

In comparison to the baseline model, which included no intervention approach, the mean probability of illness per serving and the mean number of illness cases annually were considerably ($p < 0.05$) lower for each of the three intervention strategies that were used (data not shown). The percentage reduction is depicted in Table 4. This QMRA demonstrated how well the assessed intervention options decreased the probability of STEC-related illness and fresh cut lettuce-associated sickness.

TABLE 4. THREE INTERVENTIONS AND THEIR EFFECT ON ANNUAL NUMBER OF CASES FOR SCENARIO A

Intervention	Reduction (%)
Reduce initial *E. coli* level to 1 log cfu/ml	63
Ionising radiation applied after packaging	70
Washing at home	65

3.4. Importance analysis

Spearman's rank order correlation was used to determine how sensitive the baseline model outcomes were to input values and model parameters. The results show that the mean number of annual cases was most sensitive to retail storage temperature, home storage temperature, the initial prevalence of STEC in irrigation water, and cross-contamination during processing (Fig. 4). These results are supported by a previous study [17] that also found that the probability of illness was influenced by storage temperatures along the lettuce production chain in a baseline scenario. Since fresh-cut lettuce is typically consumed raw, pathogen growth during postprocessing storage may expose consumers to higher levels of infections during consumption. This is because fresh-cut lettuce is prone to pathogen contamination throughout the supply chain. This research showed that maintaining a sufficient cold chain and controlling temperature is crucial for minimising the risk associated with STEC growth during the postprocessing lettuce storage.

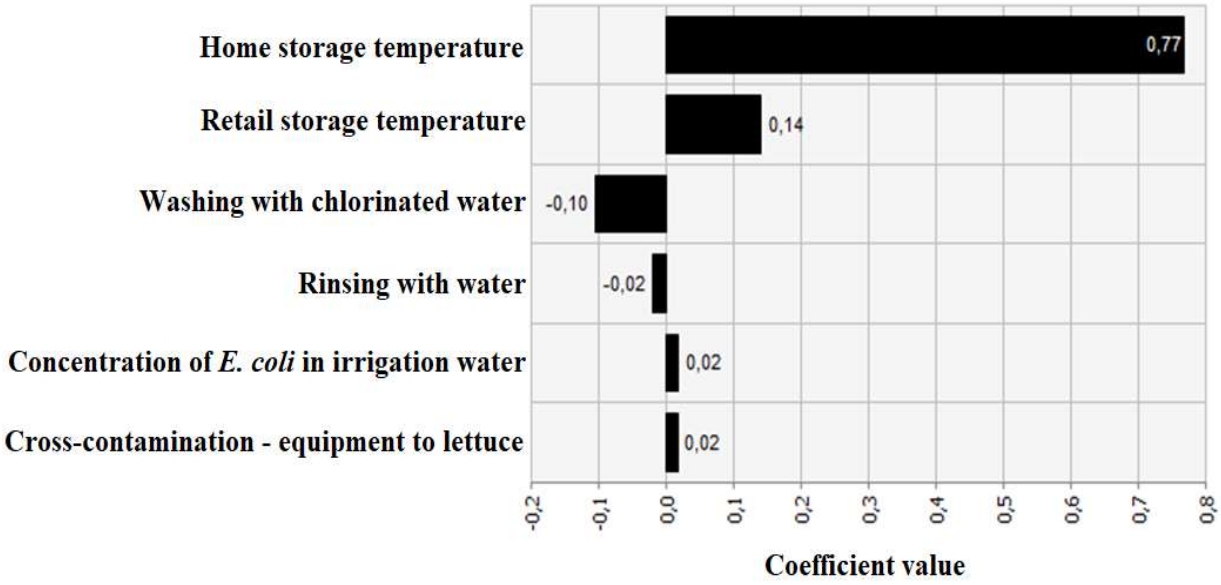

FIG. 4. Sensitivity-analysis between probability of illness and factors along the value chain (scenario A).

The built QMRA model has a number of flaws and data gaps found that could have an impact on the model's output. For instance, it is still uncertain how much STEC was initially present in irrigation water and how long fresh cut lettuce is stored at retail. Because a time–temperature profile was missing, the risk model did not account for the transportation of lettuce from the farm to the processing facility. Assumptions were assigned in case of data gaps.

3.5. Safety of lettuce supply chain

A comprehensive food safety approach for lettuce involves managing risks across the entire supply chain, from farm to fork, to control pathogens like STEC. This system begins with Good Agricultural Practices (GAPs) and Good Hygienic Practices (GHPs) at the primary production stage to minimize contamination from soil, water, and handling [42]. In processing and manufacturing, it incorporates Hazard Analysis Critical Control Points (HACCP) current Good Manufacturing Practices (cGMPs) and sanitation procedures to identify and control potential contamination points. These Pr-Requisite Programmes (PRPs) are foundational, as effective HACCP implementation depends on their correct application. By applying these layered safety protocols, the lettuce supply chain in South Africa aims to ensure that only produce with minimal STEC risk reaches consumers [42].

Current studies in South Africa have highlighted the microbiological inadequacy of irrigation water that is used for irrigation [2–4, 16]. Furthermore, samples of lettuce collected at the harvest and retail stage revealed that levels of *E. coli* were above those stipulated by national authorities. This study identified the need for continued training in areas presented in Table 5 to ensure supply of safe lettuce to consumers. Implementing GAPs and HACCP systems will be of immense importance in reducing the risk of STEC in lettuce in South Africa.

TABLE 5. AREAS WHERE GAPS, CGMPS, GHPS AND HACCP SYSTEMS CAN BE IMPLEMENTED IN THE LETTUCE AGRI CHAIN TO REDUCE THE RISK OF STEC CONTAMINATION

Stages along the production chain	Issues to be addressed using GAPs, cGMPs, GHPs or HACCPs
Farm activities	Water quality
	Soil management
	Worker hygiene
	Animal control
	Flooding
	Sanitation of Equipment
Postharvest	Facility design and maintenance
	Water used in postharvest operations
	Field containers

Stages along the production chain	Issues to be addressed using GAPs, cGMPs, GHPs or HACCPs
	Condition and sanitation of transportation vehicles
	Employee hygiene
Value addition	Wash water
	Packaging
Distribution	Sanitation of vehicles
	Cooling facilities
	Temperature monitoring
Consumer handling	Storage condition and time
	Cross-contamination

In South Africa, the application of PRPs such as GAPs, GMPs and GHPs at farms and retail of lettuce is regulated and enforced by the government through the Department of Agriculture, Forestry and Fisheries (DAFF) now the Department of Foresty, Fisheries and the Environment.

3.6. Monitoring and sampling plans

An effective monitoring plan is dependent on an effective sampling plan. Sampling is an essential tool for food safety management [43]. Based on the result from the case study and published literature [1, 17, 20] irrigation water is a significant risk factor in the lettuce food chain. Due to the low prevalence of STEC in water, generic *E. coli* can be used as an indicator organism in the sampling plan. A surveillance sampling plan in accordance with Eq. (4) is thus ideal

$$n = (Z2 \times P \times (1 - P)) / L2 \quad (4)$$

Where; n is the number of samples; Z is 1.96 for 95% confidence level (1.645 for 90%); P is the estimate of prevalence [assumed to be 50% (0.50)]; L is accuracy or absolute error (set at 5%).

Sampling can take place at the irrigator, at the source or reservoir, or adjacent abstraction stations. Relatively little advice is available to producers regarding how to react to water quality findings. The systematic sampling can be repeated twice yearly (rainy and dry season). Thus, DAFF (former) can conduct monitoring and surveillance programmes for water used to irrigate fresh produce. The information gathered from these surveys can guide methods to purify water before use for irrigation. For example, farmers can treat the water employing solarisation to reduce levels of *E. coli* before irrigating fresh produce.

4. CONCLUSIONS

Production and marketing of lettuce without microbial decontamination stages have severe consequences for consumers. Decontamination processes such as chlorine wash and irradiation are essential steps in reducing the risk of pathogens such as STEC. The model presented in this study predicted a probability of getting ill by consumption of STEC-contaminated lettuce in South Africa, albeit with low probability values. The initial prevalence of STEC in irrigation water, home storage temperature, cross-contamination during processing, and retail storage temperature were the factors that most affected the mean number of disease cases annually. However, several limitations and data gaps of the developed QMRA model were identified which could have affected the model output. To effectively manage the safety of lettuce production in South Africa, occasional training of farmers on GAPs and procedures of fresh-cut lettuce on GHPs, GMPs and HACCP principles cannot be ignored. However, to further ensure food safety, good hygiene among consumers is required. Incorporating field production and the entire farm-to-fork production and supply chain for lettuce, the developed QMRA model explicitly modelled cross-contamination during processing. This could offer risk managers and policymakers a methodical approach to considering the crucial variables along the lettuce food chain and estimating the impact of suggested risk mitigation interventions.

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INVESTIGATING POTENTIALLY TOXIC ELEMENTS (PTES) CONTENT IN BREAST MILK AND COMPLEMENTARY FOOD FROM TANGERANG, INDONESIA

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Abstract

Breast milk provides significant nutritional and health benefits to infants. The WHO recommends exclusive breastfeeding for the first six months of an infant's life and breastfeeding with appropriate complementary foods until at least two years old. Although breast milk is the natural and optimal nutrition for infants, it can also be a pathway for transferring toxic environmental contaminants from mothers to their infants. Complementary foods (CF) can also be a source of exposure to harmful chemicals such as PTEs. Potentially toxic element pollution is a severe environmental problem. These chemicals can affect the child's growth and health. Accurate monitoring of metal concentrations in different samples is essential to minimise health risks from exposure to such toxic substances. For this purpose, due to the low concentration of heavy metals in food, a highly selective and sensitive analytical method is required. Therefore, nuclear analytical techniques, including neutron activation analysis (NAA) and total reflection X ray fluorescence (TXRF) were used to determine the composition of chromium (Cr) cobalt (Co) copper (Cu) and lead (Pb). The results showed that the distribution levels of elements in breast milk for normal infants were in the order Cu>Pb>Co>Cr; for stunted infants it was in the order Cu>Pb>Cr>Co. The order of elemental concentration in complementary foods for both normal and stunted infants was Cu>Cr>Co>Pb. Given the increasing exposure to PTEs, it is imperative to monitor their concentration in food. Further studies are required to assess potential risks to infants from these contaminants.

1. INTRODUCTION

Breast milk is the primary source of infant nutrition, providing essential elements, proteins, fats and carbohydrates for normal body growth. The American Academy of Paediatrics and the WHO recommend exclusive breastfeeding for the first six months. These entities also recommend the provision of safe and appropriate complementary foods and continued breastfeeding for up to two years or beyond [1–3]. However, breast milk and complementary foods may not be free of hazards. Contamination of breast milk and complementary foods is widespread and results from decades of environmental pollution caused by toxic chemicals. The extensive exposure to PTEs from various sources, such as fossil fuels, industrial byproducts, atmospheric deposition, and agricultural land, has led to environmental pollution. This is a matter of significant concern, as some investigations have found these toxicants in human breast milk, suggesting potential health effects on breastfed infants [2, 4, 5].

Studies have demonstrated that lead can be transmitted from mother to foetus through the placenta and from mother to infant through breast milk. This and other pollutants can negatively affect developmental and physiological processes of fetuses and neonates. Furthermore, because of their underdeveloped metabolic capacity, developing infants may accumulate higher concentrations and prolong the excretion of these toxins [6]. The data on comprehensive studies of PTEs content in breast milk and complementary food in Indonesia were still limited, and due to the toxicity of elements, their adverse effects on public health and the environment, and the low concentration of PTEs in food, it is essential to measure their levels accurately. Trace elements and PTEs in breast milk and complementary foods from several countries were analysed using different methods. These techniques include atomic absorption spectrometry (AAS) ion chromatography (IC) inductively coupled plasma mass spectrometry (ICP-MS) proton-induced X ray emission (PIXE) NAA and TXRF.

The NAA is a non-destructive, adaptable, sensitive, multielement, simultaneous, selective, and nanogram-order detection method for sample research. Thus, NAA is suitable for qualitative and quantitative analysis of

multiple elements in samples such as food. TXRF can be used to verify food safety, and it is competitive with other approaches in several application areas. The TXRF limits of detection for PTEs are comparable to those of AAS, while the former approach is more sensitive regarding total sample volume. Another feature of TXRF is the ability to analyse samples without the need of digestion.

The amount of sample required for TXRF analysis is much less than that required for AAS and ICP techniques. Each technique has its strengths and limitations, and their combined use provides a comprehensive understanding of PTEs contamination in breast milk and complementary foods [7, 8]. Therefore, this research aimed to determine the heavy metal content in breast milk, complementary food consumed by children in polluted areas in Indonesia, using nuclear analytical techniques. In this study, focus was on the Tangerang area because, based on previous research, Pb levels in the Tangerang area are higher than in other cities in Indonesia [9].

2. MATERIAL AND METHODS

2.1. Materials

The materials used were of the highest quality and/or grade suitable for analysis. The materials used include the Standard Reference Material (SRM) specifically National Institute of Standards and Technology (NIST) 1548a Typical Diet and NIST 1549a Whole Milk Powder; demineralised water with a resistivity of 18.2 $M\Omega.cm^{-1}$; Gallium ICP standard $Ga(NO_3)_3$ in HNO_3 2–3% 1000 mg/l; Siliconizing solution in isopropanol; Triton X-100 for gas chromatography; quartz glass (reflector); ICP multielement standard solution VI; polyethylene vial 0.3 ml, and HNO_3 65%.

2.2. Instrumentation

An NAA and TXRF were used. Further analyses were carried out on a gamma spectrometer equipped with a high purity germanium (HPGe) detector with relative efficiencies of 25% and a multi-channel analyser (MCA). Germanium detectors are semiconductor diodes comprising a P-I-N structure, wherein the intrinsic (I) region exhibits sensitivity to ionizing radiation, particularly X rays and gamma rays [10]. The γ ray spectra were analysed using the Genie 2000 software. In addition, the samples were analysed using a benchtop TXRF system consisting of a 50 W X ray tube with a Mo tube, multilayer monochromator, a silicon drift detector (SDD). Each measurement was carried out for 1000 seconds using quartz sample carriers as sample holders and reflectors.

2.3. Sample collection and preparation

The samples of breast milk (n=57) and complementary foods (n=200) were carefully collected from the fifteen sites in Tangerang Banten, Indonesia (Tangerang district urban area) with 50 normal infants and another 50 stunted infants. Tangerang is a populated urban area with significant industrial and vehicular activities that have been increasing, especially after the detection of elevated lead concentrations in early 2001 [9].

2.4. Preparation sample

Each type of food was individually weighed, considering only the edible portions, and then blended using a titanium blade blender. The resulting smooth and homogeneous food samples were weighed again, placed into small tubes, and stored in a freezer: once frozen, the food sample-tubes were placed on a freeze dryer set at -85°C. The drying process took 72 hours until the samples reached a constant weight. The dried samples were then ground into a fine powder using a Teflon mortar and pestle and placed into polyethylene containers [11].

2.5. Water content determined

This study also assessed the water content in breast milk samples and complementary foods. The samples were weighed before being crushed by a titanium blade blender. Water was introduced and quantitatively monitored during the crushing process. After crushing, the samples were weighed again and stored in the freezer. Following freeze drying, the food samples were measured in terms of weight [12]. The water content was calculated using Eq. (1)

$$\text{Water content} = \frac{b-c}{b-a} \times 100\% \quad (1)$$

Where *a* is the weight of the blank cup, *b* is the weight of the sample and cup before drying; *c* is the weight of the sample and cup after drying

2.6. Determination using NAA

Approximately 40–50 mg of dried sample, SRM NIST 1548a typical diet, SRM NIST 1549a whole milk powder, was carefully placed into a 0.3 ml polyethylene vial and securely sealed through a heat process. The samples were irradiated with SRM and standard multi-elements for 2 hours at a thermal neutron flux of 10^{13} n.cm^{-2} in rabbit system facilities of the Multipurpose Reactor, G.A. Siwabessy, Serpong. After decay for a month, samples, SRM, and ICP standards were counted for ±60000 seconds using a gamma spectrometer with HPGe detector (25% relative efficiency) and MCA. The spectrum was analyses using an appropriate software [12].

2.7. Determination using TXRF

Samples and SRM were weighed 100 mg, placed in a vial, and then dissolved homogeneously in 1% aqueous triton to make a 5 ml solution. Approximately 10 µl of internal standard gallium solution was added and stirred using a vortex for 10 seconds. An aliquot (10 µl) of the homogeneous solution was pipetted and deposited onto a sample carrier with a siliconized surface to form a droplet. The deposited samples were dried on a hotplate to form a thin layer. Each sample was analysed for 1000 seconds using a 50 kV molybdenum (Mo-K) anode as the X ray source for TXRF [13].

2.8. Quality control

Quality control was performed using SRM NIST 1548a typical diet and SRM NIST 1549a whole milk powder. At each analytical step, the samples and SRM were treated similarly. The results were compared with their certified values and assessed for accuracy and precision.

3. RESULTS AND DISCUSSION

3.1. Characteristics of infants

Samples were collected from fifteen sites in Tangerang Banten, Indonesia, for breast milk (*n*=57) and complementary foods (*n*=200). The sites are Curug Wetan (Curug) Cibadak (Cikupa) Cikupa Permai (Cikupa) Cukanggalih (Curug) Dukuh (Cikupa) Kadu (Curug) Kutabumi (Pasar Kemis) Pangadegan (Pasar Kemis) Pasar Kemis, Sindangsari (Pasar Kemis) Sukaasih (Pasar Kemis) Sukabakti (Curug) Sukamantri (Pasar Kemis) and Talagasari (Cikupa). The characteristics of the infants and their characteristic sex and age from the age of 6 months to 24 months are shown in Table 1 and Figs 1 and 2.

TABLE 1. THE CHARACTERISTICS OF INFANTS

Category/Parameter	Mean ± SD	Range
Normal		
Age (month)	15.9±4.3	6.4–23.4
Weight (kg)	9.5±1.5	6.7–14.3
Height (cm)	77.1±4.5	67.4–85.5
Stunting		
Age (month)	17.1±4.1	7.4–23.6
Weight (kg)	8.2±1.0	6.1–10.1
Height (cm)	73.7±3.8	65.0–80.6

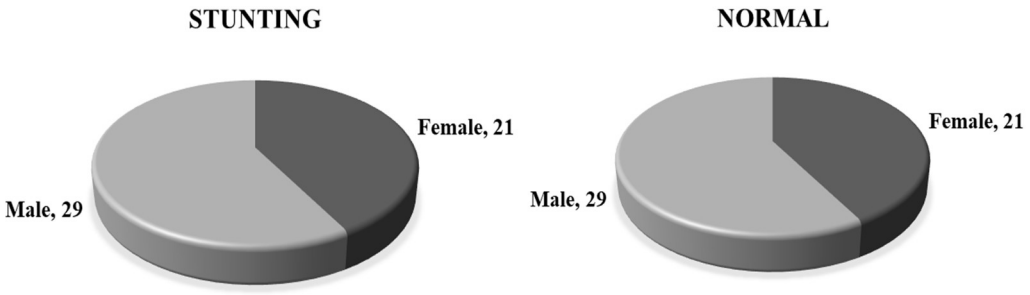


FIG. 1. Gender characteristics from age 6–24 months for normal and stunted infants.

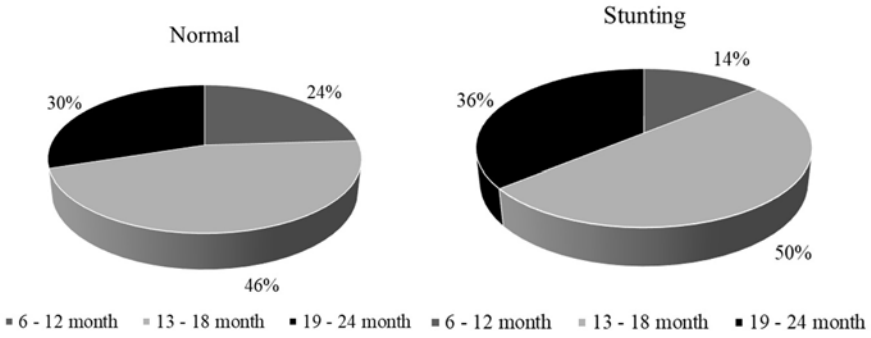


FIG. 2. Percentage of age characteristics for normal and stunted infants.

3.2. Water content

The average water content of breast milk samples from the normal and stunted categories was 85.4 ± 7.6% and 86.9 ± 3.4%, respectively. The water content in this study was as reported elsewhere [14] where the average water content of breast milk was found to be 87–88%. The composition of human breast milk is dynamic and evolves in response to the infant's changing requirements. For instance, the initial milk expressed (foremilk) is thinner and contains a higher concentration of lactose, which quenches the baby's thirst. Subsequently, the hindmilk is creamier and includes a significantly higher fat content to meet the baby's requirements [6, 14].

Determining the water content in complementary foods for infants and toddlers is essential for understanding their hydration and nutrient intake. This study used the duplicate diet method, which involves collecting the food and drink the child consumes over 48 hours. Each portion of food and beverage is duplicated exactly as it was consumed. Health professionals can ensure that infants and toddlers receive the proper hydration and nutrition necessary for their development by accurately measuring and understanding the water content in duplicate diets. The average water content of the sample from the normal and stunted categories was 69.1 ± 9.5% and 71.7 ± 6.8%, respectively.

3.3. Quality control

The whole milk powder material, SRM NIST 1548a typical diet and SRM NIST 1549a was used for quality control (QC) to evaluate the precision and accuracy of the analytical methods used and satisfactory results are presented in Table 2. Analytical accuracy, described as % recovery, was 70–125%, while analytical precision, defined as % CV, was < 0.67 $CV_{Horwitz}$. Both analytical accuracy and precision were acceptable according to AOAC guidelines [15, 16].

TABLE 2. QUALITY CONTROL ASSESSMENT USING SRM NIST 1548A TYPICAL DIET AND SRM NIST 1549A WHOLE MILK POWDER

Certified Reference Materials	Elements	Certificate value	Result	Result		Acceptable	
		mg/kg	mg/kg	Accuracy	Precision	Accuracy	0.67 $CV_{Horwitz}$
SRM	Fe	35.3 ± 3.77	33.8 ± 0.14	96%	0.4	80–115	6.3
NIST	Zn	24.6 ± 1.79	21.6 ± 0.26	88%	1.2	81–115	10.9

Certified Reference Materials	Elements	Certificate value	Result	Result		Acceptable	
1548a Typical Diet	Co	0.028	0.03 ± 0.001	107%	4.1	70 – 125	18.4
	Cu	2.32 ± 0.16	2.61 ± 0.2	113%	7.7	75 – 120	9.4
SRM	Fe	1.85 ± 0.73	1.85 ± 0.12	100%	6.5	75 – 120	9.8
NIST	Zn	33.8 ± 2.30	32.87 ± 0.33	97%	1.0	81 – 115	6.3
1549a Whole Milk Powder	Cu	0.638 ± 0.049	0.51 ± 0.05	80%	9.8	75 – 120	11.9

3.4. Concentration of PTEs in breast milk and complementary food

The elemental concentrations of breast milk and complementary food samples taken from fifteen regions measured by NAA and TXRF are presented in Figs 3 and 4. Concentrations of Cr, Co, Cu, and Pb were observed as the PTEs in all the samples analysed.

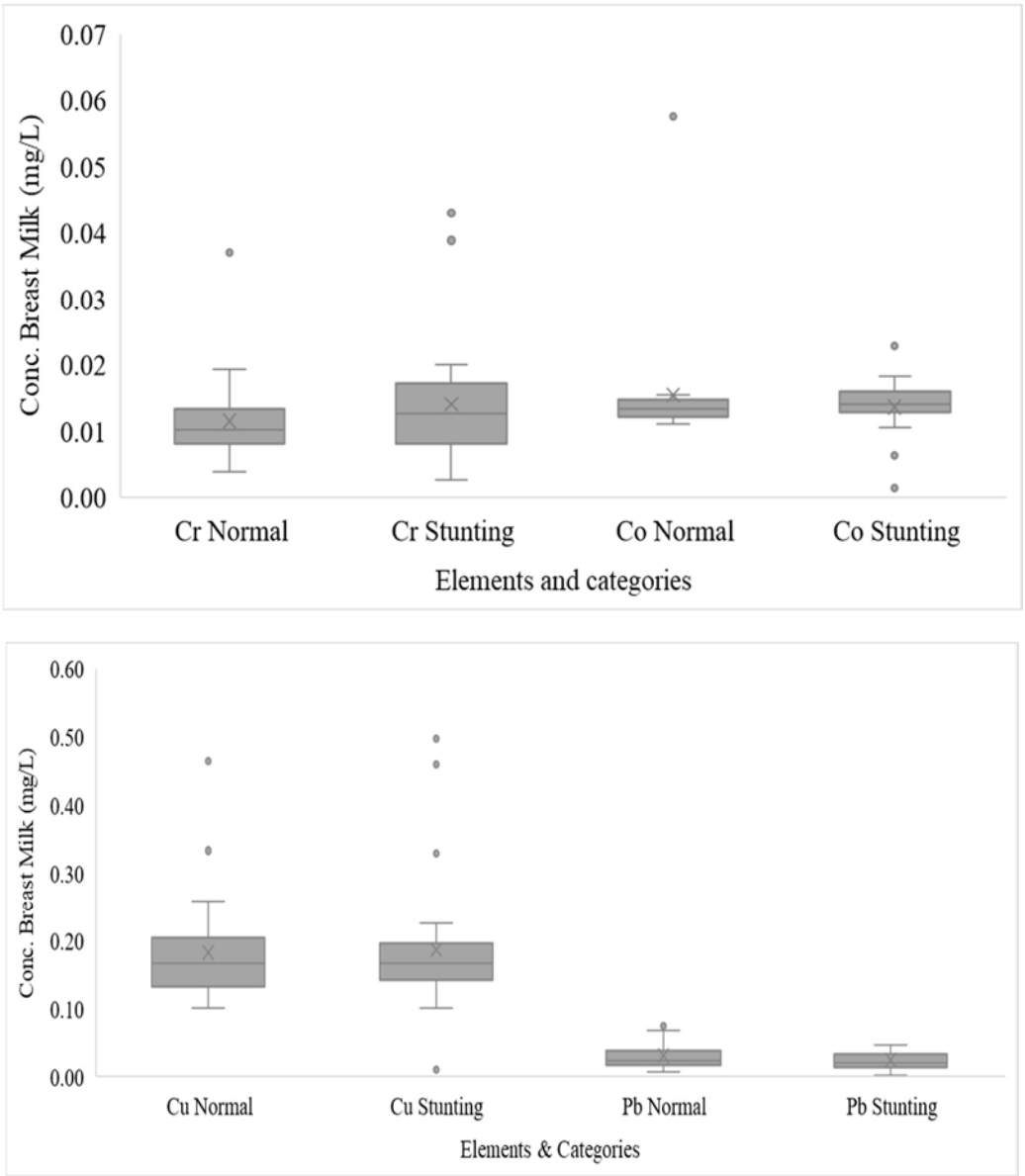


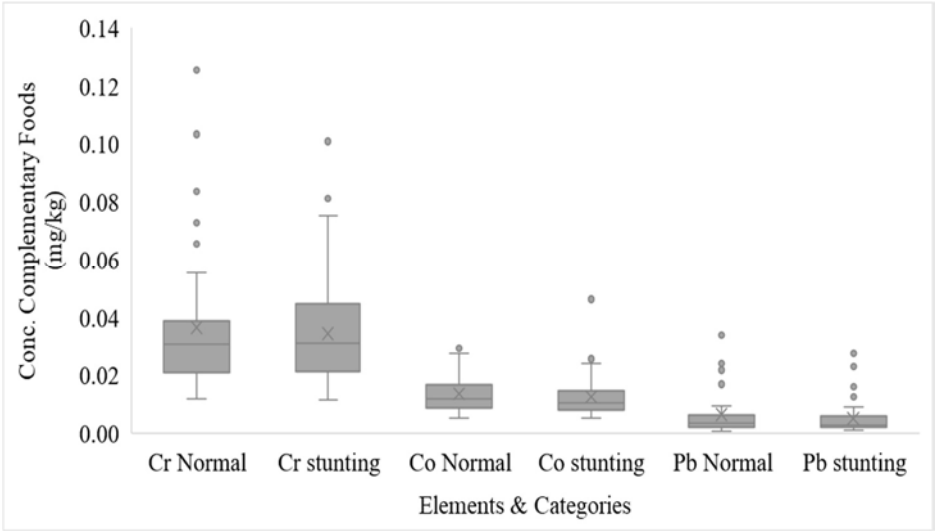
FIG. 3. The concentration of Cu, Pb, Cr, and Co on normal and stunting in breast milk.

Levels of the elements in breast milk for normal infants were in the order Cu>Pb>Co>Cr with concentrations in the range 0.100–0.464 mg/l; 0.007–0.084 mg/l; 0.011–0.058 mg/l and 0.004–0.037 mg/l and stunted infants were in the order Cu>Pb>Cr>Co with concentrations in the range 0.010–0.497 mg/l; 0.002–0.072 mg/l; 0.003–0.043 mg/l; and 0.001–0.023 mg/l. Figure 3 shows the breast milk Cu level ranged from 0.100 mg/l to 0.464 mg/l for normal infants and from 0.01 to 0.497 mg/l for stunted infants. The mean level of Cu found in the current breast milk study was lower than previous studies in Abeokuta, Ogun State, Nigeria (<LOD to 0.550±0.011 mg/l) with an average value of 0.173±0.127 mg/l [1] while the high value in the sample was above the WHO acceptable range (0.18–0.31 mg/l).

High levels of Cu are associated with liver toxicity, dementia, disorientation effects, anxiety, oxidative damage, and neurotoxicity due blood brain barrier (BBB) damage [1, 17, 18]. The BBB is made of microvascular endothelial cells that line cerebral capillaries, penetrating most mammals' brains, spinal cord, and other organisms with a well-developed central nervous system. The BBB is critical in regulating the influx and efflux of biological substances essential for the brain's metabolic activity and neuronal function. Therefore, maintaining the functional and structural integrity of the BBB is crucial for maintaining the homeostasis of the brain microenvironment [19]. Lead concentrations in human breast milk of normal and stunted infants exceeded the WHO acceptable range (0.002–0.005 mg/l). Lead concentrations were higher than those found in Pretoria, South Africa, and exceeded other in Lebanon (0.018 mg/kg) [5, 17].

The concentration of lead in this study was still lower than the lead concentration in Slovakia (4.7 mg/kg) and the acceptable range for infant formula milk in Indonesia is 0.1 mg/kg [5, 17, 20]. Previous studies from different countries have reported that the lead concentrations in breast milk vary between 0.0005 mg/l and 0.1265 mg/l. In some studies, lead levels in breast milk are higher in polluted urban centres than in less polluted areas. For example, in urbanised areas of Italy, the lead concentration in breast milk was 0.1265 mg/l, while in another region it was 0.0046 mg/l [21]. The results were also found to be higher than those reported for other countries, such as Nigeria (0.012–0.059 mg/l) Morocco (0.023–0.063 mg/l) Cyprus (0.0012–0.0015 mg/l) and Spain (0.005–0.016 mg/l). However, the result was lower than the lead in breast milk in Brazil (~0.531 mg/l) [6]. This comparison with other countries underlines the global nature of the issue. Lead is one of these dangerous PTEs, which is not known to have any biological role in humans and air pollution remains the main lead exposure route [21].

Chromium (Cr) was detected in samples at the median levels in the breast milk of Cr in the study were higher than those described in Pretoria, South Africa, which reported a median level of 0.0005 mg/l. However, Cr levels in breast milk reported from Abeokuta, Ogun State, Nigeria and western Iran (Republic of) were much higher, ranging from < LOD to 0.940±0.007 and 0.011 mg/l to 0.128 mg/l, respectively [1, 3]. The highest values for this element in breast milk samples exceed the acceptable range and the published guideline values established by the WHO (0.0008–0.0015 mg/l) [17]. Figure 4 shows that the range concentration of Co from the breast milk samples in Tangerang was higher than in Pretoria, South Africa, and infant nutrition formula in Nigeria as follows < LOD to 0.0003 mg/l and 0.002–0.010 mg/kg, respectively. It has been observed that the concentration of Co in breast milk tends to increase during lactation to accommodate the infant's growing requirements, as it produces an increasing number of antibodies over time. The deficient levels of Co in another study may be attributed to samples being collected within a week of birth when the demand for this element still needed to be added [17].



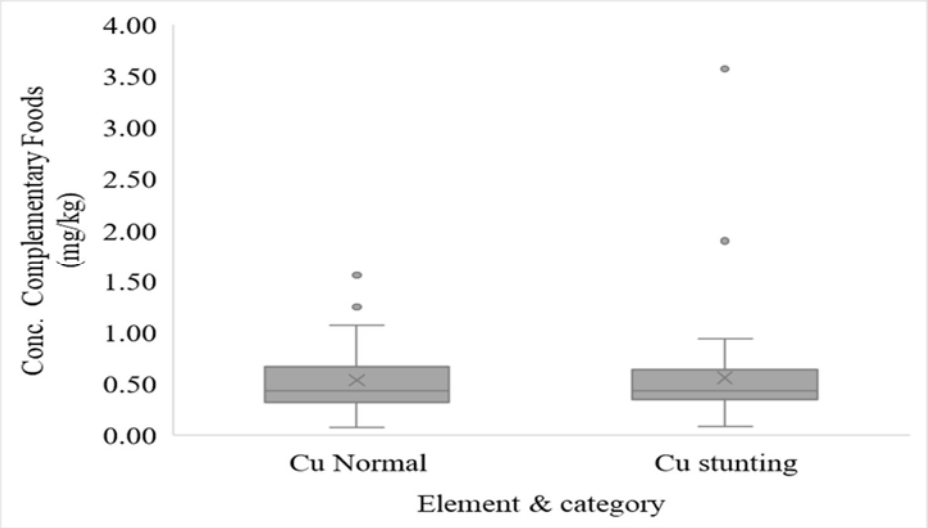


FIG. 4. The concentration of Cu, Cr, Co, and Pb on normal and stunting in complementary foods.

The elemental concentrations of complementary food samples from fifteen districts measured by NAA and TXRF are presented in Fig. 5. Concentrations of Cr, Co, Cu, and Pb were observed in all the samples studied. The concentrations in normal infants were in the order 0.073–1.566 for Cu> 0.011–0.126 for Cr>0.005–0.030 for Co > 0.001–0.034 mg/kg for Pb. The order of concentration in stunted infants was Cu (0.087–3.571 mg/kg) >Cr (0.011–0.101 mg/kg) >Co (0.005–0.046 mg/kg) >Pb (0.001–0.028 mg/kg).

The Cu content of complementary foods in normal and stunted infants ranged from 0.073 mg/kg to 1.566 mg/kg and 0.087 mg/kg to 3.571 mg/kg, respectively. The median of Cu levels for normal and stunted infants are 0.435 mg/kg and 0.431 mg/kg. The median Cu levels in the complementary food samples from normal and stunted infants were below the acceptable limits compared to the recommended limit of 0.9 mg/kg [18]. Copper (Cu) is a PTE, but it is also an essential trace metal. Adequate levels of Cu are associated with brain health and energy, cofactor function, brain and fetal development and growth, bone health, optimal metabolism of iron, glucose and cholesterol metabolism and various enzymes. Based on the results of another research, Cu correlates with immunological enhancements and antioxidant processes [5, 18].

The median concentration of chromium in complementary foods for normal and stunted infants, were 0.0306 mg/kg and 0.0311 mg/kg, respectively. The median values were below the acceptable limit of the WHO dietary guideline values of 0.0500 mg/kg. Although higher chromium concentration was observed in breast milk in this study, total chromium concentration was measured and not Cr (VI). Humans require chromium in trace amounts for optimal health. Although the exact mechanisms of chromium's actions in the body are not well understood, it is recognized for its role in enhancing insulin action in addition to the metabolism and storage of proteins, carbohydrates, and fats. Chromium is typically found in foods at very low concentrations, and the body absorbs only a small percentage of it, ranging from 0.4% to 2.5%. The element exists mainly as the biologically active Cr (III) and potentially harmful Cr (VI) [22].

Figure 4 shows that the range of Co concentration in complementary foods was 0.005–0.030 mg/kg for normal infants and 0.005–0.046 mg/kg for stunted infants. The Co concentrations in complementary foods were below the recommended limits (0.005–0.040 mg/kg/day) [18]. Cobalt (Co) is a crucial element; the health benefits of Co are linked to the formation of cobalamin (vitamin B12). Nevertheless, high levels of Co are linked to inflammatory diseases, hypersensitivity reactions and deficiencies in the nervous, cardiovascular, and endocrine systems. This research is of utmost importance in understanding the role of cobalt in infant nutrition and its potential health implications [17, 18, 23]. The highest Pb concentrations in complementary foods of both normal and stunted infants (0.034–0.028 mg/kg) were lower than the permissible standard for lead in complementary foods according to the WHO (0.01 mg/kg) Indonesia (0.3 mg/kg) and Singapore (0.2 mg/kg). The presence of metals in breast milk and complementary foods can be due to a range of factors and sources [17, 20].

4. CONCLUSIONS

This study evaluated the level of PTEs exposure of infants aged 6–24 months through breast milk and complementary foods in Tangerang, Banten using nuclear analytical techniques. The selected heavy metals were observed in varying concentrations in human breast milk and complementary food samples. Nuclear analytical

techniques (especially NAA and TXRF) gave satisfactory results in assessing PTEs content in breast milk and complementary foods. For the PTEs Cr, Co, Cu and Pb, the concentrations in human breast milk and complementary foods of both normal and stunted infants exceeded the WHO's acceptable range. However, the concentrations are similar to those found in other countries and are within the acceptable range in Indonesia for Pb. This study shows the applicability of nuclear analytical techniques to determine PTEs levels in breast milk and complementary foods. This is also relevant in assessing the nutritional status of infants.

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CHEMOTHERAPY AND CONTAMINATION-RISKS IN LIVESTOCK PRODUCTION SYSTEMS IN WEST, NORTH-WEST AND NORTH CÔTE D'IVOIRE

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Abstract

A descriptive study on therapeutic practices and the potential risk of contamination with the chemicals used, in farming systems in the West, Northwest and North regions of Côte d'Ivoire, was conducted between 2016 and 2022. Overall 152 farms including 111 transhumant and 41 sedentary near major towns, were included. The study showed that all the farms receive veterinary assistance through vaccination campaigns and Government intervention during disease outbreaks. Only 28% of these farms used the services of animal health professionals, with the rest self-medicating, using traditional means and commercial medicines including antimicrobials, anti-inflammatory agents and synthetic acaricides, albeit without necessary precautions to avoid intoxication as well as ensuring proper waste management. This may expose breeders/farmers, animals, and environment to contamination, sometimes resulting in dermal and respiratory diseases as well as development of resistance. Employing a One Health approach could help challenges as identified in this study to safeguard, human, anima and environmental health.

1. INTRODUCTION

The management of pollutants is a major global concern. According to the World Bank , waste production was projected to increase from 2.01 billion tonnes in 2016 to 3.4 billion tonnes in 2050 [1]. Urbanization, economic development and population growth are considered to be the main sources of waste production management of which is considered by national and international policies [2–7]. However, 33% of this waste is currently poorly managed through open dumping or burning [1]. In addition to these production sources, organic waste (agricultural by-products) and chemical waste (phytosanitary and veterinary products) produced in farming systems are also known sources of concern. While organic waste is fairly well managed through recycling, particularly in agriculture [8, 9] this is not the case for chemical waste, especially in Africa's low-economy countries [10].

In Côte d'Ivoire, while organic waste from farming systems is well managed [11] the management of chemical waste (phytosanitary and veterinary products) represent a major issue in farming systems [10, 12] although its use is regulated by Ivorian legislation [13–16]. This poor implementation of animal health legislation has led to a disorderly approach to prophylaxis particularly in the use of veterinary products. This situation exposes breeders to contamination risk and contributes to environmental pollution. This is one of the main reasons for low livestock production, which covers only 44.6% and 9.3% of national meat and milk consumption, respectively [17]. In order to better understand use of these veterinary products and their consequences in animal production systems, the present study proposed to characterize livestock production systems; describe the use and management of veterinary products in livestock production systems; and describe the management of veto product waste and its consequences in livestock production systems.

2. MATERIAL AND METHODS

2.1. Study areas

The study was undertaken from 2016 to 2022 in seven administrative regions of Côte d'Ivoire: Tchologo, Poro and Bagoué (North) Folon, Folon, Kabadougou (Northwest) Bafing and Tonpki (West). Côte d'Ivoire is a West African country and lies along the Gulf of Guinea between longitudes 2°30' and 8°30' West and latitudes 4°30' and 10°30' North [18]. The seven northern and northwestern regions of the study area are situated at the interface of the Sahelian and tropical zones of West Africa. This area features savannah vegetation characterized by trees, grasses and hardy shrubs, with gallery forests lining the watercourses. The main agricultural activities in

this savannah region are farming (food crops, vegetables, sugar cane, cotton, etc.) and livestock rearing (cattle, small ruminants, poultry among others) [19, 20].

The western regions (Bafing and Tonpki) are a cosmopolitan zone, forming a transition between the forest vegetation of southern Côte d'Ivoire and the savannah vegetation of north-western Côte d'Ivoire. Food and cash crops (cocoa, coffee, oil palm, etc.) and livestock are the main agricultural activities in these regions (Bafing and Tonpki). These agropastoral activities are complemented by transhumant livestock from neighbouring countries in the Sahel zone to the north of Côte d'Ivoire (Mali and Burkina Faso) and the west (Guinea) in search of natural pastoral resources and livestock markets [21, 22].

2.2. Study conduct and data collection

Sampling was carried out in three to eight villages/farms depending on the department size. The following criteria were taken into consideration when choosing them: (a) the distance between the villages to cover the department, (b) the existence of cattle in the farm, (c) farm's accessibility; and (d) consent of the local population to participate in the study. The data were collected using a survey conducted twice. The first, a presurvey, enabled selection of farms and elaborating the questionnaire. The survey questionnaire focused on characterizing the production parameters of the animal breeding system (breeder profile, feed management and technical assistance etc) and the management of the veterinary products and its residues on the farms (products used, sources of supply, management of veterinary products and their residues and problems encountered etc). The second consisted of interviewing breeders selected for the need of data collection.

2.3. Statistical analysis

To describe the different farm system parameters and animal health control as well as the veterinary products residues management, data collected were subjected to elementary descriptive analysis (mean, standard deviation and frequency etc.) and to the Pearson Chi (χ^2) comparison test. An appropriate software was used for the various analyses.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Sociodemographic profile of the breeders

Cattle breeders (n=152) aged 5–67 years old were interviewed with 42.76% being married, native Ivorian citizens, and 57.24% are non-natives of Burkinabe, Malian and Guinean nationality. Most of the respondents did not have formal education or training on animal husbandry techniques. The livestock sizes varied between 20 and 150 cattle. Of the 152 farms, 41 were sedentary and belong either to private owners or to community owners. The sedentary farms raise livestock as a secondary activity and have employees to manage the farms. The hundred and eleven other farmers practice transhumance system and they come from Burkina Faso, Mali and Guinea.

3.1.2 Characteristics of farming systems

The cattle breeding system in the seven regions (Poro, Tchologo, Bagoué, Kabadougou, Folon, Bafing and Tonpki) is the traditional type (extensive and transhumance) depending on natural pasture occasionally supplemented with agricultural and/or agro-industrial products and coproducts. The study showed that transhumant breeding belongs exclusively to breeders of mostly Peul ethnic groups from neighbouring countries to the north of the Côte d'Ivoire (Burkina Faso, Mali and Guinea). These breeders leave the Sahelian regions of their country for the northern regions of the Côte d'Ivoire during the dry season to look for livestock markets and/or pasture and water. Once in Côte d'Ivoire, these transhumant breeders spend years moving from one area to another or settle in a region. The sedentary breeders practice an extensive system. The sedentary farms, mainly community farms, are managed by dedicated employees and livestock keeping is a side activity. The animal health monitoring on cattle breeding of the seven studied regions is very complex. It is handled by both breeders and by veterinarians and/or private entities whose skills in animal health are not proven. This health monitoring can be distinguished in two ways (Fig. 1).

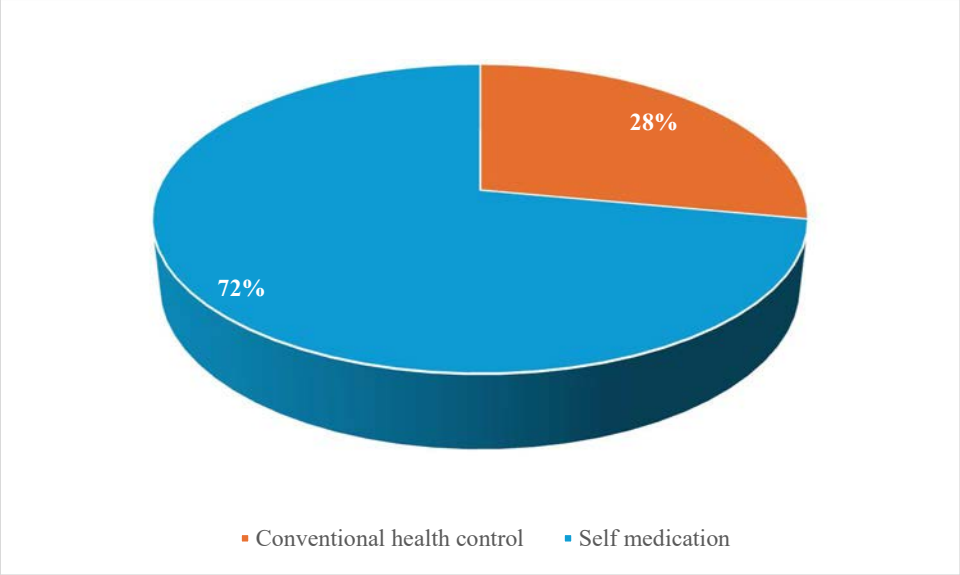


FIG. 1. Animal health control approaches observed in 7 regions studied.

Nonprofessional use of medication is the most predominant (72%) and involves local know-how based on empirical knowledge, mixtures of natural products and other chemical products and is more common in localities bordering Burkina Faso, Mali and Guinea. For tick control, the breeders use a variety of products such as: aqueous and hydroalcoholic extracts of local plants (*Zanthoxylum anthologies* bark, *Zanthoxylum gilletii* leaves); insecticide and herbicide used in cotton cultivation (CYPALM 186 (profenofos 150g/l and cypermethrine 36g/l) CYPALM 336 (profenofos 300g/l and cypermethrine 36g/l) TALSTAR 100 (Bifentrine 100g/l); and chemical products used to control termite (XILIX 1000 insecticide/anti-termites; XILIX GEL CURATIF insecticide/anti-termites).

For trypanosomoses control, the breeders use mainly antimicrobial capsules sold on the market without a veterinarian's guidance. The conventional animal health monitoring practice consists of interventions by the Ivorian government through services of the Ministry in charge of animal production which includes vaccination campaigns and treatments for animal or zoonotic diseases declared endemic and on the other hand the use of synthetic acaricides and trypanocides for ticks and trypanosomoses control, respectively. The study showed that in conventional control, veterinary prescriptions are not followed rigorously.

3.1.3 Veterinary products used on farms

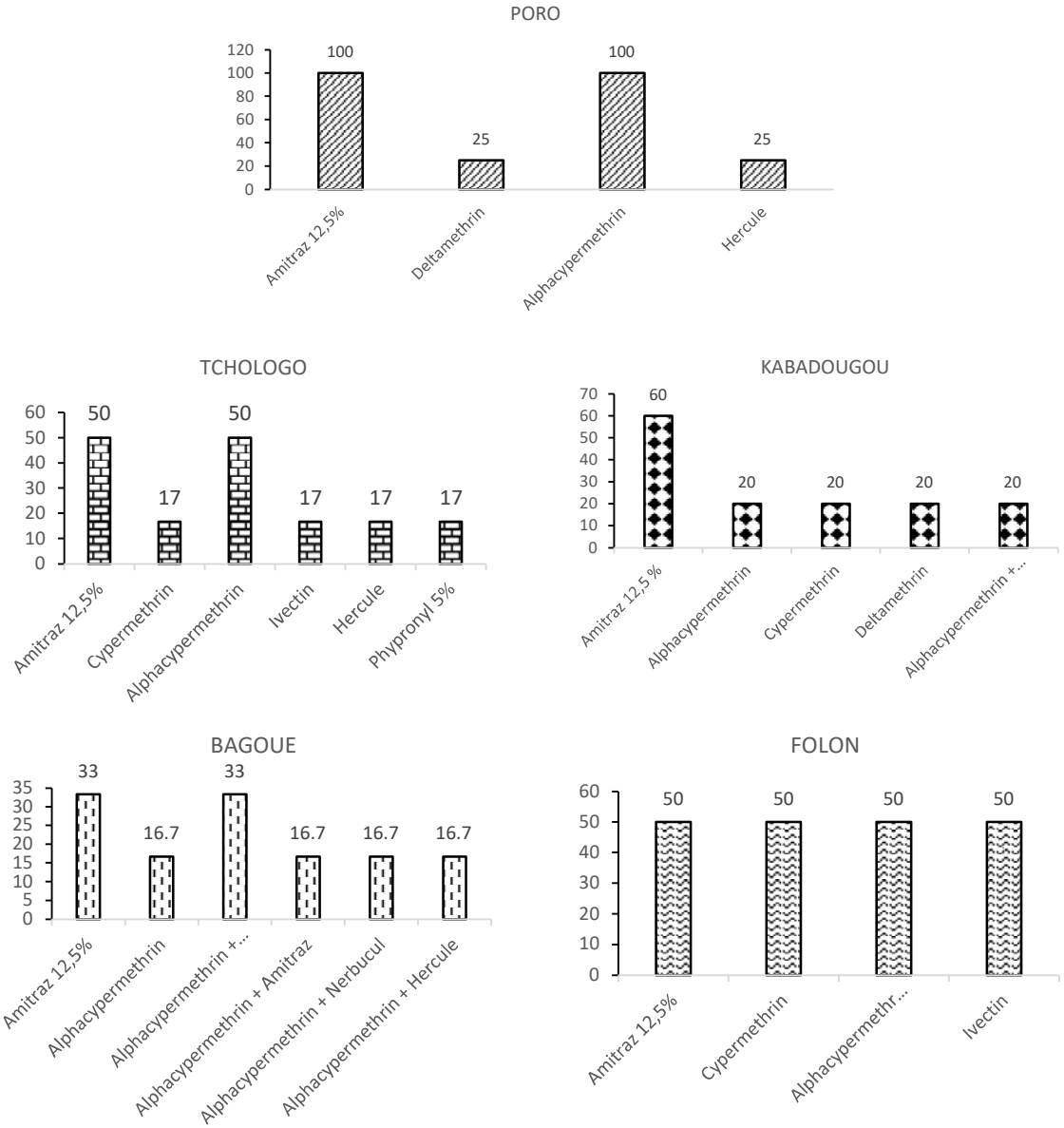
The veterinary products used in tick control and their frequency of use in the seven investigated regions showed that amitraz 12.5% and alphacypermethrin were the most used on the surveyed farms. Amitraz 12.5% was used by all breeders interviewed, whereas alphacypermethrin was used on 70% of the farms. However, alphamethrin + hercule, alphacypermethrin + nerbucul, alphacypermethrin + amitraz and fipronil 5% are the least used on farms, with a frequency of 5%.

3.1.5 Veterinary products: source supply, waste management and risks of contamination

Based on the farmers' opinions, there are two sources of veterinary products. The first source is accredited and handled by Ivorian Veterinary Services Department (DSV) through the private veterinary practices or the veterinary product depots managed by accredited veterinarians. The second source is informal, including drug depots, stores and black markets. This source is managed by non-professionals primarily the transhumant breeders from neighbouring countries who are not approved by the Ivorian authorities. In all farms investigated, the study observed that inadequate storage conditions of veterinary products and other products used. Veterinary products are stored in bags or boxes or left in the open air disregarding the storage conditions recommended by the manufacturer. Also, the breeders handle these veterinary products without safety measures. The residues of these products are thrown away in household waste or lie around on the ground. The study also revealed the absence of methods/techniques for disposing of used products, which always remains in contact with animals, breeders and the soil.

Farmers indicated that they are aware of the risks associated with the use and management of veterinary products and their waste on their farms. They dermal conditions and other discomforts they encountered after

using certain chemicals. However, they considered these as occupation hazards for which they had no solutions and alternatives. Figure 2 shows the frequencies of synthetic acaricides used in the seven investigated regions. With exception of the Folon region, the use of synthetic acaricides varies in terms of frequencies within each region and from a region to another. For trypanosomoses control, the principal trypanocides used on seven regions of study as well as their active substance are shown in Table 1.



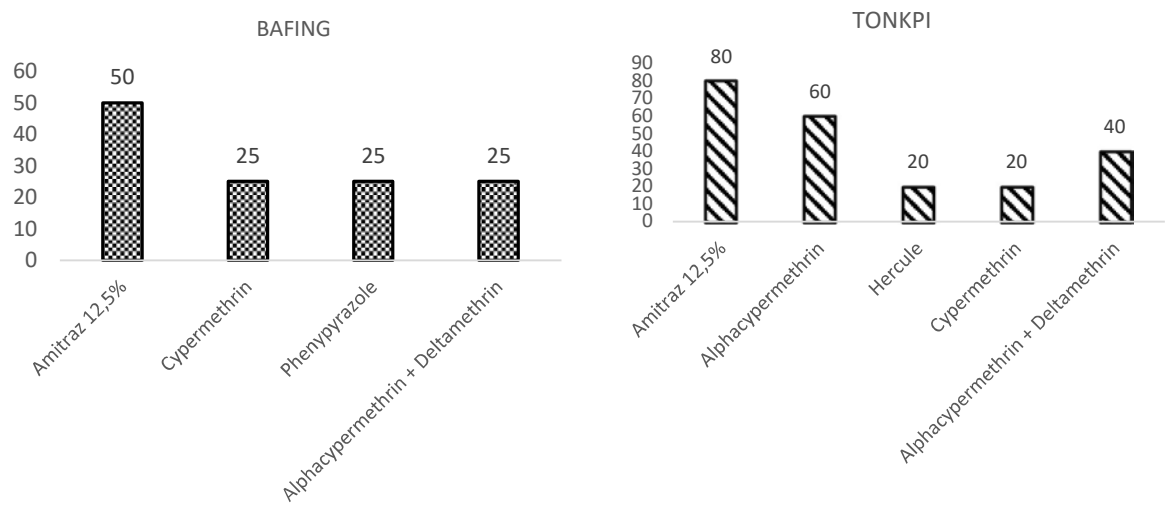


FIG. 2. Synthetic acaricides and their distribution in the seven investigated regions

TABLE 1. SUMMARY OF PRINCIPAL TRYPANOCIDES USE IN CATTLE BREEDING SYSTEM

Name of drug	Active substance
Diminazen	Diminazen aceturate
Isometamidium	Isometamidium
Trypamidium-samorin	Trypamidium
Veriben	Diminazene aceturate

The most widely used drug was trypamidium-samorin with a percentage of 73.69 % (112 farms).

3.2 Discussion

The study showed that veterinary products are used in a traditional breeding system by breeders without qualification in breeding techniques. This would explain the risk of contamination in the farms from the North, Northwest and West regions of Côte d'Ivoire. In addition, the traditional farmers investigated in the seven regions studied, do not have the technical skills to apply the instructions of technical agents supervising livestock farming, on the use the veterinary products or understand Ivorian animals' health legislation [14–16]. This is partly due to language-barriers. Nonprofessional/unsupervised medication is thus predominant.

Besides the breeders' lack of technical skills, the traditional system hinders animal health control on farms. The traditional system does not facilitate the control of animal mobility and handling, nor the rigorous monitoring of sanitary and medical prophylaxis. With the transboundary transhumance, the situation is exacerbated by the possibility of transporting diseases from one area to another, and therefore the reinfections affecting herds. Such a livestock breeding system leads to therapeutic errors, the consequence of which is the development of chemoresistance in pathogens [10, 23–25]. Farmers resort to alternative chemical products and local know-how. Although farmers affirm the success of these practices, their undesirable or toxic effects on the animal and on consumers are still poorly understood.

The present study shows the absence of good practices in the use of veterinary products and the poor management of their residues on livestock farms in the North, North-West and West regions of Côte d'Ivoire. This can be explained by several hypotheses. Firstly, the poor distribution of veterinary services throughout the country, results in limited availability of veterinary services in certain livestock areas [14, 16]. In addition, the intervention of government technical support services is limited to vaccination campaigns and cases of confirmed epidemics. This is the consequence of insufficient technical support and communication/awareness–raising among livestock farmers about livestock and animal health legislation in Côte d'Ivoire. Administrative challenges also exist.

The procedure for attaining accreditation for the sale of veterinary products is poorly understood and difficult for certain business operators to access. The drug market is dominated by a small number of individuals who sell these products at exorbitant prices and often cover a small area of operation. Informal operators obtain their supplies as best they can through the smuggling trade. As a result, the market is full of fake veterinary products [10, 20]. The mismanagement of chemicals including their supplies could affect breeders (such as skin effects) after the using of certain veterinary products or resistance against drugs with significant consequences [26, 27]. Improper handling and disposal of chemicals can also microfauna, microflora and water [10].

4. CONCLUSIONS

Livestock farming in the North, North-West and West regions of Côte d'Ivoire is a traditional system dominated by transhumance farmers with no technical skills in animal husbandry. This leads to poor use of veterinary products and a lack of efficient management of their residues in farm products. The consequence of this situation is the contamination risks in livestock production systems which compromises animal and farmers health in livestock systems. For this reason, the present study suggests the need to initiate a project to control the risks of chemotherapy-related contamination, integrating the One Health approach in agricultural production systems; review the breeding system which will facilitate the animal health control; to strengthen the technical assistance system for livestock farms; train and strengthen the technical capacity of farmers; and strengthen communication/awareness-raising systems for livestock farmers on legislation about livestock farming.

ACKNOWLEDGEMENT

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ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* SPP ISOLATED FROM POULTRY SAMPLES IN MOROCCO

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Abstract

The present work was conducted to understand the antimicrobial susceptibility of *Salmonella* spp in various poultry samples (caecum and meat) harvested from slaughterhouses and traditional markets in Rabat-Sale Kenitra region, Morocco. Out of 207 poultry samples, 31% were positive with 35% found in caecum samples and 33% in meat samples. The finding highlighted a significantly higher occurrence of *Salmonella* spp in meat samples harvested from traditional markets (38%) than in slaughterhouses (24%). The results of the antimicrobial susceptibility testing indicated that all tested strains exhibited a high resistance toward nalidixic acid (91%) ciprofloxacin (90%) tetracycline (78%) and ampicillin (44%). Others were trimethoprim (28%) chloramphenicol (10%) colistin (7%) gentamicin (7%) amikacin (6%) meropenem (3%) tigecycline (3%) ceftazidime (1%) and cefotaxime (0%). Most isolates (97%) were resistant to at least one antimicrobial and 41% were resistant to three or more major antimicrobials. This study has opened avenues for future research, including the investigation of serotyping, genotyping for resistance genes, and the identification of virulence-genes. To enhance the assessment process, also pioneered the use of Stable Isotope Labelling by Amino Acids in Cell Culture (SILAC) in combination with Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF) to enable rapid differentiation between sensitive and resistant strains using $^{13}C_6$-$^{15}N_2$-labeled amino acids.

1. INTRODUCTION

Salmonella spp., from the family *Enterobacteriaceae*, are facultatively anaerobic gram-negative associated with numerous foodborne outbreaks [1]. Salmonellosis, a significant foodborne illness, poses a major public health concern worldwide with over 20 million reported cases and 150,000 deaths annually [2]. Various animal species such as poultry and their products serve as the primary sources and reservoirs of these bacteria [2]. Infectious disease control in poultry farms often includes use of antimicrobials whose imprudent application increases the chances of antimicrobial resistance [3]. The study aimed to investigate the antimicrobial resistance of *Salmonella* spp., isolated from poultry samples collected from traditional markets and slaughterhouses in the Rabat Sale Kenitra region, Morocco.

2. MATERIALS AND METHODS

2.1. Bacteriological analysis

Poultry samples (207) were collected from traditional markets, and slaughterhouses in Rabat Sale Kenitra region, Morocco from January to September 2023 (Table 1).

TABLE 1. NATURE OF SAMPLES AND THEIR ORIGIN

Origin	Nature of samples		
Slaughterhouse	Turkey meat (36)	chicken meat (26)	caecum (69)
Traditional market	Turkey meat (38)	chicken meat (38)	

The investigation of *Salmonella* spp was conducted according to ISO 6579-1:2017 [4] and confirmed using a MALDI ToF by placing colonies into a disposable target plate and then coated with 0.1 µl of a matrix solution containing α-cyano-4-hydroxycinnamic acid. The prepared target plate was then inserted into the MALDI-ToF

mass spectrometer, and the acquired peptidic spectra were compared with the extensive MALDI Biotyper library capable of identifying over 2900 species. The comparison was facilitated using an appropriate software.

2.2 Determination of the antimicrobial minimum inhibitory concentration

All Salmonella strains isolated were tested for antimicrobial susceptibility toward thirteen antimicrobials using EUVSEC3 Sensititre plates. A suspension of inoculum (10 µl) adjusted to 0.5 McFarland was added to a cation-adjusted Mueller Hinton broth. Then 50 µl of the suspension was dispensed into each well of a 96 well microplate using an automated dispenser. The microplate was then sealed and incubated at 34–36°C for 18 hours.

3. RESULTS AND DISCUSSION

3.1. Occurrence of *Salmonella* spp., in poultry samples

This study revealed a 31% occurrence of *Salmonella* spp. in poultry samples, with 35% found in caecum samples and 33% in meat samples confirmed by MALDI-ToF MS analysis using peptidic spectra. The contamination rate in this study was similar to findings in other countries such as Algeria (34%) [5] Bangladesh (25%) [6] and Nigeria (25%) [7] but lower than rates in a 2016 study of laying farm hens in the same region in Morocco (50%) [8] and in Spain (73.2%) Portugal (79.5%) and Poland (77.2%) [8].

The findings revealed that *Salmonella* spp., contamination rates were significantly higher in turkey meat, reaching 28% in slaughterhouses and 53% in traditional markets. In contrast, chicken meat exhibited lower contamination rates of 19% in slaughterhouse and 24% in traditional market samples. There was a disparity in the presence of *Salmonella* spp., among meat samples obtained from traditional markets (38%) and slaughterhouses (24%). It is possible that poultry slaughterhouses adhere to hygiene measures that prevent carcass contamination unlike the markets.

3.2. Antimicrobial susceptibility testing

Out of 68 isolates, 91% exhibited resistance nalidixic acid and 90% to ciprofloxacin. Resistance to quinolones or fluoroquinolones typically occurs due to disruptions in bacterial DNA metabolism due to mutations in bacterial DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) genes, as well as active efflux mechanisms. These mutations prevent antimicrobial agents from binding to their intended topoisomerase targets, inhibiting their antimicrobial effects [9]. This resistance rate aligns with a study conducted in Brazil, where the resistance rate for nalidixic acid (NAL) was 95% [10]. However, a study conducted on laying farm hens in Morocco [8] showed relatively low resistance rates to nalidixic acid (61%) and ciprofloxacin (CIP, 25%). The resistance to tetracycline (TET) was notably high (78%) as reported elsewhere [11] and higher than in a previous Moroccan study [8].

The mechanism of this resistance is most commonly associated with active efflux. Among numerous genetic determinants, encoding efflux pumps tetA, tetB, tetC, tetD, tetE, and tetG are predominately reported [12]. As demonstrated in Fig. 1, resistance to ampicillin was at 44% but generally low or absent for others: trimethoprim (TMP, 28%) chloramphenicol (CHL, 10%) colistin (7%) gentamicin (GEN, 7%) amikacin (EMI, 6%) meropenem (MERO, 3%) tigecycline (TGC, 3%) ceftazidime (CAZ, 1%) and cefotaxime (CTX, 0%).

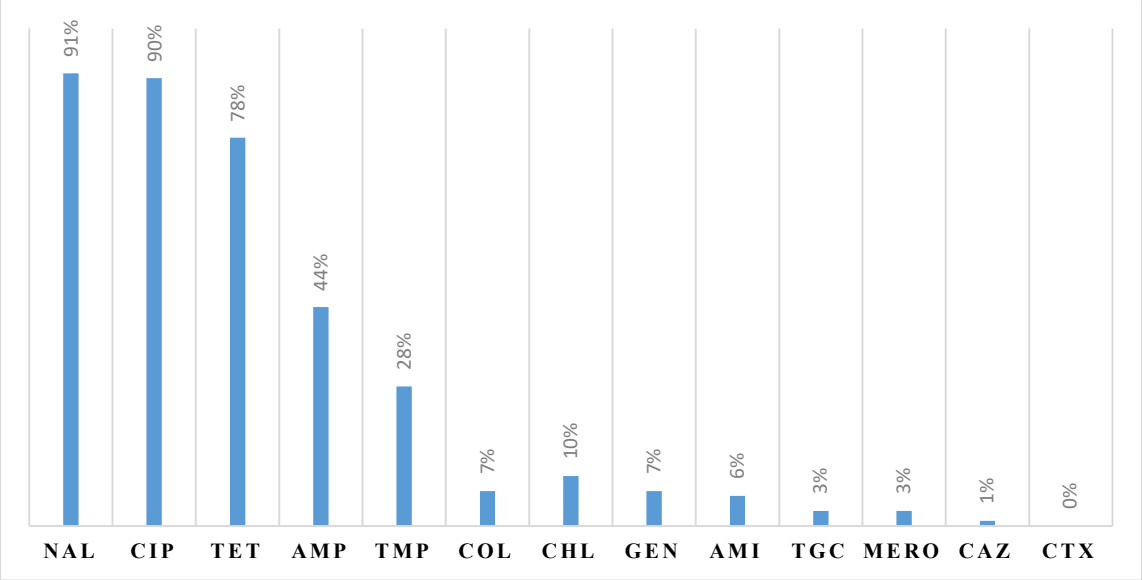

FIG .1. Antimicrobial resistance profile of Salmonella spp., isolated.

Nearly 97% of the isolates were resistant to at least one antimicrobial, and 3% showed no resistance; 41% are resistant to three or more major antimicrobial families such as fluoroquinolones, tetracycline and penicillin.

4. CONCLUSIONS

This study found high rates of antimicrobial resistance to ciprofloxacin and nalidixic acid. These findings are crucial for doctors as they can guide them in the selection of appropriate treatments, thereby preventing therapeutic failure and contributing to better infection management. Serotyping of *Salmonella* isolates and associated genotyping to identify resistance genes, along with screening for virulence genes, would be valuable for advancing understanding of the mechanisms underlying antimicrobial resistance and the virulence factors associated with these bacteria. Furthermore, the application of SILAC combined with MALDI-ToF Mass Spectrometry offers a rapid means of distinguishing between sensitive and resistant strains using $^{13}C_6$-$^{15}N_2$-labelled amino acids.

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SCREENING OF OXYTETRACYCLINE RESIDUES IN ANIMAL PRODUCTS COLLECTED FROM SOME STATES IN SUDAN

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Abstract

The presence of oxytetracycline (OTC) residues in food of animal origin in parts of Sudan has been investigated. Muscle, milk, liver and kidney samples of camels, cattle, goats and sheep were collected from slaughterhouses and farms in 2021 covering three States of Khartoum, El Gezira and North Darfur. Analysis was carried out using HPLC with a UV/VIS detector with OTC detected in 45.1% of the tested samples, El Gezira State showed the highest contamination percentage. However, percentages of 75%, 66.7% and 39% were found in Khartoum State for kidney, muscle and liver respectively, in El Gezira 82.1%, 79.7% and 57.4% were reported for muscle, liver and kidney, in North Darfur the presence of OTC residue were higher in muscles 90% than liver 36.4% and milk 16.56%. Nearly 39% of the samples were above the maximum recommended residue level, 75% in Khartoum with 100% of muscles, 77.8% of liver and 66.7% of kidney. For El Gezira, 20.2% of the samples were above the maximum residue level (MRL) with 45.5% of muscles 18.2% of liver and 3.7% of kidney; in North Darfur 67.5% were over MRL with 75% of the liver and 66.67% of the muscles and milk. The study shows wide use of OTC as antimicrobial of choice and highlights the need for an effective monitoring and awareness programme.

1. INTRODUCTION

Globally the demand for food is increasing and thus the livestock and agricultural sectors need to be expanded. The use of veterinary drugs in the animal sector is very important for disease control. Antimicrobials have been widely used in animal production as therapeutic agents, growth promoters and to maximize economic returns [1, 2]. Some of the commonest antimicrobials used in veterinary medicine are beta-lactams, macrolides, aminoglycosides and tetracyclines [3]. Animal sectors consume more than half of the antimicrobial drugs marketed in the USA, with tetracyclines accounting for 70% [4]. Oxytetracycline is widely used in the animal sector for therapeutic, prophylactic or growth promoting purposes [5]. Abdul Sajid et al., [6] reported the presence of tetracycline, ampicillin, streptomycin and aminoglycosides residues in poultry meat. Many farm workers mention the wide uses of OTC as a drug of choice in animal sector in Sudan [7, 8]. This extensive and uncontrolled usage can lead to residues in animal products, and the consumption of contaminated food may lead to adverse effects to the consumers health that include carcinogenicity, mutagenicity, bone marrow toxicity, allergy [9] and presence of resistant pathogens [10].

To protect human health, MRLs of pharmacologically active substances in foodstuffs of animal origin have been established such as by Codex [11] with JECFA's support [12] set the MRL for OTC in meat, liver and kidney to be 200 µg/kg, 600 µg/kg and 1200 µg/kg, respectively. Many sensitive methods were optimized and validated for the detection and determination of different antimicrobial residues in food and food products such as microbial tests, ELISA, biosensor- and chromatography-based techniques [2, 13]. Existing data on the residual levels of OTC in various types of foods are scarce in Sudan. Most of the research already done had employed the inhibition test for antimicrobial residues detection [1, 7, 14–16].

This study aimed at monitoring OTC residues in milk and animal tissues (muscle, liver and kidney) collected from markets, farms and slaughterhouses in Sudan by HPLC) using a simple and cost-effective extraction method. The study also aimed to introduce routine application of chromatographic techniques in the detection of residues in food matrices in the country.

2. MATERIALS AND METHODS

2.1. Study area and samples

Three States — Khartoum, El Gezira and North Darfur in Sudan — were covered. The 366 samples were collected in 2021 as follows: Khartoum: 3 muscle, 23 liver, and 12 kidney samples from cattle; El Gezira: 28 muscle, 69 liver, and 47 kidney samples from cattle and sheep; and North Darfur: 10 muscle, 11 liver, and 163 milk samples from cattle, camel, sheep and goat. These were collected from markets, slaughterhouses and dairy farms in sterile containers stored in ice boxes and were transported under refrigerator condition to the Central

Veterinary Research Laboratory of the Department of Radioisotopes and Immunology, where they were then kept at -20°C until analysis.

2.2. Chemicals and reagents

Oxytetracycline (OTC) HCl, methanol and acetonitrile (both HPLC grade) and nitric acid were used along with 0.45 µm nylon filters. Other items included oxalic acid dehydrate and ultrapure water.

2.3. Stock solutions and working standard

Stock of standard was prepared in methanol at 1 mg/ml after correcting for purity. Six different concentration levels of working standard solutions (50 µg/l, 100 µg/l, 200 µg/l, 400 µg/l. 800 µg/l and 1000 µg/l) were freshly prepared by diluting volumes of the stock standard solution in 5 ml volumetric flasks with methanol to the mark.

2.4. HPLC analysis

The presence of OTC residues was detected and quantified by HPLC as described by Hamide et al., [17]. The system consisted of a gradient pump, UV-Vis detector, a vacuum degasser, a column compartment oven, manual injector (20 µl loop) power stream module. A C18 (5 µm, 150×4 mm) analytical column was used with an isocratic separation achieved using a mobile phase consisting of oxalic acid solution 0.1M (75%) methanol (5%) acetonitrile (20%) with flow rate of 1 ml/min, wavelength 360 nm, column oven temperature of 30°C.

2.5. Sample preparation and extraction procedure; statistical analysis

The samples were homogenized in a blender. This included (2 ± 0.01) g of cured tissue (muscle, liver or kidney) or (2 ± 0.01) ml of milk placed in a 50 ml falcon tube. After adding 0.1 g citric acid, 1 ml nitric acid 30%, 4 ml methanol and 1 ml deionized water, the mixture was then vortexed. The content was, kept in an ultrasonic bath for 15 min, and centrifuged for 10 min at 5300 rpm. The supernatant was filtered through a 0.45 µm nylon filter and 20 µl of the solution was injected into the HPLC and analysed. Descriptive methods were performed using the Statistical Package for Social Scientists software version 20.

3. RESULTS AND DISCUSSION

A small number of studies using chromatographic techniques to measure drug residues in food have been reported in Sudan. Oxytetracycline is the veterinary drug most used in animal farms in Sudan. The present study examined OTC residues in samples of animal origin and revealed that 45.1% of the tested samples had OTC residues (Fig. 1) in percentages of 72.7%, 65.8% and 21.7% in El Gezira, Khartoum and North Darfur, respectively. These findings are higher than those reported in poultry tissues in Khartoum State [14] and consistent with other studies in sheep [7] and poultry [15, 16].

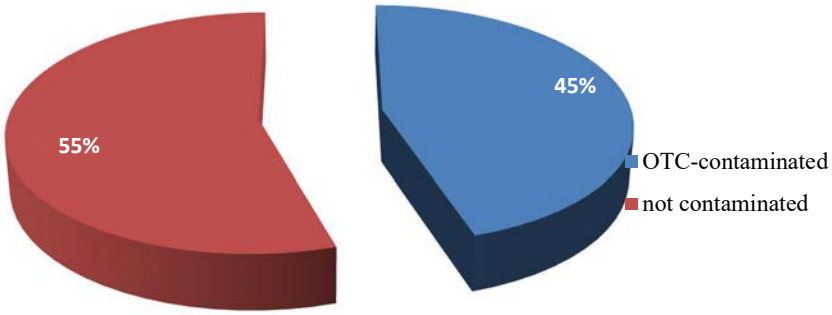

FIG. 1. Overall percentages of samples with oxytetracycline residues.

The muscles showed positive OTC percentages of over 50% in all states with range between 1195–2296.0 µg/kg (ppb) in Khartoum, 15.15–29574 ppb in El Gezira and 128–7643 ppb in North Darfur. The percentages in liver were 79.7, 39 and 36.4 in El Gezira, Khartoum and North Darfur, respectively, and ranges were 444–

8553 ppb in Khartoum, 38–20714 ppb in El Gezira and 450–1488 ppb in north Darfur. The frequency of the residues in kidney was 75% and 57.4% in Khartoum and El Gezira States and in the range 458–2466.6 ppb in Khartoum and 124–1575 ppb in El Gezira.

Milk samples collected from North Darfur State revealed 16.6% of non-compliance. The frequency in cows was 21%, with levels in the range 215–720 ppb; camel 10.9%, range of 39.074–215.838 ppb; sheep 28.6%, and range of 24–216 ppb; and goat frequency of 8.8%, range 58.6–110 ppb. Higher percentages of the residues in the three States (Table 1) were consistent with findings reported by Tasneem et al., [16] who also found no significant difference between tested tissues. Other studies in sheep tissues [7] reported significantly higher prevalence of OTC residues in sheep muscle samples compared to liver, while higher amounts in liver and kidney samples were reported in broilers [18]. Moreno and Lanusse [19] reported higher levels of antimicrobial residues in animal offal (liver, kidney and fat) than in muscles. These may be attributed to the general lack of awareness among food animal producers on the correct way of using veterinary drugs [8] including failure to observe recommended withdrawal periods [20].

TABLE 1. PERCENTAGES AND RANGE OF OXYTETRACYCLINE RESIDUE LEVELS BY SPECIES, MATRIX IN THE THREE STATES

State	Species	Sample type and No	%	Range (ppb)
Khartoum	Cattle	Muscle (*n*=3)	66.7	1195.0–2296.0
		Liver (*n*=23)	39	444–8553
		Kidney (*n*=12)	75	458–2467
El Gezira	Cattle	Muscle (*n*=11)	54.5	20.2–29574
		Liver (*n*=11)	100	511.8–20713.85
		Kidney (*n*=11)	82	345– 808
	Sheep	Muscle (*n*=18)	94	15.2–246
		Liver (*n*=58)	76	38–605
		Kidney (*n*=36)	50	124–1575
North Darfur	Cattle	Muscle (*n*=10)	90	128–7643
		Liver (*n*=11)	36.4	450–1488
		Milk (*n*=62)	21	215–720
	Camel	Milk (*n*=46)	11	39–216
	Sheep	Milk (*n*=21)	28.6	24–216
	Goat	Milk (*n*=34)	8.8	58.6–110

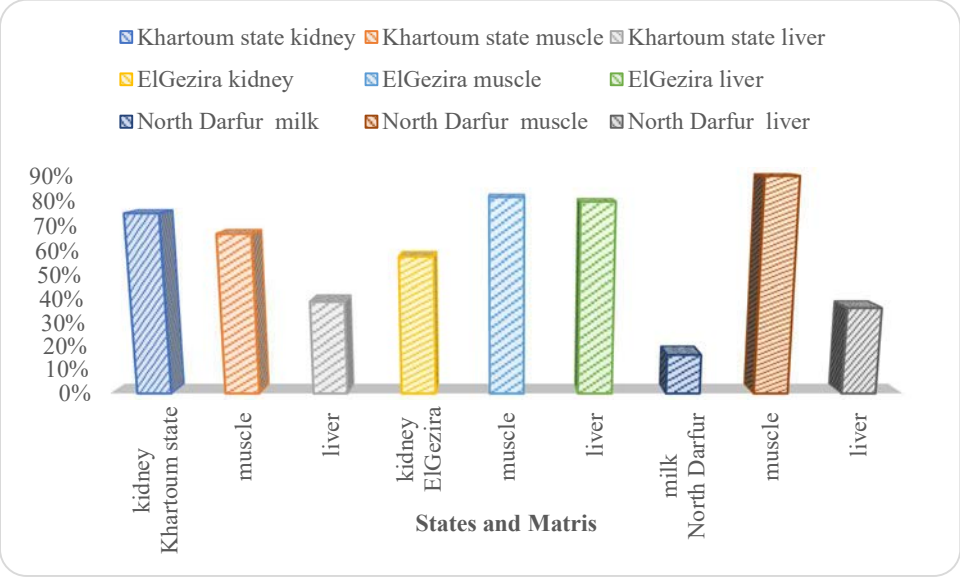

FIG.3. OTC-positive samples in Khartoum, El Gezira and North Darfur States.

Figure 3 shows that 38.7% of the tested samples exceeded the respective Codex MRLs [21]. In Khartoum State, 75% of the results were not compliant (100% of muscle, 77.8% of liver and 66.7% of kidney samples). In El Gezira State, 20.2% of the samples had OTC residues over the MRL (45.5%, 18.2% and 3.7% of the muscle,

liver and kidney samples, respectively) while, in North Darfur State 67.5% of the samples tested exceeded the MRL, with 75% of the liver and 66.7% of the muscle and milk samples considered as non-compliant (Fig. 4 and Table 2). Similar findings were reported by Tasneem et al., [16] when testing poultry tissues for OTC residues in Khartoum State. However, Ahmed et al., [7] in his study on sheep, found that 28.6% of the samples had detectable OTC levels above MRL. The reported results demonstrate higher values as compared to those reported in Nigeria [22], Thailand [23] and Canada [24] indicating a widespread misuse of veterinary drugs by food animal producers in the country.

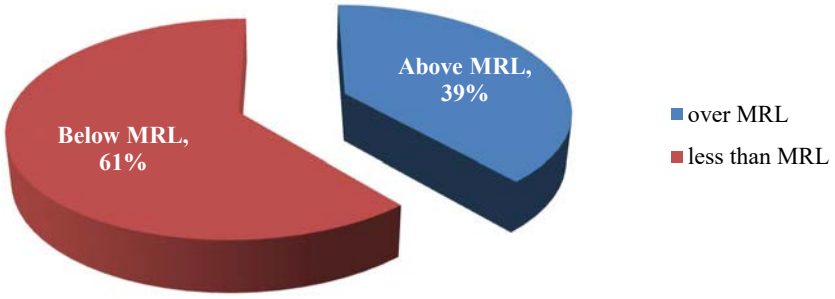

FIG.4. Percentages of samples where levels exceeded MRL for OTC.

The levels of residues above acceptable levels are shown in Fig. 5.

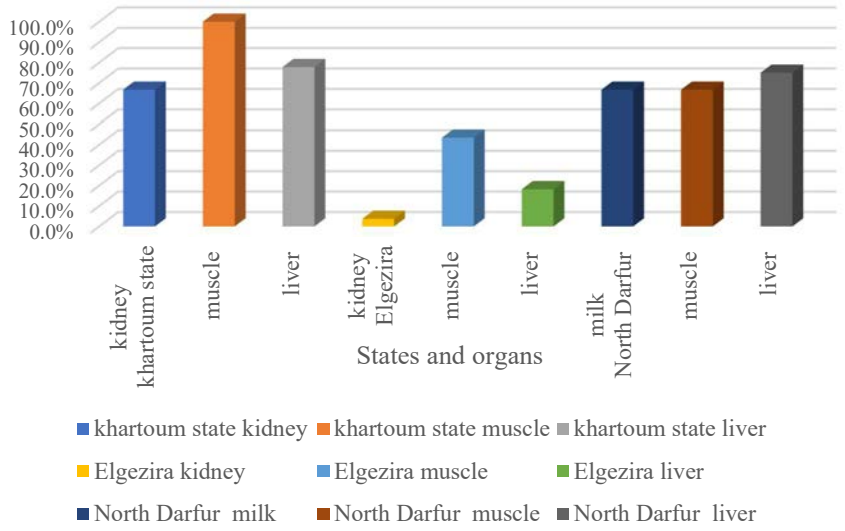

FIG.5. Percentages of non-compliant samples in Khartoum, El Gezira and North Darfur States in Sudan.

TABLE 2. PERCENTAGES AND NUMBER OF NON-COMPLIANT SAMPLES BY SPECIES AND MATRIX IN THE THREE STATES

		Cattle	Sheep	Goat	Camel
Khartoum	Muscle	100% (2/2)	—	—	—
	Liver	77.8% (7/9)	—	—	—
	Kidney	66.7% (6/9)	—	—	—
El Gezira	Muscle	83.3% (5/6)	29.4% (5/17)	—	—
	Liver	81.2% (9/11)	2.3% (1/44)	—	—
	Kidney	0% (0/9)	5.6% (1/18)	—	—
North Darfur	Muscle	66.7% (6/9)	—	—	—
	Liver	75% (3/4)	—	—	—
	Milk	84.6% (11/13)	50% (3/6)	33.3% (1/3)	60% (3/5)
Total		68.1% (49/72)	11.8% (10/85)	33.3% (1/3)	60% (3/5)

Antimicrobial abuse in animal farms is the most important cause of the high prevalence of residues in food of animal origin. Risks from consuming contaminated food could be carcinogenicity, allergenicity, alteration of the intestinal flora and antimicrobial resistance among various other toxic effects, [15, 25, 26]. Poor practices in rearing and treating animals together with the absence of proper legislation to monitor and control the production system compromise food safety.

4. CONCLUSIONS

The presence of OTC residues has been reported in a range of animal products collected from different parts of Sudan following use of a UHPLC. These included kidney, liver, milk and muscle samples from camels, cattle, goats and sheep. A large number of these matrices/drugs exceeded the recommended MRL. Further investigations and introduction of modern sensitive techniques to cover other antimicrobial categories and states throughout the country is needed. To ensure safety of animal-based food products in the country there is an urgent need for the introduction of effective and reliable monitoring programmes and control systems at different production levels.

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ANIMAL PRODUCTION AND DRUG-USAGE PRACTICES IN SUDAN

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Abstract

A study was conducted to gather basic information about the management system (practices in rearing and treatment) applied by animal owners in parts of Sudan. Questionnaire (*n*=500) were administered among respondents in three States of Khartoum, White Nile and North Darfur. Most of the respondents had small or medium size farms and used open rearing systems. The information collected revealed that about two thirds of the owners did not keep production records. They also indicated that they did not use growth promoters or additives. Almost half of the responses agree with the availability of veterinary services in the three regions but two third considered them too expensive. Thus, most of the owners treat their animals by themselves. Most farmers (84%) in North Darfur keep the medicine in a suitable place compared to 42% in the White Nile and 16% in Khartoum State. Commitment to prescriptions were in the order 89.7% in Khartoum State, 57.7% in North Darfur and 28.7% in the White Nile State. Observation of the withdrawal period varied from 49.7% in Khartoum to 36.6% in the White Nile and 16% in North Darfur.

1. INTRODUCTION

Animal resources in Sudan contribute to food security and act as store of value and wealth and means of access to power in rural areas and represent 47% of official agricultural exports [1, 2]. The use of drugs in animal production is unavoidable. A great deal of concern has been demonstrated over the last decades about the presence of chemical residues, mainly veterinary drugs and pesticides, in the food from animal origin [2, 3]. These residues affect public health and food safety and can lead to the emergence of antimicrobial resistance [4]. Unacceptable residue levels in animal product have been associated with improper farm management practices such as failure to maintain a valid veterinarian-client-patient-relationship, inadequate treatment records, failure to identify and withhold treated animals from slaughter, or not following labelled directions including withdrawal periods, dosage, duration of treatment, and route of administration. These challenges seem most common in developing countries [3, 5]. Nevertheless, in Sudan, adequate information is lacking hence this study where a questionnaire was designed to provide realistic data from Khartoum, North Darfur and White Nile on animal owner practices, knowledge in rearing their animals and the use of veterinary drugs and pesticides.

2. MATERIALS AND METHOD

2.1. Study design: questionnaire and data analysis

Questionnaires (*n*=500) were administered to respondents including animal owners and veterinarians in three States in the Sudan namely Khartoum (*n*=207) North Darfur (*n*=143) and White Nile (*n*=150). The questionnaire was designed to collect basic information about management practices among the animal owners and to evaluate their knowledge about availability of veterinary services, drug-usage, availability, storage, cost, withdrawal period as well as practices in treating animals and knowledge of the risks posed by unsafe animal products. The questionnaires were completed by direct interviewing of farm owners. Descriptive statistical analysis was performed.

3. RESULTS AND DISCUSSION

Management practices and good relationship between food producers (animal owners) and veterinarians is one of the most important aspects in minimizing the risk of residues and contaminants in food of animal origin. Most of the respondents owned small or medium size herds and operated open rearing system (Table 1) as the natural pasture is the most common feeding regime in rural area. Information collected about management practices revealed that 78.3% and 82.7% of the animal owners at Khartoum and North Darfur States, respectively, did not care about keeping records and they agreed about the use of antimicrobials for treatment and prevention. They also responded that they did not use growth promoter or additives (92.3% in Khartoum and 93.7% in North Darfur State). No information was obtained from White Nile, probably because the question was misunderstood (Table 1). Similar information was reported in previous studies [3, 4].

TABLE 1. FARM MANAGEMENT'S PRACTICES IN THREE STATES (KHARTOUM, NORTH DARFUR AND WHITE NILE)

	KHARTOUM			NORTH DARFUR			WHITE NILE		
Farm size	S	M	L	S	M	L	S	M	L
	29%	50%	21%	49.29%	19%	31.69%	47%	46%	7%
Rearing System	Open	Closed		Open	Closed		Open	Closed	
	70%	30%		67.4%	32.6%		100%	0%	
Records keeping	Yes	No		Yes	No		Yes	No	
	21.7%	78.3%		17.3%	82.7%		[a]	[a]	
Growth promoters	Yes	No		Yes	No		Yes	No	
	7.7%	92.3%		6.3%	93.7%		[a]	[a]	

Note: *S = Small (1–30 head) M = Medium (30–70 head) L = Large (more than 70).*

[a]*No information/response gathered.*

Almost half of the respondents agree with the availability of veterinary services in the three regions (Table 2). Whether these offer adequate services, is not clear since another study observed a lack of laboratory facilities for most veterinary professionals [6]. The drugs and services are too expensive as reported by about two third of the respondents (Table 2). A similar study reported that the cost is a barrier in obtaining culture and sensitivity tests to guide antimicrobial therapy [7]. Most owners thus treat their animals themselves (Table 2) as reported by other authors [1, 3, 8].

When calculating drug doses, two thirds of the owners in Khartoum and North Darfur States consider the body weight of the animals, while only one third does so in White Nile state. On storage condition of the drugs, 84% of the respondents in North Darfur kept the medicine in a suitable place compared to 42% in the White Nile and only 16% in the Khartoum State. The consideration of the withdrawal period before slaughtering treated animals or selling animal products varied from 49.7% in Khartoum, 36.6% in the White Nile and 16% in North Darfur, with 68.8% of the respondents reporting that they used animal product after one day of the treatment of the animals (Table 2). This was an indication of the limited knowledge about antimicrobial usage, withdrawal periods and the risk posed by the consumption of food containing hazards [9]. Several farmers use antimicrobials for disease prevention rather than treatment [4].

Commitment to using prescriptions was reported in Khartoum State by 89.7% of the respondents followed by 57.7% in North Darfur and 28.7% in the White Nile State (Table 2). However, other studies [5, 10] reported that veterinarians believed antimicrobials are used by producers to treat a variety of animal diseases without veterinary consultation. Another study [11] found that most farmers (86%) were not concerned that overuse or misuse of antimicrobials in animals could result in antimicrobial resistance.

TABLE 2. OWNER-RESPONSES ON TREATMENT PRACTICES IN THREE STATES

	Khartoum		North Darfur		White Nile	
	Yes	No	Yes	No	Yes	No
Availability of veterinary services	100%	0%	44%	56%	53%	47.3%
Drug Cost	100%	0%	83.7%	16.3%	68.7%	13.3%
Self-practices in treatment	79.4%	20.6%	62%	38%	60%	40%
Dose calculation	64.5%	35.5%	64.8%	35.2%	28.7%	71.3%
Storage suitability	16%	84%	82%	18%	42%	58%
Commitment with prescriptions	89.7%	10.3%	57.7%	42.3%	28.7%	71.3%
Withdrawal period consideration	49.7%	50.3%	16%	84%	36.6%	63.4%
Drug residues knowledge	58.6%	41.4%	16%	84%	60%	40%

Oxytetracycline is reported as the drug of choice by the animal owners followed by tetracycline, tylosin, penicillin and ivermectin which agrees with previous report [3, 12]. Another study found that 93% of the drugs used were procaine penicillin and oxytetracycline. It also noted that there is underdosing and inappropriate timing of drug administration [13]. Such practices contribute to the increasing trends of the antimicrobial resistance [14].

4. CONCLUSIONS

The study collected information on the practices among animal owners that could enhance unsafe food supply and the emergence of antimicrobial resistance. There was lack of knowledge about proper drug usage and withdrawal periods as well as about understanding the risk associated with the consumption of food with drug residues. Attention of policy makers and consumers among other stakeholders is needed to ensure implementation of food safety control programmes.

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OPTIMIZATION OF IN-HOUSE RECEPTORS FOR ANALYSISIS OF MULTI-RESIDUES/CONTAMINANTS BY RADIO RECEPTOR BINDING ASSAY

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Abstract

Improved in-house receptors (IHRs) were developed for mixed contaminants analysis using ^{14}C- and ^{3}H-labelled tracers. The IHRs were isolated from non-immunized poultry liver by applying different cell separation techniques. The IHRs pellet produced after serial centrifugation (at 40000g) showed a mixture of polypeptides at 20–100 kilodaltons (kDa) an important attribute for multiresidue analysis. Affinities (based on control points) of selected analytes such as chloramphenicol and aflatoxin B1, B2, M1 analysis in wheat and milk for the IHRs were found to be comparable to commercial receptors. Using wheat as a test matrix, the IHRs showed clear affinities in testing three spiked mixed chemical hazards with control points of 3645 and 2877 counts per minute (come) for aflatoxin B1 (10 ng/ml (ppb)); 8922 and 6581 come for aflatoxin B2 (10 ppb); 7998 and 7677 come for chloramphenicol (0.3 ppb) as analysed by operator 1 and operator 2 respectively. To facilitate technology transfer, the IHRs were lyophilized using starch as an excipient, talc as a softener and sodium carboxymethylcellulose (SCMC) or Hydroxymethylpropylcellulose (HPMC) as binders. Preliminary studies showed that IHR-SCMC tablet showed better performance for aflatoxin M1 analysis in milk with positive control (PC) average of 314 counts per minute (cpm) for 6 replicates. None of the responses exceeded ±20% of the PC averages (377–251 cpm). The Negative Control (NC) average was 340 cpm for 3 replicates. The control point was 377 cpm.

1. INTRODUCTION

Chemical hazards such as aflatoxins, antimicrobials, hormones, heavy metals and/or pesticide residues in food and feed are a threat to human health. Infants are considered a sensitive and vulnerable population group, because they intake more food per kilogram of body weight than adults, and their detoxification system and metabolic pathways are not fully developed [1, 2]. A study conducted using liquid chromatography high resolution mass spectrometer (LC-HRMS) determined the presence of 21 pesticides and four aflatoxins in baby food. The study noted that 68% of the samples contained at least one targeted pesticide with 10 parent compounds and one metabolite detected by suspect screening [3]. Veterinary drugs are widely used in livestock production for therapeutic, prophylaxis and in some cases for growth promotion purposes where drugs including thyreostats, anabolic hormones and β-agonists may be involved [2] though prohibited in many countries. The residues of these substances in edible animal tissues are a consumer and trade concern. Regular monitoring and control following stringent national and international regulatory requirements is needed. Many veterinary antimicrobials used in food production systems are regulated using maximum residues levels (MRLs) while a number, including chloramphenicol (CAP) as forbidden [4–5].

For the screening, quantification and confirmation, many contaminants and residues are mostly monitored individually, whereas hazards can co-exist as mixtures. Monitoring programmes for mixed food contaminants are also scarce. Although different immunochemical, chromatographic, and radiometric methods are commonly used, robust multiclass methods for analysis of mixture of contaminants and their degradation products are still very few. Some, such as the LC-MS/MS can be expensive and resource/skill-demanding thus often out of reach to some laboratories [6–10]. For strict surveillance of food contaminants on regular basis, availability of sensitive, rapid and low-cost analytical methods are mandatory to handle large sample numbers. This is important for a developing country such as Pakistan where surveillance studies have shown that about 20–25% samples of milk, bovine kidney, beef, chicken, and mutton had a higher concentration of antimicrobial residues (specifically chloramphenicol) above RPA although all fish samples were negative. Residues of CAP were detected at low levels in 11% of poultry meat [11–14].

To meet this challenge, receptor-binding assays (RBAs) are being proposed for the development of low-cost screening method for mixed food contaminants/residues in foods. The principle is based on biological receptors, which *in vivo*, can bind a variety of substances in the liver, kidney, brain, and other tissues. A major

disadvantage of this interaction is specificity since substance that are not of interest may also bind displacing bound targeted radioligand. This limitation nevertheless offers some advantageous when analysing mixed/multiple agonists [15–16]. In-house development of RBAs has been proven to be a cost-effective screening method for multiple hazards such as gentamicin, tetracycline, CAP and aflatoxins (B1, B2, M1) in food and feed [17]. The objective of the current study was to develop and optimize IHRs for mixed contaminant analysis in wheat and evaluate their performance for technology transfer using ^{3}H-labelled aflatoxins B1 and B2 and ^{14}C-labelled CAP as tracers.

2. MATERIALS AND METHODS

The liver tissue from freshly slaughtered non-immunized poultry birds (collected from local market of Faisalabad, Pakistan) was used in receptor isolation and radioreceptor binding assays. This work was conducted at the Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan. Radiolabelled standards for aflatoxin B1 (MT601 Aflatoxin-B1, [^{3}H]-1 mCi in 1 ml methanol) AFL B2 (MT636 Aflatoxin-B2 [8,9-^{3}H(N)]-1 mCi in 1 ml methanol) and CAP (MC302 EH D-three Chloramphenicol [dichloroacetyl-1,2-^{14}C]–50 µCi in 0.5 ml ethanol).

2.1. Isolation of IHRs

To improve the quality of IHRs for better specificity and affinity of mixed chemical substances in food, IHRs were prepared using two methods: (1) isolation of IHR by two step centrifugation (5000 rpm and 14000 rpm) followed by precipitation using $(NH_2)_2SO_4$; (2) isolation by serial centrifugation higher up to 40000g.

2.1.1. Isolation of IHRs by centrifugation followed by $(NH4)_2SO_4$ precipitation

Poultry liver tissue (2 g) was homogenized three times at 8000 rpm for 20 sec with a homogenizer in ice-cold 20 ml of 0.1 M phosphate buffer (pH 7.5). The homogenate was passed through a 40 µm cell strainer and sonicated for 30 min in ice-cold water (4°C). The homogenate (40 ml) was centrifuged in a 50 ml falcon tube at 5000 rpm for 30 min at 4°C to remove the connective tissue and other cell materials. Fractionation of the crude receptors was performed by salting out with $(NH_4)_2SO_4$. This involved addition of eight different saturation levels of $(NH_4)_2SO_4$ at 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% [18]. Ammonium sulphate was added to the diluted crude receptor (receptor: 0.1 M phosphate buffer; 1:10 w/v). The resultant material was refrigerated (4°C) for 30 min to facilitate salting out before purification with a refrigerated centrifuge at 14000 rpm for 30 min. The receptors prepared were stored at -20°C until used for further RBA analysis. For the affinity study, isolated receptors were tested with radiolabelled contaminants as tracers at 1.85 kBq using a liquid scintillation counter.

2.1.2. Isolation of IHRs by ultra-high centrifugation

Poultry liver tissue (3 g) was homogenized three times at 8000 rpm for 20 sec with a homogenizer. The sample was in 10 mM Tris-HCl; pH 7.5 containing 0.25M sucrose and 10 mM EDTA. A serial gradient centrifugation process of this homogenized liver tissue was performed with a centrifuge at 5000g, 10000g, 20000g and 40000g. Four fractions (1–4) cell organelles (mitochondria and microsomes) were isolated after each centrifugation. These fractions were obtained based on their molecular weight and density. Details of the IHRs isolation and development are shown in Fig. 1.

2.2. Characterization of isolated IHRs

Isolated receptors obtained from all centrifugations were characterized for total protein contents following Bradford's method [19]. The separated protein receptors were further characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis to determine their polypeptides nature [20].

2.2.1. Bradford assay

Bradford protein assay was performed for quantification of protein contents in all fractions of IHR separated at different centrifugal speeds. Each fraction of the receptor (1 µl each) was diluted in 800 µl of distilled water. Bradford reagent dye (200 µl) was added, the content measured at 595 nm after mixing and incubating at room temperature for 5 mins. The total protein content was determined using a standard curve of bovine serum albumin (BSA) in the range of 0–30 mg/l with excellent linearity (R^2=0.9947) as shown in Fig. 2. The protein concentration of each IHRs fraction was calculated using the standard curve.

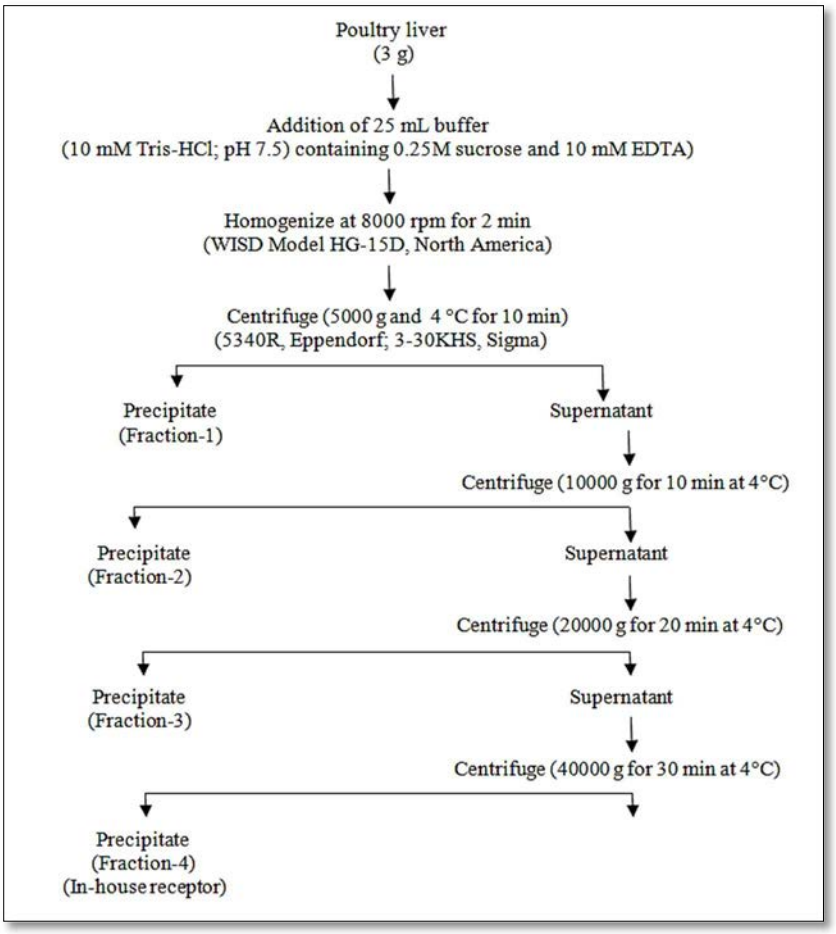


FIG. 1. Flow diagram of isolation of IHRs by ultra-high centrifugation.

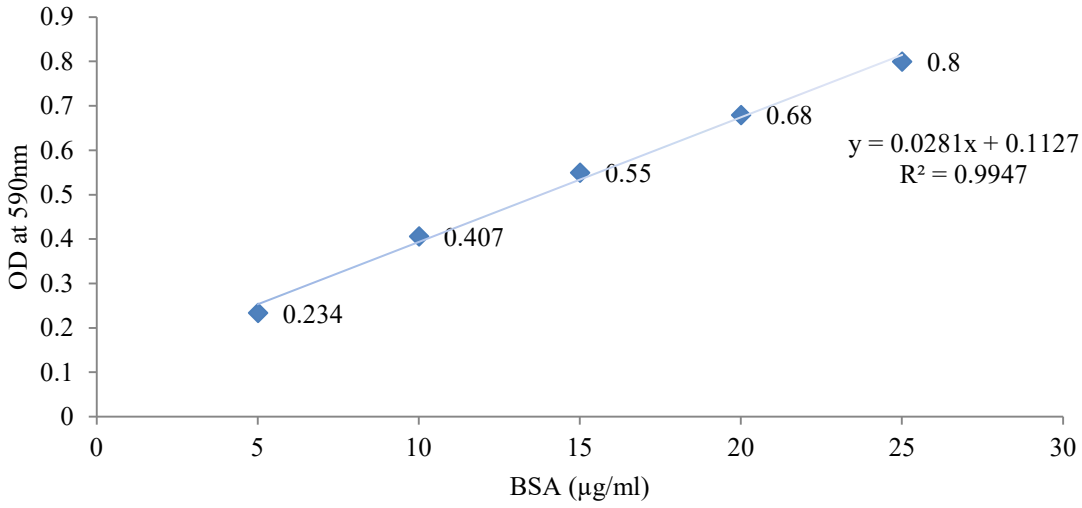


FIG.2. Bovine serum albumin standard curve for determination of total protein contents.

2.2.2. SDS-PAGE analysis

Polyacrylamide gel electrophoresis of all fractions (1–4) was performed under denaturing conditions to characterize the protein polypeptide mixtures. The protein samples were mixed with 4×loading buffer and β-mercaptoethanol. Samples were heated at 80°C for 10 mins and allowed to cool. Each sample was loaded separately in a well of gel. Electrophoresis was performed at 100V until the dye reached the end of the gel. Polypeptides of the IHR were separated by 3% stacking gel and 10–15% separating gel. Gels were stained by Coomassie Blue stain up to 16 hrs to visualize the protein bands. After staining the gels were de-stained for 16 hrs or until the background became transparent and protein bands became visible. Photographs of the gels were captured using a gel documentation system.

2.3. Preparation of wheat extract for mixed contaminant analysis

The wheat sample (50 g) was ground in a mill and mixed in a blender with 100 ml of 80% methanol in water (v/v) for 2 min. The mixture was centrifuged at 4000 rpm for 10 min at room temperature. Then 2 ml aliquots of the clear supernatant were prepared for further use in assay performance.

2.4. Radioreceptor binding assay (RBA)

Affinity of IHRs was evaluated for mixed chemical hazards spiked in wheat extracts with ^{3}H-aflatoxin B1 (AFL B1 and ^{3}H-aflatoxin B2 (AFL B2) ^{14}C-CAP as tracers. The pellet obtained after centrifugation at 40000g, having a range of polypeptides (20–120 KDa) was used as receptors to optimize RBAs for AFL B1, B2, and CAP in wheat. The receptors (20 mg) were dissolved in 400 µl distilled H_2O and treated with 100 µl each of the NC or PC for AFL B1 and B2 as well as CAP along with fifty grams of corn starch added to the mixture. The content was incubated for 2 min at 35°C for AFL and 50°C for CAP. Tritium labelled AFL B1 and B2 and ^{14}C-labelled CAP radiotracers (60 µl equivalent to 5.5 kBq) were added individually to the NC and PC. The mixtures were incubated again for 2 min (at 3°C for AFLs and 50°C for CAP) and spined in a centrifuge set at 3400 rpm for 5 min at room temperature. The pellet was dissolved in 400 µl distilled H_2O and the resultant mixture was transferred to a liquid scintillation vial with 1 ml and 2 ml wash of scintillation fluid, respectively. Analysis was performed using a liquid scintillation counter in the tritium mode for AFL and ^{14}C mode for CAP and data was recorded as cpm [21–22].

2.5. Matrix effect on IHR's affinity

In order to evaluate matrix effect, affinities of the IHRs for mixed contaminants analysis in methanol and wheat as a test matrix. For this purpose, methanol (100%) and wheat extract in 80% methanol were used as negative control (0 µg/kg or ppb). For the positive control, methanol was spiked with 15 ppb each for AFL B1, B2 and 0.6 ppb for CAP. Affinities of the IHRs for mixed contaminant analysis were further evaluated in wheat (80% methanol) and compared with methanol. For negative control (NC) wheat extract (0 ppb) prepared in 80% methanol was used directly. However, for matrix match positive control (PC) this extract was spiked with 10 ppb AFL B1 and B2 and 0.3 ppb for CAP. The NC or PC (100 µl) were analysed for the RBA using IHRs and compared with commercial receptors.

2.6. IHRs tablet-making for technology transfer

Preliminary studies were conducted to optimize the procedure for making lyophilized IHRs in the form of tablets. The selection and dosage of excipients (binders, disintegrants and softener) were standardized. A dough was prepared by uniformly mixing IHRs (20 mg) with starch (150 mg) as disintegrant and 4 mg of Sodium Carboxy Methyl Cellulose or Hydroxy methyl propyl cellulose as binder. From this dough, granules were prepared, lyophilized and subjected to a tablet press machine for tablet making. Since IHRs are temperature sensitive, talc (20 mg) was used as softener to minimize friction and temperature rise during the tablet making process and to avoid denaturing.

For reasonable thickness, stability and size of the tablet, the final weight of the tablet to be used in RBA was standardized as 200 ± 20 mg. These IHRs tablets were evaluated for aflatoxin M1 analysis (spiked at 0.5 ppb) using milk as a test matrix. The reaction mixture was measured using a liquid scintillation counter in the tritium mode and data was recorded as cpm [23].

2.7. Calculations and interpretation

The average cpm values of the six PCs and three NCs were calculated. An average plus a value of 15% and 30% for AFL and CAP, respectively was calculated and used as a CP. If the cpm value of unknown sample was higher than that of control point, the sample was negative and *vice versa*.

3. RESULTS AND DISCUSSION

3.1. Affinities of IHR fractions isolated by $(NH_4)_2SO_4$ for multiresidues/contaminants

There was an inverse relationship between the affinities of CAP for the IHRs with the increase of ammonium phosphate $(NH_4)_2SO_4$ saturation (%) from 20 to 60%. As shown in Table 1, the results indicated that at 20% $(NH_4)_2SO_4$ saturation concentration, molecular weight protein fractions exhibited better affinity for CAP (1146±29 CP) while at 40% $(NH_4)_2SO_4$ saturation concentration AFL showed better affinity with B1 at 917±76 CP and B2 at 625±12 CP. However, higher concentrations of $(NH_4)_2SO_4$ (over 60%) had very low amounts of isolated receptors which were insufficient for RBA. No other fractions were suitable for detecting all the contaminants studied at a time. This method was not further evaluated for mixed contaminant analysis.

TABLE 1. AFFINITY OF $(NH_4)_2SO_4$ ISOLATED RECEPTORS FOR LABELLED AFLs AND CAP STANDARDS

$(NH_4)_2SO_4$ (% saturation)	^{3}H-AFL B1 (5.5 kBq) 60 µl	^{3}H-AFL B2 (5.5 kBq) 60 µl	^{14}CAP (5.5 kBq) 60 µl
20	721±110^{B}	563±52^{A}	1146±29^{A}
30	708±21^{B}	574±11^{A}	814±102^{B}
40	917±76^{A}	625±12^{A}	839±144^{B}
50	493±8^{C}	438±41^{B}	640±87^{B}
60	468±11^{C}	413±47^{B}	780±115^{B}
p value	0.0034^{a}	0.0082^{a}	0.0322

Note: *Mean ± SD (n=2); LSD at alpha level 0.05; ^{a}Significant variations at 5% confidence level*

3.2. Characterization of IHRs/subcellular developed through serial centrifugations

3.2.1 Total protein contents

The protein fractions collected at higher centrifugation contained lower protein content as compared to other fractions collected at lower centrifugations. In the receptors received at 5000g, 10000g, 20000g and 40000g (fractions 1–4) the total protein content was 4.8 mg/ml, 3.2 mg/ml, 2.8 mg/ml and 2.2 mg/ml, respectively (Table 2). These concentrations were sufficient for RBA analysis of food or feed samples relative to commercial receptors for AFLs (0.28 mg/tablet) and CAP (0.12 mg/tablet) as reported earlier [17].

TABLE 2. PROTEIN CONTENT OF SUBCELLULAR/RECEPTOR FRACTIONS AT DIFFERENT CENTRIFUGATION LEVELS

Receptor description	Protein contents (mg/ml)
Receptor fraction 1 (at 5000g for 10 min)	4.8
Receptor fraction 2 (at 10000g for 10 min)	3.2
Receptor fraction 3 (at 20000g for 20 min)	2.8
Receptor fraction 4 (at 40000g for 30 min)	2.2

3.2.2. Polypeptide nature

Receptors showed a mixture of polypeptides in all four IHR fractions received after serial centrifugations as depicted in (Fig. 3) which is an important attribute for multi-residue/mixed contaminant analysis. Most of the polypeptides were in the range of 30–100 kDa. A few high molecular weight polypeptides (>120 kDa) were also

present. There was a slightly prominent polypeptide band at 55 kDa that was visible in gel electrophoresis. However, at a centrifugation speed of 40000g (Fraction 4) some polypeptide bands (20 kDa and above) were observed in low quantities. These were not seen in fractions 1–3. Therefore, fraction 4 with a wide range of polypeptides (20–100 kDa) was selected to be used in RBAs for mixed contaminants analysis. In comparison, the commercial receptors contained specific molecular weight proteins of 65 kDa and 70 kDa (but in higher concentration) for AFLs and CAP, respectively as reported earlier [17].

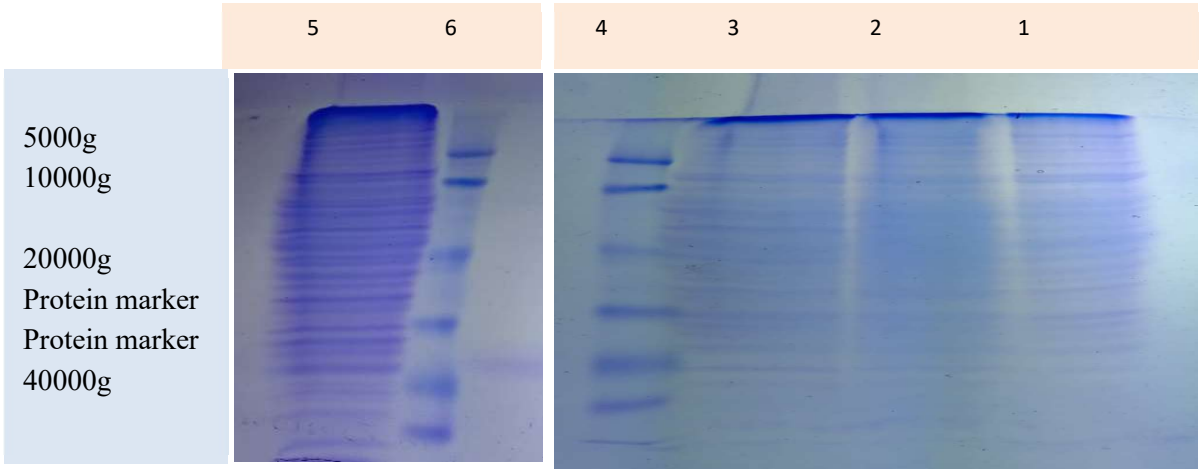


FIG. 3. Characterization of improved IHRs isolated from liver tissue by SDS-PAGE.

3.3. Performance evaluation of IHRs for mixed contaminant analysis

3.3.1. IHRs performance evaluation in methanol

Receptors (20 mg) of Fraction 4 resulting from centrifugation at 40000g and showing a wide range of polypeptides (20–100 KDa) were selected to be used in RBAs for mixed contaminant analysis. Performance evaluations of IHR for methanol and wheat extracts were conducted by two operators (Tables 3 and 4). Operator 1 reported NC (0 ppb, 100% methanol) averages for AFL B1, B2, and CAP as 2842 cpm, 5556 cpm, 4729 cpm. For the spiked methanol, the PC averages were 2408 cpm, 3502 cpm, 3620 cpm. The control points determined for AFLs and CAP were 2769 cpm, 4027 cpm, 4707 cpm, respectively. Whereas Operator 2 reported NC (0 ppb, 100% methanol) averages for AFL B1, B2, and CAP as 2593 cpm, 5091 cpm and 5868 cpm. For the spiked methanol, the PC averages were 2330 cpm, 4548 cpm and 4234 cpm. The control points determined for AFLs and CAP were 2679 cpm, 5230 cpm and 5504 cpm, respectively.

TABLE 3. CONTROL POINT FOR MIXED RESIDUES/CONTAMINANTS STANDARDS IN METHANOL USING THE INHs

Controls	Mixed residues/contaminants					
	AFL B1		AFL B2		CAP	
	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
Negative Control: (300 ml Methanol)						
NC1	2957	2563	5511	5034	4691	5648
NC2	2843	2614	5730	4926	4767	6085
NC3	2726	2601	5428	5314	4730	5872
Average NC	2842	2593	5556	5091	4729	5868
Positive Control:(methanol spiked at15 ppb each for AFL B1 and B2 (>MRL) and 0.6 ppb for CAP (>RPA)						
PC1	2460	2188	3671	5170	2871	4154
PC2	2463	2107	3526	4629	2879	4621
PC3	2409	2614	3830	4241	4554	4105
PC4	2443	2590	3830	4322	4150	4042
PC5	2394	2443	2863	4301	3711	4308
PC6	2278	2036	3290	4623	3558	4175

Controls	Mixed residues/contaminants					
	AFL B1		AFL B2		CAP	
	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
Average PC	2408	2330	3502	4548	3620	4234
15% of Av PC	361	349	525	682	1086	1270
Av PC-15%	2047	1980	2976	3866	2534	2964
Av PC +15% (CP)	2769	2679	4027	5230	4707	5504

Note: *CP=Control Point; Av= Average a± 30% PC Average CAP*

3.3.2. IHRs performance evaluation in wheat extract

Operator 1 reported NC (0 ppb, wheat extract in 80% methanol) averages for AFL B1, B2, and CAP as 3756 cpm, 9314 cpm and 8103 cpm. For the spiked wheat extract, the PC averages were 3170 cpm, 7759 cpm and 6152 cpm. The control points determined for AFLs and CAP were 3645 cpm, 8922 cpm and 7998 cpm, respectively. Whereas Operator 2 reported NC (0 ppb, wheat extract in 80% methanol) averages for AFL B1, B2, and CAP as 3269 cpm, 6146 cpm and 6523 cpm. For the spiked methanol, the PC averages were 2502 cpm, 5723 cpm and 5905 cpm. The control points determined for AFLs and CAP were 2877 cpm, 6581 cpm and 7677 cpm, respectively (Table 4).

TABLE 4. CONTROL POINT FOR MIXED RESIDUES/CONTAMINANTS SPIKED IN WHEATEXTRACT (80% METHANOL) USING THE IHRs

Controls	Mixed residues/contaminants					
	AFL B1		AFL B2		CAP	
Negative control: Wheat extract (100 ml of wheat extract in 80% methanol)						
	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
NC1	3792	3037	9631	6052	8038	6677
NC2	3693	3257	9228	5992	8012	6425
NC3	3784	3514	9083	6393	8259	6468
Average NC	3756	3269	9314	6146	8103	6523
Positive Control: (Wheat Extract: 80% methanol spiked with mixed multi contaminants (at 10 ppb AFL B1 and B2 and 0.3 ppb for CAP						
PC1	3101	2478	8096	5904	5673	6727
PC2	3179	2490	7836	5987	5958	5472
PC3	3168	2763	7909	5289	6218	5664
PC4	3034	2524	8039	5662	6734	5452
PC5	3224	2332	7358	6121	6464	5855
PC6	3313	2424	7314	5375	5865	6260
Average PC	3170	2502	7759	5723	6152	5905
15% of Av PC	476	375	1164	858	1846	1772
Av PC-15%	2694	2127	6595	4865	4306	4134
Av PC +15% (CP)	3645	2877	8922	6581	7998	7677

Note: *CP= Control Point/ Decision Limit; Av=Average± 30% PC Average CAP.*

3.4. Performance evaluation of lyophilized IHRs tablets for aflatoxin M1 analysis in milk

The IHR-SCMC tablet (Fig. 4) showed better performance than IHR-HPMC for AFL M1 analysis in milk (spiked at 0.5 ppb). The PC average was 314 cpm for 6 replicates. None of the responses exceeded ±20% of the PC averages (377–251 cpm). The NC average was 340 cpm for 3 replicates. The CP was 377 cpm. Receptors are located on plasma membranes of different body organs such as liver, brain and lungs. They mediate effects of many neurotransmitters (such as toxins, drugs and other poisonous substances). Earlier, IHRs, prepared at 14000 rpm, have proved their affinities for multiple chemical contaminants analysis making them suitable binders for food and feed analysis. The present results agree with a previous study [17] that IHRs separated from poultry liver by homogenization, sonication and centrifugation (14000 rpm) can be used for RBA screening of CAP (0.3 ppb) gentamicin (100 ppb) oxytetracycline (100 ppb) in milk. Variations in the protein content might be due to differences in buffered media and centrifugation forces [17].

However, at a low centrifugation of 14000 rpm, receptors had high turbidity due to unnecessary matrix. The receptor solution (150 mg in 400 µl) was not clear compared to commercial kit receptor (Fig. 5). Furthermore, the resulting pellet after RBA, was not very compact, an attribute essential for complete shifting of reaction mixture to scintillation vial for a successful complete count of the radiolabelled tracer (Fig. 6). The pellet was detached from the assay tube and scattered making it difficult to pour it out. This resulted in loss of reaction mixture and ultimately the radioactivity. Although the pellet-compactness was improved by $(NH_4)_2SO_4$ fractionation but due to differential affinities of IHRs for CAP and AFLs, the method involving ammonium sulfate was not found to be suitable for mixed contaminant analysis (Table 5).

TABLE 5. CONTROL POINT FOR AFLATOXIN M1 IN MILK USING IHR TABLET AND COMMERCIAL RECEPTORS

Controls	Aflatoxins M1 in Milk 0.05 ppb		
	Commercial Receptors	IHRs	IHRs Tablets
			Negative Control:
NC1	444	217	358
NC2	428	218	335
NC3	434	205	327
AV NC	435	213	340
			PC: (AFL M1, 0.05 ppb in milk)
PC1	208	151	312
PC2	227	138	338
PC3	171	150	279
PC4	181	149	330
PC5	199	158	318
PC6	196	137	308
AV.PC	197	147	314
20% Av PC	39	29	63
AV PC -20%	158	118	251
AV PC +20% (CP) /Decision Limit (CCα)	236	176	377

In the present findings, receptors obtained at higher centrifugation levels (40000g) further improved the quality of receptors for mixed contaminant analysis by removing unnecessary protein content. Mixed hazard analysis was possible even at lower concentrations (20 mg) in comparison to previous studies which were at higher concentrations (150 mg). The present studies also improved the shape of pellet, making it easier to dissolve and facilitate complete transfer of the cocktail into a scintillation vial which is mandatory for complete counting of reaction mixture.

FIG. 4. In house HR-SCMC tablet prepared from IHRs isolated at 40000g.

To overcome these challenges and improve performance of IHRs for mixed contaminant analysis, starch was added as an excipient to enhance pellet compactness. Serial centrifugation highly improved the turbidity IHRs

solution. The specific receptors for CAP and AFLs present in commercial kits were also present in all IHRs fractions although the concentrations were higher for commercial kit receptors.

The multiple centrifugation steps removed the unwanted materials resulting in a clear receptor solution especially for fraction 4 at 40000g and this was used in RBA of mixed contaminant analysis. Fraction 4 also contained the lower molecular weight proteins enhancing the range of polypeptides from 20 kDa to 100 kDa another important attribute for detecting a wider range of contaminants. Receptors prepared at high centrifugation (40000g) exhibited better pellet compactness during the RBA assay of wheat extract for multi-class contaminants/residues. Moreover, the amount of IHRs used (20 mg) was equally efficient and more effective than 150 mg of IHRs reported earlier [17]. This factor also decreased turbidity of IHRs solutions enhancing interaction of mixed contaminants/residues with improved quality IHRs.

The findings here agree with Franko et al., [24] who used the centrifugation process for the separation of cell fractions especially mitochondria. Costesec et al., [25] also isolated the rat liver tissue fractions namely nuclear content, large granules, microsomes, and final supernatant fractions using buffered sucrose. They homogenized the tissue at 1300 rpm and the resultant homogenate was centrifuged for l0 min at 1700 rpm. The cell nuclear materials (DNA and RNA) in pellets were sedimented at 1400 rpm for 10 min. Other fractions containing cell organelles were collected by increasing the centrifugation speed and the large granule fraction (mitochondrial fraction) was sedimented at 25000 rpm.

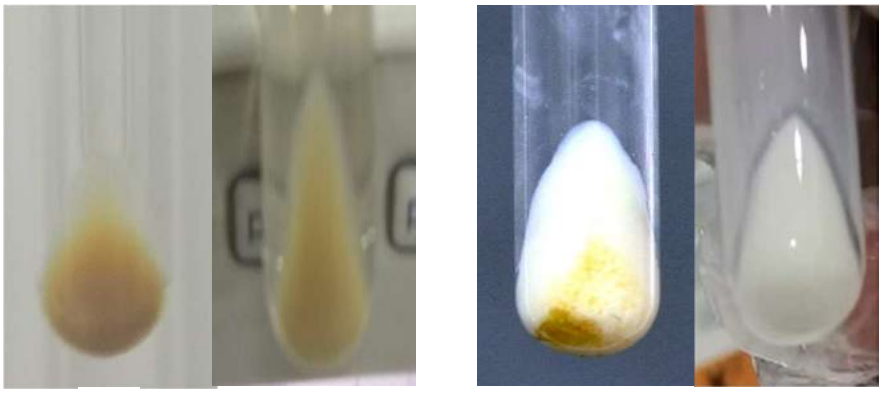
The microsomal fraction was separated from the post mitochondrial supernatant at 30000 rpm. They also reported that nuclear, large granule, microsomes, and final supernatant fractions contained 18%, 22%, 18.6%, and 39.5% protein, respectively. Similarly, Castro et al., [26] reported that Sprague-Dawley rat liver cytosol isolated by ultracentrifugation at 105000g for 1 hr at 4°C and subsequently purified with S-linked GSH-Sepharose and S-hexyl glutathione-Sepharose had protein fractions with 30–66 kDa bands.

IHR (at 14000 rpm) 150 mg/400 µl H_2O used for RBA assay

Commercial receptor tablet/ 400 µl H_2O used for RBA

IHR (at 40000g) 20 mg/400 µl H_2O used for RBA

FIG. 5. Turbidity improvement in isolated receptors at higher gyrations in comparison to commercial receptors.



A B C D

FIG. 6. Comparison of receptor pallet and compactness: A, Receptor 150 mg at 14000 rpm; B, Improved pallet receptor 20 mg at 40000g; C, low compactness of receptor 150 mg at 14000 rpm with starch; D, Improved compactness of receptor 20 mg at 40000g.

The results of the present study are also in harmony with the findings by Offiah and Adesiyun [27] who determined the AFLs B1 and B2 in raw milk samples (*n*=175) from five collection centres; pasteurized milk (*n*=37); animal feed (*n*=40) peanut (*n*=142) peanut butter (*n*=32) and peanut cake (*n*=14) samples by RBAs. Samples with a higher CP (1181 cpm) were considered negative whereas, samples with a cpm less than the CP were considered suspect. Furthermore, suspect samples were diluted with zero control standards and retested along with the NCs and PCs. For a diluted sample with a cpm greater than the CP the sample was negative, whereas for cpm less than the CP the sample was considered positive. Mukota et al., [28] reported the presence of tetracyclines, macrolides, β-lactams, aminoglycosides sulfonamides residues in fish using radioligand assay. The noted lower counts in samples spiked with radio-tracer drugs compared to blank fish samples.

4. CONCLUSIONS

The IHRs produced in this study showed clear affinities for AFL B1, B2, and CAP in methanol and wheat extracts (80% methanol) at their respective ML/RPA. The IHRs showed no matrix effect for the contents coextracted in 80% methanol in wheat when compared to 100 % methanol. The IHRs prepared at 40000g, and used in radioreceptor biding assay, are suitable for screening of mixed contaminant/residue (AFL B1, B2 and CAP) in the matrices studied. Preliminary studies also indicated that these IHRs can be produced and packaged for possible transfer to other laboratories for the same purpose. This followed lyophilization and preparation into tablets using SCMC as binder, starch as an excipient and talc as a softener. This is an area for further validation, such as the comparison with hydroxy methyl propyl cellulose as an alternative to SCMC. Future research shows great potential for rapid radiometric screening techniques that will be cost-effective to many laboratories.

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INVESTIGATING CONTAMINATION SOURCES, BIOFILM FORMATION ABILITY, AND BIOCIDE RESISTANCE OF *LISTERIA MONOCYTOGENES* RECOVERED FROM A NILE CROCODILE (*CROCODYLUS NILOTICUS*) MEAT PROCESSING FACILITY IN ZIMBABWE

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Abstract

Food production facilities have emerged as significant sources of *Listeria monocytogenes* foodborne outbreaks globally. Understanding the sources of bacterial contamination in the food processing industry is essential for preventing food borne infections. This study investigated contamination sources, biofilm-forming ability and biocide resistance of *L. monocytogenes* from a processing facility. Five sections along the production chain were sampled three times over a production year. Samples included processing water, meat products, and swabs from personal protective clothing, food-contact surfaces, and the processing environment. *L. monocytogenes* was detected in all sections of the industry except the reception area, with the highest prevalence (14.8%) found in the freezer storage warehouse. Food contact surfaces on machines were the most contaminated, accounting for 45% of all *L. monocytogenes* recovered. Isolates (20) of *L. monocytogenes* isolates were typed using Repetitive sequence-based polymerase chain reaction (REP-PCR) with REPI+II primers. Genetic-similarity analysis, revealed identical REP-PCR profiles among strains from different sources, indicating a complex genetic landscape. Additionally, the lack of source–specific profiles and the presence of multiple strains across various sections of the production chain highlight the potential for widespread contamination. All isolates showed the ability to form biofilms, with those from the skinning and evisceration sections being the most prolific. These biofilms displayed resistance to benzalkonium chloride and sodium hypochlorite at concentrations recommended by regulatory authorities. However, higher concentrations significantly reduced the biofilms, with benzalkonium chloride being the most effective. Genetic fingerprinting allows for accurate identification and differentiation of strains, facilitating the tracking of contamination sources, understanding biofilm formation, and monitoring biocide resistance. These insights are essential for creating effective strategies to prevent contamination in food processing facilities, thereby enhancing food safety and public health.

1. INTRODUCTION

Nile crocodile (*Crocodylus niloticus*) commercial farming along the Zambezi Valley in Zimbabwe dates back to the 1950s, primarily for their skins, which are exported to overseas markets. However, due to the fall in skin prices in the 1990s, crocodile meat became an alternative source of income and was later marketed as a delicacy especially in European and Asian markets [1]. The high price of traditional meat sources (e.g. beef, pork, chicken) has prompted a local demand, especially in restaurants frequented by tourists. As the demand for crocodile meat increases, the production of safe, wholesome meat and products is required to minimize the risk of foodborne infections and maintain markets.

Crocodile meat may be associated with zoonotic pathogens such as *L. monocytogenes, Salmonella* spp., and *Escherichia coli* [1]. *L. monocytogenes* is globally associated with the life-threatening listeriosis a particularly a problem in young, old, pregnant and immunocompromised individuals [2]. Due to its ubiquity, *L. monocytogenes* is readily transmitted along the food supply [3]. According to the WHO, foodborne diseases are responsible for a substantial burden of illnesses globally, with about 600 million illnesses each year due to the consumption of contaminated foods [4]. Between 2017 and 2018, a high number of listeriosis cases was reported in South Africa, resulting in what was to become the world's largest listeriosis outbreak where 1060 cases were reported [5]. Among food commodities, contaminated meat products are considered to be one of the main sources of *L. monocytogenes* infections [6]. Meat is commonly consumed and is a major vehicle of foodborne pathogens. Ready-to-eat meat products are of special concern since they are associated with a high risk of exposure to hazards [7].

Bacterial contaminants can be routinely introduced into a food processing facility through a variety of sources that include raw materials, process water, equipment, and food handlers. Subsequently, once within a food

processing facility, the bacteria can be transferred to food contact surfaces and finished products by cross-contamination. Sanitary procedures in food manufacturing facilities target the reduction and elimination of pathogens that gain access to the processing environment. However, sublethal concentrations of biocides and inadequate exposure times can result in biocide resistance and persistence of pathogens.

For *L. monocytogenes*, the ability to form biofilms is reported to be one of the ways that confer its increased resistance and persistence in the food industry [8]. Biofilm formation significantly contributes to *L. monocytogenes* survival in the food processing environment, where rigorous cleaning and disinfection routines are implemented. This has been substantiated by reports indicating that persistent strains tend to be stronger biofilm formers when compared to their sporadic counterparts [8]. Sodium hypochlorite-based biocides and quaternary ammonium compounds are used in food production systems to control the growth of microbial contaminants [9]. However, issues have been raised over the increase in microbial resistance against these widely used biocides.

An increase in *L. monocytogenes* positive cases have been noted in a Nile crocodile meat processing facility in Zimbabwe, suggesting that the food processing environment is a likely contamination source. This poses a significant burden on the food industry; challenges to food safety, as well as the country's ability to earn foreign currency through the selling of crocodile products. The aim of the study was to employ REP-PCR genomic fingerprinting to identify major contamination sources for *L. monocytogenes* in a Nile crocodile meat processing facility in Zimbabwe and to evaluate the *in vitro* biofilm-formation ability and resistance *L. monocytogenes* isolates to biocides.

2. MATERIALS AND METHODS

2.1. Sampling

A total of 248 samples were collected over a year from 31 processing control points in a Nile crocodile meat processing facility, with sampling conducted approximately once every 4 months. The samples were taken from the production process, including reception, skinning and evisceration, deboning and sanitization, slicing and packaging, as well as the freezer storage and warehouse areas. Contamination analyses was done by sampling of frozen meat, personnel protective clothing (aprons and gloves) food contact surfaces (equipment) and the processing environment (wall surfaces, floors, and drains) of three production batches at a time. The processing environment, floors, walls, drains, and equipment were examined by swabbing with sterile swabs pre-moistened in sterile phosphate buffered saline (PBS). Samples from gloves and aprons were taken by washing with 100 ml of PBS, ensuring that only the outer surfaces contacted the liquid. About 50 g of raw meat and vacuum-packed meat samples from each batch were aseptically placed into sterile stomacher bags that were closed with clips. Water samples of approximately 1 litre were aseptically put in sterile bottles. Efforts were made to sample the same specific sites at every sample collection date. All samples were properly labelled, put into a cooler box containing ice packs, transported, and processed immediately upon arrival in the laboratory.

2.2. Isolation and identification of *L. monocytogenes*

A modified ISO 11290-1:2017 [10] was used for the isolation of *L monocytogenes*. Briefly, 25 g of raw or vacuum-packed meat were stomached for 1 min with enrichment in half Fraser broth and Fraser broth supplement in a sterile stomacher 400 bag at normal speed. Swabs were stomached with 100 ml of half-Fraser broth for 1 min. The homogenized samples (25 g) were mixed with 225 ml of enrichment broth and blended using a stomacher and incubated at 30°C for 25±1 hours. After primary enrichment, 0.1 ml of broth was inoculated into 10 ml full-Fraser broth and incubated at 37°C for 24±2 hours. An aliquot (10 µl) of the culture material was sub-cultured onto *Listeria* chromogenic agar base according to Ottaviani and Agosti and Palcam agar. The agar plates were incubated at 37°C for one to two days. Presumptive *Listeria* spp., colonies were selected from the agar plates on the basis of aesculin hydrolysis and typical morphology (small, round, smooth, and grey colonies surrounded by a black zone) and sub-cultured on sheep blood agar.

2.3. Confirmation of *L. monocytogenes*

The Gram stain, catalase test, motility test at 25°C and fermentation of rhamnose, xylose and mannitol tests were performed on the suspected *L. monocytogenes* colonies (round, small, smooth, grey, β-haemolytic). Presumptive *L. monocytogenes* isolates were subjected to the analytical profile index (API) *Listeria* system for confirmation. *L. monocytogenes* ATCC 35152 was used as a positive control. The identified *L. monocytogenes* isolates were stored at -80°C in Tryptic Soy Broth (TSB) with 0.6% yeast extract and 20% glycerol. Strains were inoculated in TSB and were grown overnight at 37°C for analysis.

2.4. DNA extraction

The DNA was extracted from bacterial cells grown overnight at 37°C in TSB using the Quick-g-DNA Mini Prep Kit. Briefly, four volumes of genomic lysis buffer were added to a volume of cell suspension and mixed briefly by vortex, then left to stand at 25°C for 10 mins. The content was placed in a Zymo/spin column in a collection tube and spined on a centrifuge at 10000*g* for 1 min. The eluent was discarded and the column placed in a new collection tube to which 200 µl of DNA Pre-Wash Buffer was added. The mixture was centrifuged at 10000*g* for 1 min. Subsequently, 500 µl of g-DNA Wash Buffer was added to the spin column and centrifuged again before the column was placed in a clean microcentrifuge tube. The DNA elution buffer (50 µl) was added and the content incubated for 5 min at 25°C; further centrifuged at top speed for 30 seconds and the resultant DNA dissolved in sterile water. The concentration was determined using a spectrophotometer at 260 nm.

2.5. Genomic fingerprinting

Repetitive element sequence based PCR (REP PCR) typing was conducted using the primer systems REPI (5'-IIIICGICGICATCIGGC-3') and REPII (5'-ICGICTTATCIGGCCTAC-3') according to Jersˇek et al., [11]. The amplification was performed in a final volume of 25 µl consisting of 25 pmol of a primer (Inqaba biotec™) 200 mM of deoxynucleoside triphosphate, 0.5 U of DyNazyme II DNA polymerase, and 10×PCR buffer (100 mM Tris-HCl, pH 8.8, 15 mM $MgCl_2$, 1500 mM KCl, and 0.1% Triton X-100). A Master cycler gradient thermocycler was used. The REP-PCR was performed at an initial denaturation at 95°C (3 mins) followed by 30 cycles of 90°C (30 seconds) at 40°C (1 min) at 72°C (1 min) then at 72°C (8 mins). The PCR products were resolved on 1.5% agarose gel at 60 volts for 1 hour in 0.75×Tris acetate EDTA buffer. The bands were visualized by ethidium bromide and recorded with an imaging System. Fingerprint patterns were used to determine the genetic-relatedness of the isolates from the processing facility using the unweighted-pair group d with arithmetic mean cluster analysis.

2.6. Microtiter plate biofilm production assay

Biofilm-production was assessed in accordance with Campo-Pérez et al., [12]. *L. monocytogenes* grown in TSB at 37°C overnight, were adjusted to optical density (OD) $_{600nm}$=0.1, corresponding to 10^7cfu/ml, in TSB + 0.2% glucose. Then, 200 µl of the adjusted culture was added to each well of 96 well polystyrene plates. The plates were incubated statically at 37°C for 48 hours in a compact mini incubator with humidity saturation to allow cell adhesion and biofilm formation. After 48 hours of incubation, planktonic bacteria were removed by washing the wells three times with 300 µl of 1×PBS (pH 7.4) retaining only the attached biofilm-forming bacteria. After an additional 24 hours of incubation, the wells were washed three more times with PBS to remove any remaining planktonic bacteria.

The biofilm cell mass was then quantified using crystal violet staining. Wells filled with the medium alone served as negative controls, and the average OD values reported in control wells were subtracted from all the data. To fix the attached biofilms, 200 µl of methanol was added to each well for 15 mins. Methanol was removed, and the wells were left to dry completely. The adherent biofilm layer in each well was stained with 200 µl crystal violet (1%) for 5 mins at 25°C. Further washing was done with distilled water and crystal violet was dissolved with 200 µl of 33% acetic acid, and the OD measured at 570 nm.

2.7. Biocide resistance assays

Resistance to benzalkonium chloride and sodium hypochlorite was assessed for the three lowest and three highest biofilm forming *L. monocytogenes* isolates [13] with some modification. The disinfectants were diluted into 100 mg/l, 200 mg/l, 500 mg/l, 1000 mg/l, 2000 mg/l, and 3000 mg/l) using distilled water. After formation of the biofilm, cells that did not adhere were washed off with PBS and 500 µl of disinfectant added to the biofilms for 30 mins. Biofilms exposed to sterile water only were used as positive controls. The treatments were neutralized by adding TSB for 10 mins at room temperature, and more unbound cells removed using PBS. The biofilm cells were collected by rubbing the surfaces with two sterile swabs and then suspended in 10 ml of peptone, the content vortexed for 1 min, and aliquots of 0.1 ml serial dilutions were plated in triplicate on Tryptic soy agar plates, then incubated for 24 hours at 37°C. The cfu/ml was calculated and logarithmic reduction of viable cells due to each disinfectant determined as the difference between the logarithm of the total viable cells in biofilms not exposed to disinfectants and the logarithm of the viable cells.

2.8. Data analysis

Both one- and two- way analysis of variance (ANOVA) were performed followed by a post hoc least significant difference (5%) to determine significant differences between isolates on biofilm production and biocide resistance.

3. RESULTS AND DISCUSSION

3.1. Process control points and contamination sources of *L. monocytogenes*

A total of 248 samples were collected from 31 control points in the processing facility. From all the 5 processing sections sampled, *L. monocytogenes* was recovered in all sections except the reception section. *L. monocytogenes* was found in 8.1% (20 of the 248) of the total samples tested (Table 1). The freezer storage warehouse section had the highest prevalence of *L. monocytogenes* positive samples at 14.8%, followed by the skinning and evisceration section with a 9.7% prevalence rate.

TABLE 1. PREVALENCE OF *L. MONOCYTOGENES* IN DIFFERENT PROCESSING SECTIONS OF THE NILE CROCODILE MEAT PROCESSING FACILITY

Processing section	Number of samples tested	Number of positive samples	Percentage of positive samples (%)
Reception	29	0	0
Skinning and evisceration	93	9	9.7
Deboning and sanitisation	42	4	9.5
Slicing and packaging	57	3	5.3
Freezer storage warehouse	27	4	14.8
Total	248	20	

The most contaminated process control points were the food contact surfaces of machines, contributing 45% of all *L. monocytogenes* obtained from the processing facility. Environmental samples contributed 40% whilst 15% of the total *L. monocytogenes* were obtained from carcass samples. All the *L. monocytogenes* isolated from food contact surfaces of equipment were from processing equipment used in cutting and opening up carcasses, with the evisceration machine having the highest prevalence of 50%. Among the environmental sites, *L. monocytogenes* was isolated most frequently from drains in the deboning, and sanitization areas with a prevalence of 25%. Non-food contact surfaces, such as floors and walls from the freezer storage warehouse, had the second highest frequency of positive samples at 16.7% (Table 2). There was no *L. monocytogenes* isolated from samples in the reception section; however, an increase in the number of positive samples was noticed after the carcasses were opened in the skinning and evisceration section.

TABLE 2. DISTRIBUTION AND PREVALENCE OF *L. MONOCYTOGENES* AMONG THE DIFFERENT PROCESSING EQUIPMENT AND ENVIRONMENT

Section	Sample	Number of samples tested	Number of *L. monocytogenes* isolates	Percentage of positive samples (%)
Reception	Carcass	6	0	0
	Drain	8	0	0
	Floor	4	0	0
	Water	3	0	0
	Gloves	4	0	0
	Aprons	4	0	0
Skinning and evisceration	Cutting boards	6	0	0
	Skinner machine	9	2	22.2
	Floor	18	1	5.6
	Drain	12	1	8.3

Section	Sample	Number of samples tested	Number of *L. monocytogenes* isolates	Percentage of positive samples (%)
	Gloves	12	0	0
	Stomach contents	9	2	22.2
	Aprons	12	0	0
	Evisceration machine	6	3	50
	Water	9	0	0
Deboning and sanitization	Deboning machine	9	1	11.1
	Gloves	12	0	0
	Drain	12	3	25
	Cutting boards	6	0	0
	Sanitizer (Chlorine)	3	0	0
Slicing and packaging	Slicing machine	9	2	4.5
	Gloves	12	0	0
	Cutting boards	6	0	0
	Balance	6	0	0
	Vacuum packaging machine	9	1	11.1
	Knives	6	0	0
	Crates	9	0	0
Freezer storage warehouse	Walls	6	1	16.7
	Floor	12	2	16.7
	Frozen meat	9	1	11.1

3.2. Genetic fingerprinting of *L. monocytogenes* isolates

The agarose gel electrophoresis image of REP-PCR amplification of *L. monocytogenes* isolates is shown in Fig. 1. DNA fingerprints obtained showed diverse band patterns (Fig. 1) with isolates having between six (Lm-89) to twelve (Lm-53) bands. The size of the amplification products ranged from 300 bp to 1400 bp.

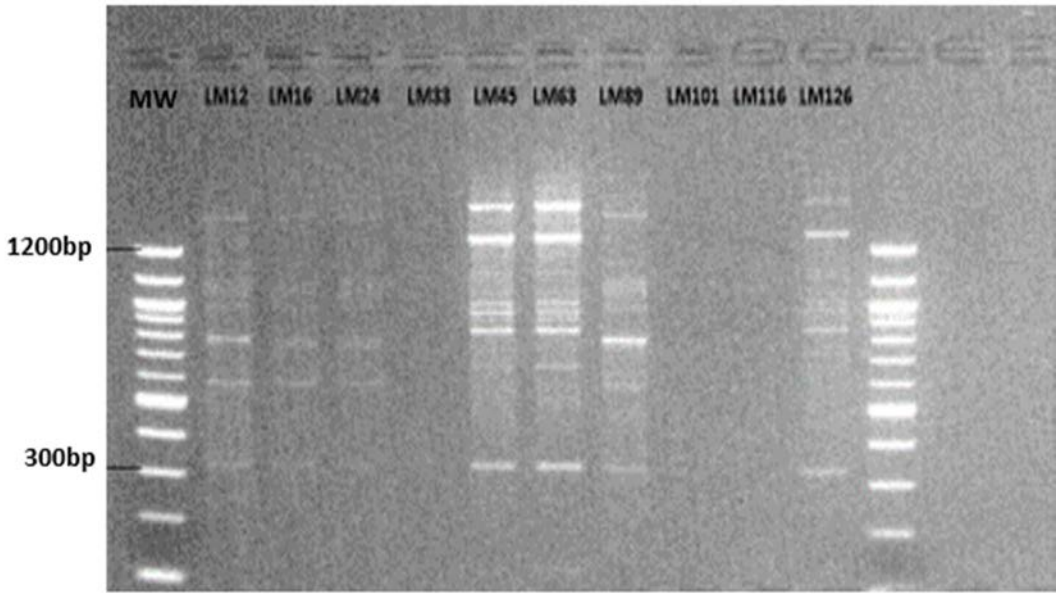

FIG. 1. Agarose gel electrophoresis image showing REP-PCR fingerprints of L. monocytogenes isolates. Lane Lm 12 to Lm 318 indicate the isolate codes. MW is a DNA molecular weight marker.

The genetic similarity analysis among the isolates is shown in Fig. 2. Two sets of isolates ((Lm-12, Lm-16, Lm-24) and (Lm-126, Lm-294, Lm-299)) from different sources had identical REP-PCR profiles. However, it was observed that no REP-PCR profile was source-specific; they were distributed throughout the production chain. The skinning and evisceration section was contaminated with five different *L. monocytogenes* strains (Lm-16,

Lm-45, Lm-126, Lm-137 and Lm-294). Meanwhile, four strains (Lm-24, Lm-63, Lm-129 and Lm-158) were present in the deboning and sanitization area. The slicing and packaging area was contaminated with two strains (Lm-89, and Lm-299). Strains Lm-12, and Lm-223 were recovered from the freezer storage warehouse. *L. monocytogenes* isolates Lm-12, Lm-16, and Lm-24 had the same REP-PCR profile although they were isolated from the different sources. This was also observed on isolates Lm-299, Lm-294, and Lm-126.

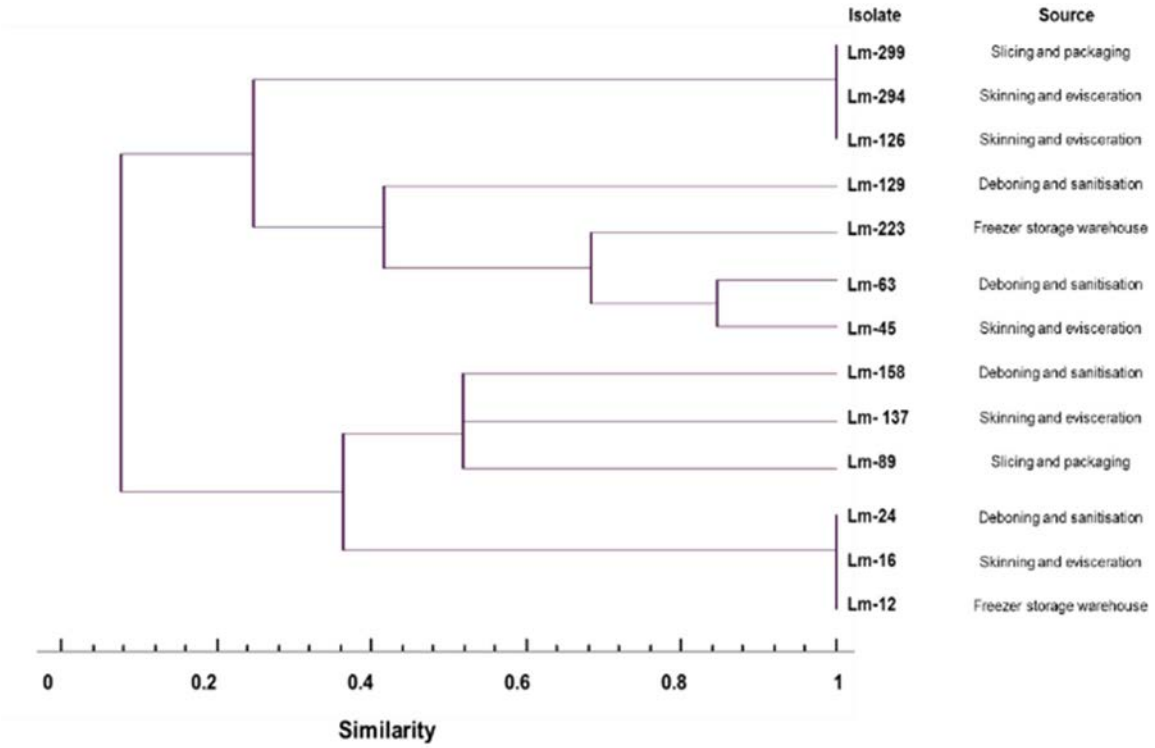

FIG. 2. REP-PCR dendrogram of L. monocytogenes isolates from different stages of the processing plant.

3.3. Biofilm formation and *L. monocytogenes* isolates

The levels of biofilm formation varied significantly ($p < 0.05$) between the different isolates, ranging from an OD_{600nm} of 0.67 to 1.89. Isolate Lm-185 from the freezer storage warehouse was the least biofilm former, while isolate Lm-271 from skinning and evisceration was the highest biofilm former (Fig. 3).

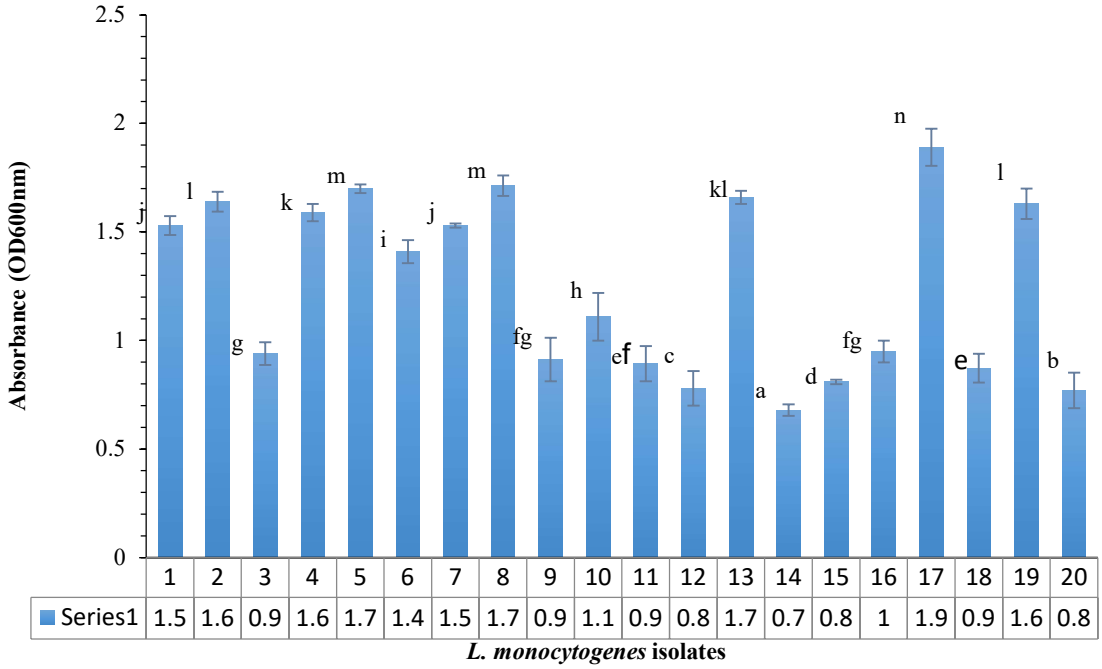

FIG. 3. Biofilm formation ability of L. monocytogenes isolates from different stages of the processing plant.
Note: *Letters above the bars indicate statistically significant differences among isolates based on Tukey's multiple comparison test. The lettering reflects a progressive increase in biofilm formation from 'a' to 'n'.*

When the biofilm formation ability of the *L. monocytogenes* isolates was analysed according to the sources of the isolates (Fig. 4) isolates from the freezer storage warehouse showed the lowest range of biofilm formation values, with a median value of 0.8. The strongest biofilm forming *L. monocytogenes* isolates were from the skinning and evisceration section with a median value of 1.6. Isolates from deboning and sanitization and from slicing and packaging had median values of 1.18 and 1.53, respectively.

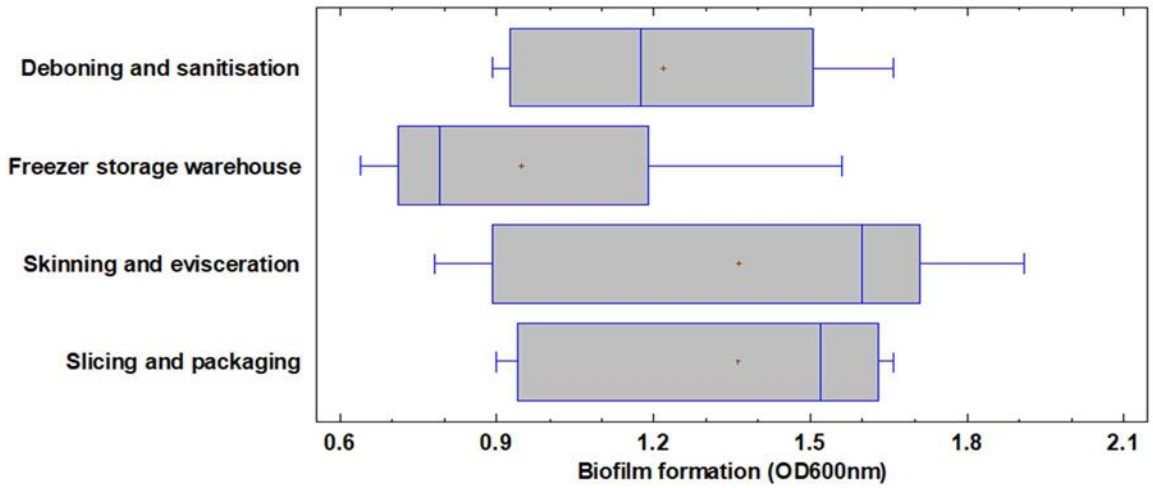

FIG. 4. Box and whiskers plot showing the median and range of biofilm formation abilities of L. monocytogenes isolates from different sections of the processing plant. Whiskers extend to minimum and maximum values and the vertical line in the box represents the median values.

3.3. Biocide resistance and *L. monocytogenes* biofilms

The biocide resistance of 24 hour old biofilms of three highest biofilm producing isolates (Lm-271, Lm-101 and Lm-45) and three lowest biofilm producing isolates (Lm-185, Lm-318, and Lm-137) was assessed against a sodium hypochlorite based disinfectant and a benzalkonium chloride-based disinfectant. The log reduction in viable cells of the biofilm after treatment with varying levels of the sodium hypochlorite is shown in Fig. 5. There was a significant increase ($p < 0.0001$) in the log reduction of biofilm cells as the concentration of hypochlorite increased. At 100 mg/l, the log reduction for all isolates was less than 2.0 $\log_{10}$ cfu/ml. At concentrations > 2500 mg/l, the log reduction was more than 5.0 $\log_{10}$ cfu/ml.

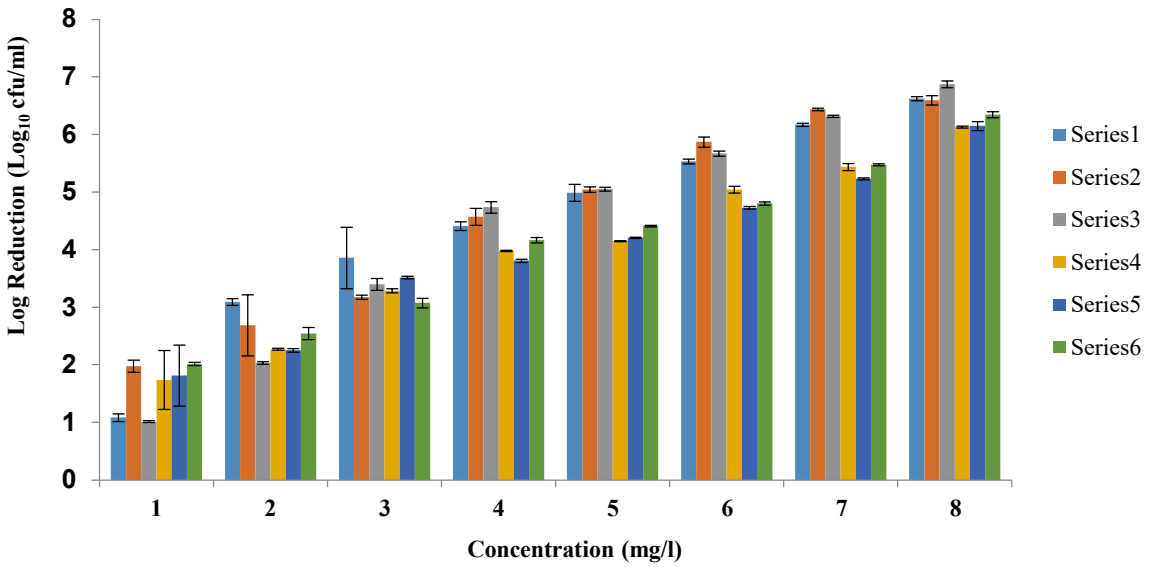

FIG. 5. Logarithmic reduction in viable cells of biofilms of L. monocytogenes isolates after 30 mins of exposure to different concentrations of sodium hypochlorite.

Both variations among *L. monocytogenes* isolates and concentrations of sodium hypochlorite had significant effects ($p < 0.0001$) on the death of *L. monocytogenes* biofilms (Table 3).

TABLE 3. VARYING EFFECTS OF DISINFECTANT-CONCENTRATION AND STRAIN VARIABILITY ON THE VIABILITY OF *L. MONOCYTOGENES* BIOFILMS

Source of variation	Degrees of freedom	*p-value*	
		QAC	Hypochlorite
Isolate	5	< 0.0001	< 0.0001
Concentration	7	< 0.0001	< 0.0001
Isolate × Concentration	35	< 0.0001	< 0.0001

There was a significant increase ($p < 0.0001$) in log reduction of biofilm cells as the concentration of benzalkonium chloride increased. At 100 mg/l, the log reduction for all isolates was less than 4.0 $\log_{10}$ cfu/ml. At concentrations > 1000 mg/l, the LR was more than 5.0 $\log_{10}$ cfu/ml. Both variations among *L. monocytogenes* isolates and concentrations of benzalkonium chloride had significant effects ($p < 0.0001$) on the death of *L. monocytogenes* biofilms (Fig. 6).

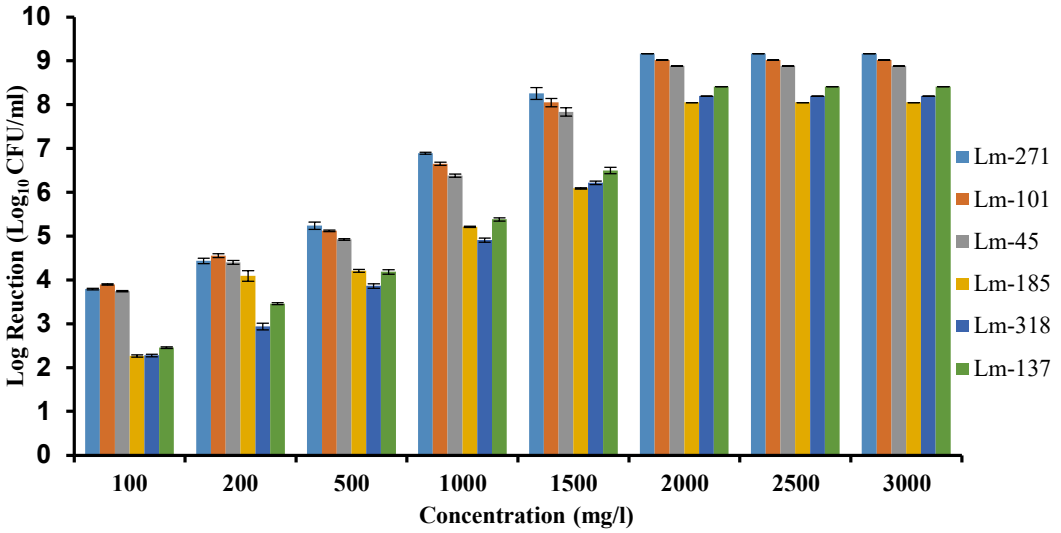

FIG. 6. Logarithmic reduction of biofilms formed by L. monocytogenes strains after 30 mins exposure to benzalkonium chloride.

L. monocytogenes remains a worldwide challenge for the food industry, regulatory agencies, and scientists across multiple fields. In 2017, the world's largest human listeriosis outbreak was reported in South Africa, with the primary source being processed meat [14]. Food production facilities are recognized as major transmission pathway, and for this study, a Nile crocodile meat processing facility was chosen because crocodile meat is now widely consumed throughout Zimbabwe. The prevalence of *L. monocytogenes* in crocodile meat is a cause of concern to the food industry and the export markets. Policies on the control of *L. monocytogenes* in ready-to-eat meat products have been effectively implemented in several countries [15]. Determination of the contamination sources for *L. monocytogenes* in food processing plants is helpful in better understanding of the epidemiology.

This study shows absence of *L. monocytogenes* in the receiving section. However, this could be attributed to proper and effective cleaning procedures applied. In contrast, *L. monocytogenes* was recovered from meat contact surfaces of machines and nonmeat contact surfaces of the environment. This could be attributed to the low efficacy of disinfectants, underdosing. infrequent application or short exposure times. The persistence of *L. monocytogenes* on grinding and cutting machines could also be attributed to the difficulty and improper implementation of cleaning and disinfection procedures on the machines due to the accumulation of meat residues and fats. Approaches to apply precise cleaning and disinfecting procedures that prevent the accumulation of organic matter are needed.

Genetically similar isolates from different processing sections and sample types suggest that a single *L. monocytogenes* subtype may be persisting and spreading throughout the facility. This suggests that contamination is not limited to a location or process, rather spread from receipt and handling of the raw material to packaging and storage. The persistence of a single subtype across the facility is likely due to the bacterium's ability to form biofilms, which makes it more resistant to cleaning and disinfection efforts [16]. Isolates from the crocodile meat processing facility demonstrated a strong biofilm-forming ability on polystyrene, a material commonly used as a food contact surface, highlighting that these surfaces can support *L. monocytogenes* attachment. Moreover, grooves and scratches in food contact surfaces can further enhance the formation of biofilms [17] which may explain persistence of the pathogen on machines, drains, and floors.

The biofilms formed by *L. monocytogenes* in the facility also showed resistance to benzalkonium chloride-based disinfectant and sodium hypochlorite, two widely used disinfectants in the food industry, even at manufacturer-recommended concentrations (50–350 mg/l for benzalkonium chloride-based disinfectant and 50–800 mg/l for sodium hypochlorite) [18]. This high level of biofilm resistance to biocides was likely due to the repeated application of sub-lethal biocide concentrations within the facility. The increased tolerance of *L. monocytogenes* biofilms to disinfectants contributes to the persistence of the pathogen. Furthermore, resistance to benzalkonium chloride based disinfectant has been reported in several studies, highlighting its growing ineffectiveness against some microorganisms [19]. Sodium hypochlorite acts on microbial membranes, oxidize sulfhydryl enzymes, hinders DNA synthesis, and inhibits protein synthesis [20]. However, as oxidizing compounds, they are readily rendered inactive depending on the availability of organic reducing material. Even at the highest concentration of 3000 mg/l used in the study, sodium hypochlorite could not eliminate all the viable

L. monocytogenes cells indicating biofilm resistance levels. Although sodium hypochlorite is a strong oxidizing agent, it has a limited diffusion ability in biofilms due to the biofilm organic layer, thus reducing its effectiveness.

Benzalkonium chloride based disinfectants are cationic surface-active agents that form electrostatic bonds with negatively charged bacterial membrane proteins, causing disruption of membrane integrity and leakage of cytoplasmic contents [21]. Although benzalkonium chloride-based disinfectant seemed to be more effective against biofilms, only higher concentrations above 200 mg/l eliminated all biofilms. However, such high concentrations are discouraged in food production. Increased efforts to ensure food security, and the production of refrigerated-food has created a higher demand for biocides in the food industry. However, the results of this study highlight the need for regular surveillance of biocide efficacy, especially in the case of those widely used in food production facilities.

The increased tolerance of biofilms to biocides used in disinfection procedures could be due to the genetic composition of the *L. monocytogenes* isolates. A study indicated that *L. monocytogenes* harbour genes that are known to contribute to biocide tolerance [22]. Members of the small multidrug resistance protein family were shown to be associated with reduced susceptibility to benzalkonium chloride based disinfectants [23]. Reactive chlorine has been shown to generate potentially carcinogenic chlorinated byproducts. Because of this, the use of chlorine has been drastically curtailed in other parts of the world, particularly in the European Union, and even in the USA, there are strict limits on levels of free chlorine in industrial waste streams [22]. New, environmentally friendly, safe and cost-effective biocides are needed along with innovative disinfection procedures.

This study, however, had some limitations. It did not investigate alternative disinfection methods beyond benzalkonium chloride and sodium hypochlorite, which leaves a gap in understanding the potential effectiveness of newer approaches, such as natural antimicrobials or bacteriophages. While the study noted the role of sublethal biocide doses in promoting resistance, it did not explore the genetic mechanisms behind this resistance, which could be crucial for developing more effective sanitation strategies. Additionally, the study lacked a thorough examination of critical environmental factors, such as temperature and humidity. These limitations highlight the need for further research to address these gaps and improve food safety practices.

4. CONCLUSIONS

The study successfully identified potential contamination sources and routes of transmission for *L. monocytogenes* in the crocodile meat processing line. The presence of genetically similar isolates across different sections of the facility indicates that *L. monocytogenes* may be spreading throughout the production process. This highlights the persistence of the pathogen, likely due to its ability to form biofilms on food contact surfaces such as polystyrene, which makes it more resistant to cleaning and disinfection. The study also revealed that biofilms of *L. monocytogenes* were resistant to commonly used disinfectants like benzalkonium chloride and sodium hypochlorite, raising concerns about the efficacy of current sanitation protocols.

To mitigate *L. monocytogenes* contamination in the crocodile meat processing facility, it is essential to enhance sanitation protocols by using higher concentrations of biocides, rotating disinfectants, and incorporating alternative strategies such as enzymatic cleaners and biofilm removal methods. Regular microbial monitoring need to be implemented, particularly in high risk areas prone to biofilm formation and wear, alongside proper maintenance of food contact surfaces. Additionally, improving equipment design to reduce crevices, ensuring the quality of treated water, and integrating these measures into the Hazard Analysis Critical Control Point plan will help systematically control risks of contamination. Staff require receive continuous training on hygiene practices and biofilm control, while a routine microbiological testing schedule is established. Exploring the use of bacteriophages as a biocide alternative, especially where resistance to chemical disinfectants like benzalkonium chloride based disinfectant and sodium hypochlorite has been observed, could provide a more effective microbial control strategy.

ACKNOWLEDGEMENTS

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EVALUATING THE DEPLETION OF AMOXICILLIN IN BROILER CHICKEN INTEGUMENTARY TISSUES

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Abstract

Unacceptable levels of drugs such as amoxicillin in animal products pose public health risks. For this reason, the 24th meeting of the Codex Alimentarius Committee on Residues of Veterinary Drugs in Foods (CCRVDF) identified the need to establish maximum residues levels (MRLs) for beta-lactams in chickens. In this context, the present study evaluated the persistence of amoxicillin in edible and non-edible chicken tissues. After a controlled study in treated broilers, amoxicillin was detected by LC-MS/MS throughout the post-treatment period at concentrations ranging from 28.63 µg/kg to 5.39 µg/kg in skin and fat, and a 5 day withdrawal time was established considering the maximum residue levels (MRLs) of the European Union (EU) of (50 µg/kg) as the cut point. In feathers, amoxicillin was detected at a concentration of 15.08 µg/kg on day 18 post-treatment, with an estimated persistence of 31 days considering the limit of quantification (LOQ) of (5 µg/kg) as cut point. These results suggest that skin and fat may act as a reservoir for residues, and feathers may pose a risk of reintroducing antimicrobials into the food chain hence the need for further research on the impact of feather processing on residue persistence. This study provides valuable scientific and regulatory insights into amoxicillin in chickens, contributing data to JECFA for establishing amoxicillin MRLs.

1. INTRODUCTION

In veterinary medicine, antimicrobials especially broad spectrum antimicrobials such as beta-lactams are one of the main therapeutic approaches for infectious diseases [1]. Among these, amoxicillin is a time-dependent antimicrobial with a broad spectrum of action against bacteria and is used in the poultry industry to control urinary, respiratory, and gastrointestinal tract infections [2–4]. While these drugs effectively treat infectious diseases, their use in food-producing animals can pose public health risks. The primary concern is the persistence of drug residues in animal products. This situation can result in several outcomes, such as promoting antimicrobial resistance and directly harming consumers and animals if residue concentrations exceed established MRLs [5, 6].

The poultry industry produces both edible and non-edible animal tissues, which are relevant in the context of residue presence and distribution. Feathers, categorized among the nonedible by-products, constitute approximately 7–10% of the bird's live weight [7]. They can be converted into meal or used as fertilizer, material for biodiesel, ingredients for bioplastics, and as feed for animals such as poultry, pigs, ruminants and fish [8]. It has been described that antimicrobials can bioaccumulate in chicken feathers, making them a pathway for residues to re-enter the food chain and the environment [8]. Alongside this study, various investigations have demonstrated the persistence of different antimicrobials in broiler chicken feathers, with higher concentrations for extended periods compared to other tissues.

This was the case for residues of flumequine, oxytetracycline (OTC) and 4-epi-oxytetracycline (4-epi-OTC) tylosin, sulfamethoxypyridazine and lincomycin [9–13]. On the other hand, skin plus fat in natural proportions represents an edible by-product of the poultry industry [14]. This product is consumed along with chicken meat directly [7]. According to data collected by the Centre for Nutritional Information on chicken meat, there are 32.4 grams of fat per 100 grams of skin [15]. This practice of consuming skin plus fat is highly significant due to studies confirming the presence of antimicrobial residues, including ampicillin, OTC, enrofloxacin and sulfadimidine in these matrices [16, 17]. In this context, a depletion study conducted on the skin plus fat of broiler chickens demonstrated that residues of enrofloxacin and its metabolite ciprofloxacin remain for longer periods after treatment compared to muscle, liver, and kidney of poultry [18]. Therefore, the ingestion of this tissue represents a potential route for the transfer of veterinary drug residues to humans.

Adverse effects of concern in humans for beta-lactams include allergic reactions in susceptible individuals and antimicrobial resistance. To minimize these risks, certain MRLs for veterinary drugs in animal products have been established by the Codex Alimentarius [6]. The European Union, through its legislation on the use of amoxicillin in production animals, has established an MRL of 50 µg/kg in edible tissues (muscle, liver, kidney and skin plus fat in natural proportions) and 4 µg/kg in milk, applicable to all food producing animals without specifying the species [19]. Meanwhile, the Codex Alimentarius specifies an MRL of 50 µg/kg for amoxicillin in

pigs, sheep, cattle and finfish, and an MRL of 50 µg/kg but does not specify limits for broilers [20]. It is for this reason that the CCRVDF, at its 24th meeting in 2018 [21] prioritized the establishment of MRLs for this antimicrobial in poultry. Feathers are not often tested for residues but may pose a risk in the food chain. In this context, it is important to determine the transfer and behaviour of beta-lactams in these animal tissues. Therefore, the objective of this study was to evaluate the persistence of amoxicillin in broiler skin plus fat and feather matrices.

2. MATERIALS AND METHODS

2.1. Experimental animals

The protocol for animal management and supervision was based on established directives and guidelines [19, 22]. The study was approved by the Institutional Animal Care and Use Committee (CICUA) of the University of Chile (Certificate No. 23643-VET-UCH). Male Ross 308 broiler chickens placed into Groups A and B, were housed, from the first day of life, in raised wire-floor batteries under controlled environmental conditions with *ad libitum* access to non-medicated feed and water. Group A was treated with a commercial formulation of 50% amoxicillin trihydrate at a therapeutic dose of 40 mg/kg for 7 consecutive days by oral gavage. Group B birds were not treated. Sample collections were conducted on days 1, 2, 5, 9, and 18 after treatment (5 sampling points).

2.2. Implementation of analytical methodologies

The analyses for the validation of methodologies and the subsequent determination of amoxicillin in broiler chicken tissues were carried out at the Veterinary Pharmacology Laboratory (FARMAVET) of the Faculty of Veterinary and Animal Sciences of the University of Chile (FAVET). The extraction methodology was developed based on a previously described method for skin and feather matrices [23]. These methods were optimized according to laboratory conditions.

2.2.1. Chemicals and reagents

The following chemicals were used: Water HPLC grade, acetonitrile HPLC grade, chloroform HPLC grade, hexane HPLC grade, formic acid HPLC grade, ammonia 25% HPLC grade, ammonium acetate HPLC grade, potassium dihydrogen phosphate HPLC grade, sodium phosphate dibasic HPLC grade, acetic acid p. a., Phosphate buffer at pH 7.5.

2.2.2. Standards and working solutions

The standard used for the analysis of experimental samples was amoxicillin (AMOX) of certified purity (96.7%). The isotopically labelled molecule, Amoxicillin-d4 (AMOX-d4) of 95% purity was used as internal standard. The intermediate solutions of AMOX and AMOX-d4 were prepared at a concentration of 2500 µg/ml in an acetonitrile (1:1) solution from a stock solution of 1000 µg/ml.

2.2.3. LC-MS/MS analysis

The skin plus fat matrix was analysed for AMOX using an HPCL-MS/MS following chromatographic separation on a C18 3.5 µm 2.1 × 150 mm column. The mobile phases consisted of 0.0032% ammonia in HPLC water and 0.0032% ammonia in HPLC acetonitrile/water 9:1 (v/v). Feathers were analysed using another UPLC-MS/MS after separation on a 1.8 µm 2.1 mm × 100 mm column. The mobile phases consisted of 0.1% formic acid in HPLC water and 0.1% formic acid in HPLC acetonitrile.

2.2.4. Sample extraction for skin plus fat and feathers matrices

For the extraction of AMOX from skin samples, 5 ± 0.1 g were weighed into a 50 ml polypropylene tube. Phosphate buffer (pH 7.5) and chloroform were added, followed by agitation, sonication, and centrifugation. The supernatant was filtered through glass wool, combined with a second batch of the phosphate extraction buffer, and then extracted with hexane. Samples were processed through columns conditioned with acetonitrile, water, and buffer, then eluted with methanol, dried under nitrogen at 30°C, and reconstituted with 0.01% formic acid and 0.2 mM ammonium acetate before filtering into vials using a 0.22 µm filter material. Feather extraction (2 ± 0.1 g) used only phosphate buffer, omitting secondary extraction.

2.2.5. Determination of amoxicillin in integumentary tissues

The day on which the amoxicillin concentration in the skin and feather matrices was equal to or below the MRL and the LOQ was identified, in accordance with the guidelines [24]. The time of antimicrobial residue depletion in the matrix was determined at a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Amoxicillin determination in broiler tissues

The withdrawal period for the skin matrix, based on a 95% confidence level and using the EU established MRL of 50 µg/kg, was determined to be 5 days. The depletion graph of the analyte AMOX in the skin matrix is shown in Fig 1. A slope of -0.3697, an intercept of 5.49 and an R^2 of 0.5811 were observed. The findings regarding feathers reveal a notable contrast with the skin matrix. Unlike skin samples, residues of the analyte were consistently detected and quantified across all sampling points.

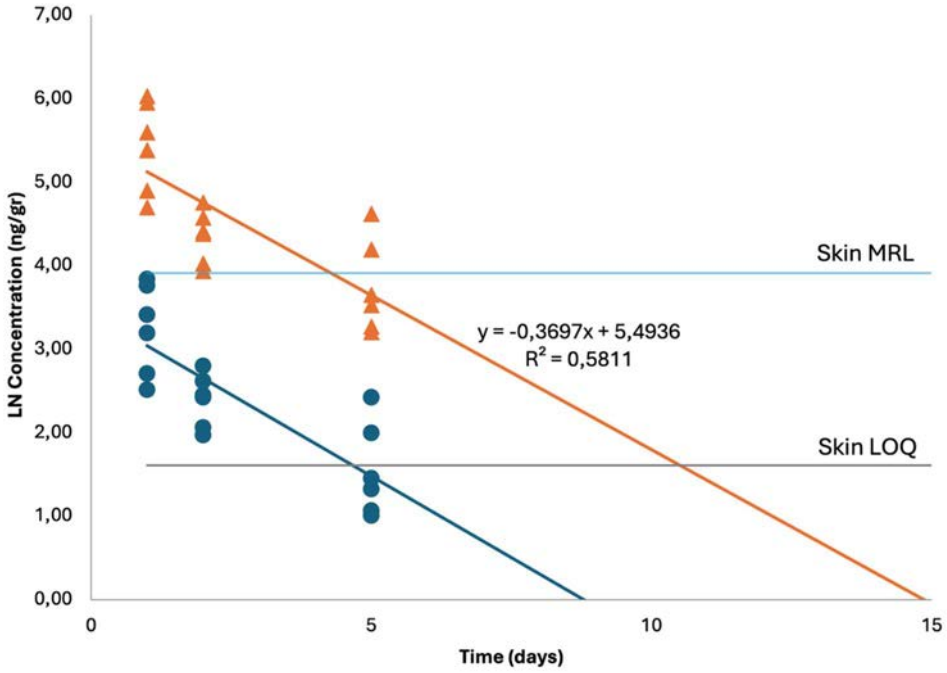

FIG. 1. Graph of amoxicillin depletion in skin plus fat. Concentrations of the analyte expressed in natural logarithm (Ln) in blue circles, and with 95% confidence in orange triangles. A line representing the MRL for skin plus fat was plotted in light blue, and the LOQ of the matrix was shown in grey.

The depletion time of AMOX in the feathers was determined at a confidence level of 95%. The MRL was stablished as the cutoff point, corresponding to a depletion period of 21 days. Additionally, using the method LOQ as a cutoff point, a withdrawal period of 31 days was determined (Fig. 2). The linear regression analysis conducted at a 95% confidence level had a slope of -0.226, an intercept of 8.49, and an R^2 value of 0.9234.

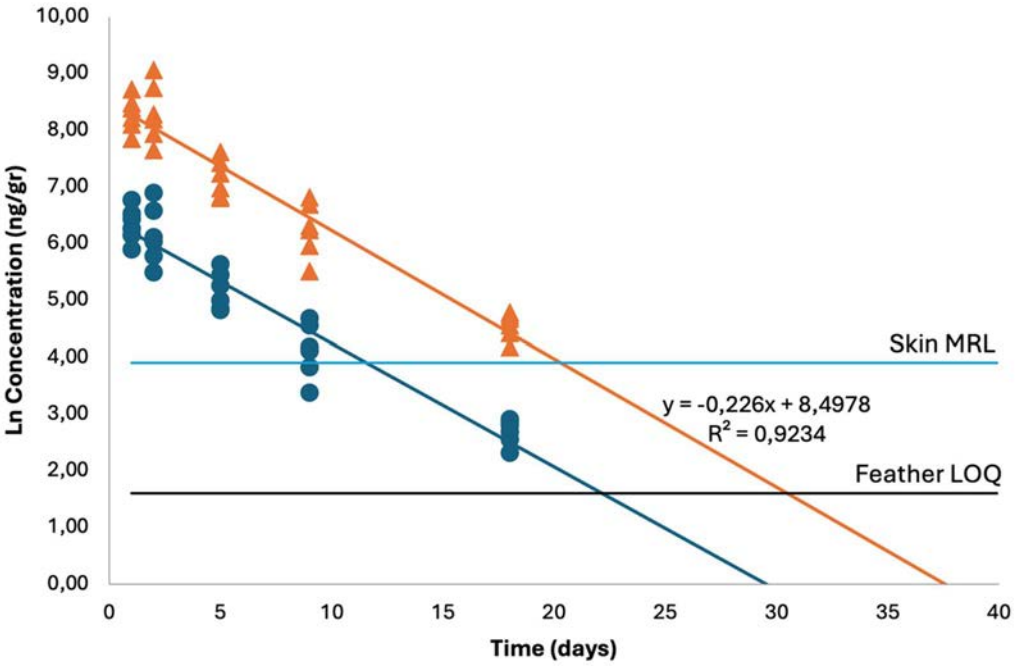

FIG. 2. Depletion graph of AMOX in feathers. Analyte concentrations expressed in blue circles, and with 95% confidence in orange triangles. A line representing the MRL for the skin plus fat was plotted in light blue, and the LOQ of the matrix was shown in grey.

3.2. Discussion

A depletion study was conducted using linear regression analysis with an upper tolerance limit of 95% confidence. From these results, a withdrawal period (WP) of 5 days was calculated for skin plus fat. This gradual decrease of the drug concentration agrees with findings from other studies on different analytes in this matrix. Residues of enrofloxacin and ciprofloxacin in the skin plus fat decreased more gradually compared to other tissues such as muscle, liver, and kidney [18]. Following oral administration of the antimicrobial at a dose of 10 mg/kg every 24 hours for 5 days, the WP for skin plus fat was determined to be 8 days, considering the European Union's MRL of 100 µg/kg. In a separate study [25] involving a group of birds that received orally a formulation of flumequine for 5 days, followed by 5 post-treatment samplings, lower concentrations of the drug were found in the skin compared to the kidney, liver, and muscle matrices analysed at the same sampling points over time, with no detection in the skin plus fat matrix from day 4 post-treatment. These findings suggest that the behaviour of antimicrobial residues and persistence can vary in skin plus fat depending on the physico-chemical characteristics of the analytes.

The high concentrations and the persistence of AMOX in feathers (21 days) could be attributed to different factors. First, the pharmacokinetic properties of AMOX's, including its low affinity to plasma proteins, result in widespread body distribution and a high intestinal absorption [26, 27]. Additionally, as feathers mature, the pulp that nourishes them is reabsorbed, which could further facilitate the residue retention in the feather matrix [11]. Another factor to consider is the potential external contamination of feathers from secretions of the uropygial gland that can contain antimicrobial residues. The uropygial gland secretes an oily substance that birds spread across their feathers during preening, providing waterproofing properties [28]. Although the use of feathers is not a real time indicator of the presence of residues in edible tissues, feathers can be an important non-invasive tool for controlling the use of prohibited drugs or drugs for off-label use in food animal production. These results lay the foundation for understanding the behaviour of amoxicillin in broiler chickens, and the persistence of these compounds in these tissues indicates that it is important to establish a continuous monitoring of beta-lactams in the poultry industry using various matrices.

4. CONCLUSIONS

The study offers a significant scientific contribution to JECFA and Codex Alimentarius for setting MRLs for amoxicillin in broiler edible tissues. It was observed that amoxicillin residues remain longer in feathers than in skin and fat in natural proportions. Therefore, feathers showed potential for bioaccumulating antimicrobials, suggesting that they could be a useful non-invasive tool for detecting veterinary drugs. Further research on feather

meal is encouraged to assess the impact of processing on the persistence of amoxicillin or other residues of importance.

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PREVALENCE OF SULFONAMIDES, BETA-LACTAMS, *SALMONELLA*, *STAPHYLOCOCCUS AUREUS* IN RAW MILK AND IRRADIATION EFFECT ON ANTIMICROBIAL-RESISTANT BACTERIA AND DEGRADATION OF ANTIMICROBIAL RESIDUES

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Abstract

The extensive use of antimicrobials in animal production can lead to the accumulation of residues in foods of animal origin and the emergence of antimicrobial resistant pathogenic bacteria, potentially causing negative impacts on human health and the environment. The present work aimed to detect the presence of sulfonamides and the β-lactam penicillin G residues in 50 samples of raw milk intended for consumption by using the Charm II radioreceptor assay (RRA) test, and to evaluate the presence of *Salmonella* and *Staphylococcus aureus* and decrease their resistance to the detected antimicrobials. The possibility of degradation of these antimicrobials by gamma irradiation was also investigated. The results indicated that sulfonamides were detected in 40 (80%) raw milk samples tested, while β-lactams (penicillin G) were detected in only 25 (50%) samples. *Salmonella* was isolated from 10 (20%) samples, while *S. aureus* was isolated from 23 (46%) samples. Out of 10 *Salmonella* isolates, four were resistant to sulfonamides. Additionally, of the 23 *S. aureus* isolates, seven were resistant to sulfonamides, and five were resistant to β-lactams (penicillin G). All gamma irradiation-absorbed doses (0.75 KGy, 1.5 kGy and 2.25 kGy) increased the sensitivity of the examined antimicrobial-resistant pathogenic bacteria. To use gamma irradiation for the degradation of antimicrobials, raw whole milk samples were individually spiked with sulfonamides and penicillin G. Analysis by HPLC indicated that gamma irradiation doses of 2.25 kGy greatly degraded the concentrations of sulfonamides (201 µg/l) and penicillin G (56.6 µg/l) spiked in raw milk by 87.0% and 42.3%, respectively.

1. INTRODUCTION

Antimicrobials are used in animal production for the treatment of bacterial and other infections or for preventing diseases and as growth promotors. The misuse of antimicrobials can lead to the accumulation of their residues in foods such as meat, poultry, milk, and eggs, with potential for negative impacts on human health and the environment. The residues and drugs used can also lead to the growing emergence of antimicrobial resistant pathogenic bacteria, severely risking public health. The accumulation of antimicrobial residues in edible food causes allergic and toxic effects in consumers, and the growth of antimicrobial resistant pathogenic bacteria [1–4].

Sulfonamides and β-lactams are the most commonly used antimicrobial in animal production because they have a broad spectrum of activity against most Gram positive and Gram negative bacteria [5, 6]. Also, *Salmonella* spp. and *S. aureus* are the most common and frequent foodborne pathogenic bacteria present in many foods including meats, fish, milk, and eggs, causing several cases of infectious diseases and food poisoning in humans [7, 8). Sulfonamides, β-lactams, *Salmonella,* and *S. aureus* is frequently found in milk and milk products [9, 10]. The presence of both antimicrobial residues and foodborne-pathogenic bacteria in milk and milk products is a greater consumer health concern.

Accordingly, accurate and rapid detection of antimicrobial residues in milk intended for human consumption is very important for controlling the emergence and spread of antimicrobial resistant bacteria. Several physiochemical, HPLC, immunological, Enzyme Linked Immuno-Sorbent Assay (ELISA) and microbiological (growth inhibition disk) methods have been used for the detection and quantification of antimicrobials in food [11, 12]. Although most of these methods are accurate and can be sensitive, they require a long time for extraction and cleanup or preconditioning before measurements and are thus costly and laborious [13]. The Charm II test is a rapid and robust radioreceptor assay technique the detection of different compounds in food products, including antimicrobials, aflatoxins, and pesticides, among others, within 20 mins [14–16]. This approach involves the use of ^{3}H or ^{14}C-labelled radiotracers that compete for the binding sites (receptor sites) along with a liquid scintillation counter which measures the amount of tracer on the binding agent and compares it with that at the control point (CP) [11, 17].

Ionizing radiation in the form of gamma radiation, electron beams, and X rays has been widely used in recent years for extending the shelf life of perishable food, reducing, or eliminating foodborne pathogens, and increasing the susceptibility of antimicrobial resistant bacteria to antimicrobial agents [18–20]. Irradiation of whole milk at doses ranging from 1 kGy to 2.5 kGy improves its microbiological properties by significantly reducing bacterial loads, inhibiting the growth of pathogenic and spoilage microorganisms, and maintaining quality during storage, with minimal impact on sensory characteristics and organoleptic properties, meeting safety requirements and complying with TRTS 033/2013. These requirements include microorganism levels, nutritional value, marking and labelling, packaging requirements, conformity assessment, and production and storage conditions for milk and dairy products to ensure that products are safe for consumption and meet health standards [21–23].

Irradiation has been developed as a promising advanced oxidation process, which is based on strong oxidizing hydroxyl radical ($OH^{\bullet}$) proven to be effective in the partial or complete degradation of antimicrobials in aqueous solutions, depending on the irradiation dose, type and concentration of antimicrobial, and matrices (Eq. (1)). The effectiveness of ionizing irradiation on the degradation of antimicrobials either in aqueous solutions or in food matrices could be explained by the generation of simultaneously highly oxidative and reductive species upon water radiolysis by irradiation which play the most important role in the decomposition of antimicrobials [24, 25].

$$H_2O \xrightarrow{\text{Gamma Radiation}} (2.6)\, e^-_{aq} + (2.7)\, OH^{\bullet} + (0.55)\, H^{\bullet} + (2.6)\, H_3O^+ + (0.71)\, H_2O_2 + (0.45)\, H_2 \quad (1)$$

Many studies have been done on the degradation of antimicrobials in aqueous solutions by irradiation [26–28]. However, very few studies have been conducted on the effectiveness of ionizing radiation on the degradation and decomposition of antimicrobials in food matrices, therefore, the aim of this study was to investigate the efficiency of gamma irradiation on the degradation of sulfonamides and penicillin G in raw milk. The degradation of antibiotic residues in food and the sensitization of antimicrobial foodborne pathogenic bacteria are the most important factors for food safety and human health. Thus, the objectives of the present study were to: 1) evaluate the presence of sulfonamide and β-lactam residues in raw milk; 2) isolate antimicrobial resistant *Salmonella* and *S. aureus* from raw milk samples; 3) sensitize the pathogens to sulfonamides and β-lactams by gamma irradiation; and 4) assess the possibility of using gamma irradiation technology to reduce these antimicrobial residues.

2. MATERIALS AND METHODS

2.1. Samples

A total of 50 raw milk samples (250 ml each) intended for human consumption were randomly collected from retail dairy product markets at different locations in Cairo. These samples were placed in sterilized polyethylene bags and transported in an ice box to the Food Microbiology Laboratory at the National Center for Radiation and Technology (NCRRT) within 2 hours. First, the polyethylene bags of the raw milk samples were aseptically opened, and 25 ml was mixed well with 225 ml of buffer peptone water for bacterial isolation. The remaining milk samples were used for the Charm II test.

2.2. Charm II procedure

2.2.1. Reagents

The antimicrobial residue test kits used contained tablet reagent receptor binders or radiotracers at less than 1.85 kBq ^{3}H for sulfonamide drug detection in milk, and the specific tablet reagent receptor binders or radiotracers containing less than 0.2 kBq ^{14}C for β-lactam drug detection in milk.

2.3.2. Charm II analyser

The Charm II 7600 system was used for the detection of sulfonamides and β-lactams in 50 raw milk samples according to the operator's manual instructions. In brief, a specific amount of the tracer radiolabelled antimicrobial was added to a binding reagent, followed by centrifugation for removal of fat and resuspension in scintillation fluid. The amount of tracer that binds to the receptor sites was counted per minute (cpm) and compared to the CP. Interpretation of the results: The negative control (samples that did not contain antimicrobials) had a cpm value greater than the CP, while the positive samples (containing antimicrobials) had a cpm value less than the CP.

2.3. Isolation of *Salmonella* and *S. aureus*

Milk samples (25 ml each) were mixed with 225 buffer peptone water (BPW) pre-enrichment medium and then incubated at one day 37°C. For *Salmonella* isolation, 1 ml of each milk pre-enrichment medium mixture was transferred to a 10 ml selenite broth tube and incubated again for one day at 37°C. Then, a loopful of each culture was streaked on the surface of a brilliant green agar plate and incubated at 37°C for 24 h. Presumptive *Salmonella* colonies appearing pink or red and surrounded by a zone of bright red were isolated. The identification of *Salmonella* isolates was based on culture characteristics, Gram staining, and biochemical tests (triple sugar iron agar, urea agar base, L-lysine decarboxylase and Voges-Proskauer tests) in accordance with ISO 6579-1993 [29].

To isolate *S. aureus*, a loopful of each inoculated isolate in BPW pre-enrichment medium that was incubated at 37°C for 24 h, was streaked on a prepared selective agar plate and incubated at 37°C for 24 h. Presumptive *S. aureus* colonies appeared to be jet black and had a white, clear halo zone surrounding them. The pure culture of each presumptive colony was streaked on nutrient agar slants and incubated at 37°C for 24 h for further characterization by Gram staining and biochemical tests including catalase test, coagulase test, oxidase test [9].

2.4 Preparation of cell suspensions for irradiation

Each bacterial isolate was inoculated in 150 ml of nutrient broth and incubated at 37°C for 24 h. Then, 10 ml of each culture was transferred to a sterilized test tube. The test tubes containing the cell suspensions were irradiated at 0.75 kGy, 1.5 kGy, and 2.25 kGy. Another 10 ml of cell suspension for each culture was not irradiation and served as a control (in triplicate for each dose). The irradiation process was performed at ambient temperature.

2.5. Sensitivity to sulfonamides and β-lactams

Cultures of sulfonamide-resistant *Salmonella*, sulfonamide-resistant *S. aureus,* and β-lactam resistant *S. aureus* isolates were grown in tryptone soy broth (TSB) at 37°C for 24 h with agitation. After that 10 ml of each culture suspension (in triplicate) was placed in a test tube and irradiated as follows: 0/control, 0.75 kGy, 1.5 kGy, and 2.25 kGy. After gamma irradiation, each culture was retested for susceptibility to the relevant antimicrobials [30].

2.6. Susceptibility tests

The antimicrobial susceptibility tests were performed by the disk diffusion method where antimicrobial-impregnated paper discs are placed on an agar plate inoculated with the bacterial strain under investigation. The effectiveness of each antimicrobial is determined by measuring the diameter of the zone of inhibition around each disc. These zones are then compared to the Performance Standard for Antimicrobial Disk Susceptibility Testing (CLSI-M100-Ed32 performance standard) [30] to classify the bacteria as susceptible, intermediate, or resistant to each antibiotic tested. In this experiment, antimicrobial susceptibility was tested using sulfonamides 300 µg, and bioanalysis penicillin G 10U.

2.8. Preparation of standard solutions and spiked milk samples

Twenty five milligrams of sulfonamides and penicillin G standards were dissolved in 10 ml of deionized water to a concentration of 2500 µg/ml. One millilitre of each antimicrobial standard solution was spiked into 9 ml of raw milk samples in 20 ml test tubes to give a final concentration of 250 µg/ml, followed by shaking for 5 min. The antimicrobial spiked milk samples were gamma-irradiated at absorbed doses of 0.75 kGy, 1.5 kGy, and 2.25 kGy. Another spiked milk sample was left without irradiation to serve as a control for comparison. Triplicate samples were used.

2.9. Extraction of sulfonamides from spiked milk samples

Spiked raw milk sample (2 ml) was placed in a 15 ml centrifuge tube and mixed well with 8.0 ml of acetonitrile, stirred, extracted for 10 min to denature proteins, and spined on a centrifuge at 14000 rpm for 5 min. An aliquot of the supernatant (6 ml) was evaporated under nitrogen flow and the remnant dissolved in 0.6 ml of 0.2 M ammonium acetate, filtered through 0.22 µm membrane syringe filters and 20 µl was injected into the HPLC for analysis as indicated elsewhere [31].

2.10. Extraction of penicillin G from spiked milk samples

Two millilitres of each milk sample were transferred to a 15 ml centrifuge tube, thoroughly vortexed, and incubated at 25°C for 3 h under mild agitation. Then, 8 ml of acetonitrile was added to allow sufficient precipitation of proteins. This mixture was centrifuged at 14000 rpm for 30 min at 25°C and then filtered through a 0.22 µm membrane filter. An aliquot of 20 µl of the resultant solution was injected into the HPLC column to determine the remaining concentrations of penicillin G [32].

2.11. Degradation of antimicrobials

2.11.1. HPLC analysis

The items used included sulfonamide standards; deionized ultrafiltration water from a local purification system; and HPLC grade, acetonitrile, methanol, ethyl acetate, ethanol, acetone, and n–hexane. Antimicrobial residue analyses were performed on an HPLC consisting of an automatic micro vacuum degasser, a quaternary pump, an autosampler, and a UV-fluorescence detector. A C18 column (4.6 mm ID × 250 mm, 80A 1.8 µm particle size) kept at 40–60°C was used to separate the analytes.

For sulfonamides, the mobile phase consisting of solutions A (acetonitrile 60%) and B (H_2O 40%) run at a flow rate of 0.8 ml/min. The column temperature was 40°C, and the detector (λ) was set at 250 nm. For penicillin G, the mobile phases consisting of solutions A (acetonitrile 60%) and B (H_2O 40%) were run at a flow rate of 1.0 ml/min. The column temperature was maintained at 40°C, and the detector (λ) was set at 220 nm. The injected sample volume was 20 µl. The correlation coefficient R of the linear relationship of the peak area against the individual antimicrobial standard concentration was calculated after three injections.

2.12. Irradiation process and statistical analysis

All irradiation processes were achieved using a ^{60}Co gamma facility at the NCRRT, EAEA, Nasr City, Cairo, Egypt. The dose rate of this source at the time of irradiation was 0.717 kGy/h, and irradiation processes were conducted at room temperature. Alanine dosimeters were used for measuring the absorbed dose. Detailed dose mapping was carried out by the Department of Radiation Protection and Dosimeter according to Egyptian Standards. All the experiments were performed in triplicate, and the standard deviation (SD) was calculated for all mean values. A one-way ANOVA was used. Differences between treatments were considered significant at $p \leq 0.05$ according to Tukey's test for multiple comparisons. Figures were plotted using an appropriate software. Error bars in figures represent standard errors.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial residues

The results obtained indicated that of the 50 raw milk samples tested, 40 (80%) were positive for sulfonamides and only 25 (50%) were positive for β-lactams (Tables 1 and 2) as detected by the Charm II test.

TABLE 1. CHARM II TEST-RESULTS FOR SULFONAMIDES IN 50 RAW MILK IN COUNTS PER MINIUTE (CPM)

Sample No.	Sulfonamides CP=2406			Sample No.	Sulfonamides CP=2406		
	Mean 3 samples, (Average of 3 replicate)		St Dev±		Mean 3 samples, (Average of 3 replicate)		St Dev±
1	296	+ve	15.10	26	868	+ve	32.0
2	2621	-ve	52.8	27	2800	-ve	78.0
3	2753	-ve	81.5	28	459	+ve	11.50
4	245	+ve	11.14	29	2970	+ve	34.6
5	337	+ve	17.09	30	569	+ve	6.00
6	351	+ve	9.07	31	2601	-ve	61.1
7	776	+ve	50.0	32	2711	-ve	92.1
8	816	+ve	35.6	33	1897	+ve	47.0
9	860	+ve	17.00	34	648	+ve	10.58
10	402	+ve	4.36	35	983	+ve	17.00
11	352	+ve	18.0	36	2016	+ve	2598
12	303	+ve	3.46	37	879	+ve	22.6
13	501	+ve	25.6	38	2912	-ve	137.5
14	471	+ve	10.54	39	2616	-ve	64.1
15	638	+ve	19.0	40	1708	+ve	7.51
16	894	+ve	39.3	41	309	+ve	4.36
17	413	+ve	6.24	42	787	+ve	68.7

Sample No.	Sulfonamides CP=2406			Sample No.	Sulfonamides CP=2406		
18	1405	+ve	49.5	43	1056	+ve	7.94
19	908	+ve	6.24	44	2808	-ve	19.3
20	760	+ve	20.0	45	1601	+ve	32.0
21	3619	-ve	93.6	46	687	+ve	14.73
22	1234	+ve	5.03	47	1609	+ve	96.5
23	1917	+ve	67.0	48	516	+ve	26.0
24	2922	-ve	68.5	49	976	+ve	18.3
25	518	+ve	12.12	50	987	+ve	10.00

Note: *The control point (CP) is the average of six standard readings. Samples with counts/minute (cpm) values greater than the CP value are considered negative (-ve) and if equal to or less than the CP, as positive (+ve) The target detection level the sulfonamide was 10 ppb sulfamethazine. The CP is the average of six standard readings.*

TABLE 2. CHARM II TEST RESULTS FOR PENICILLIN G IN 50 RAW MILK SAMPLES

Sample No.	Beta-lactams CP = 1030			Sample No.	Beta-lactams CP = 1030		
	Mean 3 samples, (Average of 3 replicate)		St Dev±		Mean 3 samples, (Average of 3 replicate)		St Dev±
1	516	+ve	25.1	26	1255	-ve	99.3
2	600	+ve	31.6	27	2534	-ve	127.0
3	136	+ve	2.00	28	568	+ve	9.17
4	171	+ve	2.65	29	1009	+ve	28.2
5	627	+ve	14.73	30	2655	-ve	98.9
6	879	+ve	57.6	31	688	+ve	13.50
7	2305	-ve	406	32	543	+ve	11.00
8	1987	-ve	298	33	226	+ve	1.000
9	1218	-ve	207	34	903	+ve	18.0
10	1305	-ve	220	35	1420	-ve	132.1
11	3100	-ve	509	36	718	+ve	16.37
12	2298	-ve	406	37	410	+ve	4.36
13	1534	-ve	169.1	38	1717	-ve	100.6
14	1375	-ve	125.0	39	532	+ve	5.00
15	194	+ve	16.00	40	917	+ve	19.7
16	1428	-ve	122.9	41	2105	-ve	84.5
17	418	+ve	7.51	42	2001	-ve	607
18	393	+ve	4.00	43	1820	-ve	104.1
19	1907	-ve	104.4	44	2002	-ve	155.2
20	1511	-ve	151.0	45	1521	-ve	78.6
21	797	+ve	32.0	46	1793	-ve	108.5
22	446	+ve	5.29	47	1521	-ve	60.0
23	603	+ve	41.6	48	726	+ve	22.0
24	3917	-ve	610	49	978	+ve	25.0
25	1320	-ve	194	50	631	+ve	5.29

Note: *The CP is the average of six standard readings. Samples with cpm values greater than the CP value are considered negative (-ve) for the drug and if equal to or less than the CP, positive (+ve); The target detection level for the β-lactams was 50 ppb of penicillin G.*

3.2. Isolation of *Salmonella* and *S. aureus*

Out of the 50 raw milk samples, *Salmonella* and *S. aureus*. *Salmonella* was isolated from 10 (20%) samples (Table 3) while *S. aureus* was isolated from 23 (46%) samples; among them, only 8 isolates were isolated from milk samples that were positive for β-lactams (Tables 3 and 6). *Salmonella* and *S. aureus* was isolated from only 6 (12%) samples. It could be concluded that *Salmonella* and *S. aureus* was found in 27 (54%) of the 50 raw milk samples tested, while 23 (46%) samples were free of *Salmonella* and *S. aureus.*

3.3. Susceptibility tests

The susceptibilities of the 10 *Salmonella* isolates to sulfonamides were tested. Table 4 shows isolates that were sensitive (4) intermediate (2) and resistant (4) to sulfonamides, respectively.

TABLE 3. *SALMONELLA SPP*. AND *S. AUREUS* ISOLATED FROM TESTED RAW MILK SAMPLES

Sample No.	Salmonella spp	S. aureus	Sample No.	Salmonella spp	S. aureus
1	+	+	29	—	+
3	+	—	32	—	+
4	+	+	33	—	+
5	—	+	34	—	+
12	+	+	36	—	+
13	—	+	37	+	—
14	—	+	39	—	+
15	+	+	42	—	+
16	—	+	44	—	+
18	+	+	45	+	—
19	—	+	47	—	+
20	—	+	48	+	—
21	+	+	49	—	+
28	—	+			

TABLE 4. SUSCEPTIBILITY TEST OF THE TEN *SALMONELLA* SPP ISOLATES AGAINST SULFONAMIDES

Isolates No.[a]	(S) ≥ 17 mm	(I) 13–16 mm	Disc content (300µg) (R) ≤ 12 mm
1	—	—	8±1
3	—	15±1	—
4	—	—	10±1
12	—	14±1	—
15	30±3	—	—
18	20±2	—	—
21	28±3	—	—
37	24±2	—	—
45	—	—	9±1
48	—	—	6±0

[a]*Isolate-number refers to the number of samples from which it was isolated; —: nothing*

The susceptibilities of the 23 *S. aureus* isolates to sulfonamides were also tested. Table 5 shows isolates that were sensitive (11) intermediate (5) and resistant (7) to sulfonamides, respectively.

TABLE 5. SUSCEPTIBILITY TEST OF TWENTY THREE *S. AUREUS* ISOLATES AGAINST SULFONAMIDES

Isolates No. [a]	(S) ≥ 17 mm	(I) 13-16 mm	Disc content (300 µg) (S) ≤ 12 mm
1	—	—	6±0
4	33±3	—	—
5	—	13±1	—
12	19±1	—	—
13	—	—	3±0
14	22±2	—	—
15	20±2	—	—
16	—	—	12±2
18	—	16±2	—
19	—	—	2±0
20	17±1	—	—
21	—	13±1	—
28	24±2	—	—
29	26±2	—	—

Isolates No. [a]	Disc content (300 µg)		
	(S) ≥ 17 mm	(I) 13-16 mm	(S) ≤ 12 mm
32	—	—	10±2
33	—	14±1	—
34	—	—	3±0
36	26±2	—	—
39	—	14±1	—
42	—	—	8±1
44	28±2	—	—
47	20±2	—	—
49	18±2	—	—

[a] *Isolate-number refers to the number of samples from which it was isolated; —: nothing*

The susceptibility tests of the 8 *S. aureus* isolates from only β-lactam positive milk samples to penicillin G were obtained. Table 6 indicates that 3 isolates were sensitive, and 5 isolates were resistant to β-lactam (referred to as penicillin G).

TABLE 6. SUSCEPTIBILITY TEST OF EIGHT *S. AUREUS* ISOLATES ISOLATED FROM BETA-LACTAM POSITIVE RAW MILK

Isolates No. [a]	Disc content (10 U) penicillin G	
	(S) ≥ 29 mm	(R) ≤ 28 mm
1	—	22±2
4	—	19±2
5	36±2	—
15	—	27±2
21	—	20±1
29	—	15±0
34	38±3	—
49	33±3	—

[a] *Isolates number refers to the number of samples from which it was isolated; —: nothing*

3.4. Effect of gamma irradiation on bacterial susceptibilities

The four sulfonamide-resistant *Salmonella* isolates, the seven sulfonamide-resistant *S. aureus* isolates, and the 5 β-lactam resistant *S. aureus* isolates were exposed to different gamma irradiation doses (0.75 kGy, 1.5 kGy and 2.25 kGy). All of these irradiated isolates were retested for their susceptibility to related antimicrobials. Figure 1 indicates that all irradiation doses used increased the sensitivity of the four *Salmonella* isolates, but at different levels, and the sensitivity increased with the irradiation doses. For example, irradiation doses of 0.75 kGy, 1.5 kGy and 2.25 kGy increased the sensitivity of isolate No. 1 by 25%, 62.5%, and 125%, respectively, while these doses increased the sensitivity of isolate No. 4 by 20%, 70%, and 110%, respectively, compared to the unirradiated control. This means that the sensitization of pathogenic bacteria to antibiotics by irradiation depends on the irradiation dose used and on the bacterial strain. The highest sensitivities of all tested isolates (four isolates) were recorded with the highest irradiation dose used (2.25 kGy).

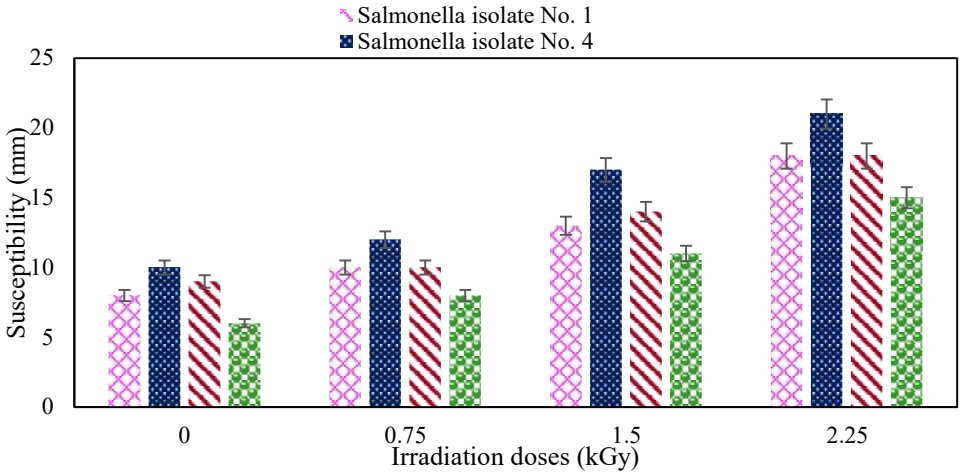

FIG. 1. Effect of gamma irradiation on the susceptibility (mm) of sulfonamide-resistant Salmonella.

The cell suspensions of the seven sulfonamide resistant *S. aureus* were exposed to the same dose of gamma radiation (0.75 kGy, 1.5 kGy and 2.25 kGy) to investigate the effect of these doses on susceptibility to sulfonamides. The results showed that the susceptibility of the six isolates increased as the irradiation doses increased (Fig. 2) while the susceptibility of only one isolate (isolate No. 32) was not affected by any of the irradiation doses used but was still constant. Compared with those of the control, the highest irradiation dose used (2.25 kGy) increased the sensitivity of isolates No. 1, 13, 16, 19, 34 and 41 by 150%, 467%, 67%, 550%, 100%, and 138%, respectively. This means the resistance of *S. aureus* isolates No. 13 and 19 to sulfonamides greatly decreased after irradiation at 2.25 kGy.

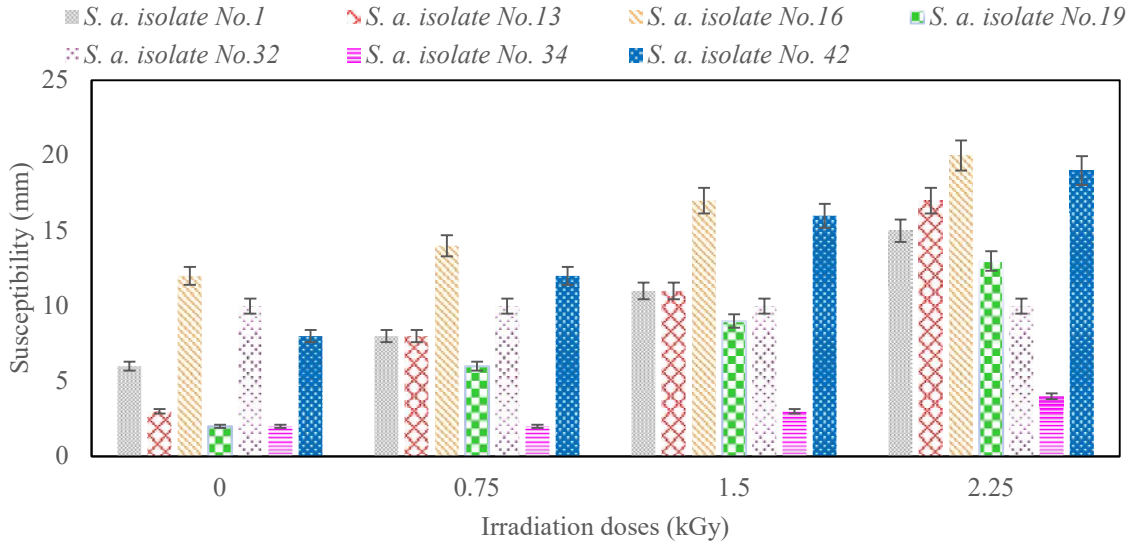

FIG. 2. Effect of gamma irradiation on the susceptibility (mm) of sulfonamide-resistant S. aureus.

The effect of gamma radiation on the susceptibility of β-lactam resistant *S. aureus* (five isolates) against β-lactams (referred to as penicillin G) was also investigated. Figure 3 shows that all irradiation doses used (0.75 kGy, 1.5 kGy and 2.25 kGy) increased the sensitivity of the examined isolates and the increase was proportional to the irradiation dose. An irradiation dose of 2.25 kGy increased the sensitivity of β-lactam resistant *S. aureus* isolates (Nos. 1, 4, 15, 22 and 29) by 50 %, 58 %, 44 %, 31 %, and 80%, respectively. It is clear from the results in Figs 2 and 3 that irradiation treatment could increase the susceptibility of *S. aureus* isolates against sulfonamides much more than against β-lactams.

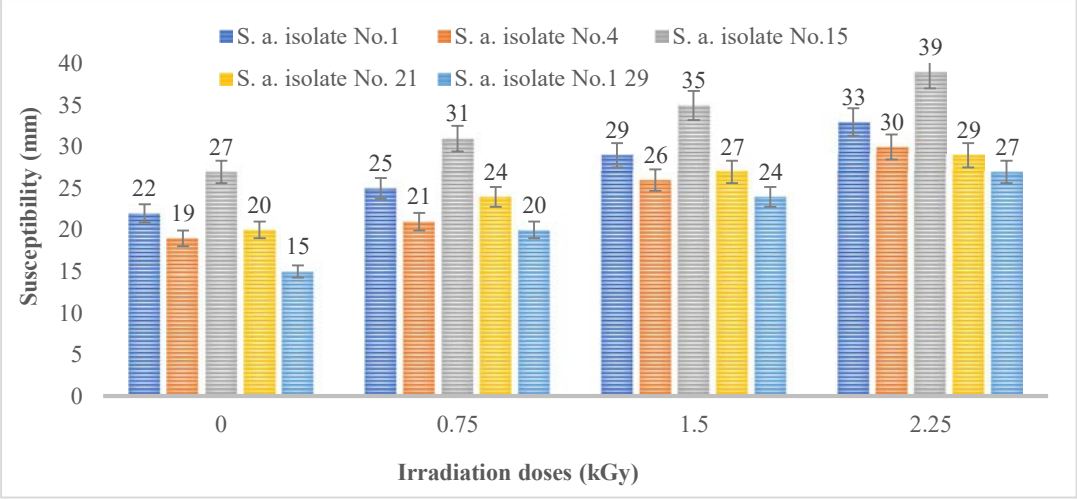

FIG. 3. Effect of gamma irradiation on the susceptibility test of β-lactam resistant staphylococcus aureus isolates.

3.5. Effect of gamma radiation on the degradation of antimicrobials

The effects of different irradiation doses (0.75 kGy, 1.25 kGy and 2.25 kGy) on the degradation of sulfonamides and penicillin G in spiked raw milk were investigated. Figure 4 shows that doses of 0.75 kGy, 1.5 kGy and 2.25 kGy reduced the concentration of sulfonamides (201 µg/l) by 20.9 %, 58.8 % and 87.0%, and the concentration of penicillin G (56.6 µg/l) by only 6.7 %, 22.6 % and 42.3%, respectively.

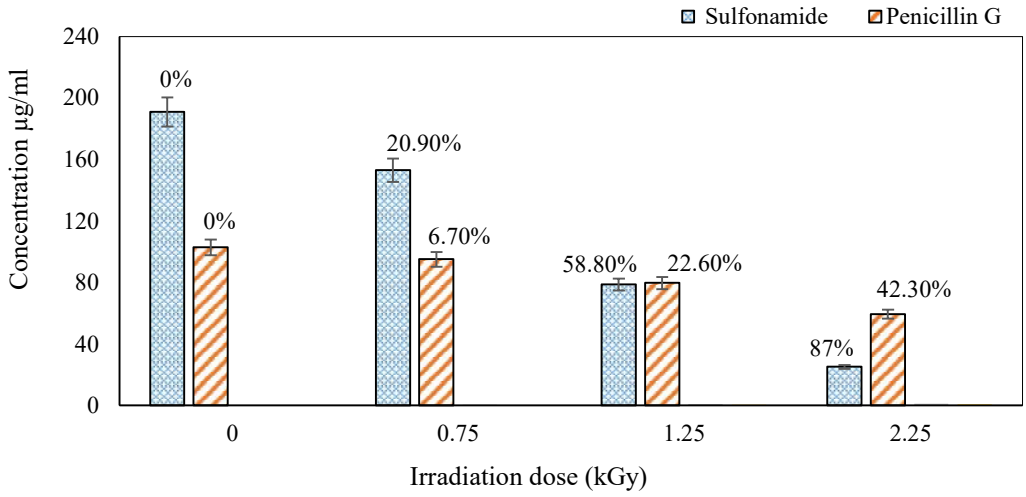

FIG. 4. Effect of gamma irradiation on the degradation of sulfonamides and penicillin G in raw milk.

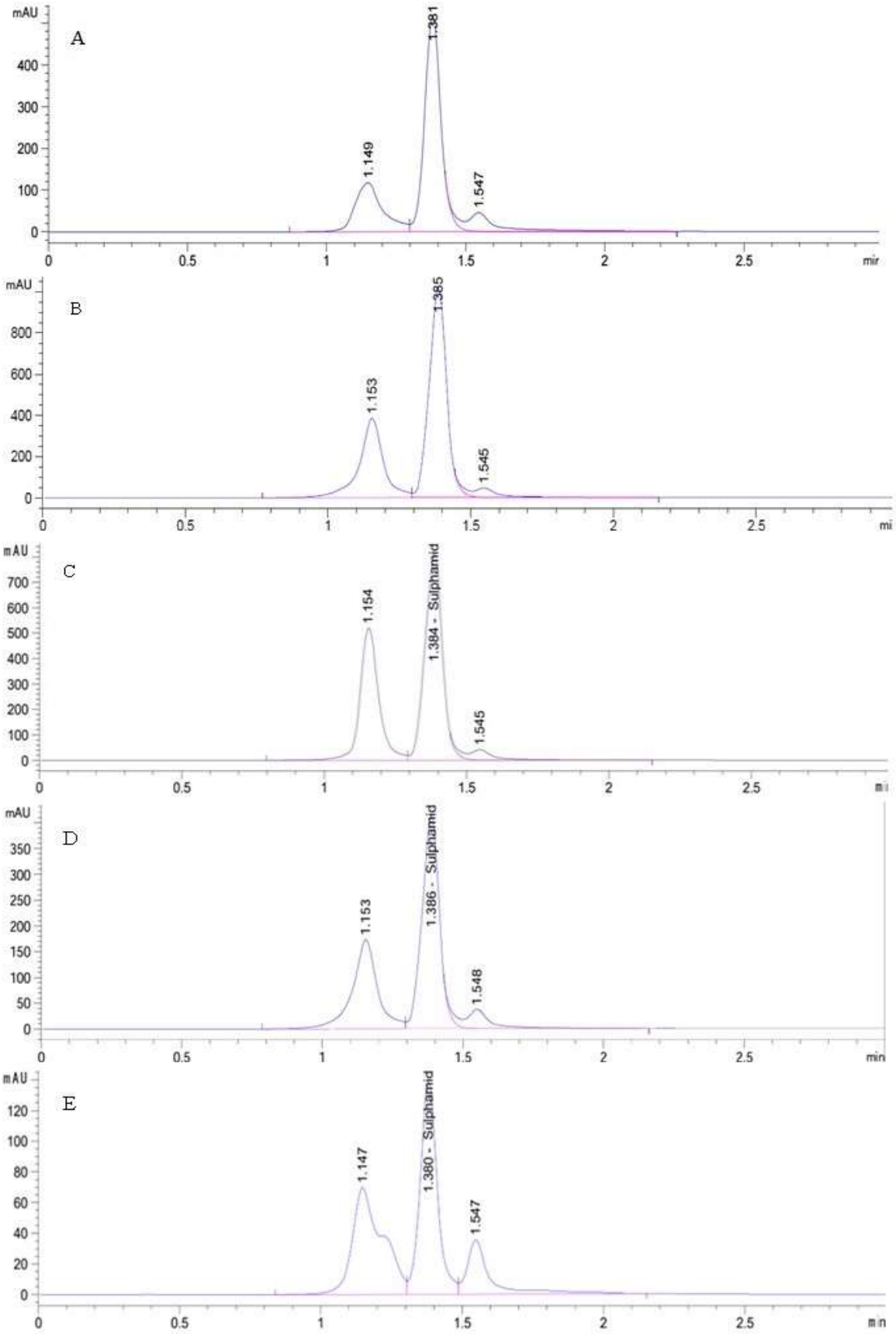

FIG. 5. HPLC chromatograph of sulfonamides standard in deionized water (A); unirradiated milk sample (control) (B) milk sample irradiated at 0.75 kGy (C); milk sample irradiated at 1.25 kGy (D); milk sample irradiated at 2.25 kGy (E).

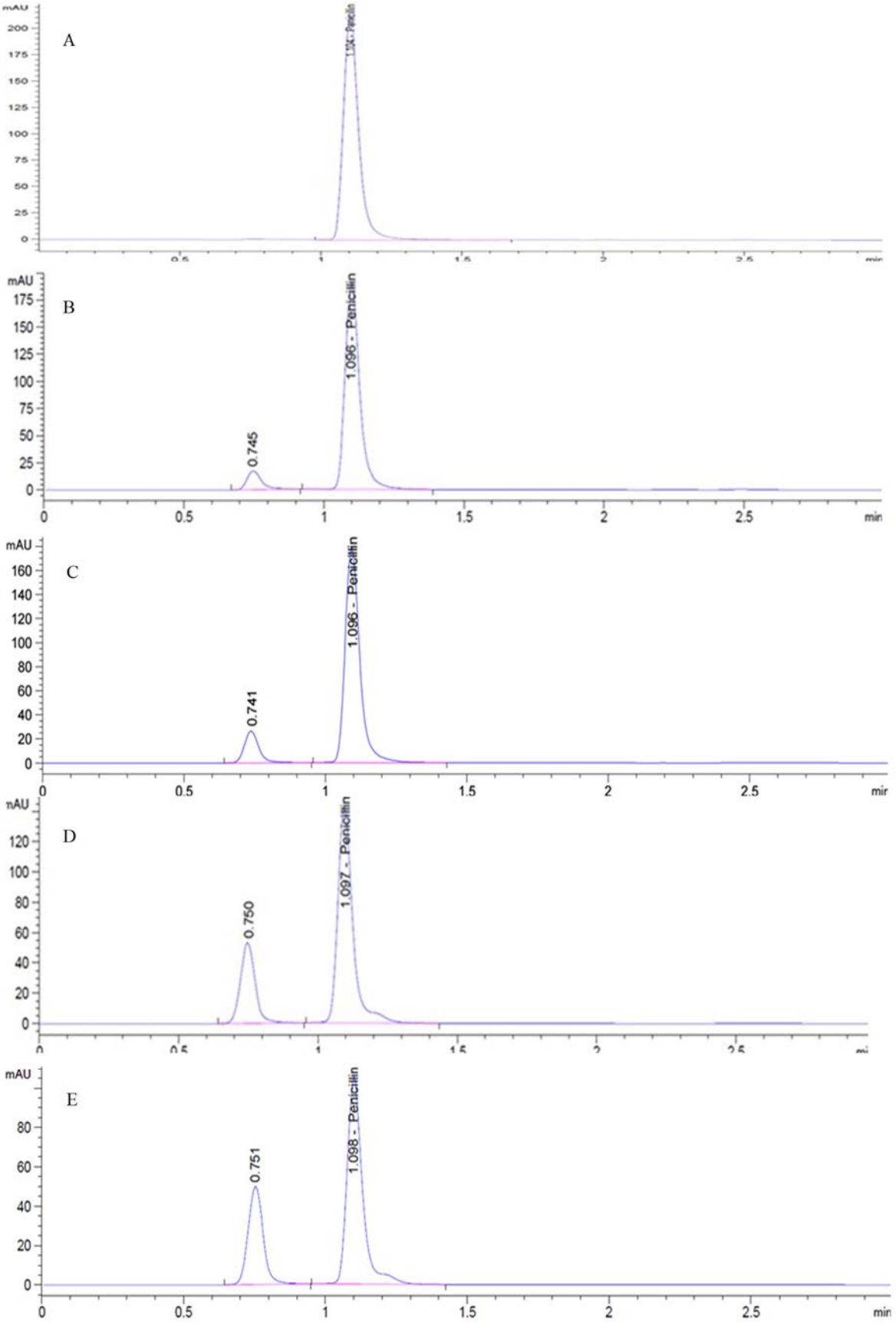

FIG. 6. HPLC chromatograph of penicillin G standard in deionized water (A); unirradiated milk sample (control) (B); milk sample irradiated at 0.75 kGy (C); milk sample irradiated at 1.25 kGy (D); milk sample irradiated at 2.25 kGy (E).

3.6. Discussion

The extensive use of sulfonamides and β-lactams for the treatment of infections in livestock as well as for growth promotion has resulted in the presence of either residue in fresh milk or milk products, potentially causing a serious risk to human health [33]. In addition, the presence of antimicrobial residues in fresh milk creates problems in the dairy industry resulting from partial or complete inhibition of starter cultures used in dairy production, inadequate ripening, and aging of cheese. Therefore, evaluating the presence of antimicrobials in fresh milk is very important. The Charm II test is proven to be a rapid and reliable radioreceptor assay for the detection of a variety of antimicrobial residues at or below the European Union maximum residue level (MRL) in a variety of foods, including raw milk, honey, eggs, meat, poultry and fish.

In the present study, 50 raw milk samples were tested for the presence of sulfonamides and β-lactams by using the Charm II technique. It was found that sulfonamides were detected in 40 (80%) samples and β-lactams were detected in only 25 (50%) samples. This means that sulfonamides are the most widely used antimicrobials in animal production in Egypt. A lower percentage of antimicrobial prevalence in raw milk was identified by others [34]. They tested 200 raw milk samples collected from dairy markets and animal farms in Saudi Arabia and Egypt. Their results indicated that only 40 (20%) samples were positive for antimicrobial residues such as tetracycline, sulfonamides, macrolides, β-lactam, etc. which is lower than the results of the current study.

Salmonella and *S. aureus* are the most common and frequent foodborne pathogens in a variety of foods, including milk and dairy products. The presence of these pathogens in raw milk causes many cases of human infections and food poisoning. In the present study, *Salmonella* was isolated from 10 (20%) milk samples of the 50 tested samples, while *S. aureus* was isolated from 23 (46%) samples. Milk and milk products are highly susceptible to contamination by a variety of microorganisms, including pathogenic bacteria, because of their high nutrient content and complex chemical composition. *Salmonella* and *S. aureus* may occur in raw milk because of its collection from diseased animals and/or due to unhygienic conditions during production, handling, and storage. *Salmonella spp.* and *S. aureus* have also been isolated from different foodstuffs, including milk, milk products, meats, poultry, fish, eggs [9, 35–38].

The 10 *Salmonella* isolates and the 23 *S. aureus* isolates that were isolated from raw milk were examined for their susceptibility to sulfonamides. Four *Salmonella* isolates and seven *S. aureus* isolates were resistant to sulfonamides. Because β-lactams (penicillin G) have limited activity against gram negative bacteria, 8 *S. aureus* isolates that were isolated from β-lactam positive milk samples were examined for their susceptibility to only β-lactams. Five isolates were found to be resistant to β-lactams. Other researchers have demonstrated similar results. Isolated *Salmonella* and *S. aureus* from poultry samples revealed that all isolates exhibited resistance to at least one or more antimicrobials and also exhibited multidrug resistance [8]. In a certain study [37] involving 108 milk samples for the detection of *S. aureus*, conducted it was determined that 25 samples (23.2%) tested positive for this pathogenic bacterium associated with food poisoning. They also isolated multidrug-resistant *S. aureus* from milk, chicken meat, beef, and eggs. In a research study, 344 isolates of ampicillin-resistant *Salmonella enterica* were obtained from a retail meat market in the USA [39].

The susceptibility of the four sulfonamide-resistant *Salmonella* isolates and the seven sulfonamide-resistant *S. aureus* as well as the five β-lactam resistant *S. aureus* strains were exposed to different gamma irradiation doses (0.75 kGy, 1.5 kGy and 2.25 kGy) to investigate the effect of these doses on their sensitivity to the related antimicrobials. In general, the results indicated that gamma irradiation treatment greatly increased the sensitivity of these pathogens to sulfonamides and β-lactams, depending on the irradiation dose, type of bacterial isolate, and type and concentration of antimicrobial. Other researchers have demonstrated similar results. A previous study [19] showed that gamma irradiation doses of 0.5 kGy, 1.0 kGy and 2.0 kGy increase the sensitivity of *S. aureus* to antimicrobials. For example, an irradiation dose of 2 kGy increased the inhibition zone to gentamicin from 7.5 mm for unirradiated *S. aureus* to 25 mm for irradiated ones. This means that the sensitivity increases by 233.3%. Gamma irradiation doses of 1 kGy and 2 kGy significantly increased the sensitivity of *Salmonella* to cephalin, chloramphenicol and gentamicin as reported elsewhere [40].

As mentioned by others [41] the susceptibility of methicillin resistant *S. aureus* to different antimicrobials increased after exposure to a gamma irradiation dose of 3 kGy. Based on other results [42] the sensitivity of *E. coli* to tetracycline and streptomycin increased after exposure to low irradiation doses. The increase in bacterial sensitivity upon irradiation could be explained by generating the highly reactive and hydroxyl radicals that damage cell membranes, proteins, and nucleic acids. This damage can render resistant pathogens sensitive by disrupting their structural integrity, leading to increased susceptibility to subsequent antimicrobial treatments [43]. The effect of ionizing radiation on the bacterial cell wall may result in the formation of channels (microcraters) in the cell wall. These channels enhance the penetration of antimicrobials through the cell wall and hence cause bactericidal effects [44].

Results in the present study indicated that a gamma irradiation dose of 2.25 kGy could reduce the concentration of sulfonamides spiked in milk by 87.0% from the initial concentration (201 µg/l) while the same irradiation dose resulted in only a 42.3% reduction in the penicillin G concentration (56.6 µg/l). These results revealed that the reduction percentage of sulfonamides was higher than that of penicillin G, indicating that sulfonamides are affected by irradiation more than penicillin G. Ionizing radiation generates reactive species such as hydroxyl radicals (OH•) and hydrated electrons (e^-_{aq}) that are pivotal in degrading sulfonamides such as sulfanilamide and sulfadiazine. These species attack the sulfonamide structure, leading to increases in the efficiency of this degradation process [27, 45, 46].

The degradation of sulfonamides through irradiation involves complex mechanisms due to the attack of hydroxyl radicals (OH•) on the C= bond, C=N bond, C-S bond, and N-S bond of the compounds and hydroxylation of the functional groups on the side ring [26]. One study [47] on the effectiveness of irradiation on the degradation of antimicrobials in aqueous matrices and found that the polar groups in sulfonamides (SA) and sulfadimethoxine (SDN) are easily affected by irradiation. According to the results of another study [24] the sulfadiazine (SD) molecule is not only present in oxidative radicals (-SO_2-) but also reductive radicals (-NH_2-NH-) therefore SD may be oxidative by OH• radicals or reductive by e^-_{aq} which results in SD removal from the aqueous solutions.

As reported elsewhere [47] electron beam irradiation at 8 kGy decreased the concentration (110 mg/kg) of sulfadimethoxine (SDA) in an aqueous solution by 94%. The same dose decreased the sulfonamide (SA) concentration (130 mg/kg) by 92.2%. Most antimicrobials at low concentrations (20 mg/kg) in an aqueous solution were completely degraded with a gamma irradiation dose of 1 kGy, but sulfamethazine required 4 kGy for complete decomposition [48]. The sulfathiazole (STZ) at concentrations of 80 and 100 mg/l in aqueous solution was degraded at electron beam irradiation doses of 3 kGy and 5 kGy, respectively [49].

Gamma irradiation was effective at degrading and deactivating penicillin G in pure water as reported by [50]. At initial concentrations of 0.27 mM, 1.34 mM and 2.68 mM, complete removal of penicillin G could be achieved at absorbed doses of 2.5 kGy, 10 kGy and 20 kGy, respectively. This means that a high concentration of antimicrobial residues in food requires a high dose for degradation. Alsager et al., [25] compared the effectiveness of gamma irradiation on the decomposition of amoxicillin, ciprofloxacin, and doxycycline residues in food matrices (milk, eggs and meat) and water. These researchers found that an absorbed dose of 7.0 kGy was sufficient to induce antimicrobial degradation (65–95%) in food, similar to that observed for aqueous antimicrobials. Gamma irradiation and electron beam irradiation have been shown to induce significant degradation of penicillin G in aqueous solutions because hydroxyl radicals and hydrated electrons play crucial roles in the degradation process, leading to the cleavage of the β-lactam ring [51–53]. It is worth mentioning that many researchers investigated the toxicity of antimicrobial radio-degradable products and reported that most of the radio-degradable products formed upon irradiation of antimicrobials were less toxic than parent compounds and had no antibacterial activity nor posed a toxic effect [25, 26, 54].

4. CONCLUSIONS

This study has demonstrated that the Charm II radioreceptor assay is rapid and reliable for the detection of sulfonamides and β-lactams (penicillin G) in raw milk. Antimicrobial resistant *Salmonella* spp. and *S. aureus* were found in the majority of the collected raw milk samples. Irradiation doses of 0.75 kGy, 1.5 kGy and 2.75 kGy proportionally increased the sensitivity of antibiotic-resistant *Salmonella* spp., and *Staphylococcus aureus* to antimicrobials. HPLC analysis indicated that a gamma irradiation dose of 2.75 kGy reduced the sulfonamides and penicillin G in spiked milk by 87.0% and 42.3%, respectively. The use of ionizing radiation (gamma radiation, electron beam) decontaminates foodborne pathogenic bacteria present in dairy products (including raw milk) to sensitize antimicrobial resistant pathogenic bacteria to commonly used antimicrobials and to reduce antimicrobials residues in these products.

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DEVELOPMENT OF TARGETED CERTIFIED REFERENCE MATERIALS FOR FOOD TESTING

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Abstract

To guarantee the quality of food products, metrologically traceable measurement to a reference value are required, the most commonly accepted being the International System of Units (SI). Metrological traceability to SI is demonstrated by means of Certified Reference Materials (CRMs) or primary measurement methods. Production of CRMs is a key activity needed to develop, improve and maintain a worldwide coherent measurement system. CRMs are used for purposes such as calibration, quality control, proficiency testing, method validation, and the assignment of values to other materials. General requirements to produce all types of CRMs are set out in ISO 17034:2016, and the detailed characterization approaches are based on ISO 33405:2024. Alerts and rejections of exported food according to the latest reports from the European Union's (EU) Rapid Alert System For Food and Feed (RASFF) are associated with the presence of pesticides, mycotoxins, heavy metals, other toxins, allergenic substances, and microbiological contamination. To control food production and minimize such rejections, CRMs are needed in associated testing and calibration laboratories testing foods such as meat, milk and dairy products, flour, olive oil, and wine among others. A range of techniques play an important role in assigning certified values to CRMs with low uncertainties. Examples include Nuclear Magnetic Resonance (qNMR) which is the starting point to assigning the purity of the CRMs for contaminants like pesticides, herbicides, mycotoxins; and Isotopic Dilution Mass Spectrometry (IDMS) with an Inductively Coupled Plasma (ICP-IDMS) or Liquid Chromatography Mass Spectrometer (IDLC-MS/MS); and Atomic Absorption Spectroscopy (AAS). The paper provides an overview of how a range of techniques are used in the certification of CRMs.

1. INTRODUCTION

International System of Units (SI) is based on a set of defining constants and associated rules, adopted by the 26th General Conference on Weights and Measures (CGPM). The International Committee of Weights and Measures (CIMP) is the monitoring arm of the International Bureau of Weights and Measures (BIPM) the organization established by the Metre Convention in 1875, through which countries deliberate on matters of measurement science and measurement standards [1]. National Metrology Institutes (NMIs) are responsible for maintaining national measurement standards and disseminating SI Units within the territory to which they are subscribed. The National Institute of Industrial Technology (INTI) was designated as the National Institute of Metrology for Argentina in 1972. Under the authority given to CIMP in the Metre Convention, a Mutual Recognition Arrangement (MRA) has been drawn up.

The CIPM MRA is the framework through which signatory-NMIs demonstrate the international equivalence of measurement standards as well as calibration and measurement certificates issued. Furthermore, it addresses the dissemination of the SI. The MRA is an important tool for helping NMIs in the development of local quality measurements in industry, health, environment, science and food safety. Dissemination of SI Units is through calibration and the provision of CRMs. The main metrological definition used in this paper can be found in the fundamentals of metrological traceability section and follows the definitions stated in the International Vocabulary of Metrology [2]. NMIs have been addressing the emerging food safety challenges within the ambit of metrology institutes, working with the scientific community, and providing reference materials and measurement procedure traceable to SI.

The EU, a key destination market for various food products, enforces stringent food safety standards. To facilitate rapid response to public health risks linked to the food chain, the EU established RASFF, enabling efficient information exchange among member states. Recent RASFF reports [3] highlight that most alerts and rejections of exported food are due to contaminants such as pesticide residues, mycotoxins, and heavy metals, leading to penalties and significant economic losses for producing countries (Fig. 1).

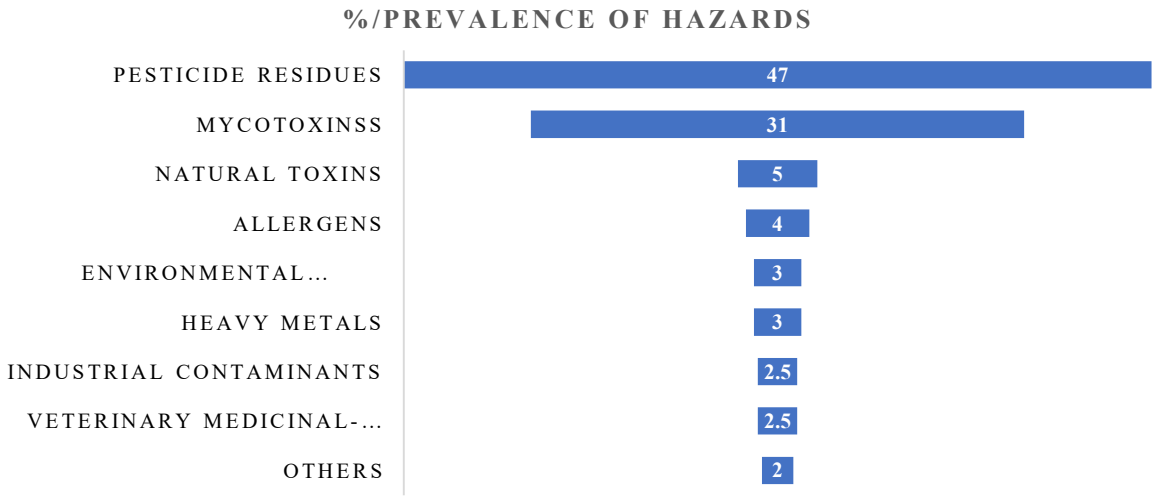

FIG. 1. RASFF alerts for food in 2024.

Testing laboratories operate under ISO/IEC 17025:2017 [4] to provide reliable results that support food safety by ensuring analytical test accuracy and consistency. This standard outline key tools and practices to achieve these objectives, including the use of CRMs for quality control and calibration purposes. Additionally, the validity of results can be reinforced through participation in proficiency testing (PT) programmes, which evaluate laboratory performance in conducting tests, measurements, or calibrations [4].

The demand for CRMs in food matrices is associated with the needs of major food industries. As already mentioned, CRMs are crucial for ensuring the competitiveness of food industries in local and global markets, as they assure quality control . For example, in Argentina, soybean and corn exports alone represented 30.5% of the country's total exports in the second half of 2023 [5]. The INTI has been producing CRMs for the food sector with an annual production of 30000 units. As an NMI, INTI is required to take part in regular key comparisons of national measurement standards to have Measurement and Calibration Capabilities (CMC) claims validated through the peer review process [6]. This process includes the approval of a reviewed quality system, usually ISO/IEC 17025:2017 [4] for calibration and ISO 17034:2016 [7] for the production and certification of reference materials. The basis of the characterization approaches for CRMs production are set in ISO 33405:2024 [8] while ISO/Guide 31:2015 [9] help reference material producers (RMP) to prepare clear and concise documentation to accompany CRM units. This article is an overview of the metrological fundament of how different techniques are used in the certification of CRMs.

1.1. Fundamentals of metrological traceability

Metrological traceability is defined as the property of a measurement result relatable to a reference through a documented unbroken chain of calibrations [1]. The RM is a stable and homogeneous material with reference to one or more specified properties demonstrating fitness-for-purpose based on a metrologically valid approach [1]. If a RM is accompanied by a certificate that provides the values of the specified properties, associated uncertainties, and statements of metrological traceability, it becomes a certified reference material or CRM [1].

The CRMs are categorized into three types: neat reference materials, calibrant solution reference materials, and matrix reference materials. A neat reference material is a single-entity substance, typically used to prepare calibrant solutions. A calibrant solution is a CRM made by mixing a diluent with one or more materials of known chemical composition (such as a neat material) and can be used as is or diluted further by the user. Matrix CRMs are materials with a composition similar to the samples being tested, containing a known amount of one or more chemical entities. These are usually homogenized natural materials with the target entities present at endogenous levels or as fortified material [1].

Neat and/or calibrant CRMs require high-purity chemicals [10, 11] for which the estimation of the mass fraction of the pure material is necessary. The mass fraction can be determined using various approaches or a combination of results obtained from two or more of these methods. One primary approach is the mass balance method, which involves determining the mass fractions of one or more impurities present in the material. The material's mass fraction is then calculated by subtracting the impurity content. Another commonly used approach is the direct assay-based method, which directly measures the purity or mass fraction of the analyte in the material. The traceability chain followed by NMIs to produce CRM is summarized in Fig. 2.

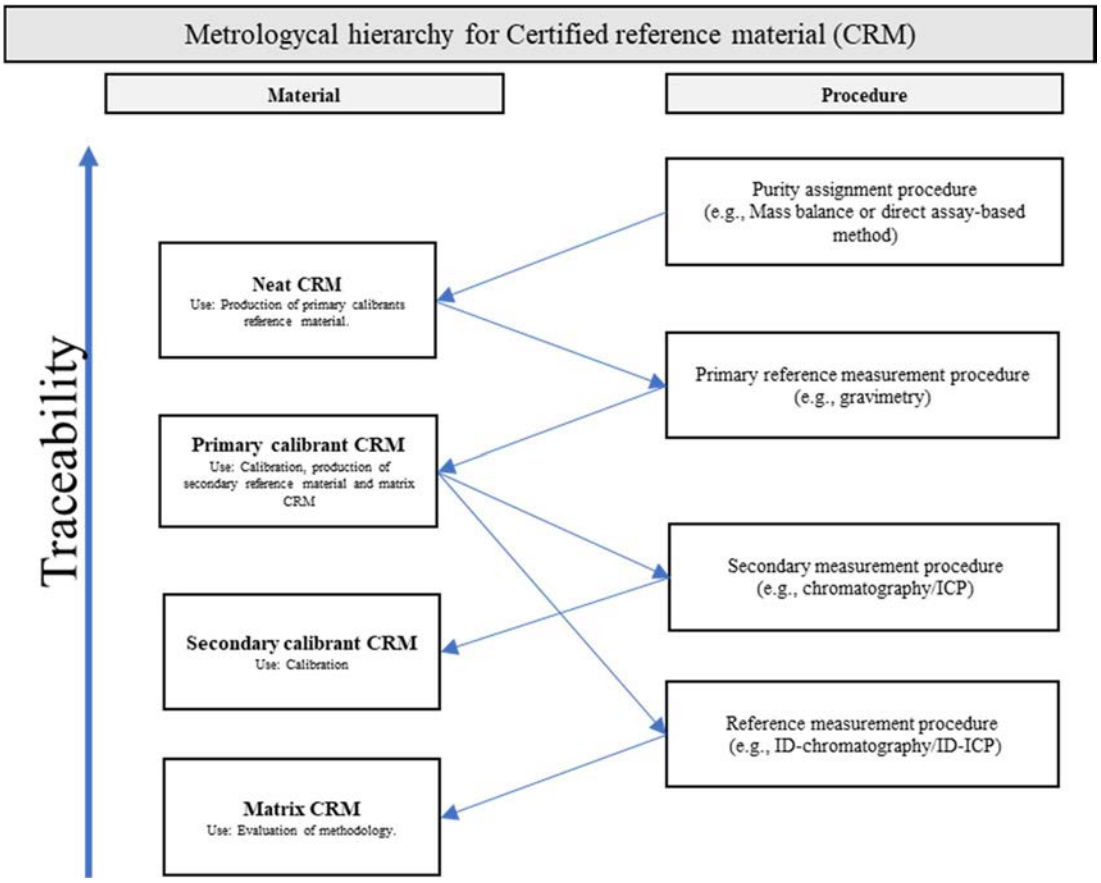

FIG. 2. Metrological hierarchy for CRMs.

2. MATERIALS AND METHODS

There are two main routes for establishing metrological traceability to SI in the certification of CRMs, the first one is a primary method realization of the unit of measurement concerned and the second one is through another NMI with relevant CMCs [12].

2.1. Primary method for unit of measurement

The qNMR and IDMS are two methods commonly used as primary methods of measurement when producing reference materials for food analysis. The qNMR facilitates purity assignment of organic neat materials and IDMS helps to characterise RM matrix. A primary reference measurement procedure does not entail its relation to a relevant measurement standard [1].

2.1.1. qNMR: A direct assay-based method for purity assignment of organic neat materials

Nuclear magnetic resonance is a spectroscopic technique used to provide rich structural information for identifying and characterizing organic molecules. The application of NMR for quantitative analysis was limited, compared to the advances in instrumentation have led to the application of qNMR in chemical metrology. Quantitative NMR methods are primary reference measurement procedures as they do not require reference material of the subject to quantification to achieve metrological traceability, according to the definition of a primary reference measurement procedure. For example, in ^{1}H-qNMR the signal intensity is directly proportional to the number of protons contributing to the resonance. The signal intensities of the protons of a molecule and the primary standard for ^{1}H-qNMR can be compared directly. Other hetero-nuclear qNMR techniques (19F-qNMR) may be used to achieve metrological traceability as well. qNMR has the advantage of not requiring analysis of impurities to assign a purity value, is a non-destructive method and has short method development times.

For the qNMR method, a gravimetrical amount of sample and the selected internal standard are dissolved in an appropriate solvent, and quantification is achieved by comparing the integral areas of a selected signal from

the analyte in interest and the one corresponding to the internal standard. The uncertainty of the qNMR method can be calculated based on qNMR processing, sample preparation, molar mass uncertainty, and the uncertainty of the purity assigned to the internal standard, between other uncertainty sources. The traceability of purity of the neat material to the SI is realized via certification for mass fraction purity calculated by qNMR and the appropriate quality assurance systems and validated qNMR method [13]. Neat materials are the higher order metrological traceability for the assigned values of derived calibration solutions, matrix reference materials, and therefore for the results of routine analysis.

2.1.2. Gravimetric preparation: a method with metrological traceability

In general, the results of a gravimetric preparation of a new CRM by diluting weighted quantities of a well characterized neat or calibrant CRM are traceable to SI through the mass calibration of the balance. Primary materials can be obtained by dilution of neat material in a proper solvent. The uncertainty of a calibrant CRM can be calculated based on the uncertainty of the neat or primary calibrant CRM and the uncertainty of the weighted procedure. Further reference material hierarchy to SI traceability and measurement procedure can be found in Fig. 2.

2.1.3. DMS: A method for reference material value assignment

Isotope dilution is an analytical method for measuring analyte concentrations, traceable to the mole. It involves mixing a sample with a spike containing the same analyte but with a different isotopic composition. The resulting isotope ratio is measured and since ID relies on isotope ratios and mass measurements, any analyte losses during treatment do not affect accuracy. ID is used in both inorganic and organic chemistry and, when combined with mass spectrometry (IDMS) allows for precise concentration determination from major to ultra-trace levels. There is extensive information regarding the use of IDMS as a primary method for the characterization of RM by IDMS [14, 15]. IDMS methods are metrologically characterized as a reference method for RM characterization since IDMS has the potential to produce analytical results of the highest accuracy and precision when coupled with LC, GC or coupled with CP.

The ICP-IDMS is important in the development of CRMs for heavy metals, and chromatographic tools such as ID-LC-MS/MS support NMIs to in producing CRMs for pesticides, mycotoxins and allergenic substances among others in food. In the CRM characterization of CRMs, the IDMS is rather limited by the uncertainties associated with ID and/or mass spectrometric measurements, mainly due to calibration standards. The calibration standards used in this method require to be traceable to SI through qNMR characterization of neat material, further reference material hierarchy to SI traceability and measurement procedure can be found in Fig. 2.

Under the authority given to CIMP in the Metre Convention, a Mutual Recognition Arrangement (MRA) established. Generally referred to as the CIPM MRA was established. This is the framework through which signature NMIs demonstrate the international equivalence of measurement standards as well as the calibration and measurement certificates they issue. The framework which also addresses the dissemination of SI units, helps NMIs in the development of national quality of measurements in industry, health, environment, science, and food safety. Determining values for secondary reference materials maybe (a) through use of other materials with similar characteristics or (b) through another CIPM MRA participant with relevant CMCs with appropriate measurement uncertainty published in the BIPM key comparison database (KCDB) or (c) through calibration and measurement services provided by the BIPM. Traceability is declared through the laboratory providing the service.

According to BIMP, CMCs often involve auxiliary influence quantities in the measurement (such as temperature when performing mass calibrations). These are not part of the main traceability path to SI and with a minor contribution to the measurement uncertainty of the CMC. Traceability of those influence quantities can either be to an institute with CMCs in the KCDB or to a laboratory accredited by a signatory to the International Laboratory Accreditation Cooperation (ILAC) Arrangement [12].

A related secondary method can be defined as an analytical technique that allow NMIs to disseminate metrological traceability using nonprimary methods of measurement, by direct comparison with a certified reference material with traceability to a primary measurement procedure. For such a comparison to be traceable to SI, producers of reference materials require appropriate CMCs, using validated methods, and must be able to demonstrate appropriate measurement uncertainty. Complementary methods such as AAS or CP without IDMS can be used as secondary methods for value assignment through these criteria.

2.2. Development of RMs with metrological traceability

2.2.1. Example of primary method realization of the unit of measurement

(a) qNMR: A direct assay-based method for purity assignment of organic neat materials—Under the BIPM-CBKT Metrology for Safe Food and Feed programme, the Consultative Committee for Amount of Substance (CCQM) aims to provide participants with a key comparison for National Measurement Institutes and Designated Institutes (DIs). These provide measurement services in organic analysis under CIPM MRA. Purity assignments of neat mycotoxin materials were the basis for CCQM-K154 comparisons. The BIPM together with metrology institutes from China (NIM) Türkiye (TÜBITAK) Argentina (INTI) Brazil (INMETRO) and Japan (NMIJ) produced and certified purity value for the neat mycotoxin reference materials [16–19] used in CCQM-K154 comparisons.

Neat materials were purchased from a commercial supplier and characterized using qNMR as primary method. For neat material characterization of deoxynivalenol (DON) in CCQM-K154c, BIMP follow a gravimetric operation using an ultramicrobalance for sample preparation, acetone-d6 as solvent, and dimethyl terephthalate (DMTP) as qNMR internal standard. For this mycotoxin, several numbers of analyte signals were selected for qNMR purity assignment. Further analytical information, spectrometer performance and specific NMR experimental parameters for this particular mycotoxin can be found in the BIPM-2022/04 guidance [16]. To have a complete characterization of the material by the BIMP. An LC-DAD-MS/MS method was developed and validated to measure the mycotoxins. The water content was measured by coulometric titration [16].

(b) Gravimetric preparation: a method with metrological traceability—During the CCQM-K154 comparisons project, the BIPM provided the participants with a primary calibrant solution of mycotoxins. The primary mycotoxin solutions were prepared gravimetrically by dissolving in acetonitrile and accurately weighing the neat material [16–19]. In CCQM-K154c, DON primary calibrant solutions were prepared by weighing the neat mycotoxin and dissolving the final solution. The BIPM tested the homogeneity of the main components and identified a minimum sample size that reduces to an acceptable level the effect of between–bottle inhomogeneity, additionally performed an isochronous stability study that found non-statistical effect for instability of mass fraction [16]. The homogeneity and stability studies were performed under ISO 33405:2024 criteria [8]. Units of primary calibrants solutions were sent to NMIs by mail in insulated boxes equipped with temperature indicators.

After the primary calibrants were obtained, under the CCQM-K154c comparisons, other calibrant solutions RMs were prepared by participants using the primary calibrants. Appropriate assignments of the property value, and associated uncertainty were conducted by participants. The INTI calibrants RM for DON were prepared using a suitable analytical balance. The calibrant solution preparation was carried outweighing the content of the primary calibrant solution into a plastic bottle and filled up with acetonitrile. Glass ampoules containing 3 ml of INTI calibrants solution were prepared. Homogeneity and stability studies were also carried out following ISO 33405:2024 guidelines [8]. The INTI calibrants RMs were sent out by the coordinating laboratory for comparison among participants after assignment of a value. The performance of INTI in the key comparison, as well as method information and gravimetric preparation of secondary calibrant RM can be found on CCQM-K154 reports [20–23].

c) IDMS: A method for reference material value assignment — Under the InterAmerican Metrology System (SIM) INTI collaborates with the National Institute of Standards and Technology (NIST) and the Food and Drug Administration (FDA) of the USA in the development of matrix CRMs for mycotoxins. The use of mycotoxins matrix reference material has been well addressed [24]. During this project, it was necessary to obtain naturally contaminated material with mycotoxins. The material chosen for this purpose was contaminated corn grains that were milled to obtain naturally contaminated corn flour. The specific characteristics of the process and the studies involved have been previously detailed elsewhere [25].

The mycotoxin content in the matrix RM was determined by LC-IDMS based on an external standard calibration curve. The external standards were neat materials with traceability to the SI by purity assignment by ^{1}H-qNMR. For the quantification of the total mycotoxin content, samples of the corn reference material with unknown mycotoxin content were spiked with a known amount of mycotoxin isotopic label standard. The samples were analysed by using a hybrid LC-MS/MS.

Two individual working calibrant solutions were gravimetrically prepared for each compound by combining aliquots of diluted calibrants RM and IS solution. The ratio of isotopically labelled to unlabelled mycotoxins in the working calibrant reflected the analyte responses in the sample extracts. An internal standard approach to calibration was used for the determination of mycotoxins. Each working calibrant was injected six times and response factors were calculated. The calculated response factors for each calibrant were averaged and were used with the respective masses of internal standard within the measured samples.

(d) Example of metrological traceability declared through another NMI having relevant CMCs — A secondary reference material of arsenic, cadmium, chromium, nickel, and lead was prepared. These elements were selected due to their relevance in routine analysis of heavy metals/contaminants in different stud matrices. In the United States of America, designating a calibration solution for elemental analysis as traceable usually means that the certified value of that solution is traceable to the certified value of the matching elemental solution from the NIST 3100 series of CRMs. The NIST 3100 series are single–element solution CRMs. As it is claimed and while accepted, the 3100 series solutions are traceable to the mole. The NIST 3100 series are themselves determined against well-characterised primary materials [26].

The SRM NIST 3100 series are used for INTI to assign value to a new secondary multielement reference material as calibration of secondary complementary non-nuclear methods such as AAS or ICP-OES. The multielement calibration solution CRM was prepared gravimetrically from concentrated individual element solutions derived from pure substances, to which deionized water and nitric acid were added to reach the final mass. The final solution was homogenized by stirring and weighed. It was then transferred to precleaned 100 ml polyethylene bottles with nitric acid and water, immediately sealed/capped and placed in cardboard boxes. The homogeneity study was performed on 10 units randomly selected from an initial batch of 100 units by ICP-OES. The stability study was conducted under storage conditions, in real time, on units selected by random sampling and analysed by ICP-OES. The assignment of certified values was determined by ICP-OES. A multielement calibration was carried out with NIST primary reference materials, and three different bottles were analysed in triplicate over four different days to determine final assigned values.

3. RESULTS AND DISCUSSION

3.1. Example of primary method realization of the unit of measurement

3.1.1. qNMR: A direct assay-based method for assigning purity values

Operators of an NMR at INTI working under a BIPM project assigned a purity value to the neat mycotoxins reference material as according to the BIPM-2022/04 guidance [16]. The direct qNMR value for the DON mass fraction in the material was estimated at 976.3 ± 3.1 mg/g [16] uncertainty budget, chemical structures for DON and related impurities, NMR spectra data, and NMR and LC parameters or data treatment can be found in the previously stated document [16]. Table 1 illustrates the assignment of the purity value of DON by direct qNMR and mass balance method.

TABLE 1. ASSIGNMENT OF PURITY TO NEAT DON REFERENCE MATERIAL

Method	Value (mg/g)	Expanded uncertainty U95% (mg/g)
Direct qNMR value	976.3	3.1
Mass balance value	980.55	2.84

Fig. 3 shows a schematic procedure for the preparation of neat material and metrological traceability to SI units.

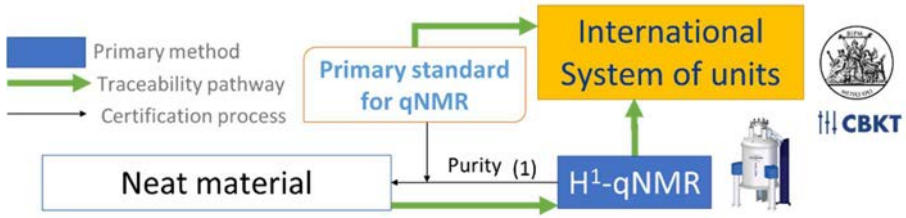

FIG. 3. Preparation and traceability.

3.1.2. Gravimetric preparation: a method with metrological traceability

After purity-assignment, BIMP developed an RM of the primary calibrant by diluting the neat materials in acetonitrile. A mass fraction of 627.1± 19.2 µg/g was calculated for the primary (DON) stock solution [16] based on the gravimetric preparation and uncertainty estimation. INTI calibrants RM preparation and specific guidelines can be found in CCQM [13–16]. Table 2 illustrates the assignment of the DON concentration value for INTI's calibrant RM as indicated in the CCQM-K154.c Key Comparison Study [16].

TABLE 2. ASSIGNMENT OF A VALUE TO THE DON CALIBRANT RM AS RECOMMENDED ELSEWHERE

INTI calibrant RM preparation	Value	Units
Primary calibrant RM concentration	627.7	µg/g
Primary calibrant RM	15646.9	mg
INTI calibrant RM final solution	179019.5	mg
Buoyancy balance LA230P	1001387	—
INTI calibrant RM concentration	54811	µg/g

Uncertainty value assignment includes primary calibrant value assignment uncertainty, gravimetric preparation, and uncertainty associated with RM stability and homogeneity. Figure 4 shows a schematic procedure for preparing calibrants CRMs and the metrological traceability to SI units.

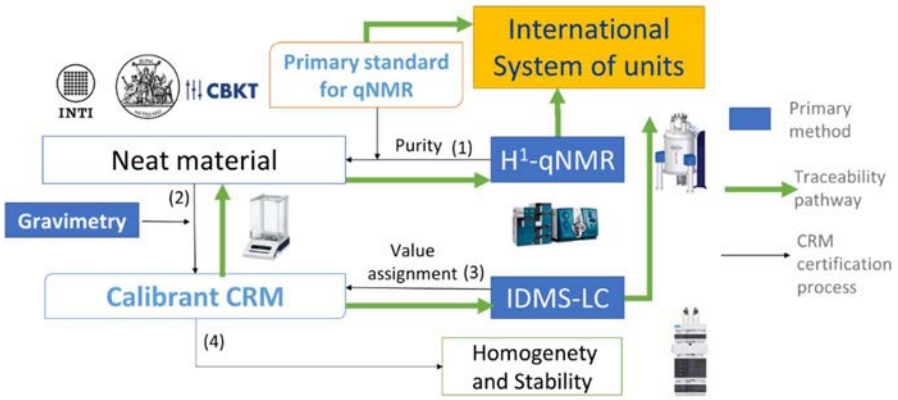

FIG. 4. Preparation of a CRM calibrant and metrological traceability to the SI units.

3.1.3. IDMS: A method for reference material value assignment

For NIST SRM 1565, mass fractions of DON were corrected for purity using the purity assigned using the qNMR method. Measurements for DON were within the reference value ranges. Furthermore, the mass fraction values for both mycotoxins were consistent with previous reference values assigned by a combination of NIST measurements with data provided by the FDA. The mass fraction (ng/g) was 466 at an expanded measurement uncertainty (U95%) of 69.

Figure 5 shows a schematic procedure for value assignment of matrix RMs and metrological traceability to the international system of units and the first units of NIST SRM 1565 for mycotoxins in corn.

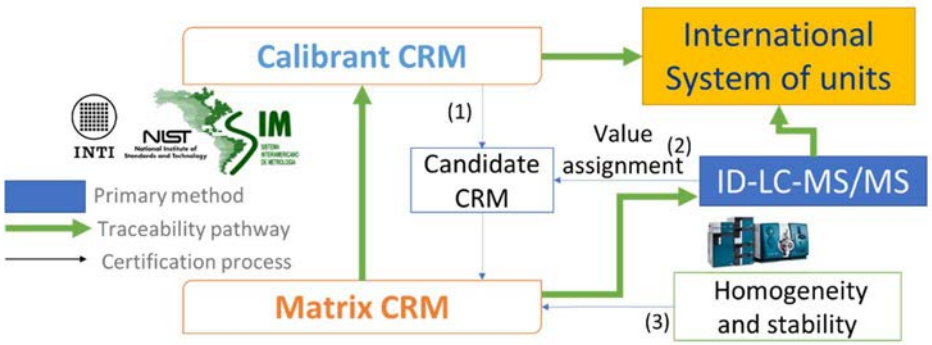

FIG. 5. Value assignment of matrix RM and metrological traceability to the SI units.

3.2. Example of metrological traceability declared through another NMI with CMCs

The RM consisting of a multielement calibration solution of arsenic (As) cadmium (Cd) chromium (Cr) nickel (Ni) and lead (Pb) at 50 mg/kg each, in water with 2–3% nitric acid, was obtained. The material was presented in a volume of approximately 100 ml in a low density polyethylene bottle. This reference material can be used as a calibration material for quantitative determinations of As, Cd, Cr, Ni, and Pb at trace levels, for the evaluation or validation of testing methods, quality assurance of measurements, and control of measurement equipment performance. The certified values for As, Cd, Cr, Ni and Pb are reported in Table 3.

TABLE 3. CERTIFIED VALUES AND EXPANDED UNCERTAINTIES FOR ELEMENTS IN CRM 011-LOT 001

MRC 011-Lot 001	Mass fraction (mg/kg)	Expanded uncertainty U95% (mg/kg)
As	50.42	1.17
Cd	47.39	1.11
Cr	49.19	1.27
Ni	49.86	1.18
Pb	49.72	1.05

The certified values of this CRM have metrological traceability to SI through the use of RM certified by an NMI and calibrated equipment in ISO 17025 accredited laboratories. The expanded uncertainty was calculated by multiplying the combined standard uncertainty by a coverage factor $k = 2$, at the confidence level of approximately 95%. Figure 6 shows a schematic procedure for preparing secondary calibrants and the metrological traceability to SI units.

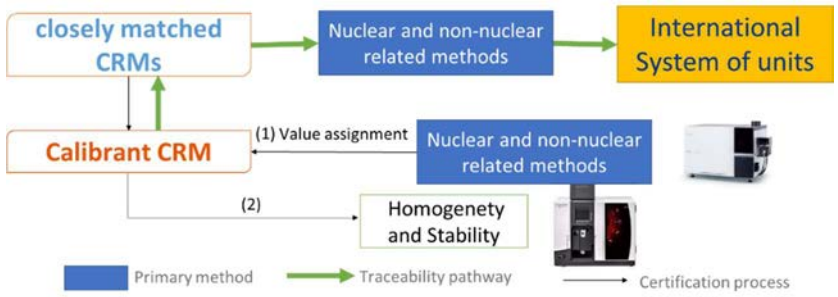

FIG. 6. Value assignment of secondary calibrants through another NMI.

4. CONCLUSIONS

Establishing metrological traceability to the International System of Units is crucial for ensuring the accuracy, consistency and reliability of measurements. Primary methods such as qNMR and IDMS offer direct and dependable approaches for assigning precise values to reference materials. These techniques play a key role in the continuous development and validation of reference materials, providing the robust metrological standards necessary for the food industry. The NMIs and the IBIPM are instrumental in maintaining these standards and ensuring that measurements are globally consistent and traceable. By ensuring traceability and accuracy, these methods, supported by the work of NMIs and BIPM, help safeguard the production of safe, high quality food products, ultimately protecting public health and reinforcing consumer trust.

ACKNOWLEDGEMENTS

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EVALUATING ANTIMICROBIAL RESISTANCE OF *SALMONELLA* STRAINS ISOLATED FROM RETAIL MEATS IN MAURITANIA

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Abstract

The increase in multi resistant pathogens responsible for infections and food poisoning is a growing concern to human and animal health services. This study involved 200 samples analysed in Mauritania between 29 May 2022 and 02 March 2023. *Salmonella* was detected using the ISO 6579 method. Antibiograms were performed on ten antimicrobial discs in accordance with the antibiogram committee of the French Microbiology Society 2021. The results showed that 9% of the samples were contaminated with salmonella spp. The *salmonella* isolated were 100% resistant to ampicillins and amoxicillin combined with clavulamic acid: 90% resistant to ceftriaxone and 85% resistant to ceftazidime. The high rates of resistance call for increased monitoring of the use of antimicrobials in animal production in Mauritania.

1. INTRODUCTION

Mauritania has significant animal resources, estimated in 2021 at 2,178,299 cattle, 1,528,122 camels, 16,055,054 sheep, 10,701,156 goats and almost 12,207,031 poultry [1]. Meat from this livestock is an important part of the Mauritanian diet but is also a highly favourable environment for the growth of microorganisms, some of which are dangerous to human health, such as *salmonella*. Atypical *salmonella* infections, which include a variety of clinical signs, contribute to the more than 600,000 deaths worldwide each year, with a high infant mortality rate in emerging countries [2]. In developing and underdeveloped countries, infection caused by *salmonella* serotypes is frequently associated with severe illness. It is often mild but can become severe in vulnerable individuals such as children, the elderly and immunocompromised individuals [3].

In Africa, there is little data on the epidemiology and antimicrobial resistance of *salmonella* [4]. However, in certain countries such as Senegal, Tunisia, Nigeria and Ethiopia, several studies carried out on various types of environmental, animal and/or human samples have revealed a fairly high contamination rate due to *salmonella* strains [5]. Endogenous or exogenous contamination of meat by antimicrobial-resistant *salmonella* strains can affect public health. It also causes significant economic losses in livestock farming and is a barrier to trade of meat and meat-based products. Thus this study on the evaluation of antimicrobial resistance of *salmonella* strains isolated from meat in Mauritania was undertaken. *Salmonella* was detected using the ISO 6579 method [6]. Antimicrobial susceptibility tests were carried out on 11 antimicrobial discs in as recommended elsewhere [7].

2. MATERIALS AND METHODS

2.1. Study area

Mauritania covers an area of 1,030,700 km². It has a 600 km Atlantic coastline to the West. There are three ecoclimatic zones. The Sahelo-Sudanian zone has a dry tropical climate in the extreme south. The Sahelo Saharan zone has a subdesert climate in the centre. The Saharan zone has a desert climate in the north. The main economic activities are agriculture, livestock farming and fishing. The study area involved five wilayas located in the centre, east and south of the country [8]. These wilayas are characterized by high population density and large livestock populations. The cross sectional study was carried out from May 2022 to January 2023.

2.2. Sampling, isolation and antimicrobial resistance testing

Sampling targeted 120 butchers and 20 abattoirs. A total of 200 samples including 43 camel meat, 75 beef, 43 goat meat and 39 mutton samples were collected. Data were collected using a global survey form and entered into an excel file. Isolation of *Salmonella* was carried out in accordance with ISO 6579 [6]. Antibiograms were determined using the Mueller Hinton agar diffusion method for 11 antimicrobial discs as recommended elsewhere [7].

3. RESULTS AND DISCUSSIONS

The findings on salmonella in the different products are summarized in Tables 1–4 and Figs 1–4.

TABLE 1. PREVALENCE OF *SALMONELLA* SPP., ISOLATED FROM RED MEATS IN THE TARGET AREAS

Study areas	Number of samples	Number *Salmonella* spp.,	Prevalence (%)
Assaba	40	1	2.5
Brakna	40	4	10
Gorgol	40	6	15
Nouakchott	40	3	7.5
Trarza	40	4	10

TABLE 2. PREVALENCE OF *SALMONELLA* SPP., ISOLATED FROM MEAT, BY SPECIES SLAUGHTERED

Species	Number of samples	Number of *Salmonella* spp	Prevalence
Camelin	43	3	7%
Bovine	75	5	7%
Goat	43	5	12%
Sheep	39	5	10%

TABLE 3. RESISTANCE OF *SALMONELLA* SPP., ISOLATED FROM RED MEAT IN THE MAURITANIAN STUDY AREAS

Antimicrobial	Assaba *n*=1(%)	Brakna *n*=4 (%)	Gorgol *n*=6 (%)	Nouakchott *n*=3 (%)	Trarza *n*=4 (%)	Total *n*=18 (%)
Ampicillin	1 (100)	4 (100)	6 (100)	3 (100)	4 (100)	18 (100)
Kanamycin	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0
Ceftriaxone	1 (100)	4(100)	6 (100)	2 (75)	4 (100)	17 (94)
Amoxicillin and clavulanic acid	1 (100)	4 (100)	6 (100)	2 (75)	4 (100)	17 (94)
Ceftazidime	0	3 (75)	5 (83)	2 (75)	4 (100)	15 (83)
Ciprofloxacin	0	0	0	0	0	0
Netilmicin	0	0	0	0	0	0

Antimicrobial	Assaba *n*=1(%)	Brakna *n*=4 (%)	Gorgol *n*=6 (%)	Nouakchott *n*=3 (%)	Trarza *n*=4 (%)	Total *n*=18 (%)
Amikacin	0	0	0	0	0	
Tobramycin	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0

TABLE 4. PERCENTAGE OF RESISTANCE OF *SALMONELLA* SPP., ISOLATED FROM MEAT ACCORDING TO SPECIES SLAUGHTERED

Antimicrobial	Bovine *n*=5 (%)	Camelid *n*=3 (%)	Goat *n*=5 (%)	Sheep *n*=5 (%)
Ampicillin	5(100)	3(100)	5(100)	5(100)
Kanamycin	5(0)	0	0	0
Gentamicin	0	0	5(0)	0
Ceftriaxone	5(80)	3(100)	5(80)	5(100)
Amoxicillin and clavulanic acid	5(80)	3(100)	5(100)	5(100)
Ceftazidime	5(80)	3(100)	5(60)	5(100)
Ciprofloxacin	0	0	0	0
Netilmicin	0	0	0	0
Amikacin	0	0	0	0
Tobramycin	0	0	0	0

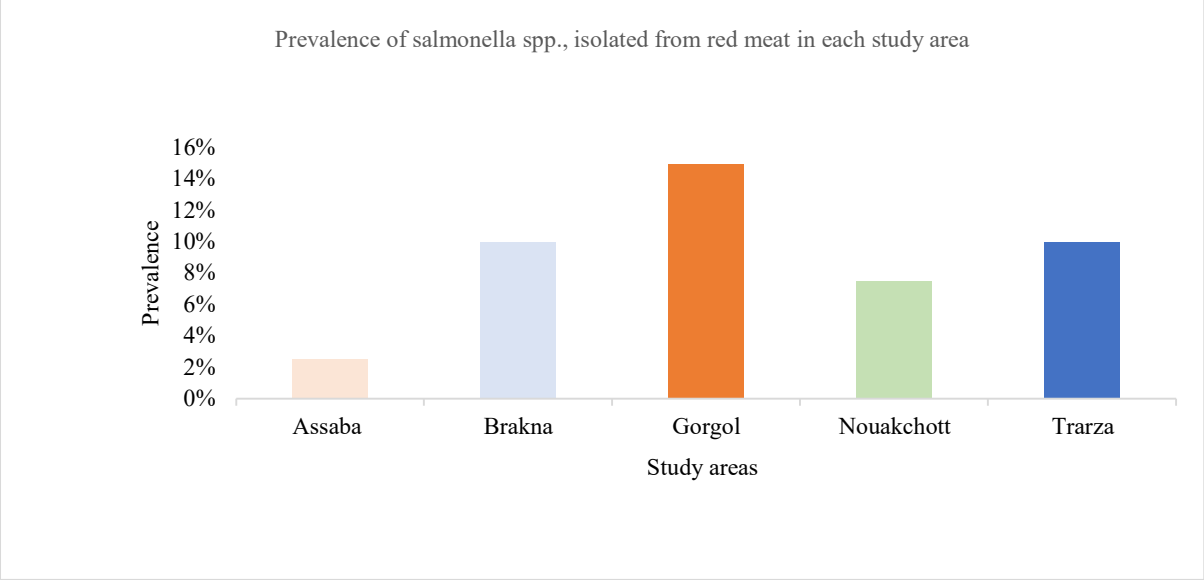

FIG. 1. Prevalence of Salmonella spp., *isolated from red meat according to study area.*

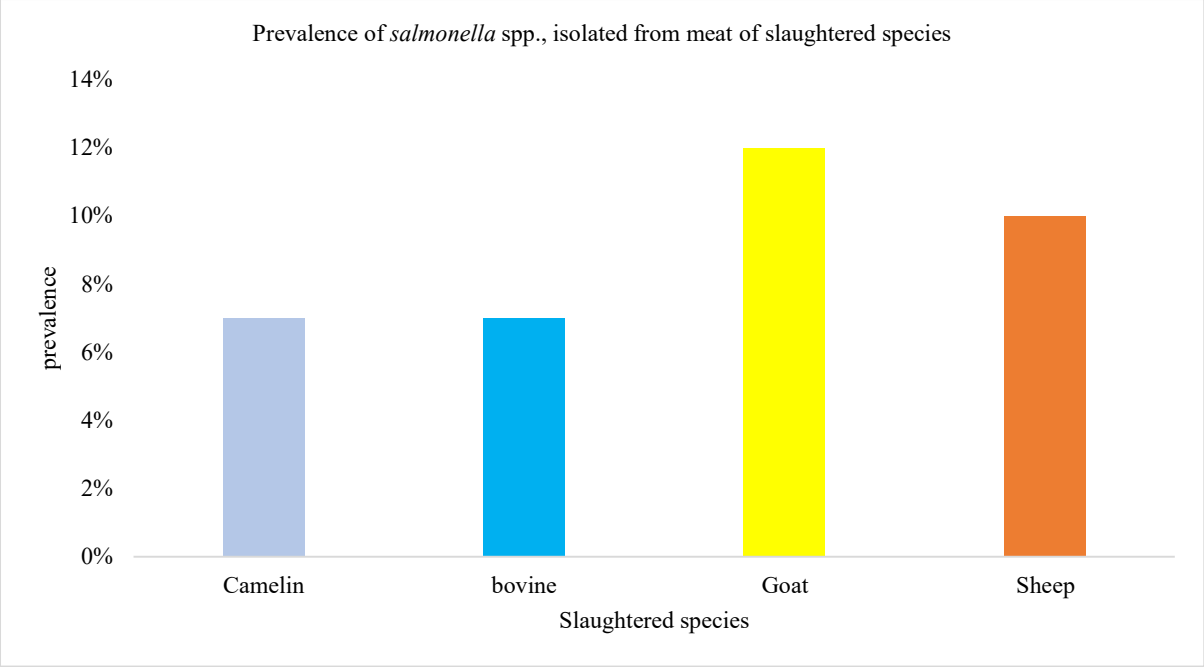

FIG.2. Prevalence of Salmonella spp., *isolated from meat of each species slaughtered.*

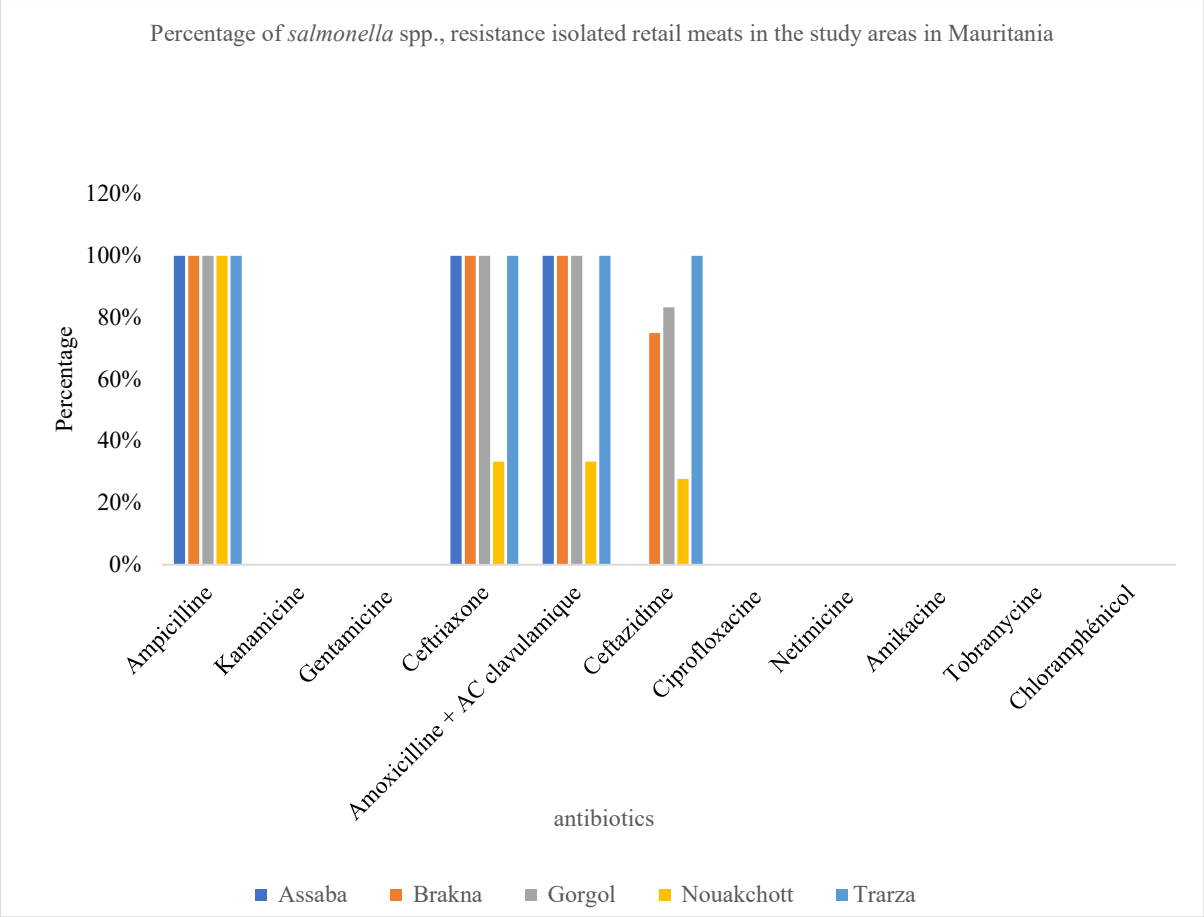

FIG. 3. Percentage of resistance of salmonella spp.*, isolated from retail meats according to the study areas in Mauritania.*

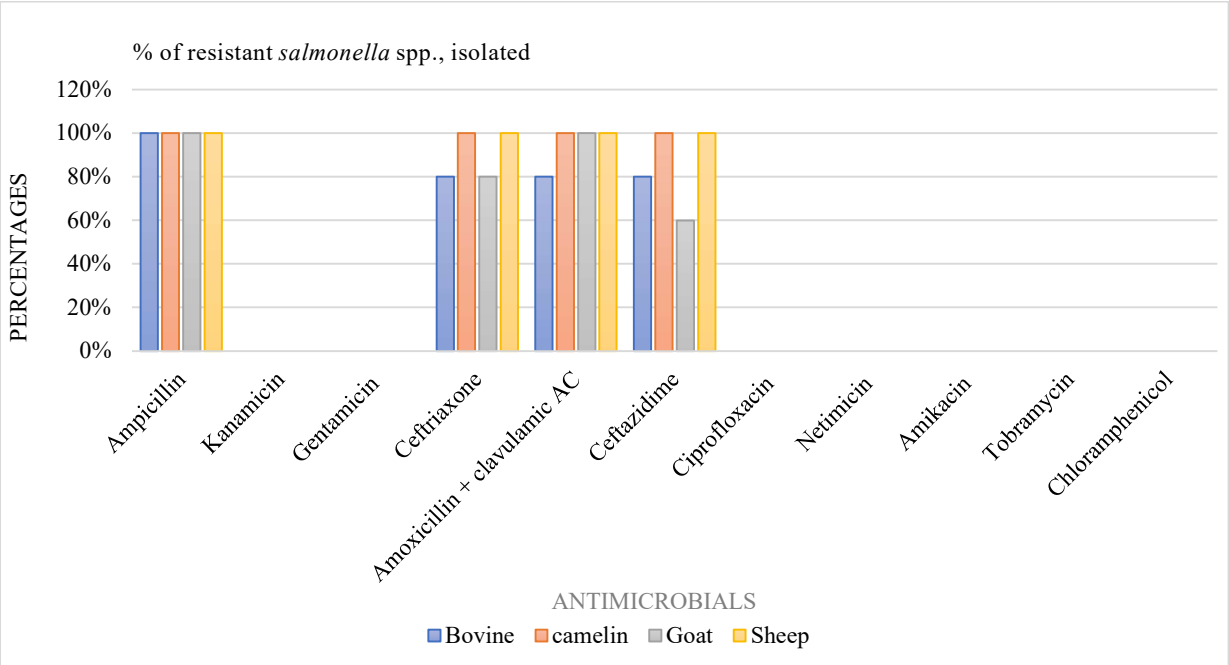

FIG. 4. Percentage distribution of resistance in salmonella spp.*, isolated according to the species slaughtered.*

The prevalence of *salmonella* spp., isolated from red meat was slightly higher in small ruminants (12% in goats and 11% in sheep) than in large animals (7% in camels and 7% in cattle). This prevalence is similar to that reported in a similar study on chicken meat in Mauritania conducted by INRSP in 2012 (unpublished data). During the experimental work, absence of a sterile sample was noted, which indicates the inadequacy of good hygiene practices at the level of slaughtering facilities, the transport chain and the marketing of meat. The majority of *salmonella* spp., isolated in the study areas were resistant to at least one antimicrobial. The highest rates of resistance were to ampicillin (100%) amoxicillin and clavulanic acid (94%) and ceftriaxone (94%) followed by ceftazidime (85%).

4. CONCLUSIONS

Resistance against the following antimicrobials: ampicillin, kanamycin, gentamycin, ceftriaxone, amoxicillin combined with clavulanic acid, ceftazidime, ciprofloxacin, netilmicin, amikacin and tobramycin was investigated in bovine, camel, goat and sheep meat. The samples were collected from the following five wilayas in Mauritania: Assab, Brakna, Gorgol, Nouakchott and Trarza. The study confirmed a prevalence rate of 9% of *salmonella* spp., in the red meat studied in Mauritania. It also determined a multidrug resistance rate of around 94% in *salmonella* spp., isolated from red meats. These results draw the attention of the control authorities in the country to the existence of antimicrobial resistance. Wider and more regular surveillance can benefit countries.

ACKNOWLEDGEMENTS

The authors thank the Office National de Recherches et de Développement d'Elevage et du Pastoralisme for supporting this study.

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A GC-MS/MS METHOD FOR DETECTING DITHIOCARBAMATES IN VEGETABLES INTENDED FOR LOCAL CONSUMPTION IN COSTA RICA

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Abstract

An analytical method was developed, validated and implemented for determining dithiocarbamates in vegetables in Costa Rica. Currently, in Costa Rica, these pesticides are not regulated by the National Phytosanitary Service (or SFE in Spanish) for the vegetables in this study. A modified European Union Reference Laboratory (EURL) method was established and validated using a 2^k experimental design to tailor it to the needs of the SFE. This work involved optimizing the conditions for sample extraction and operational parameters for the Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS). The validation followed guidelines outlined in SANTE/11312/2021. A limit of quantification (LOQ) of 0.040 mg/kg carbon disulfide (CS_2) and a linear range of (0.020–0.40) µg/ml were determined. The average recoveries of CS_2 at different concentration levels in various vegetable matrices ranged from 71% to 104%, with a standard deviation of ≤ 20%. Once validated, this method was applied to measure the pesticides in tomatoes, lettuce, celery, chili peppers, cilantro, and cucumbers from national production. These samples were collected between March and October 2023. Out of the 117 samples analysed, 21% failed to meet respective maximum residue levels (MRLs) particularly in celery. Additionally, most of the samples (55%) had concentrations lower than the LOQ.

1. INTRODUCTION

Dithiocarbamates are among the most utilized fungicides in Costa Rican agriculture, mainly due to their broad spectrum activity and low cost [1]. The main crops in which these pesticides are used are banana, beans, maize, tobacco, potato, citrus, tomato, lettuce and vegetables in general [2]. These crops are widely consumed in the country and form part of the basic Costa Rican food basket. Trace amounts of pesticides remain on crops following application and these constitute the residues [3] that could represent a health risk such as eye and skin irritation, respiratory tract irritation by inhalation, among others [4]. The MRL is defined as the maximum residue level of a pesticide at trace level, legally allowed in food (both inside the plant and on the surface) to be in accordance with good agricultural practices and is intended to make food toxicologically acceptable [3, 5]. The levels are expressed in mg of residues/kg of food [6]. Costa Rica largely follows MRLs established by the Codex Alimentarius, the United States Environmental Protection Agency (EPA) and the European Union. The decision on which MRL is applicable for crops in Costa Rica is based on local policy [7].

2. MATERIALS AND METHODS

2.1. Reagents and equipment

A certified thiram reference material (purity of 99.6%) was used to prepare the fortification solutions for both the experimental design and the analytical validation. Toluene was used as a solvent for the preparation of this standard. Mancozeb with a purity of 68.3% was also used to carry out fortification. This was prepared as a suspension using ultrapure water/acetonitrile and xanthan gum dihydrate.

A hydrolysis reagent was prepared using tin chloride, 37% hydrochloric acid and ultrapure water. The CS_2 with a purity ≥ 99.9% used as certified reference material for the preparation of the calibration curve. Isooctane was used as a solvent both in preparation of the calibration curve and in sample extraction. A freezer was used for storage of samples at -30°C while homogenization was performed with a mill operating at 2500 rpm. Heating was performed in a water bath. Subsequently, the samples were analysed by GC-MS/MS following separation with a suitable column (30 m, a diameter of 0.250 mm and a packing of 0.25 µm). The method used was adopted from an established study [8] and referenced to known MRLs [9].

This study was conducted at the Agrochemical Residue Analysis Laboratory between the first and second semester of 2023. Validation of the method included chayote vegetable as a representative matrix of group G1 (matrices with high water content) and in accordance with an established guide [10] and validation parameters recommended elsewhere [11]. After validation the method was used for routine analysis of celery, lettuce, tomato, chilli, cilantro and cucumber samples. Preparation for analysis followed established procedure [12]. Samples were frozen for 6 hours at -20°C and then homogenised in a mill at 2500 rpm.

2.2. 2^k Factorial design to determine optimal GC-MS/MS conditions

The design involved evaluating three parameters (split injection, injector temperature and oven temperature) at two different levels (split: 1:10 and 1:5, injector temperature: 90°C and 70°C and oven temperatures of 40°C and 45°C). These parameters were selected according to previous studies [1, 13]. Experiments were performed by injecting a CS_2 standard at 0.35 µg/ml in isooctane.

2.2. 2^k Factorial design to determine optimal extraction conditions

Three parameters; sample weight (5 g and 25 g) the percentage of acid present in the hydrolysis reagent (18 % and 12 %) and the volume of hydrolysis reagent added to the sample (150 ml and 60 ml) were investigated. This involved a blank chayote sample fortified at 0.040 mg/kg in CS_2. This CS_2 concentration corresponds to 0.063 mg/kg thiram. The procedure was adopted from previous studies [1, 8, 14]. Method validation followed EU guidelines 10 and the parameters included linearity (with 7 levels in a calibration curve) analytical range, LOQs and LOD, specificity, matrix effect, trueness, precision, robustness (with recoveries in routine matrix analysed) intermediate precision, uncertainty, ion ratio and retention time). Chayote was fortified with the pesticide thiram at 0.40 mg/kg CS_2 corresponding to 0.63 mg/kg thiram and 0.040 mg/kg CS_2 0.063 mg/kg thiram. The calibration curve was in a range of 0.02 mg/l CS_2 to 0.40 mg/l CS_2.

2.4. GC-MS/MS determination of dithiocarbamates in celery, lettuce, tomato, cucumber, chilli pepper and cilantro

Food samples (n=117) including lettuce (n=35) celery (n=20) tomatoes (n=33) chilli (n=12) cilantro (n=12) and cucumber (n=5) produced locally and received in the laboratory between March and October 2023 were analysed. Samples were extracted as soon as received. For each day of sample processing, a fortified quality control sample, previously tested as blank or analyte–free, as well as a reagent blank were prepared and used in a sequence. To consider such blanks as acceptable, they would not show any signal and in case of a signal, it would not exceed the LOQ by 30 %. Sample fortification was performed with thiram in most cases, at a concentration of 0.0010 mg/ml (0.040 mg/kg CS_2) and mancozeb at a concentration of 10 mg/l (0.060 mg/kg CS_2). Once the extraction was completed, the sample extract was placed in 2 ml amber vials with no space between the extract and the headspace of the vial and analysed by GC-MS/MS.

3. RESULTS AND DISCUSSION

3.1. 2^k Factorial design and GC-MS/MS conditions

Statistical analysis showed that only the split injection factor had a significant impact ($p=0.05$ and a confidence level of 95 %) effect in the analysis as can be seen in Fig. 1.

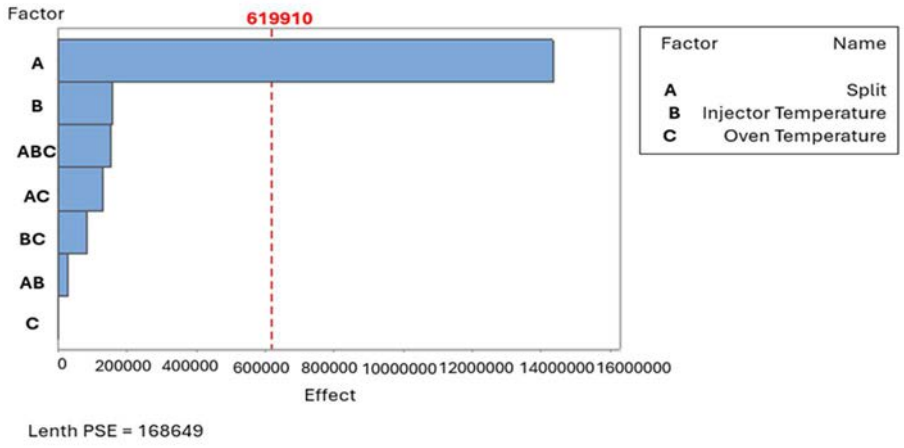

FIG. 1. Pareto diagram showing the effect of each of the parameters analysed with a reference value (red line).

Figure 2 shows the possible effect of the split-injection ratio. If a 1:10 split ratio is used, the amount of analyte entering the system is lower (0.10 ml of sample more carryovers enter the system) compared to a 1:5 with 0.20 ml of a sample enter the system. Selecting the appropriate split ratio is of great importance as in the case of dithiocarbamates [15].

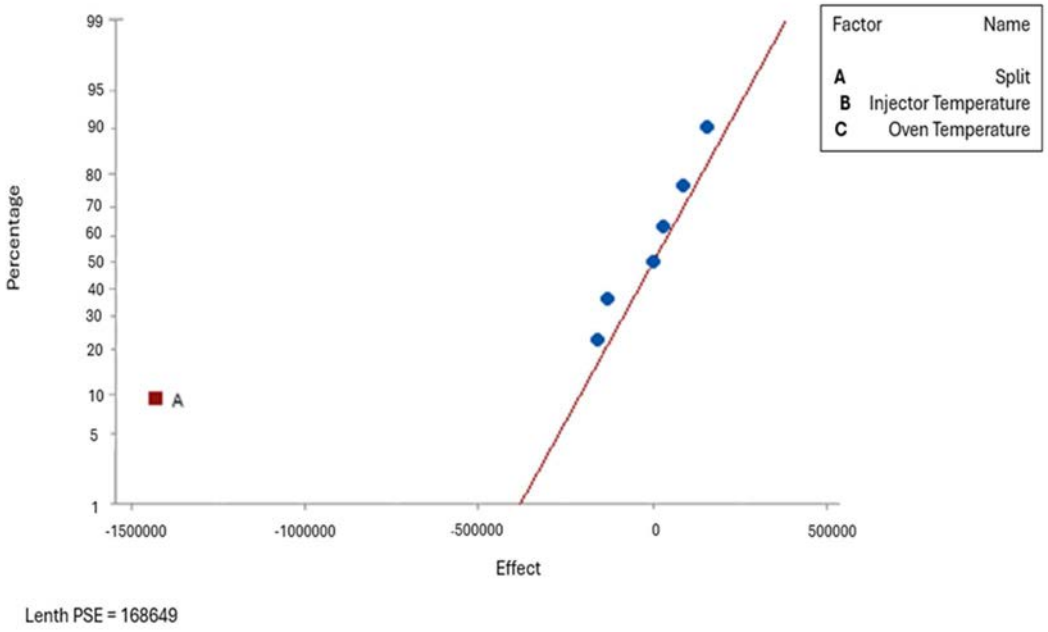

FIG. 2. Normal effects plot for the parameters analysed.

The injector temperature of 90°C was determined as ideal since the boiling temperature for isooctane is 99°C and of CS_2 is 46°C. Complete vaporization of the samples was thus deemed possible at 90°C although there is a risk of condensation in the injector [16]. The ideal oven temperature was 45°C as the chromatographic signal was higher with a lower split injection ratio.

3.2. 2^k Factorial design and extraction conditions

The ideal extraction conditions including the weight of the sample, the % v/v of HCl and the volume of the hydrolysis agent were determined based on signal intensity measure as a factor of the peak area (Gaussian shape of the chromatographic peak and with greater intensity, as shown in Table 1). Neither the Pareto diagram nor the normal effects graph was conclusive on the optimum combination of parameters to use for the extraction (Figs 3 and 4).

TABLE 1. VALIDATION PARAMETERS

	Evaluated conditions			
Experiment	Sample weight (g)	HCl percentage (%v/v) 12	Hydrolysis reagent volume (ml)	Area
1	5	18	60	682172
2	5	18	150	646499
3	5	18	60	610841
4	25	12	60	665746
5	25	12	60	632233
6	25	12	150	374332
7	5	18	150	412580
8	25		150	614507

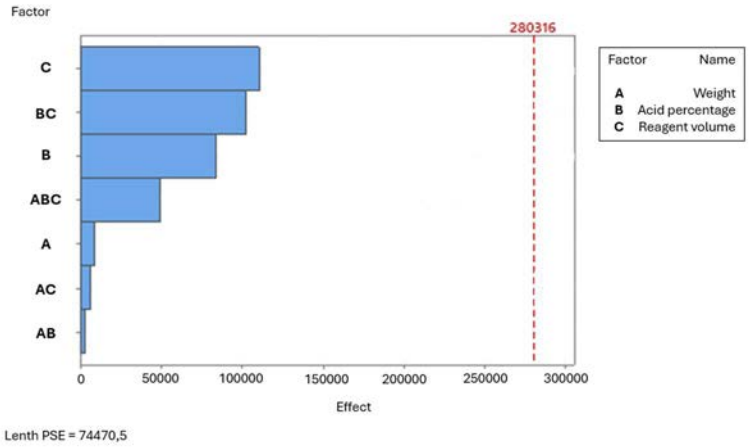

FIG. 3. Pareto diagram representing the effect of each of the parameters analysed in the experimental design.

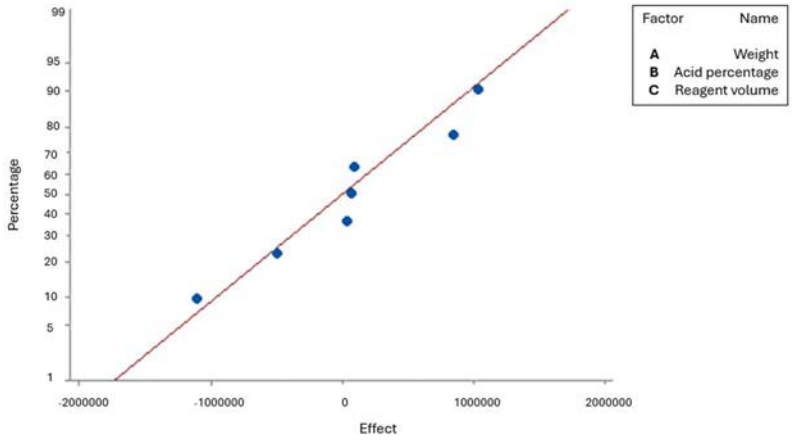

FIG. 4. Normal effects plot with the parameters analysed.

Sample weight of 5 g and 60 ml of hydrolysis reagent for digestion, with a 12% acid content were applied to reduce solvent consumption and generate less chemical waste.

3.3. Method validation

The results of successful method-validation are shown in Table 2.

TABLE 2. RESULTS OF VALIDATION FOLLOWING ESTABLISHED GUIDANCE [10]

Parameter	Value obtained	Acceptance criteria
Linearity	Residuals (-7 % to 19 %) $R^2 = 0.995$	Residuals ≤ ± 20 % $R^2 > 0.990$
Matrix effect	11 %	≤ 20 %
LOD	0.020 mg/kg CS_2	—
LOQ	0.040 mg/kg CS_2	≤ MRL
Specificity	Matrix blank: 3.0 % Reagent blank: 2.0 %	≤ 30 % of LOQ
Recovery	High level: 78 % ±48 % Low level: 88 % ±20 %	Recovery (70%–120) % Uncertainty ≤ 50 %

Parameter	Value obtained	Acceptance criteria
Linearity	Residuals (-7 % to 19 %) $R^2 = 0.995$	Residuals ≤ ± 20 % $R^2 > 0.990$
Precision (RSD_r)	High level: 9 % Low level: 15 %	≤ 20 %
Robustness	90	Recovery (60%–140) %
Precision (RSD_{wR})	18 %	≤ 20 %
Ion ratio	Complies	Precursor and/or product ion (s) peak fully overlap. Ion ratio from sample extracts has to be within ±30 % of the standards.
Retention time	Complies	±0.1 min.

Very good linearity was determined in the range 0.040 mg/kg CS_2–0.40 mg/kg CS_2 as stated elsewhere [10] ensuring a high degree of reliability in the data [17]. There was negligible matrix effect observed during the validation process. A solvent calibration curve was therefore used, the presence of endogenous or exogenous substances in the sample notwithstanding [18]. The LOD and LOQ were established in accordance with known guidelines [10] and any analyte concentration below the LOD was not quantifiable [19].

The method proved to be specific enough to determine dithiocarbamates in plant matrices with no cross-matching in matrix and reagent blanks As shown in Fig. 5, trueness was confirmed with recovery values (0.040 mg/kg CS_2 in chayote) ranging from 70% to 110% at 0.040 mg/kg CS_2 in chayote and 70–83% at 10 times the concentration and determined. The validated method was suitable for quantitatively determination of dithiocarbamates in plant matrices in group G1 [20].

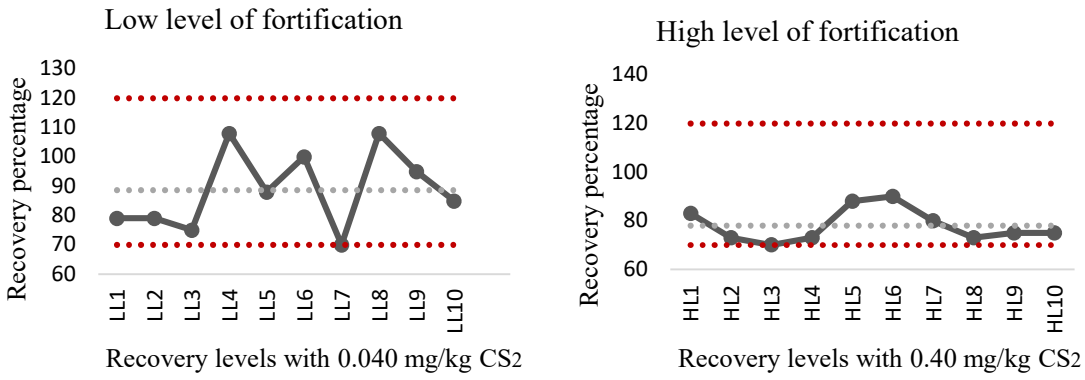

FIG.5. Control graphs of the low (0.040 mg/kg CS_2) and high (0.40 mg/kg CS_2) levels of fortification.

The method also demonstrated good repeatability and robustness as guided and demonstrated elsewhere [21, 22]. Figure 6 shows results of recovery tests performed over a period of time.

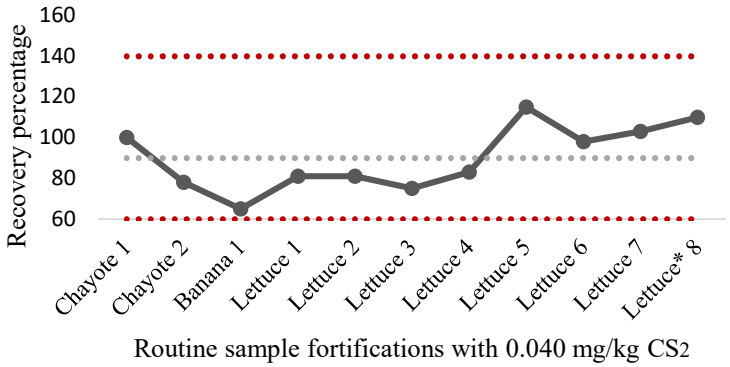

FIG. 6. Control chart with the recovery percentages.

The intermediate precision shown in routine recovery percentages, complied with followed guidelines [10] and agreed with previous studies [23]. Both the retention time and the ion ratios also met the acceptance criteria as shown in Fig. 7. The retention times for the analytes in standard and matrix were both at 4.4 mins. The qualifiers corresponding to the product ions, which have a shape, signal and retention time very similar to the analyte overlapped.

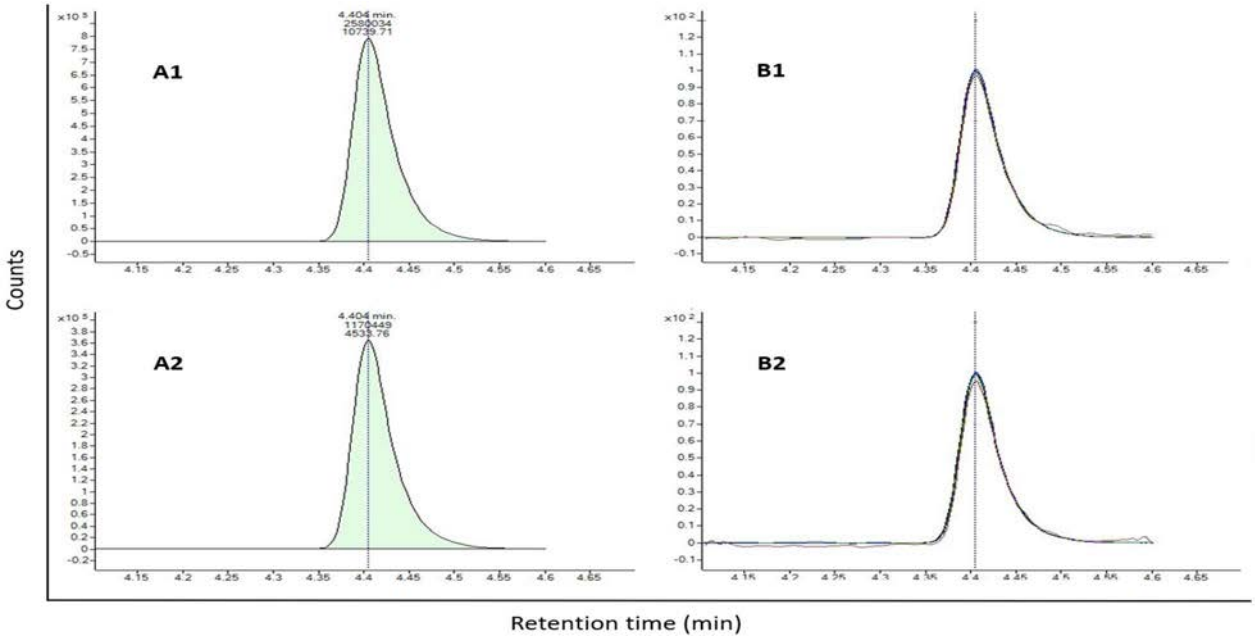

FIG. 7. A1 shows CS_2 (0.020 mg/kg) chromatographic peak at a retention time of 4.4 min; A2 is the chromatographic peak for chayote fortified at 0.020 mg/kg CS_2. Sections B1 (calibrator 1) and B2 (chayote fortified at 0.020 mg/kg CS_2) represent the qualifier ions.

3.4. Application of the validated GC-MS/MS method to determine dithiocarbamates in celery, lettuce, tomato, cucumber, chilli pepper and cilantro

Table 3 shows the results of food samples from the national production system received in the laboratory between March and October 2023.

TABLE 3. CONCENTRATIONS OF THE VARIOUS MATRICES ANALYSED AND COMPARED AGAINST MRLS

Matrix	MRL (mg/kg CS2)	Samples analysed	Non detectable residues	Detected residues <LOQ	Residues between LOQ and MRL	Residues greater than MRL
Celery	0.05	20	0	6 (30 %)	0	14 (70 %)
Lettuce	0.50	35	4 (11 %)	19 (54 %)	4 (11 %)	8 (23 %)
Tomato	2.00	33	0	17 (52 %)	16 (48 %)	0

Matrix	MRL (mg/kg CS2)	Samples analysed	Non detectable residues	Detected residues <LOQ	Residues between LOQ and MRL	Residues greater than MRL
Chilli pepper	1.00	12	0	4 (33 %)	7 (58 %)	1 (8 %)
Cilantro	5.00	12	0	4 (33 %)	7 (58 %)	1 (8 %)
Cucumber	2.00	5	0	5 (100 %)	0	0
Summary		117	4 (3 %)	55 (47 %)	34 (29 %)	24 (21 %)

As shown in Table 3, only lettuce did not contain dithiocarbamates residues above the LOQ although this matrix is known to contain residues above MRL. The findings in this could be that the pesticide was not used during production or the lettuce could have been thoroughly washed a common practice and as reported elsewhere [24]. Postharvest washing of vegetables can result in low levels or absence of residues [25, 26].

Overall, a high proportion of the samples tested (47 %) contained dithiocarbamate residues below the LOQ of 0.040 mg/kg CS_2. There were more lettuce samples (n=19 of 35) with a concentration of dithiocarbamates below the LOQ. About half (52%) of the 33 tomato samples had residue levels below the LOQ. This is a noteworthy finding since tomato is the most consumed crop in Costa Rica [27]. All cucumber samples showed residue levels below the LOQ, a welcome finding for the eleventh most consumed crop in the country [28]. The low levels could be attributed to post-harvest handling and good agricultural practices. Non-quantifiable concentrations of the pesticides were also detected in celery, lettuce, tomato, chilli pepper, cilantro and cucumber using the GC-MS/MS. A small number of chili (33%) cilantro (33%) and celery (30%) samples had dithiocarbamate residues below the LOQ (or not quantifiable) possibly due to washing off of the pesticides [29].

The presence of dithiocarbamates albeit at low concentrations could also be due to uptake from the soil. This pesticide persists in the environment for a period between 2 to 12 weeks [30]. The presence of the pesticides in 70 % of the celery suggests inadequate compliance with good agricultural practices. Celery or other foods with residue levels exceeding the MRL are a risk to because ethylene thiourea (ETU) the main degradation product of some dithiocarbamates is thought to be carcinogenic and is associated with effects on reproductive behaviour and birth defects observed in laboratory animals at high doses [31]. Since ETU is highly soluble and moderately mobile in the environment, it could easily migrate into food and/or into the crop environment [32] posing a high risk of exposure.

This study highlights the need to regularly monitor use of dithiocarbamate particularly in celery, to ensure proper application, doses, and compliance with withdrawal periods. Eight of the 35 lettuce samples exceeded the MRL, possibly due to excessive use of dithiocarbamate pesticides and/or non-compliance with good agricultural practices, adsorption on leaves of the crops of mancozeb the non-polar and most used dithiocarbamate in Costa Rica [32]. Washing would therefore not reduce the levels in lettuce [33]. Regulation and monitoring are thus imperative. The low levels of the pesticides studied in chilli pepper and cilantro below the MRL is a good finding since sweet chilli and cilantro are the 4th and 15th most consumed crops in Costa Rica, respectively [28].

Tomato, the most consumed crop, did not contain residues above the MRL although it is prone to fungal infections [33] and use of fungicides and resultant residues would be expected. Pesticides levels in cucumber, the eleventh most consumed crop in Costa Rica [27] were also low although like tomatoes it is often infested with three types of fungi [28]. The absence of fungal residue suggests the involvement of good agricultural practices [34]. The low pesticide levels could also be due to the sample collection period which was between March and October 2023 when weather/environmental conditions were not optimum for fungal growth [35]. Figure 8 shows low levels of dithiocarbamate pesticide residues in celery.

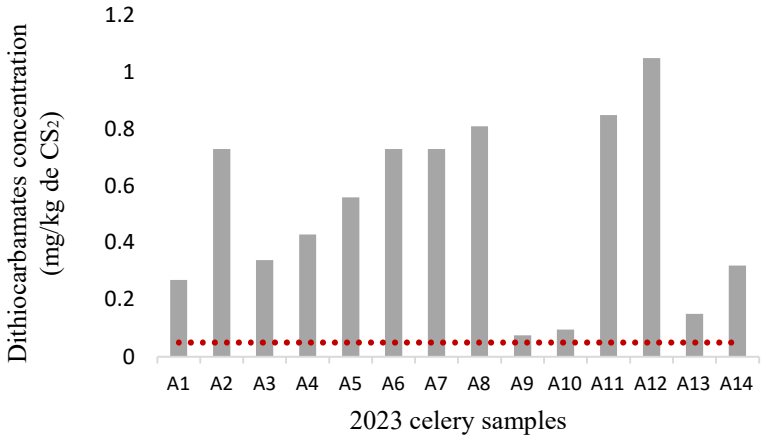

FIG. 8. Dithiocarbamate pesticide concentrations in celery above MRL of 0.050 mg/kg CS_2 (Red dotted line).

Figure 9 shows low levels of dithiocarbamate residues in lettuce although they exceed the MRL compared to celery (Fig. 8). The different (often low) residue-levels may be associated with varying prevalences of fungal infections which also influences the frequency of using the pesticide [36, 37].

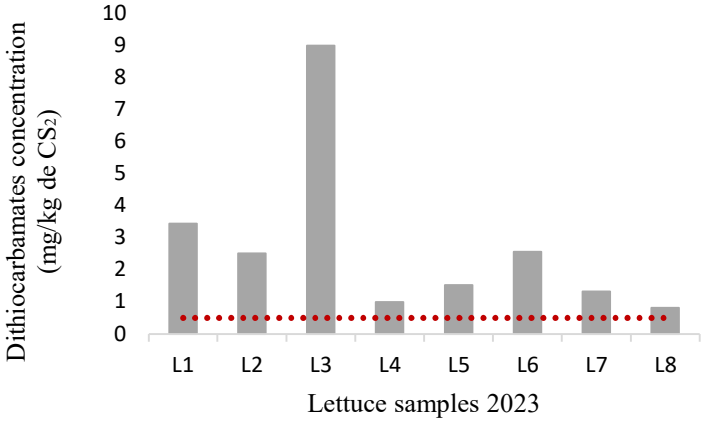

FIG. 9. Thiocarbamate residues in lettuce samples, exceeding MRL. The red dotted line represents MRL of 0.050 mg/kg CS_2. L represents each celery sample analysed.

4. CONCLUSIONS

A GC-MS/MS analytical method was developed, validated and used to determine dithiocarbamate residues in vegetables such as tomatoes, lettuce, celery, chili peppers, cilantro and cucumbers commonly consumed. in Costa Rica The method's development followed the European Commission's guidelines on analytical quality control and method validation procedures for pesticide residues analysis In food and feed. No dithiocarbamate residues were detectable in approximately half of the samples analysed (47% of the 117 samples analysed) suggesting compliance with good agricultural practices by Costa Rican producers or application of post-harvest handling practices that remove or reduce pesticides from the agricultural products. Some samples (29% of the 117) analysed contained pesticide concentrations between the LOQ and the MRL while levels for 21% exceeded the MRL. Celery was the most affected with 70% of the samples containing pesticides above the MRL.

ACKNOWLEDGEMENTS

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INVESTIGATING SELECTED PESTICIDE RESIDUES IN SEVERAL FOODS IN THE KINGDOM OF BAHRAIN (ABSTRACT)

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Abstract

A study whose findings are detailed elsewhere[1] was undertaken to ascertain the prevalence and concentration levels of certain pesticide residues in a wide range of foods consumed in the Kingdom of Bahrain. Over 500 locally produced or imported samples collected over a five-year period were analysed at the Public Health laboratory by gas chromatography tandem mass spectrometry and liquid chromatography tandem mass spectrometry following preparation by the QuEChERS method. The matrices included parsley, Bahraini legume, Bahraini basil, Bahraini rocca, Bahraini ruwaid leaves and tomatoes. Others were oils and canned products; meats such as beef and chicken; eggs; beverages such as teas; grain such as rice and nuts (e.g. cashew nuts) and a range of spices. The pesticides included several organophosphates and organochlorines. Myclobutanil was the most prevalent followed by azoxystrobin and metalaxyl. A negligible level of residues was above the maximum residue levels (as applicable) otherwise most residues were below tolerated limits or not detected at, all thus ruling out public health concerns.

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INVESTIGATING THE RISK OF IMPORTED/SMUGGLED MEAT SAUSAGES WITH *E. coli* O157:H7 IN MOZAMBIQUE

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Abstract

In Mozambique, there is a possibility that pathogens such as *E. coli* pathogenic strains could be present in meats imported from countries such as Brazil and South Africa, partly due to lack of inspection and control at boarder entry points. The objectives of this study were to assess the occurrence of *E. coli O157,* compare, retrospectively, the level of contamination of imported meat sausages from 2018 to 2023, and then, to propose actions to minimize the importation or introduction (if contamination is at the point of entry) of sausages with unacceptable levels of *E. coli O157* into Mozambique. The results showed that the risk of consuming *E. coli O157* or other pathogens through imported sausages is very low. In quantitative terms, the proportion of acceptable imported sausages was more than 69%. The majority of sausages imported from South Africa (many originating from Brazil) enter the country by smugglers in vehicles, mixed with other products, without proper cold systems which could contribute to contamination of the sausages. The risk assessment results indicated that the risk of introducing pathogenic *E. coli* through the importation of sausages into the country is very low but not entirely negligible.

1. INTRODUCTION

The WHO estimates that foodborne related illnesses cause death of an estimated 48 million people globally every year, of which 1.9 million are children under five years [1]. Some of the pathogens include *Escherichia coli O157*:H7 whose presence in food indicates recent faecal contamination and constitutes a great risk to consumers [2]. The presence of *E. coli O157*:H7 is also important to measure the sanitary condition of the product during the processing, storage, transport and use of contaminated raw material or at the beginning of the deterioration process. The bacteria could grow aerobically on plate count agar at incubation conditions varying from 30°C to 37°C [1].

According to Chingoma et al., [1], there have been concerns about the operational conditions of the sausage processing line in relation to hygiene and food safety. Most food processors handle food without aprons and with uncovered hair, long nails and bare hands; and they handle many things and food simultaneously. All these factors can be a source of food contamination. Meat sausage manufacturing is a process that allows different types of meat to undergo a series of controlled structural and chemical changes depending on formulations and attributes such as shelf-life and organoleptic properties. The sausages are processed through different stages using raw meat, fats and different spices. Some are cooked or emulsified sausages. They can be available in fresh, or in dry or cured form.

According to the American Meat Institute Foundation [3] sausages are defined as commuted processed meat products made from red meat, white meat or a blend of these together with water, binders, seasoning and preservatives. A sausage is formed (Fig. 1) in a casing, traditionally made from animal intestines but sometimes synthetic casings are also used.

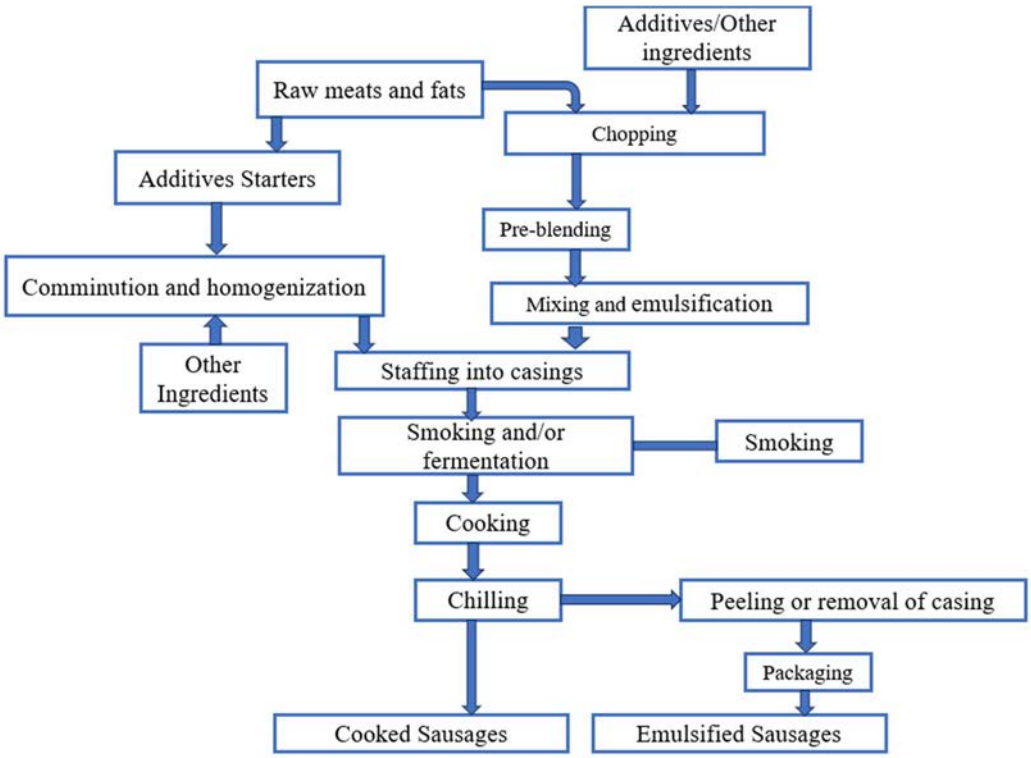

FIG.1. Schematic showing sausage processing.

Sausages can be contaminated along the whole processing line in the food plant, as well as during transportation or storage and handling at retail. Due to smuggling in Mozambique, the products are handled using small unrefrigerated vehicles from border post and reach the market in poor conditions. Mittelstaedt and Carvalho [4] reported that humans can be infected by *E. coli O157*:H7 in different ways, including water intake, contaminated food (hamburger, meats, milk, vegetables, sauces prepared for salads and mayonnaise). Consumption of meat products contaminated with faeces from infected animals during slaughtering process; consumption of the products without appropriate heat treatment during preparation; transmission from person to person, especially where hygiene conditions are not appropriate; and cross contamination, are some of the avenues for which products, equipment, environment and consumers are contaminated.

There is limited information about the contamination of sausages imported into Mozambique with *E. coli O157*:H7 hence this study that covered the period from 2018 to 2023. The focus was sausages imported from South Africa and Brazil. The objectives included evaluating the contamination levels over this six year period; conducting a risk assessment on those sausages.

2. MATERIAL AND METHODS

2.1. Study design

The research was carried out from 2018 to 2023 at the Directorate of Animal Science, Department of Food and Nutrition in Maputo, Mozambique, using laboratory results and surveillance data of chicken and beef sausages introduced in the country through smugglers from South Africa and Brazil. It is not possible to characterize which samples came directly from Brazil and which ones originated from South Africa.

2.2. Sample handling and preparation

The microbiological control of food provides for some preliminary operations such as inspecting the product before opening and preparing for the examination. The examination included expiry date, identification, filling relevant forms, checking the temperature of products and number of samples to collect, in accordance with established protocols [2]. Collected samples were transported to the laboratory using a cool box with ice. For the present study, 833 samples of sausages imported from South Africa (some originating from Brazil) were tested to determine the presence of *E. coli O157*:H7, including isolation and identification, using International Commission on Microbiological Specifications for Foods (ICMSF) methods and protocols of the Ministério da Saúde [2] international standards and FAO [5]. The samples were collected aseptically from cold chambers, packed in sterile

plastic bags, labelled and transported to the laboratory in a cool box for laboratory testing. A special software was used for data analysis including comparing of different contamination levels over the years.

Weighing was done in sterile containers, and using sterile materials, aseptically 10 g of the sample was placed in a sterile plastic bag, mixed with 90 ml of the diluent (buffered peptone water) and mixed in a Stomacher for 30–60 seconds. Three different techniques were used for bacteriological examination: Most probable number Method (MPN) with 95% confidence; *E. coli*/coliform plate/petrifilm count and *E. coli O157*:H7 strip test kit at a sensitivity of 1×10^4 cfu/g–1×10^5 cfu/g. Briefly, for the MPN method, which gives the density of viable organisms in a sample, 9 glass test tubes containing 9 ml of MacConkey broth were used. In each tube, 9 ml of buffered peptone water was used to prepare working dilutions of 10^1, 10^2 and 10^3.

The samples were incubated at 37ºC ± 0.2ºC for 48 h and all positive tubes were inoculated in 9 ml *E. coli* broth and incubated again at 44.5ºC ± 0.2ºC, using a water bath. The positive tubes were associated with colour change and gas production. The procedure included placing 1 ml of the appropriate dilution in a sterile petri dish, in duplicate. The agar (12–15 ml) was dropped on the plates that were then shaken for proper distribution of the inoculum, and after drying, the plates were inverted and kept at 37°C for 24 h. Reddish-coloured colonies were selected for biochemical tests. For the petrifilm/plate count method, a dilution of 1:10 was used directly and the plates incubated at 35ºC ± 1ºC for 24 h. Blue colonies with gas were considered positive.

2.3. Confirmation

E. coli O157:H7 strip test kits were applied to all positive tubes from the MPN test in accordance with the manufacture's protocol. The test was considered positive when two coloured lines appeared; one coloured line in the T area (test line) and one in the C (control) area. A gram test was performed and biochemical tests carried out on all suspected colonies to see the reaction.

3. RESULTS AND DISCUSSIONS

The test results are summarized in Table 1.

TABLE 1: RESULTS OF SAMPLES TESTED FROM 2018–2023

Year	Total No. of samples	No. (%) Acceptable	No. (%) Acceptable marginally	No. (%) Unacceptable
2018	42	33 (78.7)	1 (2.3)	8 (19.0)
2019	70	69 (98.6)	0 (0)	1 (1.4)
2020	213	162 (76.0)	11 (5.2)	40 (18.8)
2021	178	178 (100)	0 (0)	0 (0)
2022	191	184 (96.3)	0 (0)	7(3.7)
2023	169	163 (96.4)	1 (0.6)	5(3.0)

3.1. Risk assessment

Risk assessment is needed to predict and estimate the impact of the hazard and design the strategy for managing risk. This also facilitates risk communication. A sketch demonstrating probability of exposure is shown in Fig. 2.

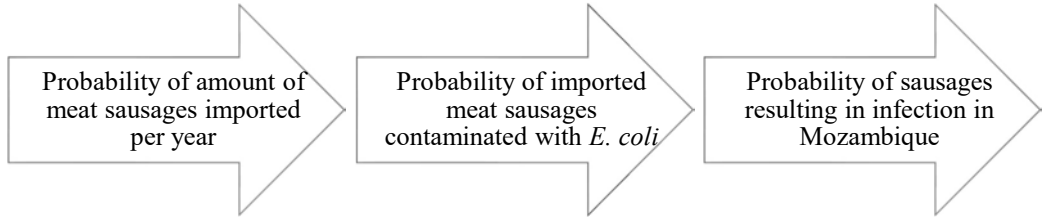

FIG 2: Probability of exposure to E. coli in meat sausages.

This study estimated the frequency of consumer exposure to sausage meat contaminated with *E. coli* O157. A scenario was developed to assess the probability of sausages being contaminated during processing (Fig. 3).

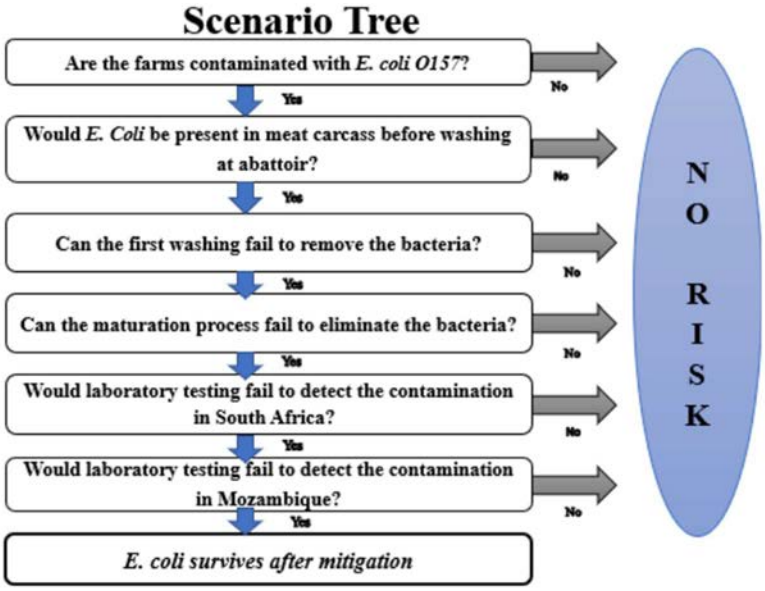

FIG. 3: Probability of product contamination from farm-to-the sausage testing.

Through risk analysis, it is possible to identify the different control points in the processing chain, the intervention options facilitating efficient management of risks. For data analysis, Monte Carlo simulation at 10000 iterations was used and the expected values at each node calculated. The number of contaminated products was calculated using a beta distribution approach. The sensitivity analysis, using the tornado graph, indicates the overall probability that the failures of not following good practices can have an impact on the risk of introducing *E. coli* in the country, with a maximum probability of 0.05% of getting contaminated products. Figures 4–6 show the models' overall probability of imported sausages contaminated with *E. coli.*

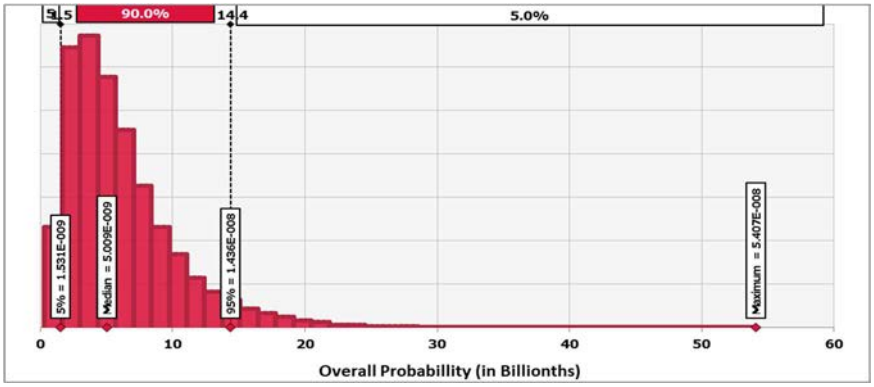

FIG. 4. The graphic representing the Modelling Uncertainty that the bacteria can cause human health problems through the consumption of imported sausage.

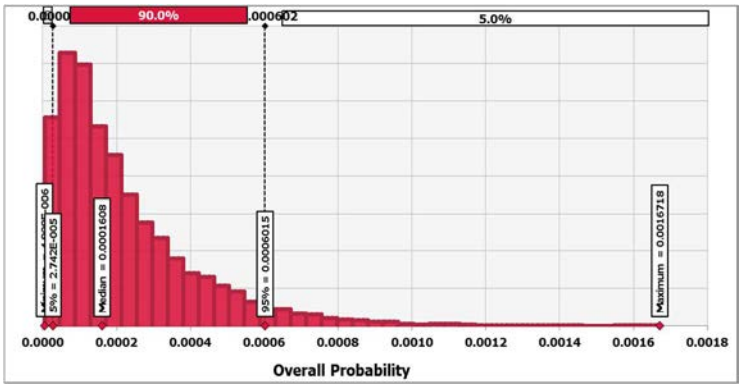

FIG.5. The number of years over which a case of consumer infection due to contamination with E. coli O157 may occur.

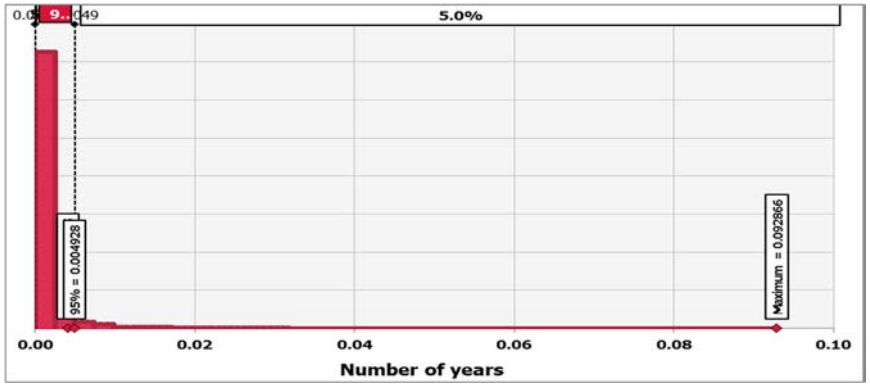

FIG. 6. Number of years in which contamination of the products due to an outbreak of E. coli can occur.

The presence of faecal coliforms may be one of the indicators of hygienic failures during processing of products, due to cross contamination by handlers, equipment or other utensils [6, 7]. According to Roberts and Greenwood [7], the presence of faecal coliforms is an indicator of an unhygienic conditions of the product; whereas a high percentage of unacceptable products (29% and 40%) means a post-processing contamination, poor sanitation or multiplication during the storage process. According to the present study, the risk of introducing *E. coli O157:H7* in Mozambique appears low. In 2019, 98.6% were compliant compared to 100% in 2021 although the levels were lowest b (19%) in 2018. It is nevertheless important that authorities continue to monitor food products to reduce any risk of infection.

Studies conducted on sausages in Harare [1] indicated that most of the samples were within acceptable microbiological quality range and only a few samples were contaminated above the standard limit. However, low the total bacterial count may be, a product is not necessarily safe. Typically, the total bacterial counts could reflect the general hygienic condition of a sample [1]. Consistent with the above report, most of samples in this study were also within the acceptable level of contamination. In 2019 for examples about 98.6% of the samples were within an acceptable range of contamination.

According to Chingoma et al., [1] the results of the enumeration of total coliforms in the samples of cooked street vended sausages ranged from 10 cfu/g to 10^2 cfu/g. In this study, the results obtained from the microbial assay indicated that 19.4% of the sampled sausages were within the satisfactory limit of 10–10^2 cfu/g [2] indicating good microbiological quality. In 2020, 213 samples were confiscated from informal traders at border posts (Mozambique and South Africa) and destroyed by officials of the inspection services. These traders were known to use poor transport and storage conditions that could result in high microbial loads [8].

Using the scenario tree and the prevalence values in each stage of the chain from farms in South Africa or Brazil (processed beef) to the laboratory testing (sausages) the simulation shows that the risk of introducing *E. coli O157* through the importation of sausages in the country exists but is low. It is nevertheless important to consider the mitigations measures applying good hygiene procedures and good manufactures procedures to avoid the cross contamination. The study noted that some consignments were shipped into the country thawed and not packed properly. The laboratory received some samples that were not frozen and protected from potential exposure to microbes.

4. CONCLUSIONS

This study investigated levels of *E. coli O157:H7* in sausages collected from 2018 to 2023. The imported sausage products were generally free of the pathogen, and this is safe for human consumption. More than 78% of the imported sausages were microbiologically acceptable. Nevertheless, it is important to ensure overall hygiene and good manufacturing practices to have safety and good quality products on the market and thereby to protect the health of the consumers. It is imperative to continue conducting this kind of surveillance at entry points and other points in the country.

ACKNOWLEDGEMENTS

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INVESTIGATING FOOD POISONING OUTBREAK CAUSED BY *STAPHYLOCOCCUS AUREUS* ENTEROTOXIN AND INCIDENCE OF STAPHYLOCOCCAL FOOD POISONING IN THE KINGDOM OF BAHRAIN

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Abstract

Staphylococcal food poisoning (SFP) is one of the most common foodborne diseases resulting from the ingestion of food contaminated with *Staphylococuss aureus* (*S. aureus*) enterotoxins. The aim of the study was to provide an overview of the incidence of staphylococcal food poisoning in Bahrain and to describe the epidemiological and microbiological investigations of a food poisoning outbreak among school members in 2018. A single-centre, retrospective study was conducted at the Public Health Laboratories (PHL) in Bahrain. All food poisoning samples received at PHL from 2013 to 2021 were included in this study. Overall, staphylococcal enterotoxin food poisoning accounted for 28.1% of confirmed food poisoning cases; Staphylococcal enterotoxin A (SEA) was the most prevalent toxin (60%). The laboratory investigation showed the presence of SEA in chicken shawarma. Also, SEA has been isolated from patients' stool samples and food handlers' hands, nose and throat swabs. Outbreak investigations revealed that poor hygiene practices during food processing and improper food handling practices accounted for this outbreak. Training workers to follow all hygienic protocols and practices for preparing, handling, and cooking food may be considered to avoid future outbreaks.

1. INTRODUCTION

S. aureus is a major cause of nosocomial and community-acquired infections [1]. The pathogenicity of *S. aureus* is due to toxins and invasive enzymes [2]. Staphylococcal food poisoning results from eating foods contaminated with enterotoxins produced by *S. aureus*. It is characterized by sudden onset of nausea, vomiting, stomach cramps, diarrhoea and fever. Symptoms usually develop within thirty mins to eight hours after eating food contaminated with the enterotoxins [3]. Twenty-eight different staphylococcal enterotoxins (SEs) and staphylococcal enterotoxin-like toxins (SEls) have been identified along with many variants [4]. The true SEs, are toxins that show emetic activity and SEls, are non-emetic toxins or toxins that have not been tested. Staphylococcal enterotoxin A (SEA) Staphylococcal enterotoxin B (SEB) Staphylococcal enterotoxin C (SEC) Staphylococcal enterotoxin D (SED) and Staphylococcal enterotoxin E (SEE) are well defined and classified as classical enterotoxins [5]. These five classical SEs contribute to 95% of SFP outbreaks, SEA being the most common comprising > 50% of SFP outbreaks [6]. Staphylococcal enterotoxins are non-glycosylated, antigenically distinct, low molecular weight (25–30 kDa) proteins consisting of approximately 220–240 amino acids that all fold into homologous globular structures [7, 8]. The genes encoding the different enterotoxins are carried and distributed by different mobile genetic elements [prophages, plasmids, pathogenicity islands, enterotoxin gene cluster (egc) and the staphylococcal cassette chromosome (SCC) [9]. The sea gene composed of 771 base pairs encodes for SEA precursor of 257 amino acid residues [10].

S. aureus is a gram-positive coccus, facultative anaerobic, non-motile, non-spore forming bacterium [11]. This bacterium produces a carotenoid pigment that appears golden, leading to the appearance of golden colonies [5]. *S. aureus* is found as a part of normal flora on the skin and mucous membranes [9]. It can be found in the air, water, dust, and excrement of humans and animals [2]. *S. aureus* can survive for prolonged periods after initial contact on hands and inanimate and environmental surfaces [12]. Staphylococcal enterotoxins are short proteins that are soluble in water and saline solutions, heat-stable, and resistant to several environmental conditions such as freezing, drying and degradation from proteolytic enzymes such as pepsin or trypsin, enabling them to function in the gastrointestinal tract after ingestion [13]. The nuc gene, which is responsible for heat resistance, is highly related to enterotoxin production and can be considered a marker of *S. aureus* enterotoxin infection [1].

Staphylococcal food poisoning can occur with any food that provides sufficient carbon and amino acid sources for *S. aureus* growth [14]. Several types of food act as optimal media for the growth of *S. aureus* such as meat, poultry, egg, dairy products, salad, bakery products and sandwich fillings [12]. *S. aureus* can grow and produce SEs over a wide range of temperatures (7–48.5°C; optimal 30–37°C) pH (4.2–9.3; optimal 7–7.5) and sodium chloride concentrations up to 15% NaCl [12]. Staphylococcal enterotoxins are transmitted to humans from food contaminated with *S. aureus* through food surfaces, food handlers, contaminated raw materials, unsuitable handling of processed food and insufficient cooling of food [4, 11]. Food handlers contaminate food through

manual contact with their noses or hands or through respiratory tract secretions [9]. Approximately one-third of healthy human populations carry *S. aureus* in their noses, which often contaminate the face, hands and fingers. Therefore, nasal carriers can act as a source of cross-contamination in food [15].

The severity of food poisoning symptoms depends on the quantity of toxin ingested and the health status of the affected person [13] and on the toxin type [9]. When the number of *S. aureus* organisms/g in the food exceed 100,000, the intoxication dose of SE is less than 1.0 µg. However, in more sensitive individuals, ingesting 100–200 ng of enterotoxin can result in symptoms [16]. Symptoms appear rapidly within 30 mins after ingesting the SE contaminated food. The incubation period is from two to seven hours, and symptoms disappear within 12 hours [9]. Symptoms are commonly characterized by abdominal pain, diarrhoea, nausea and vomiting [7]. Staphylococcal food poisoning is often self-limiting with recovery occurring one to three days after the onset of symptoms. However, symptoms may be more severe in young, elderly and immunocompromised patients [8]. Treatment is generally aimed at restoring fluid and electrolyte losses due to severe vomiting [13].

Staphylococcal enterotoxins can penetrate the small intestine through epithelial or mucus-producing goblet cells. Staphylococcal enterotoxin A stimulates the release of serotonin and histamine from mast cells. Serotonin then stimulates the vagus nerve in the vomiting centre of the brain by triggering an emetic response [14]. The release of inflammatory mediators is accountable for the local damage of the gastrointestinal tract, mostly appearing in the stomach and the upper part of the small intestine [17]. Despite strong induction of emesis, the symptoms of diarrhoea are often less apparent in SFP, which may be due to the inability of some SEs, such as SEA and SEC, to cause the fluid exudation and dilation of the small intestine [8]. Diarrhoea may happen as a result of water inhibition and electrolyte reabsorption in the small intestine [17].

This study aimed to provide an overview of staphylococcal food poisoning, identify the association of *S. aureus* enterotoxin — a major threat to food safety and the healthcare system [18] — with food poisoning outbreaks and describe the epidemiological and microbiological investigations of the food poisoning outbreak in 2018 among school members in Bahrain.

2. MATERIALS AND METHODS

2.1. Study design and study setting

A single-centre, retrospective study was conducted at PHL in Bahrain on all food poisoning samples received from 2013 to 2021. This includes the epidemiological and laboratory investigations of food poisoning outbreak in 2018 among 90 school members (87 students and three teachers). The data of all samples received from 2013 to 2021 was extracted from local database at PHL. All samples were analysed according to the microbiological criteria approved by the Gulf Cooperation Council Standardization Organization (GSO). The reference bacterial strain used in this study was *S. aureus* ATCC 6538. This study was approved by Health Research Committee in the Ministry of Health, Bahrain in January 2023.

2.2. Epidemiological investigation

A notification was received from the hospital to the public health at 1:30 p.m. that two suspects of food poisoning reported eating breakfast in school at 9:00 a.m., and five more cases were reported from another hospital at 4:15 p.m. Epidemiological investigation to collect information and samples from patient, food and food handlers was done. The Food Control Department was notified to take the necessary actions. Five samples were collected and sent to PHL for microbiological analysis. A sample from a leftover chicken shawarma and four swabs from kitchen surfaces and cutting boards (meat table, vegetable table, meat cutting board and vegetable cutting boards) were collected. Swabs were collected from the hands, nose, and throat of the four food handlers. Nine stool samples from the students were also collected.

2.3. Laboratory investigation

Microbiological analysis of the samples and detection of SEA were performed. Food samples were evaluated following established criteria [19] under the ready-to-eat foods category. The limit of *S. aureus* in the ready-to-eat foods category is equal to 20cfu/g (m value) below which is considered satisfactory and 10^2 cfu/g (M value) above which is considered unsatisfactory. Samples were analysed according to a known standard [20].

A 10^{-1} dilution of chicken shawarma was prepared in buffered peptone water (CM1049) and aliquots of the food dilution were spread on Baird-Parker agar plates (CM1127). The chicken shawarma leftover was tested for the detection of SE using an automated qualitative test for the detection of staphylococcal enterotoxins (SEA-SEE) using an enzyme linked fluorescent assay. Reconstituted extraction buffer (25 ml) was added to 25 g of food in a blender bag and centrifuged for 15 mins at 3000–5000*g* at 18–25°C. The supernatant was filtered and 500 µl of the filtrate was added to the VIDAS strip well. Environmental swabs, food handlers' hand, nose and throat

swabs, and patient stool samples were inoculated on Baird-Parker agar plates. Plates were incubated at 35°C or 37°C for 48 h ± 2 h.

Suspected colonies of *S. aureus* (black/grey, shiny colonies with white and clear zones) were counted and confirmed using the Staphaurex latex agglutination test and identified using a MALDI-ToF MS system. *S. aureus* isolates from chicken shawarma, food handler swabs, and patient stool samples were tested for the production of SEA-SED by SET-RPLA Kit. The isolates were inoculated into tryptone soya broth (CM129) and incubated at 37°C for 18–24 h. The broth was then filtered using a 0.22 mm low protein-binding membrane filter. A summary of the workflow for the laboratory investigations performed at PHL is shown in Fig. 1.

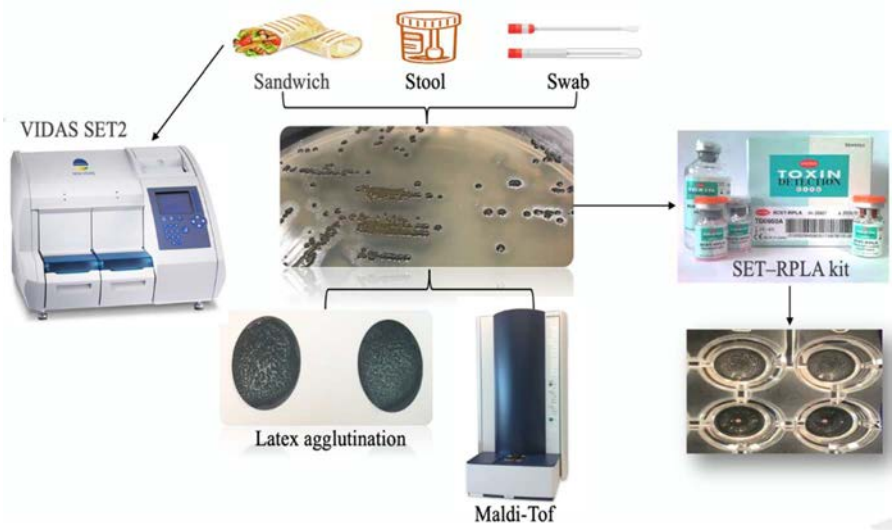

FIG. 1. General overview of analytical methods used for detection of SE.

2.4. Statistical analysis

Data analysis included the following information: number and type of samples tested, food category, number of food poisoning cases and microbiological parameters with test result. The data was analysed as number and percentage and presented in tables and graphs.

3. RESULTS AND DISCUSSION

3.1. Incidence of staphylococcal food poisoning

In Bahrain, approximately 680 food poisoning cases were reported to the Food Control Department from 2013 to 2021 (Fig. 2) with 57 cases confirmed and the causative agents involved in these food poisoning cases were isolated. *Salmonella* bacteria were the most common cause of food poisoning, accounting for 27 (47.4%) confirmed cases, followed by *S. aureus* enterotoxins 16 (28.1%) and *Bacillus cereus* enterotoxin 14 (24.6%). Among the 16 cases of SFP, 20 types of SEs were identified and SEA was the most prevalent toxin detected in 12 (60%) cases followed by SEB 3 (15%) SED 3 (15%) and SEC 2 (10%) (Fig. 3). The main factors contributing to these SFP outbreaks were poor personal hygiene, improper food handling practices and inadequate refrigeration of foods. Hot and humid weather in Bahrain increase the risk of SFP as microbial activity and spoilage of food increase. The most reported food categories involved in SFP are ready-to-eat food (61%) followed by salad and appetizers (22%) cakes and desserts (11%) and dairy products (6%) (Fig. 4). The most reported food category involved in SFP is ready-to-eat food. The disease is frequently associated with protein-rich food such as meat and meat-based products [11].

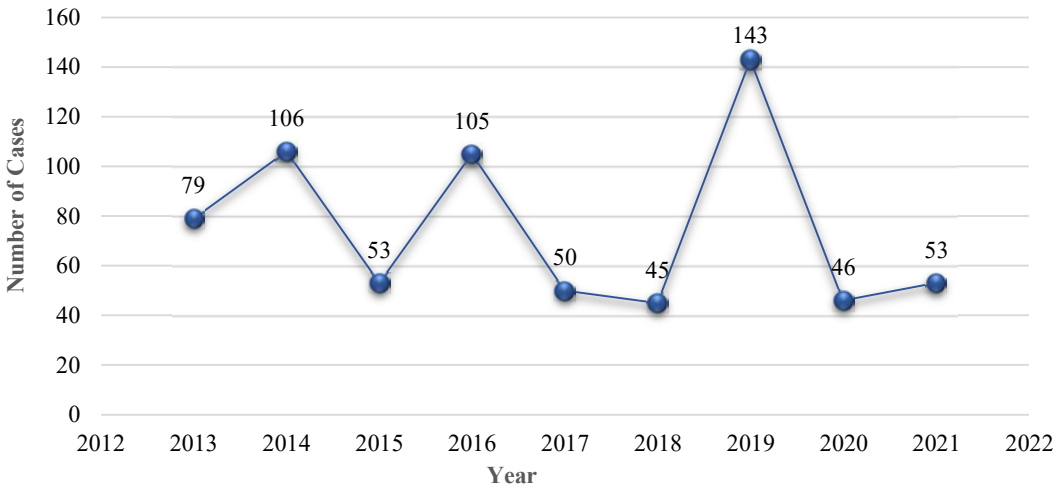

FIG 2. Food poisoning cases reported from 2013 to 2021.

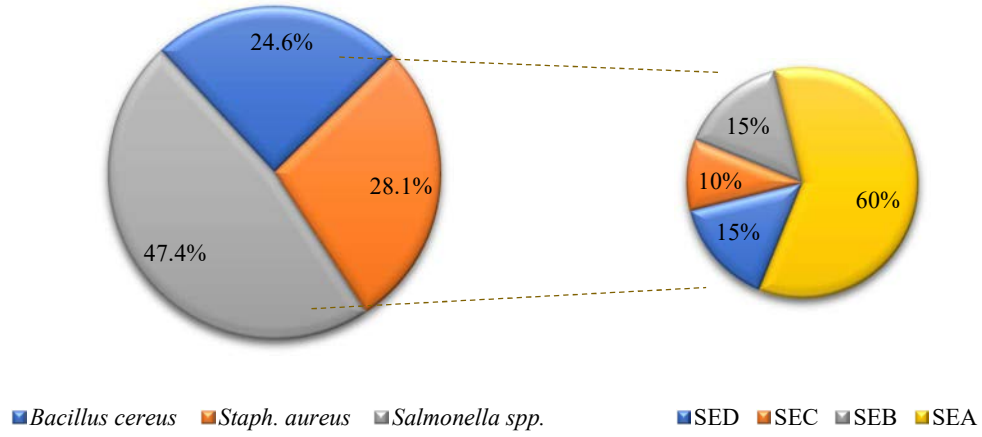

FIG.3. Causative agents involved in food poisoning with % cases in the period 2013-2021.

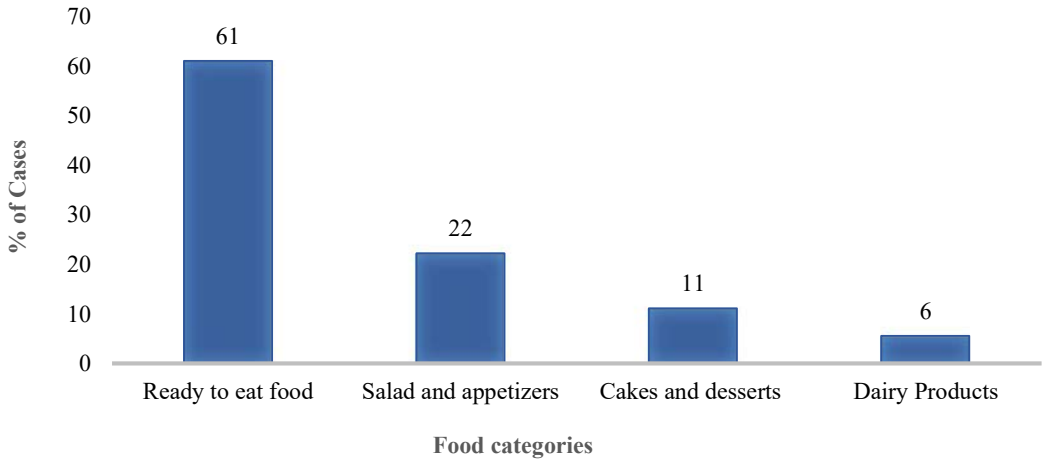

FIG. 4. Food categories involved in staphylococcal food poisoning.

3.2. Epidemiological and laboratory investigation

Three of the educational staffs and 87 students were affected by the food poisoning and 68.9% (62) were male. Most cases were between the age of 3–5 years (51.1%) followed by 9–11 years (22.2%) and 6–8 years (20%) (Table 1). Symptoms included vomiting, abdominal pain, and diarrhoea one to two hours after the students ate chicken shawarma in the school. Three cases had severe symptoms were admitted to the hospital. The school confirmed the distribution of 400 chicken shawarma sandwiches between students and some teachers: 300 shawarma chicken were given to kindergarten and first grade students and 100 to other students and teachers.

TABLE 1. DETAILS OF CASES ACCORDING TO AGE AND GENDER

Age group in years	Male	Female	Total
3-5	27	19	46
6-8	16	2	18
9-11	16	4	20
12-16	3	0	3
> 16	0	3	3
Total	62	28	90

Laboratory results indicated the presence of almost 2×10^3 cfu/g *S. aureus* in chicken shawarma, which was considered unsatisfactory according to established criteria [19]. The VIDAS SET2 test result was positive for SE in the leftover sample. Moreover, *S. aureus* colonies were isolated from swabs of three food handlers (Table 2) and stool samples from eight students. SEA was detected in chicken shawarma; food handler swabs and student stool samples.

TABLE 2. THE LABORATORY RESULTS OF FOOD HANDLERS

Food Handlers	Swabs			Staphylococcal enterotoxin (SE)
	Nose	Throat	Hand	
1	Not isolated	Not isolated	Not isolated	—
2	*S. aureus* isolated	*S. aureus* isolated	Not isolated	SEA
3	*S. aureus* isolated	*S. aureus* isolated	*S. aureus* isolated	SEA
4	*S. aureus* isolated	*S. aureus* isolated	Not isolated	SEA

The laboratory results corresponded with the symptoms that appeared on the students quickly after eating chicken shawarma, as poisoning with the bacteria isolated in the laboratory usually appears within a period ranging from half an hour to seven hours after eating contaminated food [9]. The investigation in this study revealed that *S. aureus* enterotoxin A in chicken shawarma was the cause of this outbreak. Several studies have reported that *S. aureus* counts are $>10^5$ cfu/g to be considered as toxic and produce SEs in food [4, 9, 11]. However, SEA was detected with *S. aureus* counts of up to 10^3 cfu/g in the current study. This finding was similar to a study conducted on food poisoning outbreak among children in primary school after eating lunch from a school canteen [21]. *S. aureus* was isolated from chicken floss at 10^3 cfu/g. *S. aureus* enterotoxin A was detected directly from chicken floss. A study [9] on three food poisoning outbreaks (A, B, C) that occurred in 2013 in Belgium in which SEA was detected in outbreak A with *S. aureus* at 200 cfu/g in mashed potato, and up to 10^3 cfu/g in chicken and sausage in outbreak B. This study's investigation showed that SEA was detected from nasal/throat swabs of food handlers and patient stool samples which is similar to the findings of the Belgian study.

The SFP outbreak in Bahrain was likely caused by contamination of chicken shawarma with *S. aureus* from food handlers, either through their hands or coughing and sneezing over food due to poor personal hygiene, improper handling of cooked food, improper cleaning of food preparation areas and lack of sufficient tools for preservation and storage. Medical counselling was provided to the school canteen workers who were confirmed to be infected with *S. aureus*; appropriate treatment was also provided, and coordination was made for follow-up after treatment. Coordination was made with the school to follow all hygiene instructions and good practices in

food preparation such as training workers on hygienic practices for preparing, handling and cooking food, as well as ensuring that no ill individuals could contaminate food or food contact surfaces.

Food handlers have been implicated in a large number of foodborne diseases, where *S. aureus* is one of the important pathogens often transmitted to food through food handler's nasal and hand carriage [22]. Therefore, it is important to detect healthy and asymptomatic *S. aureus* carriers among food handlers to prevent possible food contamination. Since nasal carriers frequently come into touch with hands, fingers and face, they can readily become skin carriers [23]. Iyevhobu et al., [22] conducted a study to determine the prevalence of *S. aureus* from 300 nose and skin swab samples from healthy food handlers in restaurants in Nigeria. The results revealed that 30 (10%) of the handlers were carriers of *S. aureus*, with the highest incidence of 24 (16%) from nasal swab and 6 (7.5%) from skin swab. Another study conducted involving 300 food workers from different sectors in Türkiye [23] demonstrated that *S. aureus* was isolated from 90 (30%) of noses and 84 (28%) from hands. Forty-two (33.6%) *S. aureus* strains were positive for one or more SE genes out of 125 strains and SEA was found at rates of 14.4%. Furthermore, a study conducted in Sudan on 186 food handlers who were working in different restaurants showed that SEA was the most common SE (19.4%) out of the total 93 isolated strains of *S. aureus* [24]. Alhashimi et al., [10] reported a study on 332 food handlers following SFP outbreaks in Iran Republic. The SEA gene was detected in 16 (of 100) *S. aureus* strains isolated from the handlers.

Most cases of SFP can be prevented by adequate hygiene measures and maintained cooling chains [14]. Maintaining the cold chain at temperatures below 7–10°C is essential for reducing the risk of *S. aureus* growth and production of enterotoxin in food products [5]. The permissive temperature for growth and toxin production is between 6°C and 46°C. Therefore, the ideal temperature for cooked food are above 60°C or below 5°C. Other preventive measures may be considered, such as control of raw food, proper handling, cleaning, and disinfection of equipment and tools used in food processing and preparation [12] as stainless steel, which is widely used in the food business, is one of the inert surfaces on which *S. aureus* shows a good degree of adherence and facilitates the formation of biofilm that improve the ability to tolerate disinfectants [18].

There were a number of limiting factors in this study that influenced the epidemiological investigation process and thus the isolation of the causative agent. Some cases of food poisoning have been misdiagnosed as viral gastroenteritis at hospitals, and some patients refused to provide a stool sample for laboratory testing. Moreover, there was missing data that some patients did not provide during the investigation. Some patients, especially children, forget some information such as the time of onset of symptoms, type of symptoms, and food they ate. Furthermore, most of the samples received were not leftover due to delay in acting either due to missing information or delay in reporting food poisoning cases, which reduces the chance of obtaining leftover samples, which explains the low number of confirmed cases.

4. CONCLUSION

S. aureus enterotoxin is the second common cause of confirmed foodborne outbreaks in Bahrain from 2013 to 2021 and SEA was the most prevalent toxin involved mostly in ready-to-eat food category. This study investigated the epidemiological and microbiological investigations of food poisoning outbreaks among school members in 2018. The causative agent of the outbreak was SEA-producing *S. aureus* strain isolated from chicken shawarma, patient stool samples and food handlers' nose/throat swabs due to improper food handling and poor personal hygiene with the presence of nasal carriers among food handlers. Training food handlers to follow personal hygienic protocols and food safety practices for preparing, handling, and cooking may be considered to avoid future food poisoning outbreaks. Moreover, initiating the WGS of *S. aureus* in food poisoning cases will create a qualitative shift in investigating and tracing the source of SFP outbreaks.

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ESTABLISHMENT OF A RISK-BASED SURVEILLANCE PROGRAMME OF PESTICIDE RESIDUES IN CROPS IN PANAMA

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Abstract

A risk-based pesticide residue monitoring programme has been developed for crops produced in Panama. It involves the Ministries of Agriculture and Health. Initially, to establish the number of samples, the percentage of samples above the maximum residue levels (MRL) of the previous year was used as a reference. Data from 2544 samples of 121 crops and 114 pesticides during the period 2009–2020 were considered. Several factors were considered including the acceptable daily intake (ADI) values, WHO's pesticide classification, percentage of samples above the MRL, per capita consumption, total crop area and the consumption preference. Risk was classified according to an established scoring system.

1. INTRODUCTION

Since the establishment of the pesticide residue laboratory in Panama in 2006, a surveillance programme was set up and implemented by the Ministry of Agricultural Development and the Ministry of Health. The programme used as a reference value in number of samples, as the incidence of violative samples from the previous year. This approach did not consider risk factors such as: the properties of pesticides, the ADI and annual per capita consumption among others. Risk-based monitoring programmes provide an opportunity for countries to modernize their surveillance programme under a harmonized methodology and to improve their efficiency and sustainability by applying the principles of risk analysis. Various risk ranking methods that prioritize food safety risks exist [1]. These methods are based on the concept of risk as a combination of probability of exposure and impact on human health.

All methods available for risk-ranking have their pros and cons and depending on the purpose of the ranking and time and resources available, a method can be selected to perform a risk ranking [2]. A successful risk-based monitoring programme requires political commitment and an adequate regulatory framework to support the process, designing the model based on the collection of adequate and relevant information, and periodically subjecting the model to a process of adjustment to allow for continuous improvement [3]. The purpose of establishing a risk-based surveillance programme of pesticide residues in crops is to guide official authorities regarding the hazards and foods to be sampled, prevent and reduce the risk of consuming unsafe foods while optimizing use of available resources.

2. MATERIALS AND METHODS

The risk based surveillance plan arises from the evaluation of data from 2544 samples of 121 crops and 114 pesticides collected in 2009–2020. Several factors were considered to facilitate risk posed by pesticides in food, including ADI values, WHO pesticide classification, samples above the MRL, per capita consumption, total crop area and consumption preference [4–6].

A scoring system was developed for each pesticide according to the following risk factors:

a) Toxicity: Ia=10 pts., Ib=7.5 pts., II=5 pts., III=2.5 pts., U=0 pts;
b) Solubility: Low=10 pts., Moderate= 1 pts., High=0 pts;
c) Systemic: Yes=10 pts., No=0 pts;
d) ADI (mg/kg b.w./day): <0.001=10 pts, >0.001 and <0.01=7.5 pts., >0.01 and <0.1= 5 pts.,>0.1 and <1=2.5 pts., and >1=0 pts.

Toxicity classification according to WHO latest report [3, 7] (Ia, Ib, II, III and U class, where Ia means extremely hazardous, Ib highly hazardous, II is moderately hazardous III is slightly hazardous and U is unlikely to pose acute hazard in normal use. Risk ranking was developed to classify food products based on the following risk factors and considering nutritional status [6–8]: The risk score was determined as in Eq. (1).

Total risk score=% +ve samples×% samples with residues>MRL×Annual consumption×Av pesticide score (1)

After calculating all the risk scores for each food product, the percentage (%) of each single product was determined using Eq. (2)

Percentage of product risk score=(Single product score/Total risk score)×100 (%) (2)

Where the total risk score was the sum of all individual risk scores.

3. RESULTS AND DISCUSSIONS

The exposure to pesticides was evaluated through the most consumed fresh fruits, vegetables and grains in Panama. Pesticide concentration data on most fresh fruits and vegetables consumed, daily consumption data, and population body weights were combined to estimate chronic daily dietary pesticide exposure. Ten pesticides were used in this study. A list of the most toxic pesticides found in Panama are described in the Table 1 and agree with reports and guidelines.

TABLE 1. RISK RANKING OF PESTICIDES MONITORED IN PANAMA

Substance	Toxicity	Solubility	Systemic	ADI	Risk
Mirex	Obsolete	low	no	0.0000	40
Terbufos	Ia	low	yes	0.0006	40
Carbofuran-3-hydroxy	Ib	low	yes	0.0010	38
Bromuconazole	II	low	yes	0.0100	33
Carbaryl	II	low	yes	0.0080	33
Difenoconazole	II	low	yes	0.0100	33
Dimethoate	II	low	yes	0.0100	33
Flutriafol	II	low	yes	0.0100	33
Pyrazophos	II	low	yes	0.0040	33
Chlorfenapyr	II	low	yes	0.0300	30

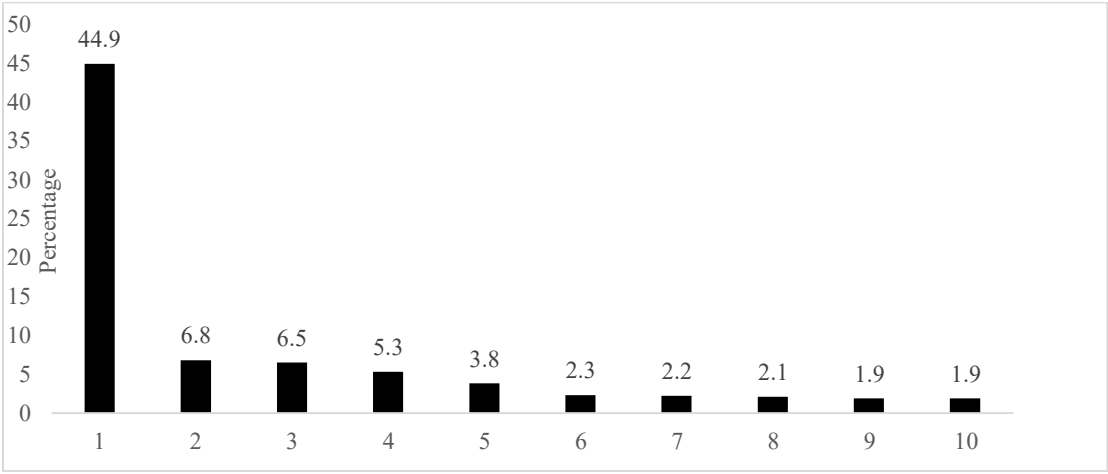

FIG.1. The crops with the highest risk considering all the risk factors in Panamá 2009–2020.

A scoring system was developed to calculate a single risk score for each food product and the contribution (percentage) of every food product over the total risk score as shown in Table 2. The exposure values were compared with the ADI values to assess the potential risk to public health that pesticides represent to Panama. The pesticides with the highest exposure were acephate at 30.9% and triazophos at 11.7% of the ADI.

TABLE 2. LIST OF THE COMMODITIES TO BE MONITORED BASED ON TOTAL RISK SCORE

Commodities	%>MRL	Consumption (kg/hab.)	Total risk (pesticides-food)	% samples to analyse
Rice	10	70.2	64.2	45
Banana	6.1	12.2	9.8	7
Pineapple	4.8	17.4	9.3	7
Avocado	30.6	2.2	7.5	5
Tomato	12.0	2.7	5.4	4
Spinach	23.8	0.9	3.3	2
Papaya	14.8	1.8	3.2	2
Chili	29.6	0.6	3.1	2
Lettuce	22.9	0.9	2.8	2
Celery	40.4	0.4	2.8	2

Initially, the surveillance programme was primarily focused on the detection of pesticides to verify compliance with MRLs, paying attention to the markets without considering the potential impact on consumers or the appreciable risk it could pose to the health of Panamanian consumers and especially the nutritional status of children [6]. This has its disadvantages given the cost of testing and monitoring foods for all pesticides and since there is no zero risk to pesticide exposure. The risk-ranking approach has been viewed as a starting point, in line with the current global trend of conducting risk-based monitoring programme. The results presented in Tables 1 and 2 facilitate identification and focus on the pesticides that pose the greatest risk, including those that are prohibited in the country. Rice, the most consumed crop, had the highest risk of exposure, despite showing few violations of the established MRLs [4, 5, 7]. This study has enabled institutions and authorities responsible for pesticide control, to redirect the pesticide residue monitoring programme, thereby optimizing the human and financial resources of the responsible institutions. However, a major limitation is the lack of updated data on dietary consumption and the absence of information for certain vegetables. These limitations present an obstacle to the implementation of a risk-based monitoring programme.

4. CONCLUSIONS

Panama has established a basis to redesign the annual pesticide monitoring programme according to the risk of each pesticide-food pairing using a selected group of crops and pesticides. Percentages calculated from this study can be used by Panamanian authorities to assign the number of samples to test for pesticides in each food crop investigated based on the pesticides' inherent risk. The exposure assessment conducted for all pesticides detected in ten targeted fresh fruits, vegetables and grains in Panama from 2009 to 2020, revealed that none exceeded the ADI value, except for mirex, where any amount detected in a sample is considered unacceptable due to associated potential health risk.

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SCREENING ANTIMICROBIAL RESIDUES IN CHICKEN MUSCLES IN DHAKA DIVISION OF BANGLADESH USING CHARM II RADIORECEPTOR ASSAY

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Abstract

In this study, 150 muscle samples from three different chicken types were screened for the residues of tetracyclines, sulfonamides, beta-lactams, and macrolides/lincosamides groups by using Charm II radioreceptor assays. The aim of this study was to support the regulatory authority in ensuring antimicrobial residue-free foods. Test samples were collected from different districts of Dhaka, and suspected samples were identified by comparing their respective radiotracer counts per minute (cpm) values with control points (CP). Detection capabilities (CCβ) were verified at 0.5 MRL level; selectivity/specificity and ruggedness for each test method were tested following guidelines set by the Community Reference Laboratories Residues (CRLs) 20/1/2010, and Commission Implementing Regulation (EU) 2021/808. Out of these 150 samples, 60 were identified as suspects, among which 14 were from more than one of the tested groups. The majority of the detected residues were tetracyclines (27.30%) followed by sulfonamides (12.70%) macrolides/lincosamides (6.00%) and beta-lactams (3.30%).

1. INTRODUCTION

Almost 20% of the proteins consumed in Bangladesh are from poultry [1]. Overall, three types of chicken are consumed for meat: (a) local/indigenous, (b) Sonali, a cross-breed between Rhode Island Red cocks and Fayoumi hens, and (c) exotic broilers. Local/indigenous and Sonali chickens are typically raised non-commercially in backyards, using a scavenging or semi-scavenging system, while broilers are reared on commercial farms. More than 58.39% of the total chickens in Bangladesh are broiler breeds [2]. The demand for poultry meat is rising in many countries including Bangladesh, which has triggered increase in poultry production, and resulting in the use of different antimicrobial drugs to fight diseases and enhance poultry production [3, 4].

Improper, unregulated use of antimicrobials is associated with residues in the edible tissues with potential health effects including but not limited to allergies in humans and spread of associated antimicrobial resistance to pathogenic microbes [5, 6]. Therefore, monitoring drug residues in food is crucial for safeguarding human health and preventing the spread of antimicrobial resistance. Different regulators have established specific MRLs to monitor and ensure safe food consumption to human, which will also facilitate international trade, protect the environment, and uphold ethical standards in the food industry [7].

Screening methods such as Four Plate Test (such as Premi test) radioreceptor assay (such as Charm II) Enzyme Linked Immunosorbent Assay (ELISA) and lateral flow assays, usually facilitate quick decision making and could be used for routine monitoring antimicrobial residues in food [8–12]. Some of the laboratories also rely largely on these screening tests, that are easily affordable and adoptable compared to the complex and expensive confirmatory instrumentation such as HPLC and LC-MS/MS may not available [4, 7, 13]. This study reports the use of a radioreceptor assay method to screen antimicrobial residues in chicken meat samples as reported elsewhere [14–17]. A previous study demonstrated that Charm II radioreceptor assays were suitable for screening antimicrobial residues in poultry eggs due to its high selectivity/specificity, repeatability, reproducibility, easy to adopt in the laboratory and affordable compared to the complex and expensive confirmatory instrumentations [3].

The study involved the analysis of chicken meat samples in Dhaka division, Bangladesh for antimicrobial residues, especially tetracyclines, sulfonamides, macrolides and beta-lactam residues.

2. MATERIALS AND METHOD

2.1. Standards, chemicals, and equipment

Chlortetracycline, sulfamethazine (SMZ) benzylpenicillin (Pen G) erythromycin A (Erythro) and methanol (purity >99%) were purchased. Others were radioreceptor assay kits for tetracycline, sulfonamides, beta-lactam, and macrolides along with other reagents such as tissue performance negative control, M2 buffer, MSU extraction buffer, scintillation fluid, 5 ml test tubes, and pH strips. The instrument used was a liquid scintillation counter and a luminometer supported by Can intronic incubator. A refrigerated centrifuge, food processor and a water bath were also used to conduct the study.

2.2. Collection of samples

Chicken samples (n=150) including 20 indigenous chickens, 54 Sonali chicken, and 76 broilers were collected randomly from various kitchen markets and supermarkets within 17 different districts of Dhaka division in Bangladesh, between 2018 and 2019. Muscle tissues from both breast and thigh were selected, cut into smaller pieces after removing excess fat. These were then homogenized in a food processor and then were stored at below -18°C until examination.

2.3. Preparation of standard solutions and control samples

Stock standard solutions (100 mg/l) of CTC, SMZ, Erythro and Pen G were prepared in separate test tubes by dissolving 0.5 mg of the respective antimicrobial powders with 5 ml methanol. Working standard solutions of 10000 µg/l for CTC, Erythro, Pen G, and 1000 µg/l for SMZ were then prepared by diluting the respective stock solutions with water and stored at 4°C before use. Homogenized chicken muscle samples, determined to be free of antimicrobial residues based on the Tissue Performance Negative Control (TPNC) test, were used as negative control/blank samples. Positive control samples were prepared by spiking the blank samples with CTC, SMZ, Erythro and Pen G at their screening target concentration (STC) level, which was set at 0.5 EU-MRL following the established EU guidelines [18, 19]. In this regard, the blank samples were spiked with CTC (50 µg/kg) SMZ (50 µg/kg) Pen G (25.00 µg/kg) and Erythro (100 µg/kg).

2.4. Sample extraction

Homogenized samples (10 g) were placed in a 50 ml polypropylene centrifuge tube and thoroughly mixed with 30 ml MSU extraction buffer. The mixture was incubated at approximately 80°C for 30 mins, and left to stand on ice for 15 mins before spinning on a centrifuge at 1750 G for 10 mins at 25°C. The supernatant was then decanted for testing, and the pH was adjusted to 7.5 using M2 buffer. The extract was then left to stand at room temperature immediately before analysis on the same day.

2.5. Validation of the Charm II radioreceptor assays

Prior to screening, the Charm II radioreceptor assay methods were adopted in the test laboratory by verifying the detection capabilities (CCβ) of the target antimicrobials CTC, SMZ, Pen G and Erythro from the antimicrobial groups of tetracyclines, sulfonamides, beta-lactams and macrolides, respectively) at their STC level (0.5 EU MRL). Selectivity/specificity, and ruggedness of each test were also evaluated following the guidelines [18, 19]. Stability testing was not conducted in this study due to availability of associated data as reported elsewhere [20, 21].

2.6. Statistical analysis

Data were statistically analysed by comparing the F-calculated value (F-calc) obtained from One way ANOVA with the theoretical F-critical value (F-crit) to check for any significant differences.

2.7. Screening of the chicken samples

2.7.1. Performance monitoring

Prior to screening the samples, a quality tests including negative and positive control standards were run each day. Typically, the negative control standards would have higher cpm values than the control point (CP) unlike the positive control standards.

2.7.2. Analytical procedure

A tablet containing the binder (binder) from a test kit was placed in a test tube. Then, 300 µl of water was added, and the contents mixed for 10 seconds. A sample extract or control sample was added, followed by another tablet containing the tracer reagent (tracer) from the same kit. The test for beta-lactams, and macrolides required additional incubation treatment before adding the tracer tablet. The solution was mixed for approximately 10 seconds and then incubated at the required temperature and time. Then, the samples were centrifuged at 1750g for the specified duration and the supernatant carefully poured off.

Deionized water (300 µl) was added and the material mixed to dissolve the resultant pellet. A scintillation fluid (3 ml) was added to the suspension and mixed well to form a uniform cloudy mixture. This mixture capped, was then counted for 60 seconds using a ^{14}C channel or ^{3}H channel on a liquid scintillation counter. The signals or responses in cpm were then compared with the respective CPs. Samples with cpms higher than the CP were considered negative while those lower than the CP were suspect positive. Suspect samples were re-tested in duplicate. The method was effectively applied in proficiency tests during the September 2019 round for the screening of sulfonamides in swine muscle and tetracyclines in chicken muscle samples, respectively.

3. RESULTS AND DISCUSSION

3.1. Verification of CCβ values for tetracyclines, sulfonamides, beta-lactams, and macrolides tests

In order to verify the CCβ for the chosen analytes at their selected concentrations, blank samples were independently spiked with CTC (50 µg/kg) SMZ (50 µg/kg) Pen G (25 µg/kg) and Erythro (100 µg/kg). Then, the scintillation counts (in cpm) of twenty blank and twenty spiked samples were analysed for five consecutive days (four samples per day). The cpm values of blank and spike samples along with the respective mean values for blank (B) and spiked samples (M) the cut-off factor (Fm) CPs, and technical threshold (T) values for each antimicrobial test kits are graphically presented in Fig. 1. The cut-off factor (Fm) represents the signal/response of a test assay, which is used for determining if the target samples contain the target analytes at or above the selected targeted concentration, while the 'T' refers to the limit for a positive result in the test method. The T and Fm values were determined using Eqs 1 and 2 following the EU guideline [18].

$$T = B - 1.64 \times SD_b \quad (1)$$

$$Fm = M + 1.64 \times SD \quad (2)$$

The SD_b and SD is the standard deviation for the cpm readings of the blank and spiked samples, respectively. Figure 1 shows that Fm < B was obtained for all of the test assays, suggesting a very low false negative rate (β error of equal to or less than 5%) which also indicates that the selected spiking concentrations can used as CCβ as reported elsewhere [18, 22, 23]. The actual CC of the tested antimicrobials may be equal to or less than the spiking concentrations since the study used only a single concentration per analyte per antimicrobial group. Reference was made to detection levels provided the Charm II kit manufacturer [24–27]. Furthermore, the Fm values, lower than the respective T values for each test assays indicated lower false positive rate (equal to, or less than 5%) and validating the assays.

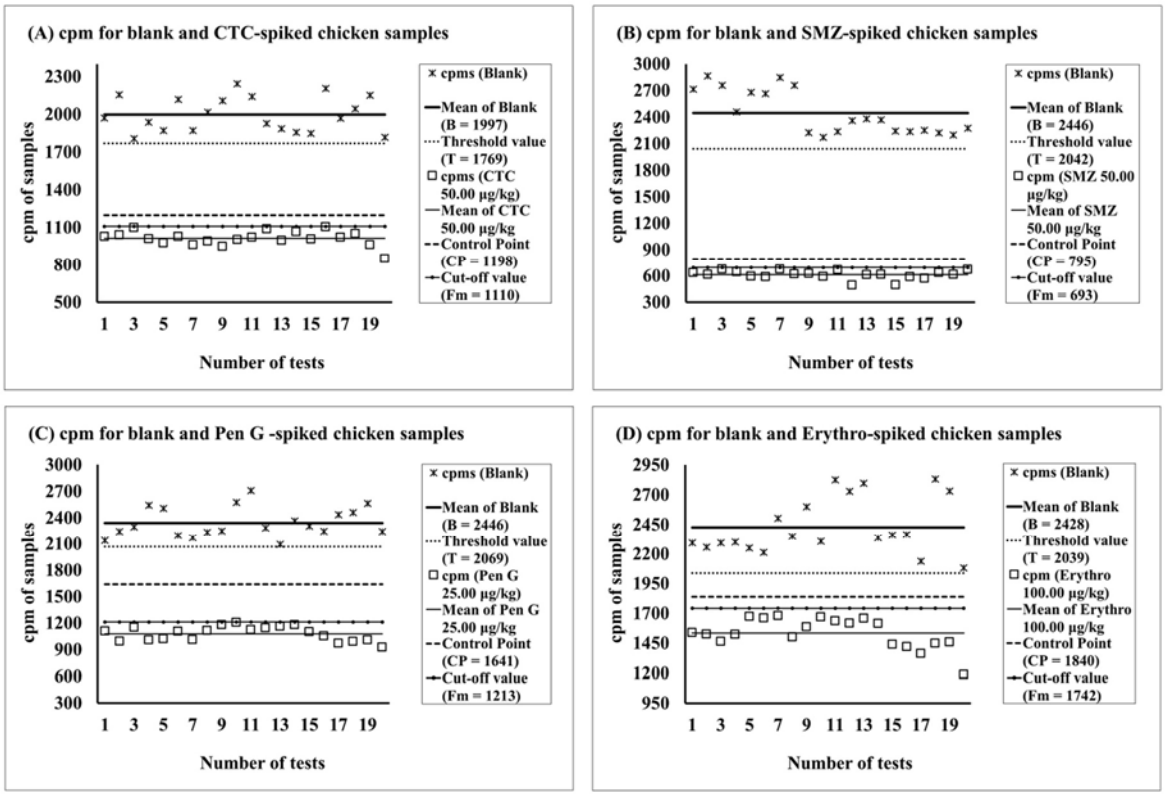

FIG.1. Distribution of cpm values for blank and chicken samples spiked with chlortetracycline (CTC) 50.00 µg/kg, sulfamethazine (SMZ) 50 µg/kg, benzylpenicillin (Pen G) 25 µg/kg, and erythromycin A (Erythro) 100 µg/kg are presented for the four evaluated Charm II kits: tetracyclines (1A) sulfonamides (1B) beta-lactams (1C) and macrolides (1D).

3.2. Suitability of Charm II CP values as cut-off level for screening purpose

The control point (CP) values for different antimicrobial tests were calculated independently using the Eqs (3–6) and according to the manufacturer [24–27].

$$CP\ (tetracycline) = B - B \times 40\% \quad (3)$$
$$CP\ (sulfonamides) = M + M \times 30\% \quad (4)$$
$$CP\ (beta\ lactams) = B - B \times 30\% \quad (5)$$
$$CP\ (macrolide) = M + M \times 20\% \quad (6)$$

The CP values for all of the test assays are lower than their respective B values, and slightly higher than the corresponding Fm values as presented in Fig. 1, which indicates that screening of chicken muscle matrix based on the CP values instead of the Fm values may reduce the chances of false negative results, while occurrences of false positive results may also increase. With CP<T, the chances for occurrence of false positive result will be lower than 5%. Therefore, the CP values can be used as a cut-off level with the probability of false positive and false negative results at less than 5%.

3.3. Specificity/selectivity

Selectivity, also known as specificity, is the ability of a test method to distinguish the target analyte from other closely related substances in a test sample. In this study, representative blank chicken samples were spiked with a mixture of nontargeted antimicrobial standards at concentrations of up to 10 times the MRL and then analysed to investigate false positive results (Table 1).

TABLE 1. TEST FOR SELECTIVITY/SPECIFICITY OF THE CHARM II TEST KITS FOR BETA-LACTAMS, MACROLIDES, TETRACYCLINES AND SULFONAMIDES

Test for antimicrobial groups	Mixed standard used and their respective spiking level at 10×EU MRL	cpm readings	Result
Tetracycline (Fm = 1110) (CP = 1198)	SMZ (1000 µg/kg); Erythro (2000 µg/kg); Pen G (500 µg/kg);	1872, 1988	Negative
Sulfonamides (Fm=693) (CP=795)	CTC (1000 µg/kg); Erythro (2000 µg/kg); Pen G (500 µg/kg);	2274, 2123	Negative
Beta-lactams (Fm=1213) (CP=1641)	CTC (1000 µg/kg); SMZ (1000 µg/kg); Erythro (2000 µg/kg);	2238, 2198	Negative
Macrolides (Fm=1742) (CP=1840)	CTC (1000 µg/kg); SMZ (1000 µg/kg); Pen G (500 µg/kg);	2453, 2683	Negative

***Note**: Fm:Cut-off value; CP=Control point.*

Blank chicken samples spiked with SMZ (1000 µg/kg) Pen G (500 µg/kg) and Erythro (2000 µg/kg) were analysed for tetracyclines, where two cpm readings obtained (1872 and 1988) were found higher than their respective Fm (1110) and CP (1198) values, indicating absence of interference of the tetracycline test results by non-targeted antimicrobials (SMZ, Pen G and Erythro). Blank chicken samples spiked with non-targeted antimicrobials showed compliance in the sulphonamide test (2274, 2123; Fm=693; CP=795) beta-lactam test (2238, 2198; Fm=1213; CP=1641) and macrolide tests (2453, 2683; Fm=1742; CP=1840) as presented in Table 1. However, the Charm II test cannot differentiate between antimicrobials from the same group.

3.4. Ruggedness

In the ruggedness study, the sensitivity of the test methods was examined by minor changes in the incubation conditions during the extraction procedure. The cpm values obtained from these variations were compared with the cpm values obtained from the regular procedure. These variations included incubation at 80 °C for 40 mins instead of standard 30 mins and then at 75°C for 30 mins instead of 80°C (Table 2). Significant differences were not found in tetracycline test for the cpm results from different incubation conditions (ANOVA, overall F-calc 0.32 < F-crit 3.68) with %CV < 4%, indicating lower variability. Similar findings were noted for sulfonamide test (ANOVA, overall F-calc 0.34 < F-crit 3.68, with %CV < 7%) beta-lactam test (ANOVA, overall F-calc 0.28 < F-crit 3.68, with %CV < 11%) and macrolide test (ANOVA, overall F-calc 0.28 < F-crit 3.68, with the %CV < 9%) as presented in Table 2. The findings demonstrate that slight changes in incubation temperature and incubation time in the testing protocol did not affect the test results.

TABLE.2. RESULTS (CPM) FOR RUGGEDNESS TEST (MINOR CHANGES IN INCUBATION TIME)

Test for antimicrobial groups	Used analyte (spiking level)	Experimental condition	Mean cpm (n=6)	SD	%CV	F-calc
Tetracyclines (Fm=1110) (CP=1198)	CTC (100 µg/kg)	incubation at 80°C for 30 mins	911.7	23.32	2.56%	0.32
		incubation at 75°C for 30 mins	904.5	26.47	2.93%	
		incubation at 80°C for 40 mins	898.5	34.68	3.86%	

Test for antimicrobial groups	Used analyte (spiking level)	Experimental condition	Mean cpm (n=6)	SD	%CV	F-calc
Sulfonamides (Fm=693) (CP=795)	SMZ (100 µg/kg)	incubation at 80°C for 30 mins	605.3	27.54	4.55%	0.34
		incubation at 75°C for 30 mins	604	32.18	5.33%	
		incubation at 80°C for 40 mins	618.7	41.44	6.70%	
Beta-lactams (Fm=1213) (CP=1641)	Pen G (50 µg/kg)	incubation at 80°C for 30 mins	1087	117.57	10.81%	0.28
		incubation at 75°C for 30 mins	1081	101.56	9.39%	
		incubation at 80°C for 40 mins	1122	77.11	6.88%	
Macrolides (Fm=1742) (CP=1840)	Erythro (200 µg/kg)	incubation at 80°C for 30 mins	1174	77.12	6.57%	0.28
		incubation at 75°C for 30 mins	1139	93.32	8.19%	
		incubation at 80°C for 40 mins	1167	84.61	7.25%	

3.5. Screening test results

A total of 150 chicken muscle samples were individually examined to identify any residues of the target antimicrobial groups (tetracyclines, sulfonamides, beta-lactams and macrolides) either below or above their STCs. Status of the suspect samples among the analysed chicken samples are outlined in Table 3 and the percentage of suspected samples among three different chicken groups are graphically presented in Fig. 2. Data presented in Table 3 indicate that 60 out of 150 (40%) samples were suspect positive, of which 14 samples contained residues from more than one of the targeted groups.

TABLE. 3. STATUS OF DIFFERENT ANTIMICROBIAL RESIDUES IN CHICKEN SAMPLES

Types of suspected chicken samples	Status of chicken samples
Total suspected samples	60 out 150 (40.0%)
Samples suspected for more than one of the targeted antimicrobial groups (multi class antimicrobial residue)	14 out 150 (9.3%)
Total tetracycline-suspected samples	41 out 150 (27.3%)
Total sulfonamide-suspected samples	19 out 150 (12.7%)
Total beta-lactam-suspected samples	5 out 150 (3.3%)
Total macrolides/lincosamides-suspected samples	9 out 150 (6.0%)

Overall, residues of tetracyclines (27.3%, 41 out of 150) and sulfonamides groups (12.7%, 19 out of 150) were more prevalent in the analysed chicken samples compared to the beta-lactam (3.3%, 5 out of 150) and macrolides/lincosamides groups (6.0%, 9 out of 150). Four indigenous chicken samples contained macrolides/lincosamide residues only (Fig. 2 and Table 4).

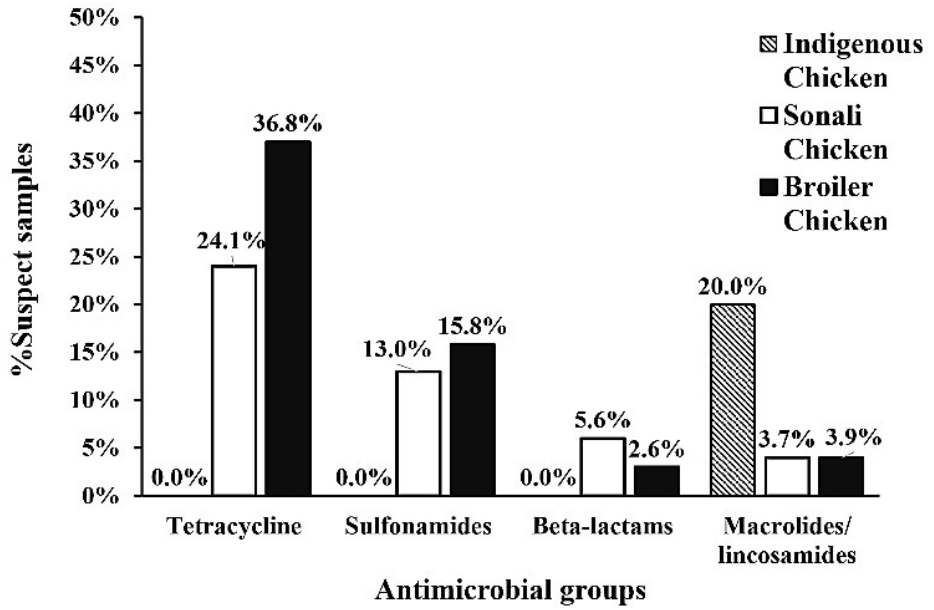

FIG.2. Screening results (in percentages) for the suspected samples among three different chicken groups.

For both Sonali and broiler chicken samples, residues of tetracycline (24.1% for Sonali and 36.8% for broiler chickens) and sulfonamides (13.0% for Sonali and 15.8% for broiler chickens) were more prevalent than beta-lactams (5.6% and 2.6% for Sonali and broiler chickens, respectively) and macrolides/lincosamides (3.7% and 3.9% for Sonali and broiler chickens, respectively).

TABLE.4. STATUS OF DIFFERENT ANTIMICROBIAL RESIDUES IN CHICKEN SAMPLES

Types of suspected samples	Indigenous chicken samples (*n*=20)	Sonali chicken samples (*n*=54)	Broiler chicken samples (*n*=76)
Total suspected samples	4 (20.0%)	19 (35.2%)	37 (48.7%)
Samples suspected of more than one antimicrobial group	0 (0.0%)	6 (11.1%)	8 (10.5%)
Total tetracycline-suspected samples	0 (0.0%)	13 (24.1%)	28 (36.8%)
Total sulfonamide-suspected samples	0 (0.0%)	7 (13.0%)	12 (15.8%)
Total beta-lactams-suspected samples	0 (0.0%)	3 (5.6%)	2 (2.6%)
Total macrolides/lincosamide-suspected samples	4 (20.0%)	2 (3.7%)	3 (3.9%)

Most (71.4%, 10 out of 14) of the multiclass antimicrobial residue suspected samples had the combination of tetracyclines and sulfonamides (Fig. 3). The other combinations were tetracyclines and beta-lactams (7.1%, 1 out of 14) tetracycline and macrolides/lincosamides (7.1%, 1 out of 14) sulfonamides and beta-lactams (7.1%, 1 out of 14) as well as beta-lactams and macrolides/lincosamides (7.1%, 1 out of 14). The suspected multi-class antimicrobial residues were in Sonali (6) and broiler (8) chickens with none in the indigenous (Fig. 3 and Table 4). For both Sonali and broiler chicken samples, majority of the residues detected were a combination of tetracyclines and sulfonamides (50.0% for Sonali and 87.5% for broilers). The other multiclass combinations detected in Sonali chicken samples included tetracycline and macrolides/lincosamides (16.7%, 1 out of 6) tetracyclines and beta-lactams (16.7%, 1 out of 6) and a combination of sulfonamides and beta-lactams (16.7%, 1 out of 6). A combination of tetracycline and beta-lactam residues was also found among the broiler chicken samples (12.5%, 1 out of 8).

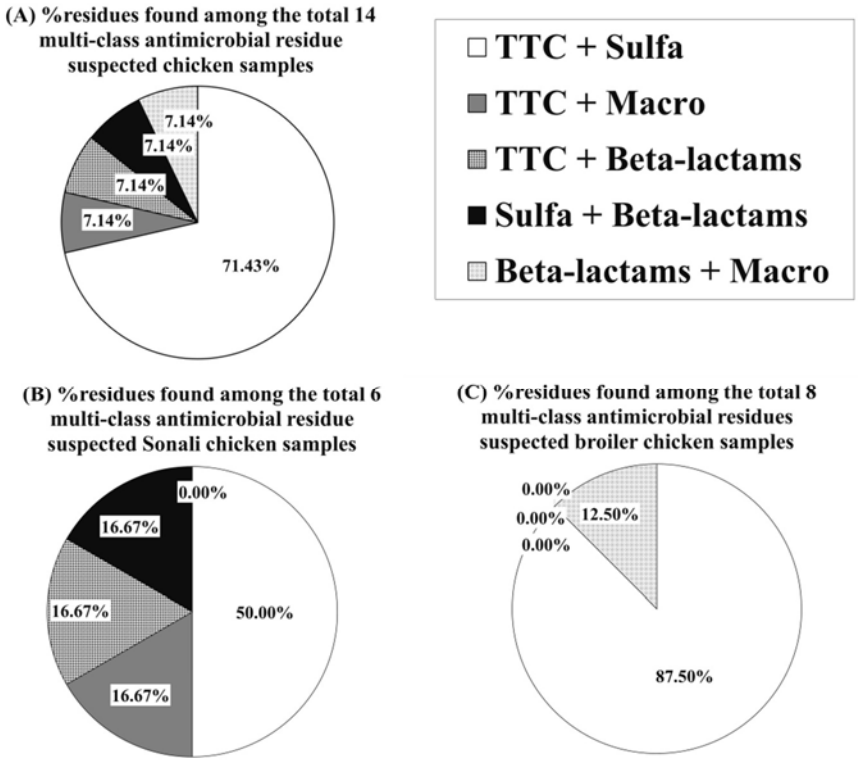

FIG.3. Results in percentages of different multi-class antimicrobial residues in (A) Total multi class antimicrobial residue suspected samples; (B) multi class antimicrobial residue suspected Sonali chicken samples, and (C) multi class antimicrobial residue suspected broiler chicken samples.

Overall, Sonali (35.2%, 19 out of 54) and broiler chickens (48.7%, 37 out of 76) had more residues than the local indigenous chickens (20%, 4 out of 20 samples) as presented in Table 4 suggesting that commercial farms tend to use these drugs more frequently than the local counterparts. This study's results agree with Kabir et al., [28] who reported higher levels of antimicrobial residues in broiler chicken samples compared to local chicken samples. Higher percentages of tetracycline and comparatively lower percentages of beta-lactams were also reported in a study conducted with chicken samples in Bangladesh [29] which supports the current study's findings. Wide use of tetracyclines and sulfonamides are also reported in other countries [10].

High levels of tetracycline residues, primarily oxytetracycline and chlortetracycline, in poultry samples was found in a study conducted in Kuwait [12]. Previous surveys also support this study's findings that tetracyclines and sulfonamides are frequently used in most of the low to middle scale commercial poultry farms in Bangladesh, along with other antimicrobials, including aminoglycosides, beta-lactams, quinolones, macrolides, polypeptides, and amphenicols [3, 30]. Misuse of various antimicrobials, improper licensing and failure to observe withdrawal periods have been reported in developing countries [28]. Edible tissues of chickens treated with antimicrobials and slaughtered without maintaining the drug withdrawal period may contain antimicrobial residues [10, 31–33] although the disposition of drugs is influenced by a range of factors [34].

Poultry meat is thought to be an affordable source of animal protein for low-income families, an alternative to beef and mutton in terms of nutritional value and economic viability [35]. Given the continued use of drugs to ensure poultry production, testing and monitoring of resulting residue is necessary as reported elsewhere [36]. Also, for a clear understanding of the prevalence of antimicrobial residues in chicken muscles, additional studies involving samples from various regions of the country are necessary. Further research is recommended to examine the presence of antimicrobial residues in other food matrices.

4. CONCLUSIONS

Chicken muscle tissues from Bangladesh were screened using a verified radioreceptor assay technique to detect veterinary antimicrobial residues, including tetracyclines, sulfonamides, beta-lactams, macrolides and lincosamides. The study found that 40% of the analysed samples were suspect positive for different antimicrobial residues, and 14 of the total suspected samples (n=60) were also found to contain multi class antimicrobial

residues. The majority of the residues detected were from tetracyclines and sulfonamides groups with a minor presence of beta-lactams, and macrolides/lincosamides groups. It was observed that samples from the commercial farm-raised broiler chickens and some of the Sonali chickens had relatively higher levels of antimicrobial residues than the locally raised indigenous chickens. Findings from this study may be useful to the relevant authorities in establishing a regular drug residue monitoring programme. Screening methods are cost-effective, rapid and easily adaptable to any laboratory. They could be a suitable tool for routine monitoring purposes, especially in low-income or developing countries where expensive confirmatory analytical instruments may not be readily available.

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PREVALENCE OF SELECTED CHEMICAL HAZARDS IN FOOD AND FEED FROM CERTAIN REGIONS OF PAKISTAN

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Abstract

Surveillance studies were conducted for mycotoxins, including total aflatoxin (AF) Aflatoxin M1 (AFM1) deoxynivalenol, ochratoxin and patulin, as well as the antimicrobials chloramphenicol, nitrofurans, tetracycline, penicillin, quinolones and β-lactams. A total of 350 selected feed and food samples including spices, tea, honey, apples, cereals, bovine milk, beef, mutton, poultry and fish) were collected from Faisalabad, Peshawar, Islamabad, Rawalpindi, D.G. Khan, Jhang, Lahore and Swat Districts. Some samples (*n*=180) were analysed by using the Enzyme Linked Immunosorbent Assay (ELISA) and the rest (*n*=170) by radioreceptor binding assay (Charm II) techniques. Results indicated that 37% feed samples and 6% food samples (cereals, spices and tea) were found positive for aflatoxin at maximum limit (EU MLs) 20 µg/kg and 10 µg/kg, respectively. Milk samples were 18% positive for AFM1 at the EU MLs of 0.05 µg/kg. While 30% feed and 35% of the cereals, spices and tea tested positive for deoxynivalenol (DON) at the EU MLs for DON followed by the EU MLs 1250 µg/kg, 1750 µg/kg and 1250 µg/kg in unprocessed wheat, maize and rice, respectively. Patulin was found in 2% of honey samples, while none was detected in apples about the EU ML 50 µg/kg. Ochratoxin was found in 45% of the feed samples at the EU ML 5 µg/kg No levels were detected in food samples. Less than 5% of eggs, milk, chicken, mutton, beef and fish were found positive for quinolones, tetracycline and β-lactams at EU maximum residue levels (MRLs) 1100 µg/kg and 50 µg/kg, respectively. This baseline information may be helpful for consumers, farmers/producers, health specialists, policymakers and other associated stakeholders.

1. INTRODUCTION

Food contamination by mycotoxins and commonly used antimicrobials is a national food safety challenge and international concern for which effective strategies for regular analysis, prevention and control are needed. Control can be adopted by following strict regulations such as those issued by the European Commission. The prevalence of antimicrobials and mycotoxins in food and feed is associated with health effects [1, 2]. The present study reports use of nuclear and related methods to measure chemical contaminants and residues in certain unprocessed and ready-to-eat products. Pakistan is a dairy producer an area of very high socioeconomic importance in Asia [3].

Antimicrobials are used in the dairy industry to enhance production [4, 5] and many, such as quinolones, beta-lactams, and tetracyclines, are associated with a wide range of adverse effects [5, 6]. Some like chloramphenicol and nitrofurans are prohibited due to chronic health effects in the consumer including cancer, nerve and immunological problems in the case of chloramphenicol [2, 4–7]. Five frequently used antimicrobials in Pakistan include tetracycline, penicillin, quinolone, chloramphenicol and nitrofuran metabolites nitrofurans metabolites such as 3-amino-2-oxazolidinone (AOZ) 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) semi carbazide (SEM) [5, 6, 8, 9]. The use of these drugs and their effects is cause of concern [4, 5, 10, 11]. Pakistan has established regulations governing the use of antimicrobials in food-producing animals [6].

Mycotoxins, especially Total aflatoxins (B1, B2, G1, G2 and M1) ochratoxin, patulin and deoxynivalenol (DON) are common in Pakistan's staple foods such as cereals, nuts, spices, apples, feed and dried fruits and call for the same attention as antimicrobial residues [2, 12]. Pakistan's hot and humid environment favours mycotoxin production in food and feed [12–14]. Products such as maize, wheat, rice, peanuts, and dried fruits are susceptible to mycotoxin-contamination. Conditions such as high humidity and improper ventilation promote fungal growth and mycotoxin production. Mycotoxins can lead to acute or chronic health effects, including liver damage, immune suppression, and even cancer [15, 16]. Pakistan has regulations in place to monitor mycotoxin levels in food and feed. The prevalence of mycotoxin contamination in local food can be tackled by following relevant FAO/WHO/Codex and European Union guidelines [3, 17, 18].

The Pakistan Veterinary Medical Council and the Drug Regulatory Authority of Pakistan (DRAP) regulate the use of veterinary drugs, including antimicrobials, in food. Pakistan Standards and Quality Control Authority have limited resources to enforce their regulations and laws effectively, notwithstanding [19]. While there are

concerted efforts to address chemical hazards in food as reported in poultry products and other food items in Pakistan [19, 20]. Although there are variations in standards and regulations, such as in the Punjab Food Authority (PFA) and the Sindh Food Authority (SFA). The ELISA and Charm II techniques are screening techniques used to determine food contaminants and residues. The former is an antigen-antibody, semi-quantitative analytical technique thus far used for pesticides, allergens, mycotoxins, antimicrobials and microbial toxins [21]. It is known for its high sensitivity and specificity, among other benefits [6, 8]. Charm II is a portable, field-deployable, radioreceptor assay technique used for detecting a range of chemical hazards such as antimicrobials, pesticides, mycotoxins and other chemical residues in food and feed [22, 23]. Both are semi-quantitative screening techniques [23, 24].

2. MATERIALS AND METHODS

2.1. Sampling and sample preparation

Food and feed samples (n=350) were collected from Punjab, KPK and Federal areas of Pakistan. These included nuts, rice, wheat, feed, mixed feed, honey, milk, tissue (meat, chicken and beef) and fish. Target areas included open local markets of Faisalabad, Kalam, Sawat, Peshawar, Lahore, Jhang, Dera Gazi Khan, Chistian, Islamabad, Rawalpindi, Sialkot and Multan. A total of 180 samples were analysed by ELISA and 170 by radioreceptor binding assay. There were 60 milk and tissue samples, including chicken, beef, mutton and fish and 20 each of dry fruits, cereals, spices, honey, tea, apples and feed, all randomly collected. After collection, milk and tissue samples were labelled correctly and preserved at 4°C, while the cereal and feed samples were stored at room temperature.

2.2. Determination of antimicrobial residues by ELISA

2.2.1. Monitoring of tetracycline residues in tissues (chicken, fish, mutton and beef)

Commercial ELISA kits with a detection limit of 5.8 ng/g, with a specificity of 100% for tetracycline and recovery of 85%, were used for analysis of the drug in chicken, fish, mutton and beef tissues (n=20 each). These kits were also standardized using the provided standards of 0 ng/ml, 0.03 ng/ml, 0.08 ng/ml, 0.22 ng/ml, 0.68 ng/ml, and 2.04 ng/ml. All tissue samples were extracted following manufacturer's instructions. One gram of defrosted, properly chopped tissue sample was placed in a polygon tube, and 1 ml of diluted diluents (with wash buffer) was added. These samples were then homogenized for 30 seconds and, after vortex-mixing for 30 seconds, centrifuged for 10 min at 4000 rpm (2880g) at 25°C. A supernatant (200 µl) was collected in glass tubes and diluted with 200 µl of diluent (with wash buffer) ready for assay preparation.

2.2.2. Monitoring penicillin residues in tissue (beef) and bovine milk

Commercial ELISA kits with a detection limit of 2.5 ng/g for tissue and 0.08 ng/g for milk; specificity of 100% for penicillin and a recovery of 85% were used for quantitative analysis of milk and beef tissues (n=20 each). These kits were also standardized using accompanying standards of 4.0 ng/ml, 2.0 ng/l, 1.0 ng/l, 0.5 ng/l, 0.25 ng/l, 0.125 ng/l and 0 ng/ml. All tissue and milk samples were extracted following a manufacturer's protocol. One gram of milk sample was placed in polygon tubes. After vortex-mixing for 3 seconds, 250 µl of milk was collected in glass tubes, diluted with 250 µl of dilution buffer, and vortexed again for 3 seconds, ready for assay. One gram of defrosted, properly chopped tissue sample was placed in polygon tubes, and 4 ml of distilled water was added. These samples were then homogenized for 30 seconds, centrifuged for 10 mins at 2000g and 25°C, and 50 µl of the supernatant was collected in a glass tube and diluted with 350 µl of dilution buffer, ready for assay.

2.2.3. Monitoring quinolone residues in tissues (chicken/mutton)

Commercial ELISA kits with detection limits of 4.0 ng/g, specificity of 100% for quinolone and recovery of s 85% were used for quantitative analysis of chicken and mutton tissues (n=20 each). These kits were standardized using accompanying standards of 0 ng/ml, 1 ng/ml, 3 ng/ml, 9 ng/ml, 27 ng/ml and 81 ng/ml. All tissue samples were extracted following manufacturer's instructions. One gram of defrosted, properly chopped tissue sample was placed in polygon tubes, and 70 % methanol was added, followed by homogenization for 30 seconds, centrifugation for 10 mins at 5000g and 25 °C after which 100 µl of supernatant was to glass tubes and diluted with 100 µl of distilled water, ready for assay.

2.2.4. Monitoring of chloramphenicol (CAP) residues in tissue (beef) and bovine milk

Commercial ELISA kits with the detection limit of 0.025 ng/g, specificity of 100% for CAP and a recovery of 85% were used for quantitative analysis of milk and beef samples (*n*=20 each). These kits were standardized using accompanying standards of 0 ng/ml, 0.025 ng/ml, 0.1 ng/ml, 0.2 ng/ml, 0.4 ng/ml and 1.6 ng/ml. Two grams of milk samples were placed in polygon tubes, 3 ml of distilled water was added, and the content vortexed for 3 seconds. Two grams of defrosted, properly chopped tissue samples were placed in polygon tubes, 8 ml of ethyl acetate, and tissue samples (as in milk samples) were added. These mixtures were then homogenized for 10 min and then centrifuged for 10 min at 4000g at 25°C before 2 ml of the supernatant was collected in a glass tube placed in a water bath at 50°C and dried under a gentle nitrogen flow. The dried milk samples were diluted with 0.5 ml of dilution buffer and vortexed for 30 seconds, while the dried tissue samples were diluted with 0.5 ml hexane and after vortex-mixing for 30 seconds and centrifuged for 10 min at 4000g and 25°C. A solution (80 ml) was then transferred into a glass tube, as reported elsewhere [25].

2.2.5. Monitoring of nitrofuran metabolites AOZ and AMOZ in tissue (chicken/fish)

Commercial ELISA kits with a detection limit of 0.1 ng/g for fish and 0.05 ng/g for chicken tissue, with the specificity of 100% for AOZ, AMOZ and recovery of spiked samples calculated 85%, were used for quantitative analysis of chicken and fish tissues. These kits were also standardized using provided standards of 0 ng/ml, 0.025 ng/ml, 0.05 ng/ml, 0.1 ng/ml, 0.2 ng/ml, 0.5 ng/ml and 0 ng/ml, 0.05 ng/ml, 0.1 ng/ml, 0.25 ng/ml, 0.5 ng/ml, 1 ng/ml for AOZ and AMOZ, respectively. All tissue samples were extracted according to manufacturer's instructions. One gram of defrosted, properly chopped tissue sample was placed in polygon tubes, and 4 ml of distilled water was added. These samples were homogenized for 30 seconds and 0.5 ml of HCl 1M added. A derivatization reagent was added to the fish (100 µl) and chicken (200 µl) samples, followed by vortex mixing for 1 min and incubation at 37°C for 16 hours in the dark. After incubation, 5 ml of the assay buffer solution (10×diluted with distilled water) 0.4 ml of 1M NaOH, and 5 ml of ethyl acetate were added to all samples, the content centrifuged for 10 min at 3000 rpm and 25°C after mixing for 1 min. A 2 µl of the supernatant was collected into glass tubes and evaporated to dryness at 50°C under a gentle stream nitrogen; 1 ml of n-hexane and 1.6 ml of washing buffer (10×diluted with distilled water) added, the content mixed on a vortex for 1 min. Finally 50 µl of lower layer was collected for assay.

2.2.6. Samples extraction for analysis of nitrofurans metabolites (SEM) tissue

Commercial ELISA kits with a detection limit of 0.1 ng/g, with specificity of 100% for Semicarbazide and recovery of 100%, were used for quantitative analysis of tissues (*n*=20 each). The kits were also standardized using accompanying standards of 0 ng/ml, 0.02 ng/ml, 0.06 ng/ml, 0.18 ng/ml, 0.54 ng/ml and 1.62 ng/ml. Representative tissue samples weighing 5 g were homogenized with 4 ml of distilled water, 0.5 ml 1M HCl, and 100 µl 2-Nitrobenzaldehyde for 2 min. The content was incubated at 70ºC in a water bath for 20 min and 5 ml of 0.1 M K_2HPO_4, 0.4 ml of 1M NaOH and 6 ml of ethyl acetate added. These were centrifuged at 4000g for 10 min and 25ºC, dried and 3 ml of ethyl acetate layer in a new tube dried using nitrogen gas. The residue was dissolved in 2 ml n-hexane and 1 ml centrifuged at 4000g for 10 min at 25ºC with 50 µl used for the assay.

2.3. Determination of mycotoxins in food and feed by ELISA

2.3.1. Sample preparation for analysis of patulin in honey and apple

Commercial ELISA kits with a detection limit of 0.1 ng/g, specificity of 100% for patulin, and recovery of > 90% were used for quantitative analysis of food samples (*n*=20 each). The kits included standards (0 ng/ml, 0.01 ng/ml, 0.3 ng/ml, 0.9 ng/ml, 2.7 ng/ml and 8.1 ng/ml). Honey samples (1 g) were diluted with 4 ml of distilled water and 50 µl used for assay. Juice samples (1 ml) was diluted with 4 ml distilled water and centrifuged at 4000g for 5 min at 20–25ºC with 50 µl used for assay.

2.3.2. Sample preparation for analysis of ochratoxin in food and feed

Commercial ELISA kits with a detection limit of 0.025 ng/g, a specificity of 100% for ochratoxin, and a recovery rate of 100% were used for analysis of food and feed samples (*n*=20 each). The standards (0 ng/ml, 50 ng/ml, 100 ng/ml, 300 ng/ml, 900 ng/ml and 1800 ng/ml) were used. The ground food/feed samples (5 g) were mixed with 100 ml of 0.13 M sodium hydrogen carbonate buffer, shaken for 15 min, filtered and centrifuged at 3500g for 15 min at 25ºC. An aliquot of 50 µl was used for assay.

2.3.3. Sample preparation for analysis of deoxynivalenol in food and feed

Commercial ELISA kits with a detection limit 224 ng/g, specificity of 100% for deoxynivalenol and a recovery of 85%, were used for quantitative analysis of the food and feed samples (*n*=20 each). The standards (0 ng/ml, 7.5 ng/ml, 15 ng/ml, 30 ng/ml, 60 ng/ml and 120 ng/ml) were used. The food/feed samples (95 g) were mixed with 100 ml distilled water and shaken for 3 mins, followed by centrifugation at 3000g for 10 mins at 25ºC and filtered through a filter paper before 100 µl was collected for assay.

2.3.4. Sample preparation for analysis of total aflatoxins in food and feed

Commercial ELISA kits with a detection limit of 1.3 ng/g for feed and 0.8 ng/g for grains, with specificity of 100% B1, 46 % G1, 28% G2, 26% B2 and recovery rate of 100%, were used for quantitative analysis of food and feed samples (*n*=20 each). The kits were also standardized using accompanying standards at 0 ng/ml, 0.02 ng/ml, 0.06 ng/ml, 0.2 ng/ml, 0.6 ng/ml and 1.5 ng/ml concentrations. Ground food/feed samples (5 g) were mixed with 25 ml of 70% methanol, vortex for 3 min, then centrifuged at 4000g for 10 min at 25ºC. The supernatant (300 µl) was transferred to a 2 ml tube containing 900 µl solution-C and then 50 µl was used for assay.

2.3.5. Sample preparation for analysis of aflatoxin M1 in milk

Commercial ELISA kits with a detection limit of 0.025 ng/g, a specificity of 100% for aflatoxin M1 and a recovery rate of 100% were used for quantitative analysis of milk samples (*n*=20). The kits were standardized using accompanying standards at 0 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml and 80 ng/ml concentrations. The skimmed milk samples (5 ml) were centrifuged at 3500g for 10 min at 10ºC and 100 µl of the resultant extract used for assay.

2.4. ELISA and analysis of antimicrobials and mycotoxin

Following the ELISA plate layout, 50 µl or 100 µl of standards and samples were added in triplicate, followed by 50 µl or 100 µl of enzyme conjugate to all wells used. Plates were covered with a sealer and incubated in the dark at room temperature. The plate was washed 3 times with 1×rinsing buffer (20×concentrated) using an ELISA washer. After washing, 100 µl of the substrate solution was added, and the plate incubated at room temperature. Finally, 50 µl or 100 µl of a stop solution was added to each well, and optical density was measured at 450 nm. The ELISA kits were standardized by using a range of standards. Calibration curves were constructed from optical density and relative absorbance (RA) and used to calculate the inhibition concentrations (IC20 and IC50). For validation studies, recoveries (%) were calculated by spiking known negative tissues. The RA was calculated for both standards and samples using Eq. (1). The RA of unknown samples was interpolated on the standard curve to calculate the concentration of unknown samples.

$$RA\ (\%) = (B)\ (B_0)\text{-}1\ 100 \quad (1)$$

Where B is the absorbance of standards or samples; B_0 is the absorbance of zero standard

Samples were analysed at 450 nm. The optical density was recorded and further used to calculate the relative absorbance (%) which was used to construct the calibration curve (Figs 2 and 3) [11].

2.5. Radioreceptor assay (Charm II) of antimicrobials and mycotoxins

A control point (CP) or cut-off value was calculated by using the average counts per minute (cpm) values of the six positive controls (PCs) and three negative controls (NCs). The average of the PC and NC was calculated and 15% or 20% of the PC value was added to determine the CP. If the cpm value of the unknown sample was higher than the PC, such a sample is negative and vice versa [22]. A sample extract was mixed with the binder and an appropriate quantity of [^{3}H] labelled aflatoxin (tracer). The analyte in the sample competes with the tracer for binding sites. A predetermined CP compares the amount of the bound tracer. The threshold between a positive and negative sample is the CP. Aflatoxin concentration in the sample decreases as tracer concentration increases. The concentration of aflatoxin in the sample increases with decreasing tracer quantity. A binder is any material or protein that interacts with or binds explicitly to a sample's target component or analyte. Analyte-labelled materials, typically fluorescent or radioactive, enable sensitive and precise detection. They indirectly quantify or track their presence by producing a measurable signal that correlates to their presence or concentration.

2.6. Determination of antimicrobials and mycotoxins by Charm II

2.6.1. Analysis of total aflatoxin in feed and food using Charm II kit

Food/feed extracts were prepared according to the method protocol OM-139-016 [25]. This involved weighing 50 g of the feed/grain sample and blending with 100 ml of 80% methanol for 30 seconds. After 10

seconds 2 ml of the extract was transferred to a new test tube and centrifuged for 3 min at 3400 rpm. For the assay run, binder tablet was added, followed by 1 ml of distilled deionized water and 400 µl each of NC or PC sample extract spiked with aflatoxins. The mixture was incubated for 5 min at 35°C alone and further with a tracer for 5 min at the same temperature, before centrifugation at 3400 rpm for 5 min. The resulting pellet was dissolved in 400 µl of distilled water, placed in a liquid scintillation vial, and added 2 ml of a scintillation fluid. Measurement was conducted on a liquid scintillation counter using the ^{3}H channel [25, 26]. The results are summarized in Table 1.

2.6.2. Analysis of aflatoxin M1 in milk by Charm II radioreceptor assay

Milk samples were prepared using an established protocol OM-200-009 [27]. Raw, mixed, pasteurized, homogenized, skim and powdered cow's milk samples were analysed for aflatoxin M1 by following EU ML as 0.025 µg/kg [27]. A Solution of AF (300 µl) to 6.5 to 7.5 ml milk sample and/or control standard, centrifuged for 5 min and cooled to 4±2°C. A binder tablet was dissolved in 300 µl of distilled deionized water, and 5 ml of each NC or PC sample extract was spiked with aflatoxin. The content was incubated for 5 min at 35°C for aflatoxins. After adding the tracer tablet with 1.85 kBq of [^{3}H]-aflatoxin M1, the content was incubated for 5 min (at 35°C) spined on a centrifuge for 5 min at 3400 rpm and the pellet dissolved in 400 µl of distilled water, 2–3 ml of scintillation fluid added and counting performed [27]. The test results are shown in Table 1.

TABLE 1. MONITORING OF AFLATOXINS IN MILK AND FEED USING CHARM II KITS

(Islamabad and Rawalpindi)

Aflatoxin M1 in Milk (OM-200-008)				Total Aflatoxin in feed (OM-139-016)			
PC	cpm	NC	cpm	PC	cpm	NC	cpm
PC-1	231	NC-1	427	PC-1	244	NC-1	305
PC-2	152	NC-2	324	PC-2	239	NC-2	303
PC-3	166	NC-3	459	PC-3	193	NC-3	443
PC-4	157	Sum	1210	PC-4	258	Sum	1051
PC-5	198	Average	403	PC-5	225	Average	350
PC-6	164			PC-6	240		
Sum	1068			Sum	1399		
PC Average	178			PC Average	233		
20% PC average	35.6		80	20% PC average	47		70
+20%	213.6		484	+20%	279		420
-20%			322	-20%	186		280
CP (cpm)			322	CP (cpm)	279		
Range	178–213		322–484	Range	186–279		183–276

2.6.3. Analysis of CAP residues in tissue using Charm II kits

Samples were thawed and cooled before testing. Each sample (20 g) was homogenized in 20 ml MSU extraction buffer and then incubated set at 80±2°C for 45 mins in an ice water bath and later centrifuged at 3300 rpm for 10 mins. The sample extract pH was maintained at 7.5. A binder tablet was dissolved in 300 µl of distilled deionized water, and then 4 ml each of NC or PC sample extract spiked with CAP added. A tracer tablet with less than 1.85 kBq of [^{3}H]-CAP was then added and the mixture incubated for 3 mins at 50°C before centrifugation at 3400 rpm for 5 mins. The resulting pellet was dissolved in 400 µl of distilled deionized water and mixed with 2 ml of scintillation fluid before measurement [28]. The results are summarized in Table 2.

2.6.4. Testing CAP residues in milk using Charm II kits

Milk samples were prepared according to and established protocol using a reference detection level of 0.1 µg/kg [29]. Cold milk samples were brought to room temperature and centrifuged to remove a thick layer on top. A binder tablet was dissolved in 300 µl of distilled deionized water and then treated with 5 ml each of NC or PC skimmed milk samples. A tracer tablet containing less than 1.85 kBq of [^{3}H]-CAP was added in the mixture, incubated for 2 min at 50°C and centrifuged at 3400 rpm for 5 min. The resulting pellet was dissolved in 400 µl of distilled water and the content was transferred to a liquid scintillation and 1–2 ml of scintillation fluid was added before measurement. The test results are shown in Table 2.

TABLE 2. MONITORING OF CAP AND BETA-LACTAM RESIDUES USING CHARM II KITS

(Islamabad and Rawalpindi)								(Sialkot, Lahore and Faisalabad)			
CAP in milk				CAP in tissue				Beta-lactams in eggs			
PC	cpm	NC	cpm	PC	cpm	NC	cpm	PC	cpm	NC	cpm
PC-1	379	NC-1	629	PC-1	299	NC-1	610	PC-1	1116	NC-1	1596
PC-2	369	NC-2	589	PC-2	328	NC-2	541	PC-2	1266	NC-2	1550
PC-3	344	NC-3	602	PC-3	309	NC-3	559	PC-3	1245	NC-3	1608
PC-4	335	Sum	1820	PC-4	238	Sum	1710	PC-4	1236	Sum	4754
PC-5	370	Average	607	PC-5	307	Average	570	PC-5	1168	Average	1585
PC-6	318			PC-6	287			PC-6	1130		
Sum	2115			Sum	1768			Sum	7161		
PC Av	353			PC Av	295			PC Av	1194		
20% PC Av	70.5			20% PC Av	59			15% PC Av	179.1		
+20%	423			+20%	354			+15%	1373.1		
-20%	282			-20%	236			-15%	1014.9		
CP (cpm)	423			CP (cpm)	354			20% PC Av	239		
				CP (cpm)	304			CP (cpm)	435		

2.6.5. Testing tetracycline and β-lactam residues in eggs using Charm II kits

Egg samples were prepared following the manufacturer's protocol for tetracycline and beta-lactam and research elsewhere [30–32]. The kit can detect penicillin G at 50 ng/g. The process included pouring ~10 ml of the blended egg sample and 0.5 ml MSU multi-antimicrobial concentrate standard in a tube and heated in boiling water for 6 min. The MSU Extraction Buffer (30 ml) was added, the samples homogenized for 30 seconds, followed by centrifugation for 5 min at 3300 rpm. The opaque top layer was decanted with a binder tablet dissolved in 300 µl of distilled deionized water and 2 ml each of NC or PC or sample extract added. A tracer tablet consisting of 1.85 kBq of [^{3}H]-tetracycline and 0.2 kBq of [^{14}C]-penicillin was added and the mixture was incubated for 2 min at 55°C for β-lactam (35°C for tetracycline). The resultant pellet was dissolved in 400 µl distilled water, 2–3 ml of scintillation fluid added and counted. Table 3 shows results of Sialkot, Lahore, Faisalabad, Islamabad and Rawalpindi samples analysed.

TABLE 3. TESTING OF TETRACYCLINE IN MILK, TISSUE AND EGGS USING CHARM II KITS

Tetracycline CP in Milk (OM-2-006)				Tetracycline CP in Tissue (OM-23				Tetracycline CP in Eggs (OM-5-003)			
PC	cpm	NC	cpm	PC	cpm	NC	cpm	PC	cpm	NC	cpm
PC-1	337	NC-1	660	PC-1	273	NC-1	576	PC-1	372	NC-1	610
PC-2	291	NC-2	643	PC-2	269	NC-2	442	PC-2	353	NC-2	609
PC-3	345	NC-3	684	PC-3	219	NC-3	491	PC-3	363	NC-3	615
PC-4	306	Sum	1987	PC-4	222	Sum	1509	PC-4	361	Sum	1834
PC-5	315	Average	662	PC-5	269	Average	503	PC-5	359	Average	611
PC-6	302			PC-6	275			PC-6	367		
Sum	1896			Sum	1527			Sum	2175		
PC Av	316			PC Av	254			PC Av	363		
23% PC Av	73			15% PC Av	38			15% PC Av	55		
23%	389			15%	292			15%	418		
-23%	243			-15%	216			-15%	308		
CP (cpm)	389	CP (cpm)	435		51			20% PC average	73		

Tetracycline CP in Milk (OM-2-006)				Tetracycline CP in Tissue (OM-23				Tetracycline CP in Eggs (OM-5-003)			
PC	cpm	NC	cpm	PC	cpm	NC	cpm	PC	cpm	NC	cpm
				CP (cpm)	304			CP (cpm)	435		

2.6.6. Analysis of tetracycline residue in tissue using the Charm II technique

Tissue samples were prepared according to the manufacturer's protocol [33] for tetracycline at EU MRL 100 µg/kg. Tissue samples were thawed and kept cool before testing. Then 10 g of the tissue sample was homogenized with 0.5 ml MSU extraction buffer, incubated at 80±2°C for 45 min; rested on ice and centrifuged at 3300 rpm for 10 mins. The sample pH was maintained at 7.5. A binder tablet was dissolved in 300 µl of distilled deionized water followed by 4 ml each of NC or PC or sample extract and a tracer tablet at 1.85 kBq of [^{3}H]-tetracycline. The mixture was incubated for 3 mins at 35°C and centrifuged at 3400 rpm for 5 mins with the pellet dissolved in 400 µl distilled water, dissolved in 2 ml wash of scintillation fluid before counting [33]. The CP for tetracycline was calculated (Table 3).

2.6.7. Monitoring tetracycline residues in milk by Charm kit.

Milk samples were prepared per method protocol OM-156-003 with detection capability at EU MRL 100 µg/kg [34, 35]. Milk samples were thawed, centrifuged and cooled frozen samples before testing. While milk samples were centrifuged. For the assay run, binder tablets were dissolved in 300 µl of distilled deionized H_2O and then treated with 5 ml each of NC or PC skimmed milk samples. The added tracer tablet contained less than 1.85 kBq of [^{3}H]-tetracycline. The content was incubated for 3 mins at 35°C, spined on a centrifuge at 3400 rpm for 5 mins and the pellet was dissolved in 400 µl distilled H_2O, mixed with 2–3 ml of the scintillation fluid before counting and the CP for beta-lactam calculated (Table 3).

3. RESULTS AND DISCUSSIONS

3.1. Prevalence of antimicrobials in different regions of Pakistan

The use of tetracycline and penicillin is common in Lahore and several samples were found positive in the past [28]. Beef muscles contained a maximum residue concentration of 0.70 µg/kg and 6.4 µg/kg in two samples, while all the bovine milk and muscle samples were found negative by following EU MRL of 4 µg/kg for milk and 50 µg/kg for tissue. The ELISA tests for bovine milk, mutton, and beef tissue showed that 5% bovine milk was suspect positive for penicillin in the range of 0.26 µg/kg to 0.70 µg/kg below EU MRL. Tetracycline residues ranged from 5.76 µg/kg–6.40 µg/kg and 3.7 µg/kg–10.8 µg/kg, respectively. Tetracycline residues have been investigated by others [35, 36].

Tetracycline was found in chicken at a concentration of 20.8 µg/kg by ELISA. It was observed that in 5% of the milk samples and 3% of egg samples, 10 % of the samples contained tetracyclines in the range of 20–48 µg/kg. Similar studies using the Charm II technique reported the screening of tetracycline in Kuwait, where 5% of poultry and 18% of milk were suspect positive and confirmed by LC-MS/MS [36, 37]. Other studies using quantitative techniques such as the HPLC have been used to detect tetracycline residues in food samples including tissue and milk [11.

Screening with ELISA showed that less than 5 % of the egg and tissue samples were positive for quinolones. Results indicated that one chicken sample and two mutton samples were positive at 230 µg/kg and 520 µg/kg, respectively. Overall, 5% of mutton samples had quinolones from 520 µg/kg to 100 µg/kg, all above MRLs. Also, 5 % of mutton samples contained tetracycline and quinolones in the ranges of 3.40 µg/kg to 3.60 µg/kg and 51 µg/kg to 520 µg/kg, respectively. The findings are in agreement with related studies in Ankara, Türkiye, where chicken and beef samples contained high levels of quinolones [38]. Samples were analysed for chloramphenicol. Results indicated that all milk and tissue samples were found negative by following the EU's reference point of action (RPA) of 0.15 µg/kg [1]. None of the samples were found to be positive by both ELISA and Charm II techniques as this is a banned drug [8].

Nitrofuran metabolites are protein-bound drugs and often found in gelatine of animal origin [1, 39, 40]. Results indicated that all tissue samples were negative for AOZ metabolite, and the concentration was negligible according to the EU RPA of 0.5 µg/kg for all food matrices. Similarly, furaltadone-AMOZ and semicarbazide were not detected above the EU RPA level of 0.5 µg/kg [1].

3.2. Prevalence of mycotoxins in different regions of Pakistan studied

Samples collected from various cities in Pakistan, as mentioned were successfully analysed by ELISA and Charm II. Aflatoxins and other mycotoxins are associated with a range of health effects of concern [15, 16]. Total aflatoxin levels ranged from low to 1.75 µg/kg in all cereals, spices and tea samples, although only one each from the Swat region was above 10 µg/kg. For all food samples, 35.3% had AFL concentrations higher than 10 µg/kg, while 37% of feed samples had levels greater than 20 µg/kg as reported elsewhere [17, 18]. Aflatoxin occurs frequently in Pakistani milk samples due to their presence in feed [41–43]. The current study found 18 % of milk samples to contain AFL M1 based on the EU ML 0.05 µg/kg. This is a risk to consumers, including children.

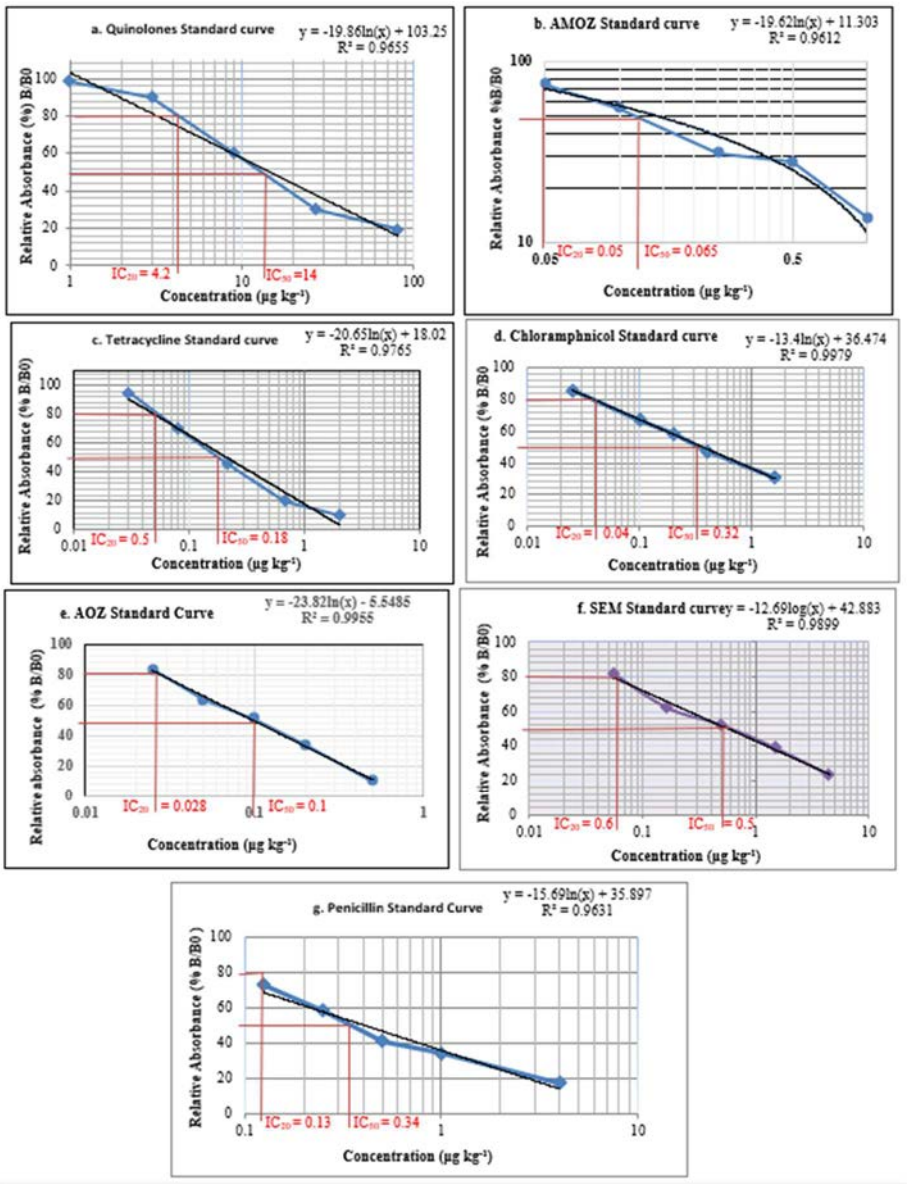

FIG. 1. Standard curves for antimicrobials (a) Quinolones (b) AMOZ (c) Tetracycline (d) CAP (e) AOZ (f) SEM (g) Penicillin.

Patulin content was not detected in all apple samples collected from Swat Valley in October. While in honey samples, its range was 12 µg/kg to 33 µg/kg. Ochratoxins were found in the range 0.49 µg/kg to 4.88 µg/kg range in all cereal and spices samples. Ochratoxin was found positive in 45 % of feed samples by following EU ML of 100 µg/kg as shown by other researchers [17, 18]. Deoxynivalenol was detected in 38% of food and 31% of feed samples following EU ML limits of 900 µg/kg. Of the cereal samples, 12.5% were positive, while 62.5% showed no DON levels. In all spices and tea samples, the range of DON in samples collected from Swat Valley was 30 µg/kg to 2659 µg/kg. Overall, 35 % of food samples were positive in the range 1750 µg/kg and 1250 µg/kg

for unprocessed wheat and cereals, respectively [17, 44]. Meanwhile, 30 % of feed samples were positive with 1750 µg/kg as reported elsewhere [45]. Aflatoxin, ochratoxin and DON frequently also occur in other regions partly due to changes in climatic conditions [45].

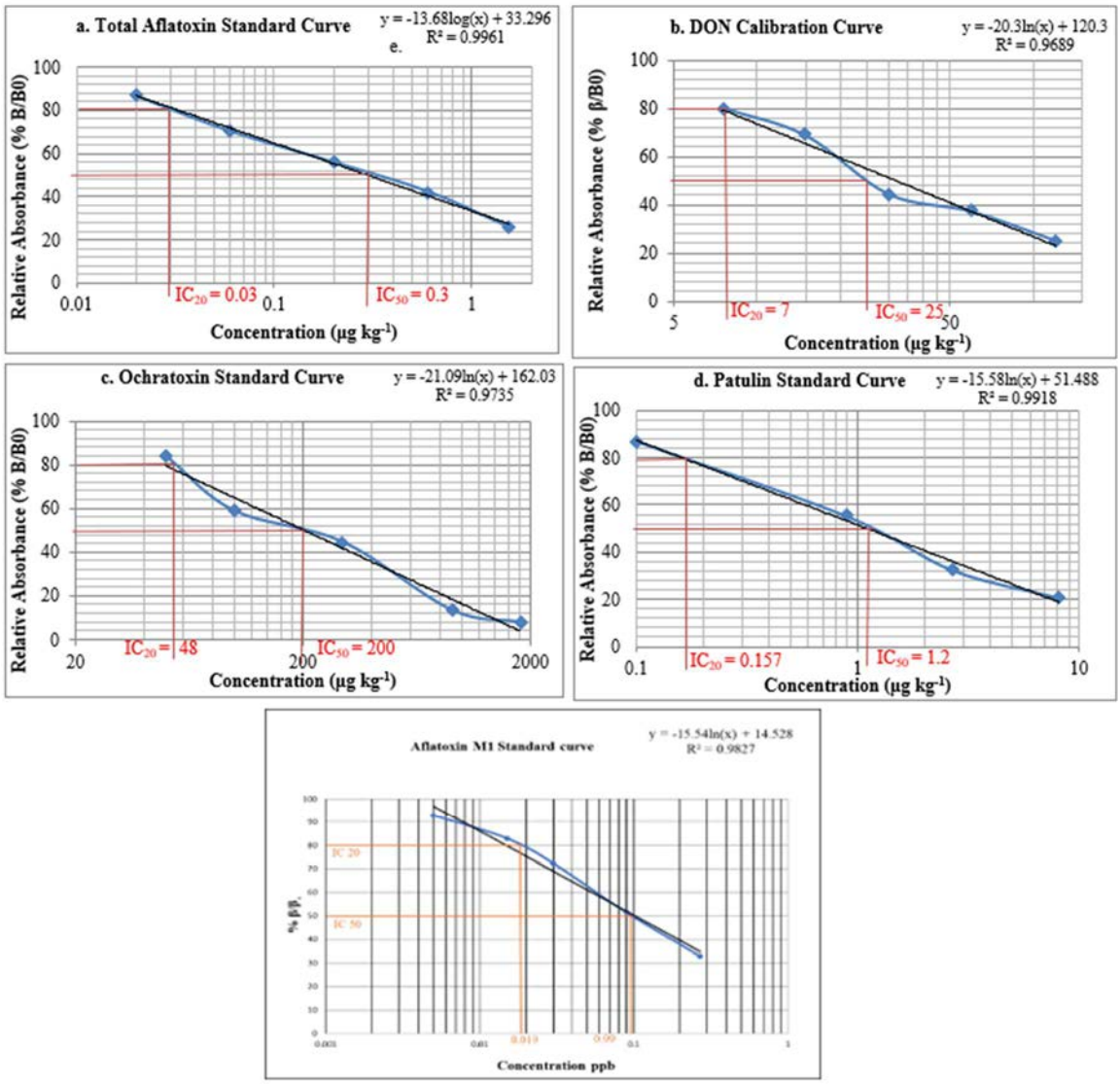

FIG. 2. Standard curves for mycotoxins (a) Total Aflatoxins (b) DON, (c) Ochratoxin, (d) Patulin, (e) Aflatoxin M1.

Figure 3 summarizes the overall trend of antimicrobials and mycotoxins detected.

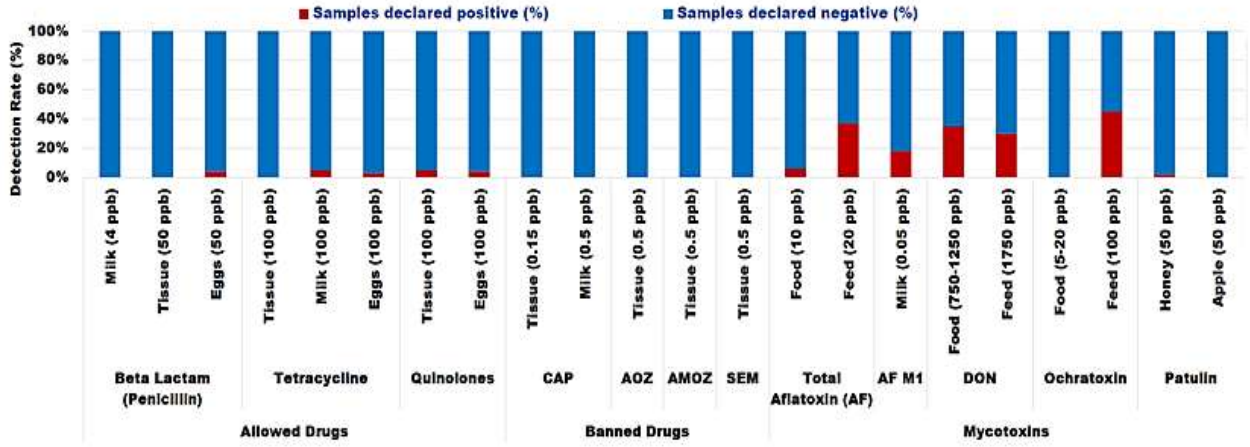

FIG. 3. Prevalence of food contaminants/residues (%) in various food matrices analysed by Charm II and ELISA.

The presence of veterinary drugs and mycotoxins in food and feed calls for routine testing and robust safety management practices in a country.

4. CONCLUSIONS

Two screening techniques have been successfully used to determine levels of selected antimicrobials and mycotoxins in food and feed samples collected from a number of regions in Pakistan. While prohibited drugs such as chloramphenicol and nitrofurans were not detected above reference points of action and only 5% of the foods contained violative levels of the residues, routine surveillance of foods and feed is essential in safeguarding consumers from potentially higher levels since chemicals are still used in food production. Food and feed are still susceptible to mycotoxins as up to 45% of the samples tested contained the toxins. The radioreceptor assay presents an easy-to-use tool for countries to apply to ensure consumer safety.

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SCREENING OF SELECTED DRUG RESIDUES AND AFLATOXINS IN MILK AND ANIMAL FEED IN MYANMAR

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Abstract

The screening of selected antimicrobials and aflatoxin M1 (AFM1) in targeted animal products and feed is reported in a study conducted by the Veterinary Assay Laboratory. Using the Charm II radioimmunoassay, 557 feed and milk samples were analysed with 229 feed samples testing positive for tetracycline, while 146 were negative. Out of 45 samples of raw materials for animal feed tested for beta-lactams, 9 samples were positive. Out of 226 feed samples tested for macrolide, 26 were positive. The aflatoxin B1 levels measured in import, export and grass feed samples for dairy cows were below the action level of 20 µg/kg (ppb) while feed samples from small scale dairy farms exceeded the level. Six milk samples from grass-fed cows were negative for aflatoxin M1, while 55 samples from a milk collecting centre in Yangon region were positive. Out of 142 milk samples tested for antimicrobial residues, 52 were positive 52 and 90 negative. The Charm II radioreceptor assay is reliable, tests multiple matrices for a wide variety of residues and offers appropriate detection capabilities to assess compliance with Codex maximum residue levels (MRLs).

1. INTRODUCTION

The misuse and overuse of antimicrobials in animals can result in harmful residues and also contribute to emergence of antimicrobial resistance. The World Health Organization (WHO) [1] recommends reducing use of medically important antimicrobials in food production. Myanmar has followed WHO's guideline with the Ministry of Agriculture, Livestock and Irrigation issuing the Notification number (4/2020) to control and restrict the use of antimicrobials as growth promoters. The Veterinary Assay Laboratory under the Livestock Breeding and Veterinary Department monitors antimicrobials in animal feed and food. Aflatoxins such as B1 and M1 are of concern, with consumers being exposed to aflatoxin M1 in milk as a result of metabolism of aflatoxin B1 in feed [2]. Misuse of antimicrobials and the presence of mycotoxins such as aflatoxins in food and animal feed can have negative impact on public health [3, 4]. Bacteria may develop resistance to the antimicrobials while aflatoxins are reported to be carcinogenic. Antimicrobial residues in foods of animal origin are thus a concern and hence the need to establish laboratory testing and monitoring programmes. This study was undertaken as part of the Veterinary Assay Laboratory under to detect antimicrobial residues and mycotoxins in feedstuffs and animal products.

2. MATERIALS AND METHODS

A survey was conducted where 557 samples of complete feed, raw feed and milk were collected from the Yangon region during the period 2022–2024 and tested. A radioreceptor assay instrument (Charm II system) was used in the study. Animal feed samples were analysed immediately upon arrival at the laboratory with samples ground (and sieved) to 1 mm particle size. Milk samples were collected at farms and milk-collecting centres in Yangon region. Milk was submitted cool (2–6°C) or frozen in plastic disposable bottles before assay. Frozen milk was thawed thoroughly. Tests were conducted following established manuals [5–13].

3. RESULTS AND DISCUSSION

Among the 61 milk samples analysed for AFM1, 6 samples from grass-fed cows were negative, while 55 samples from the milk-collecting centres were positive. For the samples tested for drug residues 142 were milk with 52 suspect positive and the rest (90) negative. The frequency of tetracycline residues in animal feed was 59.46%, beta-lactam residues was 20% and macrolide 11.5%, respectively. The corresponding percentages for tetracycline residues in milk were 29%; 56% for beta-lactam residues; and 51% for sulphonamide as well as 25% for chloramphenicol and other amphenicols. The distribution of aflatoxin B1 in animal feed and the veterinary drug residues are shown in the Tables 1 and 2 and Fig. 1.

TABLE 1. SUSPECT-POSITIVE FEED SAMPLES FOR ANTIMICROBIAL RESIDUES AND AFLATOXINS

Residues/mycotoxins	Detected levels(µg/kg)	%positive
Tetracyclines	200–800	59.46
Beta-lactams	150–3800	20
Macrolides	1000–2500	11.5
Aflatoxins	5–40	—

Note: *— = nothing detected*

TABLE 2: DETECTED LEVELS OF RESIDUES AND AFM1 BY CHARM II

Residues/mycotoxins	Detected levels (µg/kg)
Tetracycline	20–100
Beta-lactam	4–30
Chloramphenicol, other amphenicols	1–50
Sulfonamides	30–500
Aflatoxin M1	0.025

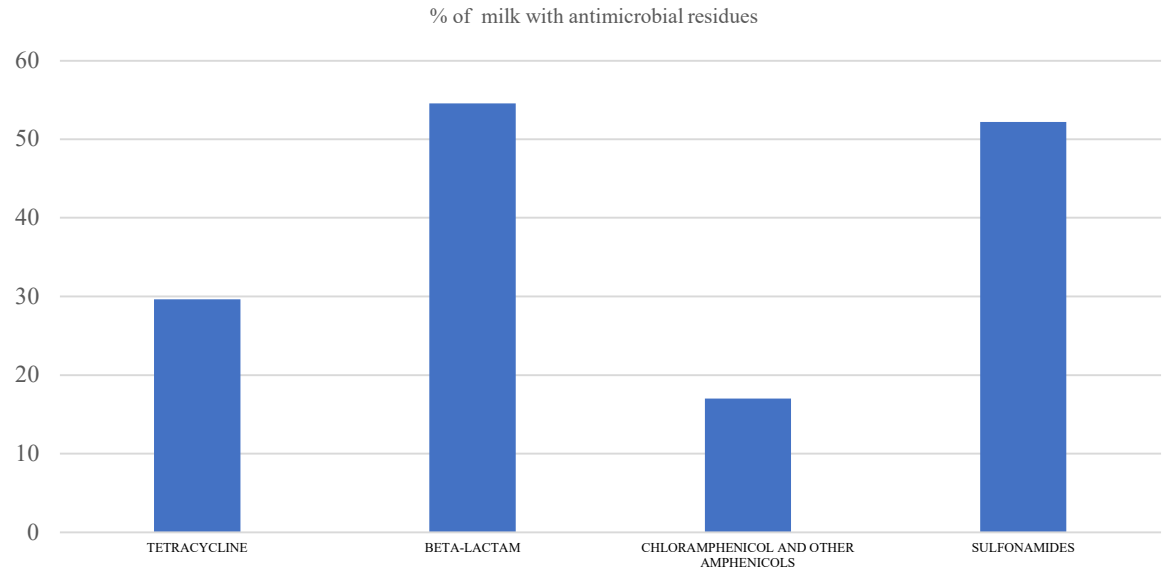

FIG 1. Antimicrobial residues in milk.

The screening for drug residues and mycotoxins in milk and animal feed is essential in ensuring the safety of animal products, maintaining animal health and complying with international food safety standards [14]. Employing effective sample collection, advanced analytical techniques, and adherence to regulatory guidelines will mitigate risks associated with food and feed hazards. Furthermore, establishing robust quality assurance practices will ensure the reliability and accuracy of results, fostering consumer confidence in dairy products and animal feed. The findings agree with a previous report in Tunisia that found 84.4% of feed samples contaminated with Aflatoxin B1 which also found AFM1 in milk [15]. The presence of drug residues in this study is consistent with previous findings [16].

4. CONCLUSIONS

The results of this study show that milk from grass fed cows was free of AFM1 unlike that from milk collecting centres. This suggests that the collections may include milk from animals that consumed feed contaminated with aflatoxin B1. The use of grass as feed for dairy cattle is encouraged, potentially reducing the risk of exposing the public to aflatoxins. Food and feed are still commonly exposed to residues based on the screening test results in this study. More of such studies, routine testing and monitoring including confirmatory

analyses is encouraged. To combat antimicrobial resistance, evidenced based data of antimicrobial residues come from monitoring of residues and contaminants which will be provided to policy makers for the implementation of standard setting, rules and regulations, directives for antimicrobial usage in livestock farms for consumer health and national action of combating antimicrobial resistance.

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ESTIMATION OF COMMITTED EFFECTIVE DOSE DUE TO RADIOACTIVITY IN PLANT-BASED MILK AVAILABLE IN KUWAIT MARKETS USING GAMMA AND ALPHA SPECTROMETRY SYSTEM: LEVELS OF POLONIUM-210 (^{210}PO) IN COMMERCIALLY OBTAINED PLANT-BASED MILK

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Abstract

Different radionuclides, both natural and human-made can be transferred to foods and beverages through pathways such as air, water and soil. Environmental pollutants and radioactive contaminants such as radioisotopes of polonium, lead and caesium can be found in milk and plant-based alternatives. Therefore, measurement of radionuclide concentration levels (activity concentrations) and calculation of committed effective dose arising from natural and human-made radioactive elements in foods is essential to assess the potential implications for human health. The research objective was to investigate the presence of artificial radionuclides such as radiocaesium (^{134}Cs and ^{137}Cs) and natural radionuclides such as isotopes of potassium (^{40}K) radium (^{224}Ra, ^{226}Ra and ^{226}Ra) and polonium (^{210}Po) in four commercially available plant-based milk products. Only the natural radionuclides ^{40}K and ^{210}Po were detected above limits of detection. The committed effective annual dose that might be received by a member of the general population from consuming ^{210}Po at the levels found, were calculated for each product. The committed effective doses from ^{210}Po at the levels found in plant-based milk were calculated and they were found to be relatively low in coconut milk, oat milk, almond milk, and soy milk (15.4 µSv/y, 21.4 µSv/y, 8.9 µSv/y and 15.0 µSv/y, respectively). It was concluded that the committed effective dose from ^{210}Po at the levels found can be considered as acceptable (much less than the 1000 µSv/y public dose limit).

1. INTRODUCTION

Humans, like other mammals, drink milk as their first food because it contains all the basic substances needed for rapid development and growth. As food, milk consists of many microelements and nutrients such as calcium, chloride, magnesium, phosphorus, potassium and sodium, and a number of proteins, but samples have also been found to contain toxic metals like lead and cadmium [1] and may also contain radionuclides. The food industry has been transformed by the introduction of plant-based alternatives to animal products and consumer demand has risen in recent years. According to the health and nutrition survey [2] people choose a dairy-free diet for ethical reasons or health reasons; as animal derived milk can be difficult to digest by many, and its lactose content may be unhealthy for some, which plays in favour of dairy alternatives. Cow milk allergies, lactose intolerance, calorie concerns, the incidence of hypercholesterolemia and a growing desire for vegan diets have all led consumers to seek cow milk alternatives.

In 2017, the term ‘vegetarian’ became popular in Kuwait, prompting the opening of more specialist vegan eateries, restaurants with clearly labelled vegan alternative menus, supermarket shelves with more vegan items, and cafés serving dairy-free and plant-based alternatives to milk [3]. Plant-based milk alternatives (‘plant-based milks’) are fluids that result from the breakdown of plant material (cereals, legumes oilseeds, nuts) that have been extracted in water and further homogenized so that a stable emulsion results with lipid phase droplets of size distribution in the range of 5–20 µm suspended in water, which imitates cow’s milk in appearance and consistency [4]. Although there is no clear description or classification of these plant-based milks in the scientific literature oat milk, almond milk, coconut milk and soy milk were selected for this study, as these products are imported into Kuwait and are widely available for consumption.

Radiation is emitted by naturally occurring and human-made radionuclides that are present to some degree in the environment and can therefore sometimes be detected in foods because they transfer into foods and beverages through various environmental pathways. Exposure to ionizing radiation emitted by radionuclides in food (internal exposure) gives rise to a committed effective ingestion dose. Monitoring and if necessary, controlling radioactive elements in the environment and in foods is critical for managing the levels of radiation to which human beings are exposed, whether directly or indirectly. Aside from natural radionuclides, a variety of manufactured radioactive materials have been introduced into the biosphere as a result of human activities including nuclear weapons tests and as a result of nuclear and radiological accidents. At the time of writing, almost every country in the gulf area has either a civil nuclear power programme in progress or under consideration. For

example, Iran Islamic Republic of launched its Bushehr Nuclear Power Plant in 2011, the Barakah Nuclear Power Plant opened in 2019 in the United Arab Emirates and preoperational nuclear power plants and associated radiological surveys have been conducted in the Kingdom of Saudi Arabia [5] as part of its plans to establish a civil nuclear power industry.

The IAEA, FAO and WHO have suggested that in the absence of any specific information on radionuclides present in foods nationally, there are several key radionuclides (^{210}Po, ^{210}Pb, ^{228}Ra, ^{226}Ra, ^{137}Cs, ^{134}Cs, ^{90}Sr and ^{14}C) that have to be investigated in food monitoring programmes [6]. These radionuclides are generally found to contribute almost all of the radiation dose from the ingestion of radionuclides in food. This study therefore aimed to include as many of these radionuclides as possible. ^{40}K was included because it occurs naturally and is found in all foods. Therefore, levels of the artificial radionuclides (^{134}Cs, ^{137}Cs) and natural radionuclides (^{40}K, ^{224}Ra, ^{226}Ra, ^{228}Ra and ^{210}Po) in several commercially available plant-based milks available in Kuwait were studied, and an assessment of the committed effective dose received by the general population due to consuming plant-based milks was also undertaken as part of this research [7].

2. MATERIALS AND METHOD

2.1. Samples

A selection of plant-based milks from various plant group types (oat, almond, coconut and soy) were obtained from shops in Kuwait. Samples were removed from their packaging and bottled in cylindrical glass containers (400 ml screw top jars) with triplicate subsamples. Samples were properly sealed to prevent any radionuclides escape and left for about one month in a fridge (at ~4°C) to reach equilibrium between radium and its daughters.

2.2. Gamma spectroscopy

During the gamma acquisition process, the activity of gamma radiation is measured in becquerel per litre (Bq/l). Measurements of radioactivity were taken using a high purity germanium (HPGe) detector. The detector is coupled with a 1000 inspector multichannel analyser, which has a relative efficiency of 100%. An appropriate software was utilized for gamma spectrum data acquisition, analysis and reporting. The samples were measured for a long time in order to accumulate a substantial amount of spectrum data points and resolve radionuclide peaks, while minimizing measurement errors. Simulation software LabSOCS that is compatible with the data-manipulation software, was used to generate an efficiency calibration curve for sample geometry keeping attention on any parameter that can affect the activity concentration calculation like sample density, source absorber and source to detector distance.

Thus, the simulation calibration curve generated was used to calculate the activity concentrations of different gamma emitter radionuclides per their presence on the gamma spectra and their branching ratio. To verify the derived efficiency calibration for the detector, an internal calibration sample was prepared in the Radiation Measurements Laboratory (RML) of the Kuwait Institute for Scientific Research by spiking a certified mixed radionuclide source with a known amount of water. This calibration sample was prepared in the same way as the plant-based milk samples and analysed using the efficiency curve generated by LabSOCS software.

2.3. Alpha spectrometric measurement

Following the alpha spectrometry measurement step, the concentration of ^{210}Po is determined in units of becquerel per litre (Bq/l). This is achieved by the use of a radiochemical separation technique and the analysis approach outlined in the IAEA 2009 guidelines [8] as depicted in Fig. 1. The measurement was performed on a Canberra Alpha equipped with 450 mm^2 passive implanted planar silicon detectors with a resolution of 20–24 keV at 5 MeV; 15% efficiency. Samples were counted for 3–4 days to determine ^{210}Po small net peak areas of 5304 keV. Chemical recovery of 93% to 95% was determined using ^{209}Po as the radiotracer (4877 keV).

2.4. Committed effective ingestion dose

For all radionuclides except ^{40}K, an estimate of committed effective ingestion dose can be calculated using the ICRP model [9] using Eq. (1)

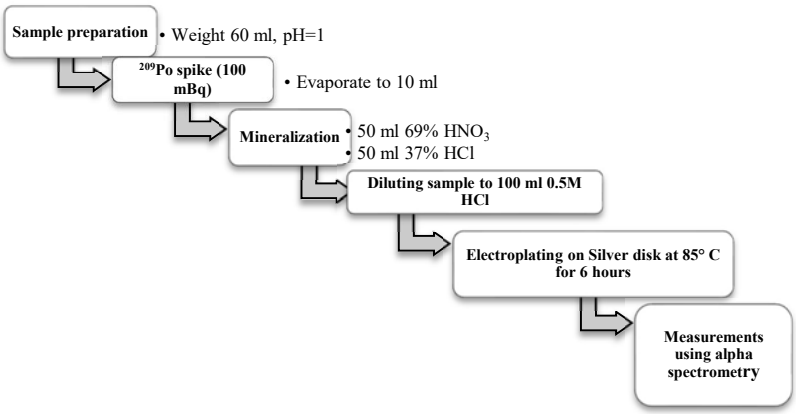

FIG. 1. The ^{210}Po radiochemical separation analytical procedure.

$$\text{Dose}\left(\frac{\text{Sv}}{\text{year}}\right) = [\text{A}]\left(\frac{\text{Bq}}{\text{l}}\text{ or }\frac{\text{Bq}}{\text{kg}}\right) \times \text{Annual intake}\left(\frac{\text{l}}{\text{year}}\text{ or }\frac{\text{kg}}{\text{year}}\right) \times \text{CEDC}\left(\frac{\text{Sv}}{\text{Bq}}\right) \quad (1)$$

Where: Dose is the annual committed effective dose due to ingestion; [A] is the activity concentration of the radionuclide under consideration; Annual intake is the amount consumed per year; CEDC is the committed effective dose coefficient for radionuclide A.

No data are available for the annual rate of plant-based milk consumption in Kuwait. The calculation of annual committed effective ingestion dose assumed that plant-based milk is consumed at the same rate as cow's milk. The FAO reported a food balance for milk excluding butter of about 235 kg/yr (approximately 235 l/y) for Kuwait in 2021 [10]. A committed effective dose coefficient of 1.2×10^{-6} Sv/Bq [9] was used to convert the annual activity intake for ^{210}Po into the committed effective dose.

3. RESULTS AND DISCUSSION

3.1. Potassium

The measured activity concentrations of ^{40}K in milk samples are shown in Fig. 2. The natural radionuclide ^{40}K was as expected detected in all samples with a maximum value of 84.3±3.7 Bq/l in soy milk imported from Belgium, and a minimum value of 16.9±1.0 Bq/l for almond milk from Italy. For comparison the natural levels of ^{40}K in liquid oat, almond and soy milk were calculated using data from 'FoodData Central', the USDA online source of food composition [11]. Multiplying the total, minimum, maximum, medium and average potassium content from the USDA database by the natural abundance of 0.000117 for ^{40}K and a specific activity of 2.6×10^{8} Bq/kg (and assuming that 1 kg was equivalent to 1 litre) the following were determined: oat milk (n=16) ^{40}K min of18.0 Bq/l, max 62.4 Bq/l, median 51.1 Bq/l and average 45.0 Bq/l; almond milk (n=14) ^{40}K min of 4.9 Bq/l, max. 22.5 Bq/l, median 19.8 Bq/l and average 14.9 Bq/l; soy milk (n=13) ^{40}K min of 32.5 Bq/l, max. 46.2 Bq/l, median 34.1 Bq/l and average 35.9 Bq/l. Data on coconut milk was not available.

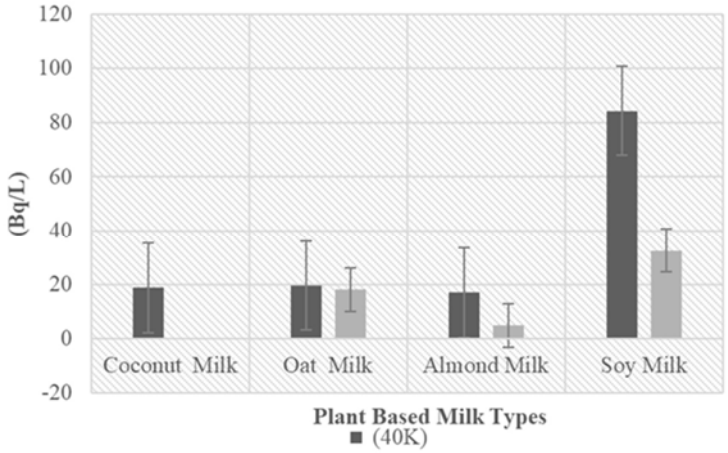

FIG. 2. The ^{40}K activity concentrations found in different plant-based milk products in Kuwait.

Potassium is reasonably uniformly distributed in the human body where its concentration is under homeostatic control. For adults, the body content of potassium is about 0.18%, and for children, about 0.2% and the United Nations Scientific Committee on the Effects of Atomic Radiation evaluate the annual effective doses from ^{40}K in the body as 165 μSv and 185 μSv for adults and children, respectively [12].

3.2. Radium and caesium

Radium radionuclides were not detected in the samples, ^{224}Ra, ^{226}Ra, ^{226}Ra-eq and ^{228}Ra activity concentrations were therefore lower than the LOD of ^{224}Ra 0.47 mBq/l ^{226}Ra 6.06 mBq/l, ^{226}Ra-eq 0.459 mBq/l, and ^{228}Ra 0.844 mBq/l (100,000 Sec, N-Type detector, 400 ml cylindrical container). Radiocaesium was also not detected above the LOD for ^{137}Cs of 0.472 mBq/l and for ^{134}Cs of 0.435 mBq/l.

3.3. Polonium

Figure 3 shows the average ^{210}Po concentrations measured in plant-based milk. Levels were found to range from 31.5±4.0 mBq/l to 75.8±5.0 mBq/l, which is low in comparison to ^{210}Po activity concentrations given in the literature for animal milk of typically 162±75 mBq/l [13]. As shown in Fig. 3, ^{210}Po activities were very low.

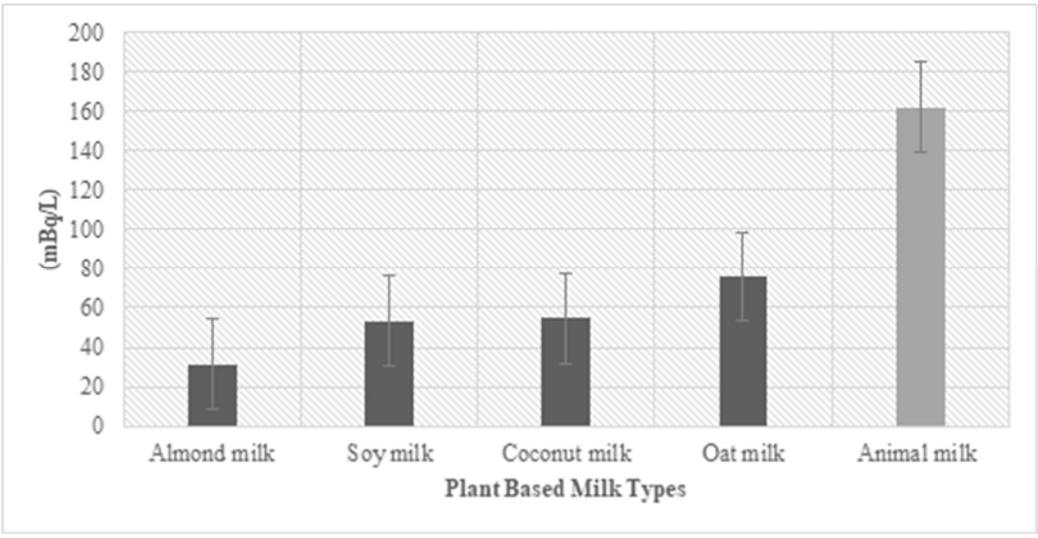

FIG. 3. The ^{210}Po activity concentrations detected in plant-based milk products available in Kuwait in comparison to a literature value reported for ^{210}Po in cow milk.

3.4. Committed effective dose

The committed effective dose for each plant-based milk product was calculated for ^{210}Po using Eq. (1) with an annual intake of 235 litres of plant-based milk alternative. The results are presented in Table 1.

TABLE 1. ^{210}Po ACTIVITY CONCENTRATIONS AND COMMITTED EFFECTED DOSE ESTIMATES

Sample	Country of manufacture	^{210}Po (mBq/l)	Dose (µSv/y)
Coconut milk	Australia	54.61 ± 4	15.4
Oat milk	Spain	75.81 ± 5	21.4
Almond milk	Italy	31.51 ± 8	8.9
Soy milk	Belgium	53.32 ± 5	15.0

4. CONCLUSIONS

Unsurprisingly, the naturally occurring radionuclide ^{40}K was detected in all samples due to its natural abundance in food. A typical annual effective dose arising from the ingestion of ^{40}K from all foods has been evaluated by the UNSCEAR as approximately 170 µSv [12]. Radium and radiocaesium were not found above the limits of detection. The committed effective dose contribution from radiocaesium and radionuclides of radium were assumed to be negligible because none were detected in the plant-based milk samples. The alpha-emitting radionuclide ^{210}Po was detected in the plant-based milk samples and the associated ingestion doses were assessed for coconut milk, oat milk, almond milk, and soy milk as (15.4 µSv/y, 21.4 µSv/y, 8.9 µSv/y and 15.0 µSv/y respectively) and can be considered as being acceptable compared to the public dose limit (i.e. much less than 1 mSv/y) [14]. Overall, the imported plant-based milk products on the Kuwait market are radiologically safe to be consumed.

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INVESTIGATING CERTAIN RADIONUCLIDES IN MAIZE AND SOIL FROM A COAL MINING REGION IN ZIMBABWE

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Abstract

Coal mining activities contribute to elevating levels of naturally occurring radioactive materials in the environment, affecting agriculture, the backbone of Zimbabwe's economy. These activities also pose a potential risk to food safety when emitted radionuclides from mines migrate to farming areas. This paper explores nuclear techniques and methodologies used to prepare for food emergencies associated with radioactivity exposure. One of the key aspects in preparing for emergencies, is the ability to constantly identify and quantify radionuclides in food from suspected radiation sources. This enables the government to monitor the levels of radionuclides in food before the population receives a high dose of radiation from contaminated food. Since soil is one of the pathways to radiation-contamination in food, and maize is the most common food crop in Zimbabwe, soil and maize samples from the mining towns were collected and tested for radionuclides using gamma spectrometry. Results showed that the tested radionuclides were within the acceptable range of approved theoretical limits.

1. INTRODUTION

Foods can be exposed to radionuclides through soil (mainly for terrestrial crops) and water for most aquatic creatures [1]. Anthropogenic activities such as coal mining can contaminate agricultural activities and therefore food. Anthropogenic activities such as coal mining contribute to environmental contamination by releasing hazardous chemicals [2]. Food grown near these mining sites can therefore be at risk of contamination, potentially negatively impacting human health and exacerbating food security. Literature reports the presence of naturally occurring radioactive materials including uranium (^{238}U) thorium (^{232}Th) their daughter radionuclides and potassium (^{40}K) in many countries due to coal mining activities [3].

All food contain some trace natural radionuclides from primordial radionuclides that are transferred from soil, water and air to plants and animals [1, 4]. Since radiation poses a serious health risk when significant quantities are ingested [5] attention through routine testing is needed to estimate background and abnormal levels. Maize is Zimbabwe's most grown crop, and it constitutes a major staple diet [6]. The cop is grown by both large and small scale farmers throughout the country.

In northwest Zimbabwe, coal mining is a major economic activity, with coal serving as one of the energy sources. Coal mining in Zimbabwe began as early as 1902, before any radiological control measures were established in the country [7]. According to literature, these mining activities increase the environmental radiation background. Research indicates that fly ash, bottom ash and boiler slag from coal power stations contribute to the high levels of radiation [8].

Radiation transmission to food occurs through various pathways, including both direct and indirect mechanisms. Indirect transfer occurs through deposition from the atmosphere with radionuclides accumulating in edible parts of plants. Direct transfer can be through soil and root uptake to all parts of a plant [9]. Crops and soil are therefore suitable samples for testing to determine radionuclide occurrence levels, hence their inclusion in this study.

2. MATERIALS AND METHODS

Twelve maize and soil samples were obtained from four distinct areas on farms in Hwange district in Zimbabwe. The samples were air-dried followed by drying in an oven dried at 70 degrees Celsius to ensure constant weight. After drying, the samples were crushed into smaller fine grains and weighed into beakers, sealed and stored for 35 days before analysis. The aim was to attain radioactive equilibrium between parent radionuclides and their daughter radionuclides. The analysis of the samples was then carried out at Radiation Protection Authority of Zimbabwe laboratory using a lead shielded high purity germanium detector interfaced to a multichannel analyser. A standard source (Eu 55 and Na 22) was used to calibrate the energy and efficiency of the detector. The samples were analysed for 21600 seconds each. The results of the spectra analyses were obtained using an appropriate software.

3. RESULTS AND DISCUSSION

Table 1 presents the radioactivity concentrations of the radionuclides thallium, (^{208}Tl) lead (^{210}Pb, ^{212}Pb and ^{214}Pb) bismuth (^{214}Bi) ^{40}K and caesium (^{137}Cs). The activity concentrations for all the radionuclides, except ^{40}K, ranged from 0.00007489 Bq/g to 0.0072 Bq/g. As expected, the activity concentrations of ^{40}K were higher than the other radionuclides, ranging from 0.0952 Bq/g to 0.1618 Bq/g. Despite the higher concentrations of ^{40}K, this is not considered a major health concern because ^{40}K can be utilized as a nutrient and is regulated by the body's metabolic processes [9]. The detection of ^{137}C in one of the samples, could be due to past nuclear fallout.

TABLE 1. CONCENTRATIONS OF RADIONUCLIDES IN MAIZE SAMPLES

Samples code	Radioactivity concentrations in Bq/g						
	^{40}K	^{208}Tl	^{210}Pb	^{212}Pb	^{214}BI	^{214}Pb	^{137}Cs
FS1	0.1125	0.0005	0.0008	—	0.0017	—	—
FS2	0.1151	0.0015	—	—	0.0002	0.0048	—
FS3	0.1618	0.0009	—	0.0008	0.0013	—	—
FS4	0.1142	0.0018	—	0.0072	0.0007	—	7.48923E-05
FS5	0.0952	0.0002	—	0.0015	0.0009	1E-05	—
FS6	0.1299	0.0022	0.0013	—	0.0005	0.0019	—

Note: *— = nothing detected; FS = food sample*

Table 2 shows the radioactivity concentrations of the soil samples analysed. The activity concentrations of the soil samples for the uranium and thorium decay chains were below 1 Bq/g. This is in accordance with IAEA standards for radionuclides of natural origin. The radionuclides measured included ^{40}K, ^{208}Tl, ^{210}Pb, ^{212}Pb, ^{214}BI, ^{214}Pb, radium (^{226}Ra) and actinium (^{228}AC). These findings have resulted in initiation of further studies to assess the soil-to-plant transfer factor of radionuclides. Both studies will help in predicting future levels of radionuclides in plants grown in soil around mining areas [10].

TABLE 2. CONCENTRATIONS OF RADIONUCLIDES IN SOIL SAMPLES

Sample code	Radioactivity concentrations in soil samples in Bq/g							
	^{40}K	^{208}Tl	^{210}Pb	^{212}Pb	^{214}BI	^{214}Pb	^{226}Ra	^{228}AC
SS1	0.643154	0.010661	0.092806	0.018563	0.021478	0.02336	0.075296	0.035121
SS2	0.303636	0.012213	0.027041	0.014892	0.020427	0.026556	0.02482	0.046728
SS3	0.729112	0.008995	0.056097	0.020742	0.018435	0.024043	0.015795	0.032746
SS4	0.173754	0.00873	—	0.016545	0.018911	0.028428	0.04396	—
SS5	0.430271	0.009639	0.010323	0.014246	0.017332	0.021391	—	0.048404
SS6	0.316588	0.009764	—	0.004273	0.017037	0.02143	0.018227	0.018704

Note: *— = nothing detected; SS = soil sample*

3.1. Effective ingestion dose

The annual effective ingestion dose is used to assess the health risk of ingesting radionuclides through food or water [11, 12]. The annual effective ingestion dose in this study was calculated for ^{226}Ra and ^{232}Th. The activity concentrations of ^{214}BI and ^{214}Pb were used to estimate the activity of ^{226}Ra. The activity of ^{232}Th as shown in Table 3 was determined by assessing ^{208}Tl. The annual effective ingestion dose of radionuclides in the maize samples was calculated using Eg. (1).

Annual effective ingestion dose =

activity concentration (Bq/kg) × annual intake (kg/year) × ingestion dose coefficient (Sv/Bq) (1)

Where the dose coefficient factors for ^{226}Ra and ^{232}Th were 0.00028mSv/Bq and 0.00023mSv/Bq, respectively as indicated elsewhere [13]. The annual consumption rate of 120 kg per person/year was used to calculate annual effective dose ingestion following a previous report [14]. The values for the annual effective ingestion dose of ^{232}Th and ^{226}Ra in the maize samples were below 0.1 mSv/year based on individual food product guidance level according to the IAEA [11] confirming previous reports about low-level radioactivity in food [1].

TABLE 3. ANNUAL EFFECTIVE INGESTION DOSE

Sample code	Dose (mSv/year)	
	^{226}Ra	^{232}Th
FS1	—	0.02282
FS2	0.08413	0.07469
FS3	—	0.04498
FS4	—	0.08891
FS5	0.01596	0.00775
FS6	0.04039	0.10802

Note: *— = nothing detected; FS = food sample*

4. CONCLUSION

A study was successfully undertaken to investigate levels of certain radionuclides in maize and soil from a coal-mining region of Zimbabwe, using a high purity germanium detector. Very low to levels were found, with none detected in many samples. The annual effective ingestion doses obtained from the analysis of the maize samples, were below the recommended limit of 0.1mSv/year acceptable level. Based on the low levels detected, there is no risk of exposure to unacceptable radiological hazards for people consuming maize grown in the vicinity of coal mines in Zimbabwe. Nevertheless, continuous monitoring is essential to ensuring preparedness for any potential radiological emergencies.

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LIST OF ABBREVIATIONS

AFL	Aflatoxin
AMR	Antimicrobial resistance
CAC	Codex Alimentarius Commission
CFU	Colony forming units
CPM	Counts per minute
CRM	Certified reference materials
FAO	Food and Agriculture Organization on the United Nations
GI	Geographical indication
HPGe	High-purity germanium detector
IAEA	International Atomic Energy Agency
IRMS	Isotope ratio mass spectrometry
KeV	Kilo electron volt
kGy	Kilogray
LEEB	Low-energy electron beam
LOD	Limit of detection
LOQ	Limit of quantification
LSC	Liquid scintillation counter
MADLI ToF	Matrix Assisted Laser Desorption Ionization Time of Flight Mass spectrometry
ML	Maximum limit
MRL	Maximum residue level
NAA	Neutron activation analysis
NMR	Nuclear magnetic resonance
PCR	Polymerase chain reaction
RBA	Receptor binding assay
RRA	Radio receptor assay
SILAC	Stable Isotope Labelling by Amino Acids in Cell Culture
STEC	Shiga toxin producing *Escherichia coli*
TXRF	Total reflection X ray fluorescence
WHO	World Health Organization

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Dalto, Y. M.	National Atomic Energy Commission, Argentina.
Etoom, M.A.	Jordan Atomic Energy Commission, Jordan
Gatti, P.	National Institute of Industrial Technology, Argentina
Hammad, A.A.I	National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Egypt
Harntaweesup, Y.	Bureau of Quality and Safety of Food, Ministry of Public Health, Thailand
Hernandez, G.	Ministry of Agricultural Development, Panamá
Hewajulige, I.G.N.	Industrial Technology Institute, Sri Lanka
Hoo Fung, L.A.	International Centre for Environmental and Nuclear Science, Jamaica
Katsande, P.	Department of Veterinary Technical Services Harare, Zimbabwe
Kocia, R.	Institute of Nuclear Chemistry and Technology, Poland
Makufa, E.	Radiation Protection Authority of Zimbabwe
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Pavlidis, D. E.	Department of Food Science and Technology, University of Peloponnese, Greece
Raad, A.	National Atomic Energy Commission, Argentina
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Thantar, S.	Department of Nuclear Technology, Technological University Kyaukse, Myanmar
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Turan, Ö.	Turkish Energy, Nuclear and Mineral Research Agency, Türkiye
Widyastuti, H.	National Research and Innovation Agency of Indonesia, Indonesia
Zarkawi, M.	Atomic Energy Commission, Syrian Arab Republic

PROGRAMME COMMITTEE, IAEA SECRETARIAT, TIMETABLE, SESSIONS AND SESSION-PARTICIPANTS

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Event Organizer:	J. Zellinger, Conference Services, IAEA Department of Management
Administrative Support:	H. Kenny, Joint FAO/IAEA Centre
	E. Makeche, Joint FAO/IAEA Centre (formerly of)
	M. Rydeng, Joint FAO/IAEA Centre

TIMETABLE

MONDAY, 27 MAY 2024

Time	Session No.	Session Title / Break	Venue
08:00–16:00		Collection of Entry Badge	VIC Gate 1
09:30-11:20		Opening Session	
09:30–09:35		Welcome Remarks	
09:35–10:15		Opening Remarks by IAEA and FAO Directors General	
10:15–10:45		Scene Setting Keynote Speech High Level Panel Discussion: 'Joining Hands for Safer Food, a Healthier and Prosperous People'	M-Plenary
10:45–11:15		Group photo (High level panel)/ Coffee/Tea Break	
11:20–12:00	Session 1	Opening Technical Session	
12:00–13:00		Group photo (all participants) and Lunch Break	
13:00–14:15	Session 2	Chemical Residues & Contaminants in Food & Feed	
14:15–14:55		Poster Session 1A (Chemical Residues & Contaminants in Food & Feed)	
14:55–17:05	Session 2	Chemical Residues & Contaminants in Food & Feed (cont'd)	
17:05–17:45		Poster Session 1A (Chemical Residues & Contaminants in Food & Feed) (cont'd)	
18:00–20:00		Welcome Reception	M-Building, Ground Floor

TUESDAY, 28 MAY 2024

Time	Session No.	Session Title / Break	Venue
08:30–9:30	Session 3	Public-Private Partnerships; Funding Agencies; the Food Industry; Policy Making	M-Plenary
09:30–10:10		Safe Food in Africa (SAFA) – A new FAO-IAEA-UNIDO Tripartite Food Safety Programme	
10:15–11:00	Session 4	Preparing for and Responding to Emergencies and Incidents Affecting the Food Supply; Measurement of Radionuclides	
11:00–11:20		Coffee/Tea Break	
		Poster Session 1B (Chemical Residues & Contaminants in Food &	

		Feed) and Poster Session 2 (Preparing for and Responding to Emergencies and Incidents Affecting the Food Supply; Measurement of Radionuclides)
11:20–13:05	Session 4	Preparing for and Responding to Emergencies and Incidents Affecting the Food Supply; Measurement of Radionuclides (cont'd)
13:05–14:05		Lunch Break
14:05–14:30	Session 4	Preparing for and Responding to Emergencies and Incidents Affecting the Food Supply; Measurement of Radionuclides (cont'd)
14:40–15:40	Side Event	Women in Nuclear, IAEA
15:40–18:10	Side Event	Exhibitor Presentations

WEDNESDAY, 29 MAY 2024

Time	Session No.	Session Title / Break	Venue
09:00–10:15	Session 5	Food and Phytosanitary Irradiation	M-Plenary
10:15–10:55		Coffee/Tea Break Poster Session 3 (Food and Phytosanitary Irradiation)	
10:55–12:15	Session 5	Food and Phytosanitary Irradiation (cont'd)	
12:15–13:15		Lunch Break	M-Plenary
	Side Event	Vienna Centre for Disarmament Non-Proliferation (VCDNP)	
13:15–15:00	Session 6	Food Authenticity and Fighting Food Fraud	
15:00–15:40		Poster Session 3 (Food and Phytosanitary Irradiation) (cont'd)	
15:40–17:35	Session 6	Food Authenticity and Fighting Food Fraud (cont'd)	

THURSDAY, 30 MAY 2024

Time	Session No.	Session Title / Break	Venue
09:00–10:15	Session 7	Standard Setting, Regulations, Metrology, Risk Assessment	M-Plenary
10:15–10:55		Coffee/Tea Break Session 4 (Food Authenticity and Fighting Food Fraud)	
10:55–12:25	Session 7	Standard Setting, Regulations, Metrology, Risk Assessment (cont'd)	
12:25–13:25		Lunch Break	

13:25–14:20	Session 7	Standard Setting, Regulations, Metrology, Risk Assessment (cont'd)
14:20–15:05	Session 8	Detection and Characterization of Pathogens in Food, AMR
15:05–15:45		Poster Session 4 (Food Authenticity and Fighting Food Fraud) (cont'd)
15:45–17:45	Session 8	Detection and Characterization of Pathogens in Food, AMR (cont'd)

FRIDAY, 31 MAY 2024

Time	Session No.	Session Title / Break	Venue
09:00–10:15	Session 9	One Health	M-Plenary
10:15–10:55		Coffee/Tea Break Poster Session 5 (Standard Setting, Regulations, Metrology, Risk Assessment) Poster Session 6 (Detection and Characterization of Pathogens in Food, AMR) Poster Session 7 (One Health)	
10:55–12:25	Session 9	One Health (cont'd)	
12:25–13:25		Lunch Break	
13:25–13:50	Session 9	One Health (cont'd)	
13:50–14:00		Award of Poster Presentations	
14:00–14:10		Conclusion and suggestions	
14:10–14:50		Closing Session	

ORAL PRESENTATIONS/ LECTURES

MONDAY, 27 MAY 2024

09:30–11:20 OPENING SESSION

Moderator: N. Mokhtar, DDG-NA

Time	Name	Designation	Title
09:30–09:35	N. Mokhtar	Deputy Director General and Head of the Department of Nuclear Sciences and Applications, IAEA	Film Introductions
09:35–09:55	R.M. Grossi QU Dongyu	IAEA Director General FAO Director General	
09:55–10:15	K.E Baipoledi	Permanent Secretary, Ministry of Lands and Water Affairs, Republic of Botswana	Global Food Safety Situation in Relation to Climate Change, Trade and One Health Approach

HIGH LEVEL PANEL DISCUSSION:'JOINING HANDS FOR SAFER FOOD, A HEALTHIER AND PROSPEROUS PEOPLE'

Time	Name	Designating Member State/Organization
10:15–10:45	R.M. Grossi	Director General, IAEA
	A. Alkhalifa	President, OPEC Fund for International Development
	K.E. Baipoledi	Permanent Secretary, Ministry of Lands and Water Affairs, Republic of Botswana
	D. Aguemon	Director, Cabinet of the Minister of Agriculture, Livestock and Fisheries, Republic of Benin
	G. Beger	Managing Director, United Nation Industrial Development Organization
	J. Pleiner-Duxneuner	Managing Director, Austrian Agency for Health, and Food Safety
10:45–11:15	*Group Photo (high level panel) & Coffee/Tea Break*	

11:15–11:20	C. Vlachou & C. Blackburn	Joint FAO/IAEA Centre	Secretaries'/Administrative Remarks

11:20–12:00 SESSION 1: OPENING TECHNICAL SESSION — M-Plenary

Chairperson: A. Strasser, France

Time	Paper No.	Name	Designating Member State/Organization	Title of Paper/Presentation
11:20–11:35		S. Pillai	USA	Irradiation, Microbiology, Public Health, and Trade - the Interconnectedness
11:35–11:50		M. Lipp	FAO	Diet Vs Food - Safeguarding Consumer Health
11:50–12:00		Discussion		
12:00–13:00		*Group photo (all) & Lunch Break*		

13:00–17:45 SESSION 2: CHEMICAL RESIDUES & CONTAMINANTS IN FOOD & FEED — M-Plenary

Chairperson: M. V. Cesio, Uruguay

Co-Chairperson: C. Vlachou, Joint FAO/IAEA Centre

Time	Paper No.	Name	Designating Member State/Organization	Title of paper/presentation
13:00–13:15		V. Bartkevics (Keynote)	Latvia	Advances in Testing and Monitoring Residues and Contaminants in Food
13:15–13:30	70	T. Chigiya	Zimbabwe	An Assessment of Veterinary Drug Residues in Beef and Chicken from Zimbabwe: National Monitoring Program
13:30–13:45	160	G. Liftinger	Austria	Heavy Metals and Arsenic in Residue Control of Animals in Austria - an Overview and Interesting Results
13:45–14:00	154	B.S.N. Al Salhi K. Alfarsi B. Alrawahi	Oman	Determination of Heavy Metals in Selected Fruits and Vegetables Collected from Local Market in Muscat, Sultanate of Oman
14:00–14:15	150	U. Maqbool J. Sasanya S. Shah I. Chughtai M. Mumtaz M. Yasin G. Hussain	Pakistan	Nuclear Techniques to Enhance Mixed Contaminants Analysis in Food and Feed
14:15–14:55	*Poster Session 1A (Chemical Residues & Contaminants in Food & Feed)*			
14:55–15:10	206	B. R. Fernando K.G.A.D. Abesooriya B.C.J. Fernando A.R.C. Gunasena P.W. Jayasooriya R.M.S.K. Karunarathne M.M.A.W.P. Mudannayake	Sri Lanka	Safety Concerns of Animal Originated Food in Sri Lanka due to Antimicrobial Residues and Aflatoxins
15:10–15:25	303	S. Panigrahi C. V. Srinivas S. N. Bramha M. Margret L. Srivani S. Chandrasekaran B. Venkatraman	India	Food and Radiological Safety Assessment Based on the Activity Concentration of Radionuclides in Seafood from Klapkkam, India
15:25–15:40	282	S. Herrera A. Montenegro R. Cabanzo E. R. Sánchez Y.R. Giraldo C. A.Vargas García	Colombia	Quantification of Cadmium in Cocoa Beans by Laser Ablation coupled with Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) via Pressed Pellets

Time	Paper No.	Name	Designating Member State/Organization	Title of paper/presentation
15:40–15:55	151	Md. H. Islam D. Chakma M. J. Islam Md. Nazrul Islam P.M. Billah A.S.M. Saifullah K. S. Chironjit	Bangladesh	Screening Antimicrobial Residues in Chicken Muscle in Bangladesh Using Charm II Radio-Receptor Assay Technique
15:55–16:10	177	K. Nasr Ep Kachroudi M. Samaali C. Tissaoui Z. A. Berriche L. Chouba F. Boujelbane	Tunisia	Evaluation of Polonium-210 and Dose Assessment in the Bivalve Venerupis Decussate from Tunisian Coastline
16:10–16:25	29	B. Katati	Zambia	Prospecting the BioControl of Fusarium's Fumonisin from Maize Phyllosphere
16:25–16:40	33	G. García C. M. A. Hernández L.C. Longoria	Mexico	Microplastics Extraction and Identification from Commercial Samples of Edible Mussels (*Mytilus Galloprovincialis*)
16:40– 16:55	42	S. Y. Fazaeli Hosseininezhad G. Shahhosseini A. Neisi J. J. Sasanya P. Ashtari S. F. Zenjanab	Iran Republic of	Depletion Imaging using Radionuclides; a Novel Method for Evaluation of Depletion of Veterinary Pharmaceuticals using Radioisotopes
16:55–17:05			Discussion	
17:05–17:45		*Poster session 1A (Chemical Residues & Contaminants in Food & Feed) (cont'd)*		
18:00–20:00 Building – Ground Floor		*Welcome Reception*		M-

TUESDAY, 28 MAY 2024

M-Plenary

08:30–09:30 SESSION 3: PUBLIC-PRIVATE PARTNERSHIPS; FUNDING AGENCIES; THE FOOD INDUSTRY; POLICY MAKING

Chairperson: G. Molnar, UNIDO

Co-Chairperson: E. Dupouy, FAO

Time	Paper No.	Name	Designating Member State/Organization	Title of Paper/Presentation
08:30–08:45		D. Ihedioha (Keynote)	African Development Bank (AfDB)	The African Development Bank and Food Safety in Africa

08:45–09:00	G. Molnar (Keynote)	United Nations Industrial Development Organization (UNIDO)	Modernizing Food Control Systems through Innovative Public-Private Partnerships

PANEL DISCUSSION, Q& A

Time	Name	Designation
09:00–09:30	D. Ihedioha	AfDB
	G. Molnar	UNIDO
	K. Onul	The International Finance Corporation (IFC)
	Y. Siddiqi	Asian Development Bank (ADB)
	N. El-Haj	IAEA-Nuclear Sciences and Applications

09:30–10:10	E. Dupouy J. Sasanya G. Molnar Partners	FAO Joint FAO/IAEA Centre UNIDO Stakeholders	Safe Food in Africa (SAFA) – A new FAO-IAEA-UNIDO Tripartite Food Safety Programme Proposal Presentation and discussion

10:15–14:30	SESSION 4: PREPARING FOR AND RESPONDING TO EMERGENCIES AND INCIDENTS AFFECTING THE FOOD SUPPLY; MEASUREMENT OF RADIONUCLIDES	M-Plenary
Chairperson:	R. Newkirk, WHO	
Co-Chairperson:	D. Ihedioha, AfDB	

Time	Paper No.	Name	Designating Member State/Organization	Title of Paper/Presentation
10:15–10:30		E. Dupouy (Keynote)	FAO	Comprehensive Food Safety Emergency Preparedness for Rapid Response and Effective Management
10:30–10:45	240	M. Sanad	Bahrain	Investigation on Food Poisoning Outbreak Caused by Staphylococcus aureus Enterotoxin A and Incidence of Staphylococcal Food Poisoning in the Kingdom of Bahrain
10:45–11:00	242	S.S. Hosseini A. Attarilar F. Keshavarz E. Mirrezaei M. Nazmabadi M. Salehi	Iran Republic of	Determination of Po-210 in Iranian foodstuff

11:00–11:20			*Coffee/Tea Break* *Poster Session 1 B (Chemical Residues & Contaminants in Food & Feed) and* *Poster Session 2 (Preparing for and Responding to Emergencies and Incidents Affecting the Food Supply; Measurement of Radionuclides*)	
11:20–11:35	219	E. Makufa	Zimbabwe	Preparing for Emergencies and Incidents Affecting the Food Supply-Coal Mining Towns in Zimbabwe
11:35–11:50	2	S. Alnaaimi A. Aishah A. Omar	Kuwait	Estimation of Committed Effective Dose of Radioactivity in Plant-Based Milk Available in Kuwait Markets Using Gamma and Alpha Spectrometry System
11:50–12:05	78	J. Lubbe M. Van Rooy	South Africa	NMISA Supports Food Safety through Radioactivity Testing of Food, Feed & Water
12:05–12:20	97	P. Pavlenko V. Kashparov	Ukraine	Testing Various Countermeasures to Reduce Radioactive Contamination of Freshwater Fish
12:20–12:35	171	M. Samaali K. N. Ep Kachroudi W. Leghribi Z.A. Berriche F. Boujelbane	Tunisia	Uranium Natural Levels in Bottled Mineral Water Produced in Tunisia
12:35–12:50	67	J. A. Guerrero Dallos	Colombia	Pesticide Residue Analysis Laboratory an Example of Collaboration between Member States and IAEA
12:50–13:05	128	V.K. Gouws M. Madhuk M. Mathuthu R. D. Mavunda	South Africa	Assessment of Norm in Fruits and Vegetables from Hartbeespoort, Mafikeng and Pretoria Markets
13:05–14:05		*Lunch break*		
14:05- 14:20		O. Guzman Lopez Ocon	IAEA	Management of Exposures Due to Radionuclides in Food other than during a Nuclear or Radiological Emergency: New IAEA-FAO-WHO Guidance
14:20–14:30		Discussion		

14:40–18:10	SIDE EVENTS	
Time		
Moderator:	R. Attia, Joint FAO/IAEA Centre	
14:40–15:40		Women in Nuclear, IAEA
Moderators:	B. Maestroni, Joint FAO/IAEA Centre A. Mihailova, Joint FAO/IAEA Centre (Formerly)	
15:40–18:10		Exhibitor-Side Event

WEDNESDAY, 29 MAY 2024

09:00–12:15	SESSION 5: FOOD AND PHYTOSANITARY IRRADIATION	M-Plenary
Chairperson:	A. Strasser, France	
Co-Chairperson:	C. Blackburn, Joint FAO/IAEA Centre (Formally)	

Time	Paper No.	Name	Designating Member State/Organization	Title of Paper/Presentation
09:00–09:15	05	M. Lacroix (Keynote)	Canada	Cross-Linked Gelatine-Riboflavin-Based Film Incorporated with Essential Oils and Silver Nanoparticle by Gamma-irradiation: A novel Approach for Extending Shelf-Life of Meat
09:15–09:30	302	A.M. Benita H. Widyastuti	Indonesia	Effect of Gamma Irradiation and Vacuum-Packaging on Shelf-Life Extension of Ethnic Food Gudeg
09:30–09:45	143	I. Hewajulige M. Jayasinghe R. Pitipanaarachchi N. Gunesekara M. Samarananayake G. Madurakanthi H. Mahanama	Sri Lanka	Development of Granola Bars using Locally Available Raw Material as Emergency Food and Evaluating the Effect of Gamma Irradiation (Co 60) on Nutrition and Sensory Attributes
09:45–10:00	21	F. Ocloo J. Agyei-Amponsah S. Fiadey U. Gryczka	Ghana	Comparative Effects of High Energy Electron Beam and Gamma Irradiation on Microbial Quality African Nutmeg (*Monodora Myristica*) Powder
10:00–10:15	161	F. Kuntz A. Strasser F. Van der Stappen A. V. Rivadeneira	France	Low, Medium and High Energy X rays or Electron Beam, which is the Optimal Irradiation Technology for Phytosanitary Application? A Dosimetry and Monte Carlo Simulation Approach
10:15–10:55	*Coffee/Tea Break* *Poster Session 3 (Food and Phytosanitary Irradiation)*			
10:55–11:05	212	S. Stutchbury P. Dethier	Canada	Industrial E-beam and X ray Systems for Food Irradiation-Mevex Latest Developments
11:05–11:20	273	O. Acuna J. Elster S. Pillai	USA	Ensuring Food Security and Food Safety by Examining the Feasibility of

				Implementing E-Beam/X ray Technologies
11:20–11:35	318	V. Simoes Dias C. Caceres G. Hallman M.J. B. Vreysen S. Myers I.V. Gomes	FAO/IAEA	Harmonization of Phytosanitary Irradiation Treatments
11:35–11:50	192	G. Diano G. Abrera C. Deocaris D. R. Montefalcon E. Sabado K. M. Tardecilla	Philippines	Exploring the Gut Microbiome of Mass-Reared Super Worm (Zoophobas Morio) in the Philippines: Insights into Bioactivity and Radiosensitivity
11:50–12:05	153	S. Cabo Verde B. Pinheiro J. Madureira U. Gryczka	Portugal	Extraction of Bioactive Compounds from Food using Low Energy E-Beam
12:05–12:15		Discussion		
12:15–13:15		*Lunch Break*		

12:15–13:15	SIDE EVENT		
Paper No.		Organization	Title
		Vienna Centre for Disarmament Non-Proliferation (VCDNP)	The Future of Food: Globalizing Access to Technologies

13:15–17:35	SESSION 6: FOOD AUTHENTICITY AND FIGHTING FOOD FRAUD		M-Plenary
Chairperson:		C. Elliott, United Kingdom	
Co-Chairperson:		M. Lees, France	

Time	Paper No.	Name	Designating Member State/Organization	Title of paper/presentation
13:15–13:30		G. Marechal (Keynote)	Belgium	EU Activities in the Fight Against Agri-Food Fraud

13:30–13:45	213	C. Terro N. E. Amenzou L. Bontempo M. Eddabdouby M. J. Hudobivnik B. K. Seljak D. Mazej R. Modic B. Moncef M. Ogrinc N. Ogrinc D. Potočnik A. Simčič	Slovenia	IsoFoodTrack-Stable Isotope and Multi-Elemental Tool for Determination of Saffron Traceability as Selected Commodity
13:45–14:00	104	J. Geist A. Gadelmeier	Germany	Development of a Database with Stable Isotope Data sets for Egg Authenticity at the National Reference Centre for Authentic Food (NRZ-Authent)
14:00–14:15	144	A. Li A. Abrahim X. Yang S. Kelly	China	Potential of Non-exchangeable Hydrogen Stable Isotopic Fingerprint for Honey Authenticity
14:15–14:30	252	M. O. Varrà L. Husáková P. Iacumin J. Patočka M. Rossi A. Ianieri E. Zanardi	Italy	Detecting Fraud in Cephalopods using Decision Rules based on Carbon and Nitrogen Isotope Ratios and Rare Earth Elements
14:30–14:45	193	S. Pianezze N. Barilaro L. Bontempo S. Carpino V. D. Martino D. Masuero M. Perini	Italy	Stable isotope Ratio Analysis to detect synthetic citric acid addition to Italian tomato sauce
14:45–15:00	311	A. Kambikkanath B. Bharath N. Karunakara	India	Quantification of Natural and Synthetic Constituents in Edible and Essential Oils through Determination of ^{14}C Specific Activity by Liquid Scintillation Counting
15:00–15:40	*Poster Session 3 (Food and Phytosanitary Irradiation) (cont'd)*			
15:40–15:55	*274*	D.A. Werner	France	Exploring the equivalence of high and low Field NMR Instruments to Assess Pepper Authenticity

15:55–16:10	328	G. P. Sabin P. G. Celso D.L. Dantas de Freitas L. Ferreira de Aguiar H. Vitória de Oliveira Silva	Brazil	Enhancing Coffee Sector Integrity through Multispectral Microscopy
16:10–16:25	237	T. A. Bagawath Arachchige K. Binduhewa S. M. N. K Thilakarathne C. K. Dissanayake A. M. R. W. S. D. Rathnayaka	Sri Lanka	Differentiation of Tea based on Country of origin Using the FT-IR Pattern Recognition Method
16:25–16:40	203	R. Duft J. Griffin	United Kingdom	Development of LC-MS/MS Proteomics and Lipidomics Methods to Differentiate Meat Species for Food Authenticity
16:40–16:55	87	D. Pavlidis S. Balafas A. Mallouchos G.J. Nychas	Greece	Volatilomics for Authenticity Assessment of Raw Red Meats
16:55–17:10	105	R. M. H. Raja Nhari N. F. K. Mokhtar A. M. Hashim J. H. Soh	Malaysia	Detection of Pork Adulteration Using Lateral Flow Immunoassay Targeting Porcine-Specific IgG
17:10-17:25		P. Xiao	China	China's Food Fraud Regulation in the Digital Age: A Close Look at a Landmark Case During the Pandemic
17:25–17:35		Discussion		

THURSDAY, 30 MAY 2024

09:00–14:20 Chairperson: Co-Chairperson:	SESSION 7: STANDARD SETTING, REGULATIONS, METROLOGY, RISK ASSESSMENT S. Cahill, CAC H. Heinzen Gonzalez, Joint FAO/IAEA Centre		M-Plenary

Time	Paper No.	Name	Designating Member State/Organization	Title of Paper/Presentation
09:00–09:15		S. Wearne (Keynote)	Codex Alimentarius Commission	You have a Stake in Setting and Implementing International Food Safety Standards and Guidelines.

09:15–09:30	155	M. Hachinohe K. Kubo M. Ogihara M. Yoshiyama T. Hirayama H. Matsuoka T. Sato Y. Urashima	Japan	Research on Honey Production to Reduce the Risk of Radiocesium Contamination in Decontaminated Farmland in Fukushima
09:30–09:45	186	G. GAJSKI A. Domijan K. Hercog M. Štampar M. Gerić M. Sokolović B. Žegura	Croatia	Toxicity of Mycotoxins Deoxynivalenol and Zearalenone alone and in a Binary Mixture
09:45–10:00	121	M. L. Pareja C. Pérez S. Pereyra A. Pérez	Uruguay	Winter Cereals Production Status in the Northwestern Part of Uruguay regarding Food Safety
10:00–10:15	204	X. Xu L. Huang Y. Pan L. Wang L. Wen	China	Disposition of Tritium Labeled Diaveridine in Pigs, Broilers and Rats
10:15–10:55			*Coffee/Tea Break* *Poster Session 4 (Food Authenticity and Fighting Food Fraud)*	
10:55–11:10	130	J. Cornejo E. Pokrant P. Cortés B. Pinto F. Suazo M. Maturana A. Flores C. Vergara A. Maddaleno	Chile	Amoxicillin Evaluation in Broiler Chickens: Public-Academic Collaboration in the Framework of an International Project to Provide Data for the MRLs Establishment
11:10–11:25	141	A.S.M. Saifullah Md. J. Islam C. K. Shaha Md. H. Islam Md. N. Islam D. Chakma Md. M. B. Prince	Bangladesh	Residue Depletion Kinetics and Withdrawal Time Estimation of Doxycycline in Tissues of Sonali Chicken following Multiple Oral Administrations
11:25–11:40	22	R. Ismail	Egypt	The Importance of Black Seed Oil and the Challenges to the Establishment of International Standards
11:40–11:55	335	H. Heinzen Gonzalez N. Gérez M. V. Cesio Cesconi	Uruguay	Monitoring for Risk Assessment Studies of Cucurbitaceae Species Sold in the Uruguayan Market

11:55–12:10	57	S. Issaka A. Asamoah A. Gibrilla	Ghana	Risk Assessments of Polycyclic Aromatic Hydrocarbons (PAHs) in Repeated Use Vegetable Oils and Finger Foods in Ghana
12:10–12:25	194	T. G. Angeles B.J. Barba C.G. Causapin M. Cebujano P. Gonzales A. Mendoza A.Y. Salen	Philippines	Cyanotoxin Risk Assessment (CRA): Preliminary Study on Utilizing Isotopic Techniques for Food Safety and Water Quality Management of Freshwater Lake Systems in the Philippines
12:25–13:25		*Lunch break*		
13:40–13:55		D. Marko (Keynote)	Austria	Mycotoxins, Risk Assessment, Toxicology
13:55–14:10		J. LeJeune	FAO	Microbiological Risk Assessment and JEMRA
14:10-14:20		Discussion		

14:20–17: 45 SESSION 8: DETECTION AND CHARACTERIZATION OF PATHOGENS IN FOOD, AND ANTIMICROBIAL RESISTANCE (AMR) M-Plenary

Chairperson: P. Ellitson, Namibia

Co-Chairperson: J. Sasanya, Joint FAO/IAEA Centre

Time	Paper No.	Name	Designating Member State/Organization	Title of paper/presentation
14:20–14:35		J. Nakavuma (Keynote)	Uganda	The Beauty and Hustle of Pathogen Detection in the Face of Global or Hitherto Ignored Challenges
14:35–14:50	261	S. Pillai S. V. Patchametla S.K. Singamneni K. Narayanan	USA	Rapid Screening of Viral Food Pathogens using a Single Stage Pooling Method
14:50–15:05	66	B. M. Ondieki	Kenya	Detection And Characterization of Pathogens in Food
15:05–15:45		*Coffee/Tea Break* *Poster Session 4 (Food Authenticity and Fighting Food Fraud) (cont'd)*		
15:45–16:00	228	E. Wagner	Austria	Did a Shortage of Knowledge on *Cronobacter Sakazakii* Lead to a Shortage of Infant Formula?

16:00–16:15	64	J. Klinsoda	Thailand	Application of 16S Ribosomal RNA Gene Sequencing Microbiome Innovation in Food Safety and Quality Assurance
16:15–16:30	36	P. Mafirakureva J. Mbanga B. Saidi	Zimbabwe	Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens with Colibacillosis in and around Harare, Zimbabwe
16:30–16:45	347	M. Mataragas	Greece	Comparative Genomic Analysis and Antimicrobial Resistance Profile of Enterococci Strains Isolated from Raw Sheep Milk
16:45–17:00	101	A. O. Obadina I. E. Martins	Nigeria	Global burden of Antimicrobial Resistance: Monitoring and Evaluation of Ready-to-Eat Vegetables
17:00–17:15	348	N. Andritsos	Greece	Characterization and Antimicrobial Resistance of *Listeria monocytogenes* Isolated During Microbiological Testing of Various Foods
17:15–17:30	180	X. Mkhize	South Africa	Detecting Microbial Levels and Food Environment Management for Indigenous Crops sold by Informal Traders in the Central Market of Durban, South Africa
17:30–17:45	90	P. Katsande	Zimbabwe	An Investigative into Contamination Sources, Biofilm Formation Ability and Biocide Resistance of Listeria Monocytogenes Recovered from a Nile Crocodile (*Crocodylus niloticus*) Meat Processing Facility

FRIDAY, 31 MAY 2024

09:00–14:10 SESSION 9: ONE HEALTH M-Plenary

Chairpersons: S. Darkaoui, Morocco

Co-Chairperson: J. LeJeune, FAO

Time	Paper No.	Name	Designating Member State/Organization	Title of paper/presentation
09:00–09:15		W. Markotter (Keynote)	South Africa	What the Transdisciplinary Nature of One Health Means: Opportunities and Challenges
09:15–09:30	277	J. Kwon	Republic of Korea	Management of Antimicrobial Resistance for Food and Environmental Safety: Complementary Approach beyond Gaps between Research and Regulation
09:30–09:45	187	Y. Harntaweesup S. Mhudmah S. Wajasit	Thailand	Surveillance of Antimicrobial Residues in Aquatic Animals in Central River Basin in Thailand–a One Health Approach
09:45–10:00	190	V. Ntuli	South Africa	Quantitative Risk Assessment of Shigatoxin Producing *Escherichia coli* Associated with Consumption of Contaminated Lettuce: A Case Study in South Africa
10:00–10:15	327	K. Morgan	USA	Risk-informed Approach to One Health Resilience
10:15–10:55		*Coffee/Tea Break/* *Poster Session5 (Standard Setting, Regulations, Metrology, Risk Assessment)* *Poster Session 6 (Detection and Characterization of Pathogens in Food, AMR)* *Poster Session 7 (One Health)*		
10:55–11:10		V. Owino	IAEA	Characterizing Exposure to Food-Borne Contaminants and Potential Link to Nutrition
11:10–11:25	264	B. Aslam	Pakistan	Tracing Antimicrobial Resistance in the Food Supply Chain: From Farm to Fork
11:25–11:40	227	K.E. N'Goran N.B. Aboly H.K.G. Sonan U. Bahi	Cote d'Ivoire	Chemotherapy and Contamination Risks in Livestock Production Systems in the West, North-West and North regions of Côte d'Ivoire: How the One-Health Approach can Contribute to Anticipating the Contamination Risks

11:40–11:55	173	W.Y.N. Syahfitri N. Adventini D. P. Dwi Atmodjo E. Damastuti S. Kurniawati I. Kusmartini D. D. Lestiani M. Santoso D.K. Sari P. Wardhani	Indonesia	Heavy Metal Content and Risk Assessment in Breast Milk and Complementary Food from Tangerang, Banten
11:55-12:10	132	W Rumbeiha M. Clapham F. Lima B. McNabb S. Radke D. Schrunk L. Tell K. Watson	USA	Tissue Bromide Residues in Cows and Calves Experimentally Exposed to Inorganic Bromide in Feed
12:10–12:25	233	W. Awad D. Ruhnau	Austria	Co-exposure to Deoxynivalenol (DON) and *Campylobacter jejuni* Increases Intestinal Permeability and Bacterial Translocation in Broiler Chickens
12:25–13:25		*Lunch*		
13:25–13:40	257	W. Al-Baker N. A. Alabdulmalik	Qatar	The Impact of International Standards on Food Safety Emergencies–Qatar Experience for FIFA 2022
13:40–13:50		*Discussion*		
13:50–14:00		C. Vlachou C. Blackburn	Scientific Secretaries Joint FAO/IAEA Centre	Award of Poster Presenter winners
14:00–14:10		J. Sasanya	Symposium Chairperson, Joint FAO/IAEA Centre	Conclusion and suggestions

14:10–14:50 CLOSING SESSION

Moderator: D. Feng, A/Director (then) Joint FAO/IAEA Centre

Time	Name	Designating Member State/Organization	Title of paper/presentation
14:20–14:35	H. Liu	DDG and Head of the Department of Technical Cooperation, IAEA	Final remarks and official closure of the symposium
14:35–14:50	N. Mohktar	DDG and Head of the Department of Nuclear Sciences and Applications, IAEA	Final remarks and official closure of the symposium

POSTER SESSIONS

MONDAY, 27 MAY 2024

14:15–14:55
17:05–17:45

POSTER SESSION 1A: CHEMICAL RESIDUES & CONTAMINANTS IN FOOD & FEED

M-Building, M01

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
7	I. M. Fernández Gómez M. Valdes Ramos	Cuba	CONRADALIM: Cuban Database of Radioactive Contaminants in Food
31	M. Oueslati F. Gharbi	Tunisia	Measurement of Radioactivity in Tunisian Drinking Waters
32	A. S. Traore A. Traore	Mali	Assess the Contamination of Milk Imported into Mali by Radionuclides, Aflatoxin M1 and Melamine
34	J. Kateregga P. Waiswa E. Wampande	Uganda	Abamectin and Dichlorvos Residues in Meat, Tissues and Milk of Cattle from Selected areas of Gomba District, Uganda
40	P.A. Gatti O. Acosta F. Raco S. Rillo M. Simon	Argentina	Development of Certified Reference Materials (CRMs) in Food Matrices using Nuclear Techniques
60	M. Zarkawi	Syrian Arab Republic	Detection of Tetracycline, Chlortetracycline, Doxycycline and Oxytetracycline Veterinary Residue Levels in Syrian Chicken Meat, Skin and Liver Using HPLC
72	F. Nikiema	Burkina Faso	Incidence of mycotoxins in maize sold in Ouagadougou
74	T. Randriamparany N. De Borgia Randriamora	Madagascar	Evaluation of Veterinary Drug Residues in Milk in Madagascar
81	B.K. Kassaye	Ethiopia	Prevalence and Concentration Level of Aflatoxin B1 and Total Aflatoxin in Animal Feed in and around Shegar City, Oromia, Ethiopia
84	L. Miheso V. Gikera	Kenya	Detection Techniques for Microplastics and Nanoplastics in Food and Feed.
94	M. Ahmadi M. Abdollahi M. Mahrougi A. Naserian	Iran Republic of	Reduction of Antibiotic Residue in Waste Milk by Cold Plasma Method
98	A. Husen	Ethiopia	Aflatoxin in Ethiopia and Current Global Trends of Combating Aflatoxin Contamination Level in Foods with the help of Gamma Irradiation and Other Physical Methods
108	S. Thantar M. Naing	Myanmar	Determination of Elements in Milk and Dairy Products by ICP-OES Method for Food Safety
110	A. Hassan	Sudan	The Role of the IAEA in Establishing National Network for Food Safety; Lesson Learnt and Success Story

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
111	J. V. F. Nsoga J. C.M. Koule M. Ndomou	Cameroon	Human Health Risk Assessment of Polycyclic Aromatic Hydrocarbons in Selected Fish Smoked Using Various Kilns in Douala, Cameroon
113	N. Randriamora T. Randriamparany	Madagascar	Detection of Veterinary Drug Residues in Foods of Animal Origin and Animal Feed in Antananarivo, Madagascar
115	D. Javangwe	Zimbabwe	Determination of Lead (Pb) Concentrations in Drinking Water at Child Day Care Centres in and Around Harare, Zimbabwe
116	L. Mukani	Zimbabwe	Qualitative Analysis on Antimicrobials in Milk Collected from Harare
125	E. Matema	Zimbabwe	Determination of Heavy Metals in Fish from Harare Peri-urban Dams using Atomic Absorption Spectrometry
127	N.N. Andriamahenina N. N. F. Andrianirinamanantsoa M. Harinoely N. Rabesiranana L.V. Rakotozafy E.O. Rasoazanany H.N. Ravoson	Madagascar	Assessment of Heavy Metal Levels in Beef Meat from Antananarivo City Markets, Madagascar.
131	E. Salazar	Costa Rica	Development of a Novel Methodology for Detecting Dithiocarbamates in Vegetables Intended for National Consumption in Costa Rica utilizing GC/MS-MS
134	A. Coulibaly A.A. Dicko O. Camara	Mali	Radioactivity Measurement in Various Types of Rice Cultivated in Three Localities in Mali
136	W. Barrantes	Costa Rica	Six Years of Progress in Antimicrobial Residue Monitoring in Animal-Derived Foods in Costa Rica.
139	Md. S. Rahman	Bangladesh	Characterization, Source Identification and Hazard Level Assessment of Ingested Microplastics in one of the Most Important Fish Species in South Asia: Oreochromis Niloticus
149	F. Nurhaini D. D. Lestiani M. Santoso S. Kurniawati D. P. D. Atmodjo W. Y.N. Syahfitri E. Damastuti I. Kusmartini	Indonesia	Application of Nuclear Analytical Techniques for Nutrient and Heavy Metal Characterization in Local Food Commodities: A Strategy for Stunting Prevention
183	Z. Azzouz Berriche K. N. Ep Kachroudi M. Samaali	Tunisia	Validation of Charm II test as Radioimmunoassay for Screening of Sulfonamide Residues in Meat
205	X. Xu L. Huang M. Huo Y. Ou L. Sun	China	The Residue Elimination of Diclazuril in Eggs
209	M. Mumtaz I. Chughtai	Pakistan	Prevalence of Chemical Contaminants in Feed and Food

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
	U. Maqbool J. Sasanya M. Yasin		Samples, collected from Different Regions of Pakistan
245	A. Kileo	United Republic of Tanzania	The Role of Digitalization in Regulatory Control of Radioactivity Level in Food Stuffs in United Republic of Tanzania.
248	A. Babiker	Sudan	Screening of Oxytetracycline Residues in Animal Products Collected from Some States in the Sudan
250	H. I. Osman	Sudan	Monitoring of Heavy metals Residues in Milk Khartoum State Sudan
253	P. Nimbona	Burundi	State of Aflatoxin Contamination in Burundi
254	W. Attyaallah Amna Babiker	Sudan	Animal Production Sector Practices in Sudan in Relation to Drug Usage: Overview
255	G. Ramadan N. Al-Abdulmailik A. S. Mohammed	Qatar	Monitoring of Pesticide Residues in Common Cultivated Vegetables in State of Qatar and Assessment of their Associated Health risks
263	C. Kusena	Zimbabwe	Qualitative Analysis of Antimicrobials in Bovine Products Collected from Masvingo and Manicaland, Zimbabwe
266	A. Mohamed H. Abdulredha M. Alghawas E. Jassim A. M. M. Mohamed F. S. Hashem	Kingdom of Bahrain	Evaluation of Critical Pesticides Residues in Local and Imported Food Samples in Bahrain
278	J. Naouli H. Ait Bouh A. Benkdad A. Laissaoui R. Saadi	Morocco	Biomonitoring of Molluscs along the Northwest Moroccan Coast–Radiological Risk Assessment and Human Health Risk Analysis
280	G. Medoua Nama G. Kansci F. D. Paul Tatfo Keutchatang	Cameroon	Occurrence of Mycotoxins in Chicken and Eggs in Some Urban Areas of Cameroon and Population Dietary Exposure
285	M. L. Montiel Leguizamon B. Michajluk L. B. Chaparro	Paraguay	Determination of Cesium-137 as a Radioactive Contaminant in Yerba Mate (Ilex paraguariensis) Cooked Mate and Coffee in Asunción-Paraguay
292	W. Ali K. Omar S. Suleiman	United Republic of Tanzania	Safety Analysis of Animal Food Products in Zanzibar
296	Y. Salazar Chacon	Costa Rica	Beginning of the Implementation of the Database of the Data Sharing Committee of the Latin American and Caribbean Analytical Network (RALACA)
297	D. Kerekes Y. S. Chacón	Uruguay	The Future of Food Safety Data through Latin American and Caribbean Cooperation - Initiative to Establish a Regional

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
			Food Safety Data Exchange Network
304	A. Osman N. Ashkanani M. Hasan	Qatar	First Radionuclides Data and Associated Health Risks in Local Qatari Vegetables
313	M. Gonzalez Zeledón	Costa Rica	Multiclass and Multi-Matrix Method for the Analysis of Residues of Antibiotics in Foods of Animal Origin
314	J.C. España Amórtegui J. A. Guerrero	Colombia	Tiered Development of a PAH Analysis for Roasted Coffee: A Mini-Method Approach
317	D. Shao R. J. Mwamahonje W. D. Ruseruka P. W. Shirima	United Republic of Tanzania	Food Control Systems in the United Republic of Tanzania: The Regulatory Framework and the Role of the National Nuclear Regulator for the Detection and Control of Radionuclide Contaminants in the Food Chain During Importation and Exportation in the United Republic of Tanzania.
326	M. Moe Thein W. War Oo N. P. Thwin P. P. San	Myanmar	Screening of Selected Drug Residues and Mycotoxin in Milk and Feed in Myanmar
330	E. Vieira R. C. Batista Ferreira C. M. Barbosa	Brazil	Fipronil Risks to Bees

TUESDAY, 28 MAY 2024

11:00–11:20 POSTER SESSION 1B: M01

CHEMICAL RESIDUES & CONTAMINANTS IN FOOD & FEED (CONTINUED)

Paper No.	Author(s)	Designating Member State/Organization	Title of Paper
331	C. Abdelkader M. Moutaly A. Beyit	Mauritania	Study of the Prevalence of Antibiotic Residues in Red Meat Produced and Marketed in Mauritania
334	A. Reyes Hernández B.C. Orrego H. F. Ávila Z. Ng Chinkee	Panama	QuEChERS Method Optimization for Mycotoxins extraction Aflatoxins and Ochratoxin A, in Green Coffee Beans
337	F. Baroudi	Algeria	Determination of Lead and Cadmium in Food Supplements Commercialized in Algeria by Graphic Furnace Absorption Spectrometry
339	M. Azouz	Algeria	Pesticide Residues Analysis in Fresh Fruit Case of Strawberry in the Central and Eastern Region of Algeria
340	M. Mokhtari	Algeria	Pesticide Residues in Tomato and Pepper, and their Risk Assessment on Algerian Consumer

343	H. Hussan	Sudan	Simultaneous Determination of Fluopyram, Tebuconazole, Trifloxystrobin and their Metabolite Residue in Cucumber (Cucumis sativus) and Soil by a Modified QuEChERS with LC-MS/MS
349	O.A. Lucas Aguirre	Peru	Development, Validation and Application of Multiclass Isotope-Based Methods for Simultaneous Analysis of Mycotoxins, Pesticide Residues and Related Contaminants in Quinoa, Grapes and Other Selected Matrices
351	D. Mwalwayo S. Kuyeli	Malawi	Aflatoxin Contamination of Selected Mostly Consumed Commercial Products in Malawi
354	R. El Moustaid Alaoui	Morocco	The ONSSA Laboratory Network: Unyielding Guardian of Food Safety in Morocco

11:00–11:20 POSTER SESSION 2: PREPARING FOR AND RESPONDING TO EMERGENCIES AND INCIDENTS AFFECTING THE FOOD SUPPLY; MEASUREMENT OF RADIONUCLIDES M01

Screen No.	Paper No.	Author(s)	Designating Member State/Organization	Title of Paper	Link
	35	P. Mafirakureva J. Mbanga B. Saidi	Zimbabwe	Incidence and molecular characterization of lumpy skin disease virus in Zimbabwe using the P32 gene	
	357	A. Petchkongkaew	Thailand	IJC-FOODSEC: The New International Joint Research Center on Food Security for ASEAN region	

WEDNESDAY, 29 MAY 2024

M01

10:15–10:55
15:00–15:40 POSTER SESSION 3: FOOD AND PHYTOSANITARY IRRADIATION

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
28	M.A. Etoom	Jordon	Food Irradiation in Jordan
45	A. F. Abdull Razis	Malaysia	Gamma Irradiation Affects Tropomyosin Allergen, Nutritional Value, and Mineral Elements of Giant Tiger Prawn (Penaeus Monodon).
46	S. P. Shirmardi S. Y. F. Hosseininezhad M. Yeganeh	Iran Republic of	Disinfestation of Beans and Cereals Utilizing Novel Gamma Irradiators in Iran Republic of
53	V. Tenishev	Russian Federation	High Quality Radiochromic Film Dosimeters Development
56	E. Güllüoğlu	Türkiye	Food Irradiation Technology and Application in Türkiye
61	Md. M. Hasan Md. A. Hossain	Bangladesh	Comet assay: A Novel Technique for Determining the Radiation Induced DNA Damage in Insect Pests Infesting Food Grains
75	M. Al- Bachir	Syrian Arab Republic	Microbiological Safety of Irradiated Food in Syria
76	O. Turan	Türkiye	Conceptual Evaluations for Low-Energy Electron Beam (LEEB) and Comparison with Gamma Irradiation of Citrus Fruits

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
83	F. Hmaied W. Zernadji S. Jebri F. Rahmani I. Amri D. Aissaoui M. H. Trabelsi M. Yahya I. Amri	Tunisia	Effect of Gamma Irradiation and Bio preservation to Minimize Pathogens in Packaged Ready-to-Eat Salads
85	L. Villanueva Jiménez	Peru	Banana Improvement by Mutations
91	M. Y. Mansour	Syrian Arab Republic	Low Energy X Rays as a Possible Alternative to Gamma Radiation in Phytosanitary Treatments of Agricultural Products
114	U. Gryczka A. Nasreddine F. Kuntz S. Bułka	Poland	Development of Control Requirements for the Process of Low Energy Electron Beam Food Irradiation
122	A. Raad M. Constanza Cova M. S. Guerrero Martínez S. R. Vaudagna M. V. Vogt	Argentina	Gamma Radiation Effects on the Physicochemical Characteristics of Fine cut Salamín (Argentinian Dry Fermented Sausage)
124	I. W. Setya Andani A.R. Kumaraningrum I. D. Junianto	Indonesia	The Development of Irradiated Food in Indonesia: Opportunities for Using Irradiated Traditional Ready-to-Eat Food as Emergency Food and Food for the Hajj
137	E. Mejías T. Garrido C. Gomez	Chile	Effect on the Antioxidant Properties of Native Chilean Endemic Honeys Treated with Ionizing Radiation to Remove American Foulbrood Spores
152	R. J. Mwamahonje R. Suleiman	United Republic of Tanzania	Physico-chemical Properties of Fresh Irradiated Tomatoes During Storage
164	Z. Khalilov E. Muradov	Azerbaijan	Enhancing Pet Food Safety and Shelf Life
165	D. Ariyanti E. Fakhrurozi A. I. Christian	Indonesia	Characterization of Activated Chitosan Edible Coating Film Using Cobalt Gamma Irradiation Exposure and Its Effect on Pempek (Traditional Sumatran Food) Preservation
169	A. I. Christian D. Ariyanti A. I. Christian E. Fakhrurozi	Indonesia	Effect of Cobalt Gamma Irradiation on Shelf-Life Extension of Pempek Preservation (Qualitative Physical Appearance, Refer to Time needed of Expedition Intercity and/or Interisland) in Indonesia
170	R. Vazirov S. Sokovnin A. Krivonogova	Russian Federation	Surface Irradiation of Food and Agricultural Products by Low-Energy Electron Beam at the Urt-1 and Urt-0.5 Accelerators
178	D. T. Vu	Viet Nam	Advancing Food Safety and Control: The Landscape of Food Irradiation in Viet Nam
182	Y. Dalto C. Barrenechea V. Vogt	Argentina	Analysis of Radiotolerance in the Developmental Stage of Diatraea Saccharalis

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
184	M. Byambajav M. Gunaajav C. Radnaabazar N. Enkhgerel T. Amgalan J. Tserendorj L. Baasansuren	Mongolia	Shelf-life Extension of Potatoes by Low Energy X-rays
195	U.O. Madu O. Chukwu C. A. Okolo	Nigeria	Functional Properties and Proximate Composition of Vegetable Cowpea (Vigna sesquipedalis) Seeds after Irradiation
198	U. O. Madu O. Chukwu P. A. Idah K. U. Isah G. N. Nwachukwu	Nigeria	Predicting the Effect of Radiation Dose on Nutritional Composition of Nigeria Colocasia esculenta Using Response Surface Methodology
202	F. Boujelbane Ep Atta M. Samaali K.N Ep Kachroudi	Tunisia	Radioactivity Determination and Transfer Factor from Soil to Opuntia Ficus Indica in Selected Areas in Tunisia
211	Md. F. Mortuza Md. A. Hoque Md. K. Hossain Md. H. Kabir M. Khan A. Nahar Md. T. I. Samsad Md. T. I. Tanim	Bangladesh	Calibration of Radiation Dosimetry System and Optimization of Dose Delivery Process for Phytosanitary Irradiation and Food Preservation in Bangladesh
218	G. Guzik	Poland	European Intercomparison Studies as a Tool for Perfecting of Irradiated Food, White and Cane Sugar Detection Methods
225	J. Ellis O. Acuna J. Elster S. Pillai	USA	Sustaining Alternative Source Radiation Technology for Food Irradiation Services in Grid Challenged Environments
229	M. Sinche-Serra V. Ciobota L. Ramos-Guerrero E. Vera-Calle	Ecuador	Water-Soluble Keratin Hydrolysates Obtained from Chicken and Turkey Feathers Treated with Ionizing Radiations
238	A. W. Betga K. Cieśla G. Wfojciech	Poland	Biodegradable Packaging Materials with Antioxidant Activity Based on Polysaccharides –PVA System and Obtained with the Use of Radiation Methods
262	T. S. Lara-Ramos D. Mulyana S. Pillai	USA	An Attenuation Study for Optimizing Low Dose Delivery using 10-MeV Electron Beam for Food Processing Applications
268	K. Howie W. Yang R. Nayga	USA	Study Investigating the Dynamics of Consumer Response to Food Labelling
269	D. Mulyana S. Chirayath Aaron Tarone J. Welch P. Phillips J. Elster S. Pillai	USA	Engineering Pre-design Study of Low Energy Electron Beam Irradiation System for Sterile Insect Technology

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
270	A. Tomac M. P. Cenci M. C. Cova A. B. Garcia Loredo	Argentina	Valorization of Argentine Fish Resources Through Ionizing Radiation
271	A. Zaouak C. Belgacem S. Jebali	Tunisia	Assessing the Impact of E-Beam Irradiation on Diclofop Methyl Herbicide Residue in Wheat Samples and Aqueous Solutions
276	Y. Mabrouk I. Amri K. Khemissi H. Kouki	Tunisia	Combination of Gamma Radiation and Essential Oils from Two Eucalyptus Species in Managing Fungal Contamination of Stored Wheat Seeds
279	V. Ipatova U. Bliznyuk P. Borshchegovskaya T. Bolotnik A. Chernyaev E. Kozlova A. Oprunenko I. Rodin	Russian Federation	Volatile Organic Compounds as Indicators of Oxidative and Microbial-Enzymatic Processes in Irradiated Meat and Fish
283	A. Shik M. Beklemishev U. Bliznyuk I. Doroshenko T. Podrugina Y. Zubritskaya	Russian Federation	Kinetic-Based Method for the Estimation of Irradiation Doses Absorbed by Raw Chicken
294	A. L. Casañas Haasis Villavicencio L. Ronda-Flores B. Guimaraes-Negrao	Brazil	Application of Radiation Processing in Food for Food Safety Purposes: Maintaining Food Quality and Prolonging the Shelf Life of Products
300	R. B. Saragih	Indonesia	Food Irradiation in Indonesia: Integrated Approach to Ensure Security for Future Prospects in the Amid of Climate Change
305	E. Kozlova U. Bliznyuk A. Braun P. Borshchegovskaya A. Chernyaev V. Ipatova I. Mezhetova P. Ukhina I. Rodin	Russian Federation	Quantitative Assessment of Structural and Functional Changes of Proteins in Irradiated Biological Objects
306	I. Zubritckaia U. Bliznyuk P. Borschegovskaya A. Chernyaev N. Chulikova V. Ipatova A. Malyuga A. Nikitchenko I. Rodin D. Yurov S. Zolotov	Russia Federation	Inhibition of Phytopathogens and Crop Growth Stimulation Using Low Energy E-Beam and X ray Irradiation
307	S. Zolotov U. Bliznyuk P. Borschegovskaya A. Chernyaev V. Ipatova V. Khankin A. Nikitchenko I. Rodin	Russian Federation	Reconstruction of Electron Beam Energy Spectrum to Increase the Precision of Irradiation Dose Uniformity Estimate

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
	F. Studenikin		
11	L. Owaoade I. Sambo	Nigeria	Assessments of Some Common Food Products in Nigeria to Determine their Authenticity and Mitigate Food Fraud: A Basic Component of Food Control
68	R. Huque R. Frew K. McComb Y. Suzuki Mst. A. khatun	Bangladesh	Food Authentication of Milk and Rice using Isotopic and Elemental Composition
80	B. Deya	Kenya	Advancing Food Safety through Isotopic Analysis for Verification of Production and Geographical Origin
82	N. Haider	Syrian Arab Republic	Qualitative Detection of Adulteration of Some Plant-Based Spices with Materials of Plant Origin Based on DNA Barcoding
88	I. Migues Á. Otero G. Moyna F. Rivas H. Heinzen	Uruguay	Evolution of the Metabolomic Profile (1H NMR) of the Mandarin Ovary under Different Pollination Conditions
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146	C. Kukusamude S. Kongsri	Thailand	Nuclear Analytical Techniques for Verifying the Geographical Origin of Thai GI rice
168	L. Bontempo M. Paolini L. Cucinotta A. Roncone D. Sciarrone F. Camin S. Mose R. Larcher	Italy	Moscato Giallo: First Exploratory Aromatic Characterization by GC-MS/MS and Stable Isotopic Ratio Analysis of the Major Volatile Compounds
175	L. Bontempo	Italy	Promedlife: Novel Food Products for the Promotion of Mediterranean Lifestyle and Healthy Diet.
172	M. Perini S. Pianezze	Italy	Influence of the Equilibration Time and the Bath Temperature

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			on the Oxygen Isotopic Values of Different Matrices analysed through Continuous-Flow Isotope Ratio-Mass Spectrometry
188	K.M. Binduhewa C. K. Dissanayake B. A. T. Amalka S. M. N. K. Thilakarathne K. G. Nelum P. Piyasena M. A. B. Ranatunga E. N. U. Edirisinghe C. J. Ratiyala R. Chandrajith L. S. K. Hettiarachchi L. Suriyagoda	Sri Lanka	Stable Isotope and Multi-Element Profiling for Discriminating the Regional Tea in Sri Lanka
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231	E. Soares Lins R. B. Nazareno D. N. Sifuentes	Brazil	Challenges on Accommodating the Monitoring Testing Routine with Urgent, Unplanned Investigation Testing in an Official Laboratory
241	E. Mejías A. Abrahim A. Li S. Kelly	Chile	Tools for Determination of Authenticity in Chilean honey: Stable Isotopic Techniques and its Relationship with Botanical Origin and Bioactive Compounds
256	N. A. Abdulmalik G. R. Gouda A. A. Yossouff	Qatar	Modern Analytical Methods for the Detection of Food Fraud and Adulteration using Statistical Methods Over Isotope Ratios in Honey and Olive Oil Using IRMS Technique
298	L. Hoo Fung J. Antoine	Jamaica	Evaluation of Trace Elements in Turmeric and Turmeric Products on the Jamaican Market
299	A. Rivera Mondragón Y. Christopher A. De Lora H. H. F. Marín S. Hernández G. Navas	Panama	Detecting Adulteration of Commercial Honey Samples from Panama by using two HPLC methods
301	H. Widyastuti A. M. Benita D. Lasmawati T. R. Mulyaningsih I. M. Pratama B. Pratikno	Indonesia	Geographical Identification of Rice Cultivars in Different Regions in West Java using Stable Isotopes and Elements Analysis with Unsupervised Learning

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338	F. Baroudi	Algeria	Detection of Prohibited Substances Fraudulently Used in Several Food in Algeria
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355	A. Alnuaimi	UAE	Efforts of Sharjah City Municipality in Unveiling Fraud in Protein Content of Milk Powder Products
356	R. Hochegger	Austria	Metabarcoding for the Simultaneous Detection of Species in Foods
308	I. Viana Gomes Lima J. Andrade C. Caceres V. S. Dias S. W. Myers J. Sanchez A. Sobreira M. J. B. Vreysen	IAEA	Evaluation of *Drosophila suzukii* Female Fertility After Phytosanitary Irradiation Treatment Application
309	U. Bliznyuk F. Studenikin A. Chernyaev V. Ipatova A. Nikitchenko S. Zolotov	Russian Federation	E-beam Spectrum Modification Method for Improving Dose Uniformity in Irradiated Objects
319	H. Widyastuti S. A. Safitri R. Fitriana N. Lailyshofa M. A. E. Putri A. H. Saputro	Indonesia	Is Gamma Irradiation an Effective Method to Apply to Rice Grains? Study of Amylose and Anthocyanin Contents in Native Indonesian Rice
323	A. Nikalayenka E. Fedorenko L. Rozdyalouskaya	Belarus	Belorussian Experience in Food Irradiation
332	A. Raad M. Constanza Cova M. Cingolani M. Ciávaro M. Díaz J. I. Garrido M. G. Martínez C. Lires	Argentina	Coriander and Black Pepper Decontamination by Ionizing Radiation and its Impact on their Antioxidant Capacity and Aroma
350	S. Ayari	Tunisia	Evaluation of Electron Beam Treatments on Microbial and Chemical Properties of Sesame Seeds

THURSDAY, 30 MAY 2024

M01

10:15–10:55
15:05–15:45

POSTER SESSION 4:
FOOD AUNTHENTICITY AND FIGHTING FOOD FRAUD

FRIDAY, 31 MAY 2024

10:15–10:55 POSTER SESSION 5: M01

STANDARD SETTING, REGULATIONS, METROLOGY, RISK ASSESSMENT

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
55	B. Sharma Khanal	Nepal	Regulatory System on Food Safety and Quality Control in Nepal
181	M.O. Varrà G.T. Lanza L. Husáková J. Patočka M. Piroutková S. Ghidini E. Zanardi	Italy	Risk Characterization of Toxic Elements Through the Consumption of Botanical Preparations Across the European Population
167	J. Lubbe D. Prevoo-Franzsen L. Quinn M. Linsky M. Fernandes-Whaley	South Africa	The Importance of Proficiency Testing in Food Safety: An African perspective
215	J. Tinarwo B. Jackson S. Chimuti	Zimbabwe	Food Safety in Zimbabwe - An Overview
352	B. I. Checa Orrego L. Aparicio D. Gordón G. Hernández	Panama	Establishment of Risk-Based Surveillance Program of Pesticide Residue for Crop Production in Panama

10:15–10:55 POSTER SESSION 6: M01

DETECTION AND CHARACTERIZATION OF PATHOGENS IN FOOD (AMR)

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
48	A. Hammad A. Eid W. F. Khalil S. A. El-Nour	Egypt	Prevalence of Sulfonamides, β-lactam, Salmonella, Staphylococcus Aureus in Raw Milk: Irradiation Effect on Antibiotic-Resistant bacteria and degradation of antibiotic residues
51	A.F.D.S. Sumbana	Mozambique	The risk of introduction E. coli in Mozambique through the importation of meat sausages from South Africa
73	S. Chimuti, D. T. Mugadza P. Kamau, V. Ntuli	Zimbabwe	Contribution of Farm Level Hygiene Practices to Microbial Safety Profiles in the Informal Dairy Sector in Zimbabwe
65	B. Jackson R. S. Masunga	Zimbabwe	Determination of Pesticide Residues in Honey from Small Scale Producers in Harare Zimbabwe
93	H. Suseno	Indonesia	Analysis of Saxitoxin Content in Various Freshwater Biota Originating from JATILUHUR using 3H-RBA Methods

Paper No.	Author(s)	Designating Member State/Organization	Title of paper
96	M. Muslim	Indonesia	Determination of Saxitoxin Concentrations in Various Biota on Tegal Beach, Indonesia using Nuclear Applications Techniques (3H-RAB)
106	N. Ziyate Z. Soubai S. Darkaou I. lharch D. Abislim N. Aujjar	Morocco	Antimicrobial Susceptibility of Salmonella spp Isolated from Poultry Samples in Morocco
112	J. V. F. Nsoga J. C. Manz koule M. Ndomou	Cameroon	Isolation and Identification of Fungal Strains during Storage of Fish Smoked by Various Kilns in the City of Douala, Cameroon
159	G. Nijimbere F. Nitereka C. Uwizeyimana	Burundi	Street Food Sales and Food Safety in the Cities of Burundi
163	A. Mahin Md. K. Pramanik Md. A. K. Sarker Md. S. Hossain N. Nahar	Bangladesh	Simultaneous Detection of Salmonella spp., Escherichia Coli, and Listeria Monocytogenes by Multiplex PCR
166	S. Wongsrichai	Thailand	Antimicrobial Resistance Surveillance in Livestock Sector, Thailand
208	I. S. Beia	Romania	Enhancing Food Safety and Quality of Consumer Protection in Romania
234	W Awad D. Ruhnau	Austria	Contrary Effect of Deoxynivalenol (DON) and Deepoxy-Deoxynivalenol (DOM-1) on *Campylobacter jejuni* Resolved in Vitro
249	L. Alteio F. Spiege K. Kober-Rychli M. Wagner	Austria	FoodSafeR: Disentangling Factors Supporting Persistence of *Listeria monocytogenes* in Food Processing Environments
260	S. Pillai A. Anderson S. Dixon S. Ruggles K. Narayanan	USA	A Single Stage Pooling Scheme for Large-Scale Bacterial Pathogen Detection in Food Matrices
324	M. Garba Hima P. Sessou	Niger	Qualité Hygiénique Du lait Local Au Niger Et Facteurs de Risques
333	C. T. Dedah C. Abdelkader D. Diop A. B. El Mamy A. Beyit M. Sid'Ahmed	Mauritania	Evaluation of Antibiotic Resistance of Salmonella Strains Isolated from Red Meat in Mauritania
336	G. Omarch	United Republic of Tanzania	Insights of Antimicrobial Resistance and Antimicrobial Susceptibility Testing Performed at the Central Veterinary Laboratory, Dar es Salaam

10:15–10:55 POSTER SESSION 7:
ONE HEALTH

Paper No.	Author(s)	Designating Member State/Organization	Title of Paper
9	B. Atanasovska	North Macedonia	Exploring the Nexus between Climate, Microplastics, Radionuclides and Foodborne Illness in North Macedonia 'A Pathway to Ensuring Food Safety'
49	M. Gerić L. Bendix S. Božićević J. Bošnir S. Cvijetić L. Delić A. M. Domjan G. Gajski V. G. Vrhovac V. Gluščić G. Jurak J. Jurasović K. Matković T. Orct M. Peraica L. Nanić I. Rubelj D. Rašić M. V. Lovrenčić N. Š. Vidaček	Croatia	Is the Future of the Human Diet Green?–A Case of Vegetarian Diet in Croatia
54	R. Albakain S. A. Mualla	Jordan	Control of Chemical Contents of Jordanian Guava: Application on Different Geographical Origins of Jordanian Guava
58	B. K. Kassaye	Ethiopia	A systematic review and meta-analysis on aflatoxin M1 prevalence, concentration, and Analytical Techniques in Raw Cow Milk in Africa
86	C. Galdhar A. Kakad P. Alone J. Kawade P. Sapkal M. Shinde V. Mote R. Gaikwad S. Patankar V. Patrawale S. Basagoudanavar M. Hosamani A. Sanyal B.P. Sreenivasa	India	Studies on the Disposition of Technetium-99m Labelled Foot and Mouth Disease Vaccine in Guineapigs
120	G. Güngör	Türkiye	Production of New Boron Ester Compounds As Nutritional Supplementation Agents
230	E. Ghoniem	Egypt	Impact of Whey Protein a Protective Natural Antioxidants in Cisplatin Ototoxicity

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259	H. Pouraram A. Mohammadi	Iran Republic of	Comparative Analysis of the Water Footprint of Plant-Based and Common Iranian Diets: An Environmental Impact Perspective

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