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MINI REVIEW

Application of Spectroscopic Techniques in Heavy Metal Analysis for Meat Quality Assurance

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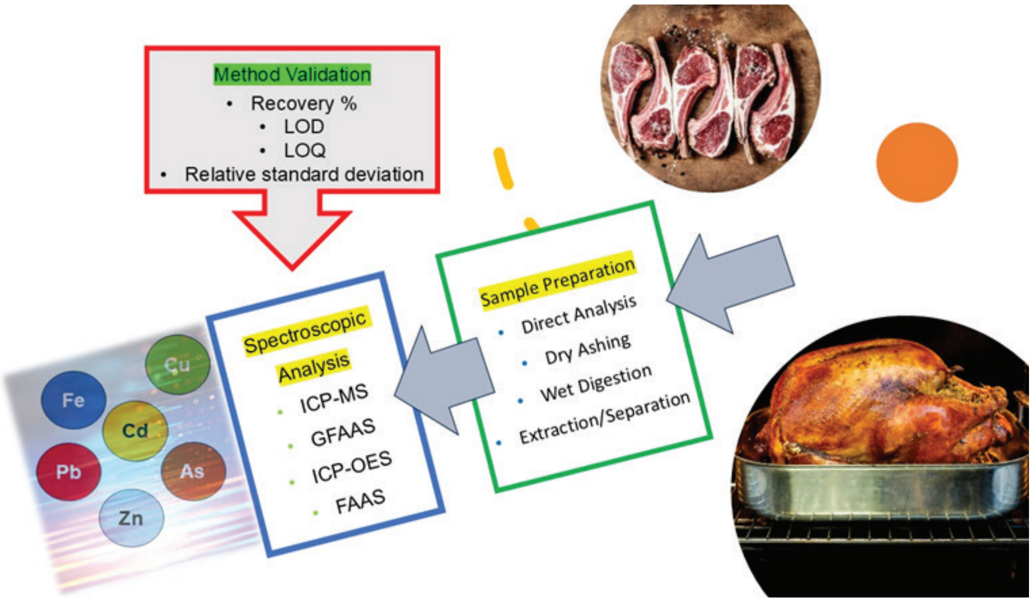

Highlights

- Spectroscopic techniques ensure accurate heavy metal determination in meat
- Sample preparation methods support the accuracy and precision of the analyses
- Spectroscopy analysis ensures meat quality by detecting both nutritional and toxic elements, aiding in both product safety and authentication
- Method validation ensures accuracy, precision, sensitivity and reliability
- Heavy metal monitoring is important for mitigating health risks in meat and meat products

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Application of Spectroscopic Techniques in Heavy Metal Analysis for Meat Quality Assurance

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Abstract: Quality assurance is a critical aspect of human food. Meat is one of the major high-quality protein suppliers to the human body and plays an essential role in our daily meals. With industrialization, heavy metals became major food contaminants leading to serious health risks. FAAS (Flame Atomic Absorption Spectrometry), GFAAS (Graphite Furnace Atomic Absorption Spectrometry), ICP-MS (Inductively Coupled Plasma Mass Spectrometry) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) are analytical methods that promise consumer safety by ensuring quality assurance of meat and meat products with their accurate and reliable analytical capacity. Their characteristics may vary with their theory of analysis and advancement of applied technology. Dry ashing, wet digestion, microwave-assisted digestion, and ultrasonic extraction like different sample preparation techniques or direct analysis after slurry preparation like simple sample preparation, are involved with spectroscopic analytical methods to prevent the sample matrix effect. These methods are validated based on parameters such as LOD (Limit of Detection), LOQ (Limit of Quantification), recovery %, relative standard deviation, and characteristic mass to ensure their reliability.

***Keywords*:** Trace element analysis; Meat quality assurance; Spectroscopic techniques; Heavy metal analysis; Food contaminants

INTRODUCTION

Meat is a crucial dietary component, rich in high-quality proteins, minerals, and vitamins. The growing awareness of the necessity of these nutrients has led to an increasing demand for meat and meat products (Maria, 2023). Consequently, maintaining the quality of meat and its derivatives becomes pivotal in ensuring consumer safety and satisfaction.

Heavy metals are defined as elements with a density greater than 5g/cm^3 (Jessica et al*.,* 2020). They are abundant everywhere, in living and non-living organisms, water, soil, air, and most ecological systems. In every biological system, they play an important role. In the human biological system, heavy metals have a diversified role in most of the functions such as biochemical reactions, immune functions, neurological functions and vice versa. There are two main classes of heavy metals, essential heavy metals such as Fe, Cu, Co, Cu, and Zn and non-essential heavy metals like As, Pb, Hg, and Cd. Although essential heavy metals are vital to human biological functions, they might be toxic if they enter the body in large quantities (Bharti & Sharma, 2022; Okewale & Grobler, 2023). Simultaneously, non-essential heavy metals are toxic to humans and other organisms even in smaller quantities if they exceed the maximum tolerable levels. Further, heavy metals accumulate in the body tissues and may cause chronic illnesses following several years (Jessica et al., 2020).

When examining heavy metals that aren't essential for biological functions, their impact becomes more critical. Pb is particularly notorious for its neurotoxic effects, specifically its damaging impact on the neurological system and cognitive development (Samuel et al., 2022). Hg may cause nervous system, enzyme, and immune disorders (Mahdi et al., 2021). Cd is a carcinogenic element to humans and also may cause kidney damage and Alzheimer's like neurodegenerative disorders (Mahdi et al., 2021). Long-term arsenic exposure may cause cardiovascular diseases, diabetes, and immune system dysfunction (El Youssfi et al., 2023).

Ingestion is generally considered the primary route of heavy metal exposure for the general population, particularly through food and water. However, inhalation, and dermal absorption can also be significant in certain conditions. Some metals such as As and Hg can be inhaled in vapour or particulate form, may absorb through the skin, especially in occupational settings (Al Osman et al., 2019; World Health Organization, 2024). With time, environmental pollution has increased and consequently, heavy metal transmission is augmented among natural systems as well as man-made food systems (Salim et al., 2023). Therefore, risks associated with heavy metals in food products, especially main food categories such as cereals, meat, milk, fruits and vegetables are severe concerns to food safety and threat to human health.

Heavy metals can enter the food supply chain through various natural sources such as soil, water, volcanic activity, etc. and anthropogenic sources such as mining industrial activities, pesticides, fertilizers, and packaging (Jessica et al., 2020; Okewale & Grobler, 2023). Awareness about heavy metal content in our foodstuff is vital for the well-being of humans. Spectroscopic methods play a crucial role in assessing heavy metals in food due to their sensitivity, accuracy, and efficiency. While existing literature extensively covers heavy metal concentrations, sources, and distributions in meat, there's a lack of focus

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on the analytical methods used for heavy metal analysis in meat. This review discusses the significance of common spectroscopic methods utilized in heavy metal analysis in meat, their sample preparation techniques, quality control and validation.

METHOD

This study was conducted to compile research articles detailing heavy metal analytical methods, focusing on their quality control and validation methods. A comprehensive literature search was conducted across databases such as Scopus, Web of Science, PubMed and Google Scholar using the following keywords:

- "heavy metals" AND "spectroscopic techniques" AND "meat quality analysis"
- "spectroscopy methods" AND "heavy metal analysis" AND "meat"
- "trace elements" AND "ICP-MS" AND "meat contamination"
- "heavy metal detection" AND "FAAS" AND "meat"
- "sample preparation" AND "spectroscopy" AND "meat"
- "graphite furnace atomic absorption" AND "meat"
- "spectroscopy methods" AND "method validation" AND "meat"
- "slurry sampling" AND "spectroscopy" AND "meat sample preparation"

Abstracts and titles which were included with keywords were investigated. When selecting articles journal, publication year, and citations were considered.

DISCUSSION

Spectroscopic methods

Spectroscopic analytical methods are pivotal in detecting and quantifying heavy metals in meat. Techniques like Flame Atomic Absorption Spectroscopy (FAAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) are the generally used spectroscopic methods. Figure 1 provides the main characteristics, advantages, limitations, and sample preparation techniques of mentioned spectroscopic methods.

In FAAS prepared samples are introduced to a flame where it goes atomization which breaks into atoms. Then atomized sample passes through a light path which enables atoms to absorb energy and become excited state. The light energy absorbed by the atoms is proportionate to the concentration of the specific element (Beaty & Kerber, 1993). FAAS is less sensitive compared to other spectroscopic methods due to the inefficiency of the burner-nebulizer system and the complex matrix of meat might affect analysis by interfering with heavy metals (Perkin Elmer, 2013; Alturiqi & Albedair, 2012). Improved sampling devices will lead to an improvement in the sensitivity of the method.

During ICP-MS analysis, Argon ICP produces singly charged ions from the elemental species present in the meat sample and then they are directed into the mass spectrometer and separated according to their mass: charge ratio (Scott & Matthew, 2019). The detector determines these separated ions. ICP-MS has exceptional detection limits with the ability to measure isotopic concentrations of elements, and multiple elements at a time (Nageswaran

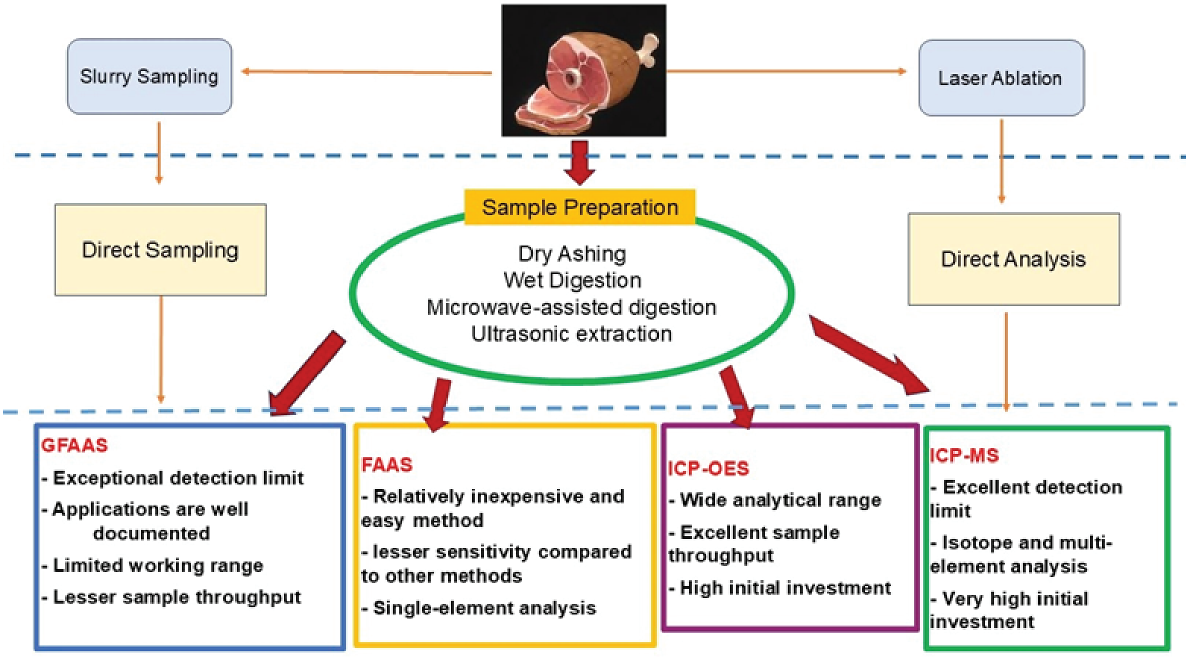

Figure 1: Spectroscopic approaches for heavy metal assessment in meat

et al., 2017). There are difficulties with determining the amount of matrix to be added and high demands in the maintenance of the instrument. Current developments assist in analyzing meat-like solids precisely by improving the long-term stability of high-matrix solutions (D'Ilio et al., 2011; Sun & Xing, 2016).

Miedico et al. (2017), analyzed trace elements in equine meat using ICP-MS. The highest concentrations were Zn, Fe, and Ca among essential elements and Al, Ni, and Sr among non-essential elements (Table 1). In another study, 43 trace elements were assessed in mechanically separated meat (MSM) using ICP-MS, revealing that MSM contained higher levels of Ca, and Fe compared to non-mechanically separated meat, whereas Mg levels were lower. The elevated levels of Ca in MSM are attributed to the presence of fragments of bone and periosteum (bone skin), as MSM is produced by mechanically removing meat from flesh-bearing bones. However, some non-mechanically separated meat products also may contain higher Ca levels due to the addition of permitted food additives like calcium ascorbate (E302) or calcium citrate (E333). The increase in Fe content in MSM is likely due to the release of haemoglobin from bone marrow during processing. Additionally, an inverse correlation between Ca and Mg was observed, with fresh meat containing higher Mg levels than MSM (Miedico et al., 2022).

Raeeszadeh et al. (2022), assessed heavy metal levels (Se, Pb, Cd, As, Co, Zn, Ni, Cu, and Cr) in sheep, beef, turkey, and ostrich meats using ICP-MS. Dried and crushed samples were subjected to acid digestion before the element analysis. Pb, Cd, As, Zn, Cu, Cr, and Ni levels were significantly higher than maximum permissible levels. Furthermore, they found a negative correlation between As and Se levels.

Bandoniene et al. (2020), conducted a study to authenticate meat products using rare earth labelling. Detection was performed using ICP-MS following acid digestion. Alternatively, laser ablation ICP-MS (LA-ICP-MS) was used, allowing for direct analysis of bone samples, as well as analysis of meat after dry ashing and pressing them into pellets (Bandoniene et al., 2020). Similarly, another study was conducted in Romania to assess the geographical influence on the isotopic and elemental composition of pork using ICP-MS and Isotope ratio mass spectrometry (IRMS) (Dehelean et al., 2023). A risk assessment based on the heavy metal concentrations revealed that none of the detected heavy metals pose a carcinogenic risk.

In a study As, Cd, Pb, Cr, Cu, Fe, Mn, Zn, and Co elements in camel meat were assessed using ICP-MS (Asli et al., 2020). Meat samples were subjected to acid digestion before the analysis. They found that Co and Zn were influenced by gender, with male camels showing higher levels than females. Additionally, younger animals (under 6 months) showed higher concentrations of Co and Zn. Except for Cd, all other elements exceeded the concentration limits set by EU standards.

ICP-OES measure the light emitted by the element introduced into the ICP source (Perkin Elmer, 2013). The emission intensity is compared to the intensities of standards of known concentrations to determine the elemental concentration of the unknown sample (Herman, 1996). A major limitation of ICP-OES is the higher initial cost of purchasing (Sudhakar et al., 2016).

Microwave-induced plasma optical emission spectroscopy (MIP-OES) is a relatively modern atomic emission spectroscopy technique used for elemental analysis. In terms of low cost, it is considered an attractive alternative for ICP-OES and ICP-MS. Further, it has high selectivity and is fast and easy to handle. However, MIP-OES faces challenges in detecting low concentration and is more susceptible to matrix interferences (De Sá et al., 2020). In a study De Sá et al. (2020), As, Cd, Cr, Cu, Hg, Pb, and Zn assessed bovine and ovine meat samples using MIP-OES. The analytes were separated and preconcentrated using solid-phase extraction (SPE), which facilitates rapid phase separation, low solvent consumption and allows for easy automation. The limit of detection (LOD) and limit of quantification (LOQ) values were lesser than ICP-MS but not low enough to meet stringent regulatory standards. The authors suggested combining preconcentration with hydride generation for arsenic and cold vapour generation for mercury to enhance the detection sensitivity.

Uluozlu et al. (2009), conducted a study to assess the trace element content of chicken products produced in Turkey. They used FAAS equipped with hydride generation (HGA) for Zn, Mn, Fe, and Cu, elements that are commonly found in higher concentrations, while using GFAAS for detecting trace levels of toxic and less abundant elements, Cu, Cd, Pb, Se, As, Cr, Ni, Co, and Al analyses. The highest concentration was Fe, followed by Cu, Zn, and Mn. The highest Cu concentration was in the skin of the chicken. Heart samples had the highest Cd concentrations while kidney samples showed minimum Cd levels. Further gizzard samples contained the highest Pb levels. Pb levels in meat, skin, and sausages were higher than the recommended levels. Liver and kidney samples had the highest concentrations of Se. Sausage products had the highest Mn levels while kidney samples held minimum levels. A study using ICP-MS to assess the concentrations of 28 trace elements in the muscular tissues of broiler chicken fed a diet containing arsenic trioxide administered. This is particularly relevant because arsenic-based drugs are commonly used in the poultry industry, making the results of this study essential for understanding how such treatments affect trace element accumulation in edible tissues (Li et al., 2017).

With GFAAS, the sample is introduced to the graphite tube directly or after the digestion process then, which is subjected to a series of temperature programs that remove solvent and major matrix components and atomize the remaining sample (Beaty & Kerber, 1993). Element concentration is determined by the light path which passes through the sample and the absorbance of excited atoms is determined by the detector. GFAAS has exceptional detection limits and can measure even very small samples (Butcher, 2021). Modern GFAAS instruments provide more robust direct methods to determine heavy metals in solid samples (Zmozinski et al., 2015).

Rudy (2015), conducted a study to measure heavy metal (Pb, Cd, Hg, and As) bioaccumulation in muscle and liver tissues of wild boar. As was measured using hydride generation atomic absorption spectrometry (HGAAS), Cd and Pb were determined using GFAAS, and Hg was measured using cold vapour atomic absorption spectrometry. All the element concentrations were below the maximum permissible levels, except Cd concentration in 2 liver samples.

Sample Preparation

Effective sample preparation is vital to precise spectroscopic heavy metal analysis in meat (El Hosry et al., 2023; Uddin et al., 2016). This process guarantees reliable outcomes by minimizing interferences, enhancing solubility, and strengthening sensitivity (Andrade et al., 2008). This section describes some common sample preparation techniques for spectroscopic analysis of heavy metals in meat.

Dry ashing is a technique that involves controlled combustion of organic matter in a sample to leave behind inorganic residues for subsequent analysis. Usually, in this method sample is exposed to a thermal decomposition with an ashing aid such as magnesium oxide or magnesium carbonate to remove organic matter. The resulting ash residues are dissolved in a suitable acid (Harris & Maurice, 2017). This method is simple and enables concentration in the solution. However, ashing aid would be problematic for trace element determination and may cause contamination (Andrade et al., 2008).

The wet digestion technique involves decomposing samples using acid or a mixture of acids. This process occurs in open vessels, tubes, on a hot plate, or within closed vessels under high pressure (digestion bombs), employing thermal or microwave heating (Andrade et al., 2008). Traditional sample preparations were time-consuming and less efficient due to spending large volumes of reagents (López-Lorente et al., 2022). Subsequently, the advent of microwave-assisted acid digestion, ultrasound-assisted extraction, slurry preparation, and direct sampling analysis provided respite, revolutionizing spectroscopic analysis with their efficiency and reduced time requirements (Éder et al., 2000)

Table 1: Trace element analysis in various meat samples using spectroscopy instruments and different sample preparation methods

Type of Meat Sample	Analytical Instrument	Elements Assessed	Sample Preparation Method	Reference
Equine	ICP-MS	Mo, V, Co, Se Mn, Cu, Zn, Fe, Ca, Pb, Cd, Hg, As, U, Sr, Sn, Tl, Sb, Cr, Ni, Be, Al	Microwave-assisted digestion of homogenized sample with HNO_3 and H_2O_2	(Miedico et al., 2017)
Bovine, ovine and fish	MIP-OES	As, Cd, Cr, Cu, Hg, Pb, Zn	Solid-phase extraction and microwave-assisted digestion with HNO_3 and H_2O_2	(De Sá et al., 2020)
Mechanically separated meat	ICP-MS	^{107}Ag, ^{27}Al, ^{75}As, ^{138}Ba, ^{9}Be, ^{44}Ca, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{56}Fe, ^{202}Hg, ^{7}Li, ^{24}Mg, ^{55}Mn, ^{98}Mo, ^{23}Na, ^{60}Ni, ^{120}Sb, ^{82}Se, ^{118}Sn, ^{88}Sr, ^{205}Tl, ^{238}U, ^{51}V and ^{66}Zn	Microwave-assisted digestion of homogenized sample with HNO_3 and H_2O_2	(Miedico et al., 2022)
Sheep, beef, turkey, ostrich	ICP-MS	Se, Pb, Cd, As, Co, Zn, Ni, Cu, Cr	Acid digestion of dried and crushed samples with HNO_3 and H_2O_2	(Raeeszadeh et al., 2022)
Camel	ICP-OES	As, Cd, Pb, Cr, Cu, Fe, Mn, Zn, Co	Microwave-assisted digestion of homogenized sample with HNO_3 and H_2O_2	(Asli et al., 2020)
Bovine	ICP-OES	Pb, Cd, Hg, Cu, Zn	Wet digestion with nitric-perchloric acid under laminar flow hood conditions	(Hashemi, 2018)
Chicken	FAAS with HGA	Zn, Mn, Fe, Cu	Microwave-assisted wet digestion with HNO_3 and H_2O_2	(Uluozlu et al., 2009)
	GFAAS	Cu, Cd, Pb, Se, As, Cr, Ni, Co, Al		
Chicken, veal, sheep, camel, fish	GFAAS (for Hg, with hydride unit)	Mg, Cu, Fe, Zn	Wet digestion with HNO_3 and $HCLO_4$	(Alturiqi & Albedair, 2012)

For meat samples, a combination of nitric and sulphuric or perchloric acid is typically used to ensure complete digestion of proteins and fats (Twyman, 2005; Sun & Xing, 2016). However, the high viscosity of sulphuric acid can pose challenges with FAAS, ICP-OES, or ICP-MS, affecting sample introduction and analysis procedures (Andrade et al., 2008).

Microwave-assisted digestion with nitric acid, nitric and hydrochloric acids without or with the addition of hydrogen peroxide is a commonly used method for the digestion of meat samples (Andrade et al., 2008; Uluozlu et al., 2009). This method saves time as well as minimizes the required strength of reagents. However, hydrochloric acid is not recommended for GFAAS analysis due to potential volatile formation (Andrade et al., 2008)

Ultrasonic extraction can serve as a valuable sample preparation technique for spectroscopic analysis, aiding in the extraction and dissolution of target compounds from meat samples (Ashley et al., 2001). Ultrasound accelerates sample preparation by reducing the concentration gradient between the solid and liquid interface, leading to the release of unstable species into the solvent. Additionally, it can augment sample surface area by eroding the solid, further aiding in the extraction process (Andrade et al., 2008).

The slurry sampling method involves preparing a homogeneous mixture or suspension of a finely ground or dissolved sample in a suitable solvent for spectroscopic analysis (Nilgun, 2007). In the context of meat analysis, slurry sampling can be utilized for direct analysis of the sample in spectroscopy (Andrade et al., 2008). A direct analysis study was conducted to assess Cd and Pb in seafood samples, by combining slurry sampling after cryogenic grinding, using GFAAS equipped with a transversely heated graphite tube atomizer with an integrated platform (Santos et al., 2002). Cryogenic grinding is used for samples with high fat content, such as animal products, or high fiber content, like vegetables. In cryogenic grinding, the sample is subjected to grinding under liquid nitrogen, ensuring proper homogenization, which is particularly vital for GFAAS that utilize tiny sample volumes (Santos et al., 2002). After cryogenic grinding, slurries were prepared directly in the autosampler cup by mixing the sample with HNO_3 acid and Triton X-100, followed by sonication. Then further mixed with tungsten carbide-rhodium modifier and $NH_4H_2PO_4$ chemical modifiers to increase pyrolysis temperature of Cd. The results showed no statistically significant difference compared to the digestion-based method.

GFAAS is the most performing method for direct analysis among others due to the absence of a nebulizer (Resano et al., 2006). It is a fast and accurate method with minimum errors due to reduced contamination and corrosion of materials. However, its sensitivity might vary in different scenarios (Andrade et al., 2008). Direct analysis of meat using GFAAS studies is scarce. However, Zmozinski et al. (2015), measured As levels in fish samples through direct analysis using GFAAS, with results showing no statistically significant difference compared to the standard reference material. Pd, Mg, and Triton-x100 matrix modifiers were added to minimize matrix effects and stabilize volatile elements (Zmozinski et al., 2015), thereby improving the accuracy and reliability of the analysis (Ajtony et al., 2008).

In the study done by Rudy (2015), lipolyzed and homogenized samples were subjected to acid digestion using nitric and hydrogen peroxide mixture (6:1, v/v), with heat applied through a pressure-microwave system. Pre-drying the sample and extracting the fat before digestion significantly reduced the amount of acid needed (Bohrer et al., 2007). In Rudy's study $NH_4H_2PO_4$ and $Mg(NO_3)_2$ were used as matrix modifiers for the determination of Cd and Pb.

Quality control and validation

Quality control and validation are vital for securing the accuracy, precision, and sensitivity of spectroscopic methods used for heavy metal analysis in meat. The accuracy of the testing was determined by comparing the results with standard reference materials (SRMs), which contain known concentrations of analytes, and by calculating the recovery percentage (Santos et al., 2002; Bazié et al., 2021). Further, the results of direct sample analysis compare with the results of the digestion-based method, or when difficult to find an appropriate standard reference material (Zmozinski et al., 2015).

Precision is assessed through replicate analysis and calculation of the relative standard deviation (Santos et al., 2002). Sensitivity is assessed using LOD and LOQ values, which establish the lowest reliable concentrations detectable by the method (Uluozlu et al., 2009). LOD and LOQ values are calculated as 3 and 10 times of standard deviation of blank samples respectively (Raeeszadeh et al., 2022). Further, characteristic mass is an important sensitivity parameter of the sensitivity, particularly in GFAAS (Santos et al., 2002). It describes the amount of element required to produce a specific absorbance signal (Beaty & Kerber, 1993).

These validation steps ensure the accuracy, reliability, and compliance of spectroscopic methods in the analysis of heavy metals in meat.

Future directions

Sample preparation is a critical step in the trace element analysis of meat due to the complex nature of the meat matrix. Advancements in this area, particularly in microwave-assisted digestion and ultrasound-assisted extraction would offer significant improvements in accuracy, speed and efficiency. Further slurry sampling and cryogenic grinding techniques represent promising ways for direct analysis without the need for complex sample preparation procedures. These techniques can simplify the analytical process by allowing samples to be in their semi-solid form, reducing both preparation time and potential contamination. The development of miniaturized and portable spectrometric devices would further revolutionize heavy metal analysis by enabling real-time monitoring and facilitating testing in remote or under-equipped laboratories. This would make heavy metal analysis more accessible and practical in various field applications. Furthermore, the combination of techniques such as laser ablation and microwave-induced plasma holds the potential to create instruments that are highly sensitive and cost-effective. These advancements would significantly enhance the quality control and safety management of food products, ensuring compliance with regulatory standards more efficiently and economically.

CONCLUSIONS

Spectroscopic analytical methods play a vital role in heavy metal analysis in the meat industry with promising sensitivity and reliability to ensure the well-being of consumers. Selection of the most suitable method depends on the sample complexity, LOD values, sample size, and number of samples to be analyzed within a specific timeframe. Advancing technologies address challenges in sample preparation, sample throughput, and background interferences ensuring safer and higher quality meat products for consumers.

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DECLARATION OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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AUTHOR CONTRIBUTION

The author confirms sole responsibility for all aspects of the study, including conceptualization, literature review, writing, and final approval of the manuscript.

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