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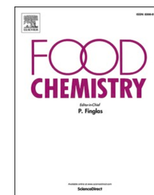

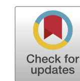

Methods for detection and quantification of gelatin from different sources

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ABSTRACT

Gelatin is a water-soluble protein obtained from the collagen of various animal origins (porcine, bovine, fish, donkey, horse, and deer hide) and has diverse applications in the food, pharmaceutical, and cosmetics industries. Porcine and bovine gelatins are extensively used in food and non-food products; however, their acceptance is limited due to religious prohibitions, whereas fish gelatin is accepted in all religions. In Southeast Asia, especially in China, gelatin obtained from donkey and deer skins is used in medicines. However, both sources suffer from adulteration (mixing different sources of gelatin) due to their limited availability and high cost. Unclear labeling and limited information about actual gelatin sources in gelatin-containing products cause serious concern among societies for halal and fraud authentication of gelatin sources. Therefore, authenticating gelatin sources in gelatin-based products is challenging due to close similarities between the composition differences and degradation of DNA and protein biomarkers in processed gelatin. Thus, different methods have been proposed to identify and quantify different gelatin sources in pharmaceutical and food products. To the best of our knowledge, this systematic and comprehensive review highlights different authentication techniques and their limitations in gelatin detection and quantification in various commercial products. This review also describes halal authentication and adulteration prevention strategies of various gelatin sources, mainly focussing on research gaps, challenges, and future directions in this research area.

1. Introduction

Gelatin is a denatured form of collagen produced from its partial hydrolysis. Collagen is found in animal skin, tendons, cartilaginous tissues, and mammalian bones (porcine, bovine, donkey, and horse), marine (jellyfish, sea cucumber, and tuna), and poultry (chicken, duck, wild, and turkey) (Rigueto et al., 2022). Gelatin has unique physiochemical properties, such as foaming, stabilizing, thickening, gelling, emulsifying, and binding; therefore, it is widely used in foods, cosmetics, and pharmaceutical products. For instance, gelatin is used in chocolates, candies, jelly, dairy products (quark cheese, whipped cream, and creamy yogurt), creams, capsule shells, lotions, and dietary supplements (Usman et al., 2023).

Commercially available gelatins are mainly produced from the mammalian species and rarely from marine species. Gelatin is mostly extracted from porcine and bovine skin (46 %, 29.4 %), bones (23.1 %), and other species' (donkey and horse hide, fish skin, etc.) raw materials (1.5 %), as represented in Fig. 1(A). Other gelatin sources include marine species (fish, jellyfish, sea cucumber, and marine snail) (AI-Temimi et al., 2021; Gaspar et al., 2019) and poultry feet and skin (Abedinia et al., 2020). Gelatin extracted from marine sources is an alternative to mammalian gelatin due to its higher availability and compliance with religious restrictions. The enzymatic hydrolysates of marine gelatin also exhibit antioxidant and antihypertensive properties. However, they have limited applications in the food industry due to their poor rheological and functional properties, darker color, and unpleasant smell. Further studies are required to improve the quality of gelatins extracted from marine species (Ranasinghe et al., 2022). Gelatin production in the global market is approximately 326,000 tons with a current market size of USD 3.6 billion, and by the end of 2027, the market size of gelatin is expected to reach USD 6.7 billion, as shown in Fig. 1(B) (Uddin et al., 2021).

Moreover, different extraction methods with maximum yield and high Bloom strength have been developed to extract gelatin from animal

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sources. The gelatin quality mainly depends on the sources and extraction method. Collagen is partially hydrolyzed using acids, bases, or enzymes to extract gelatin. Enzymatic extraction methods are more promising than chemical ones (Noor et al., 2021). Small pieces of gelatin sources (skin, bones, etc.) are soaked in NaOH solution to remove non-collagenous materials. Undesirable components are removed during this treatment, which is further processed to extract gelatin (Rather et al., 2022). When acids (hydrochloric acid, acetic acid, sulfuric acids) or alkalis (sodium hydroxide and calcium oxide) are used for pretreatment, the obtained gelatins are categorized as type A and type B, respectively (Bahar and Kusumawati, 2021). Other methods, such as ultrasound- and microwave-assisted, are also used for gelatin extraction (Alipal et al., 2021). Fig. 1(C) illustrates the gelatin extraction steps (Usman et al., 2023). Collagen comprises three polypeptide chains interconnected through hydrogen bonding, forming a triple helix. During pretreatment, acid, alkali, and temperature break hydrogen and hydrophobic bonds in the triple helix structure, splitting molecules into polypeptide chains. This cleavage converts collagen's triple helix structure into a random coil, forming gelatin (León-López et al., 2019).

Due to its multiple applications, unique properties, and vast market size, gelatin is one of the most studied ingredients in today's halal and adulteration research. Gelatin's acceptability depends on the collagen sources. According to the Laws of Islam, Judaism, and selected denominations of the Christian community, gelatin must be free from porcine sources (Tukiran et al., 2023). Similarly, the religious beliefs of the Hindu community need gelatin products free from bovine sources (Zhu et al., 2023).

Moreover, Colla Corri Asini (CCA), commonly known as donkey hide gelatin (DHG), has been a valuable medicine for thousands of years in Southeast Asia, especially in China (Zhang et al., 2023). It contains various health benefits, such as nourishing blood, enhancing immune response, improving metabolic balance, treating gynecologic diseases, and anti-oxidative and anti-aging effects. Collagen from donkey skin is hydrolyzed to amino acids and high molecular weight polypeptides, forming a solid glue. This thermally processed solid glue is DHG (Sheu et al., 2020). Similarly, deer hide gelatin prepared from the deer skin (*Cervus nippon* Temminck) is a precious medicinal material extensively used in traditional Chinese medicine (TCM) and food remedies. It is widely applied in tonification therapy to balance qi (according to TCM followers, qi is a vital energy/force from which everything is made up)

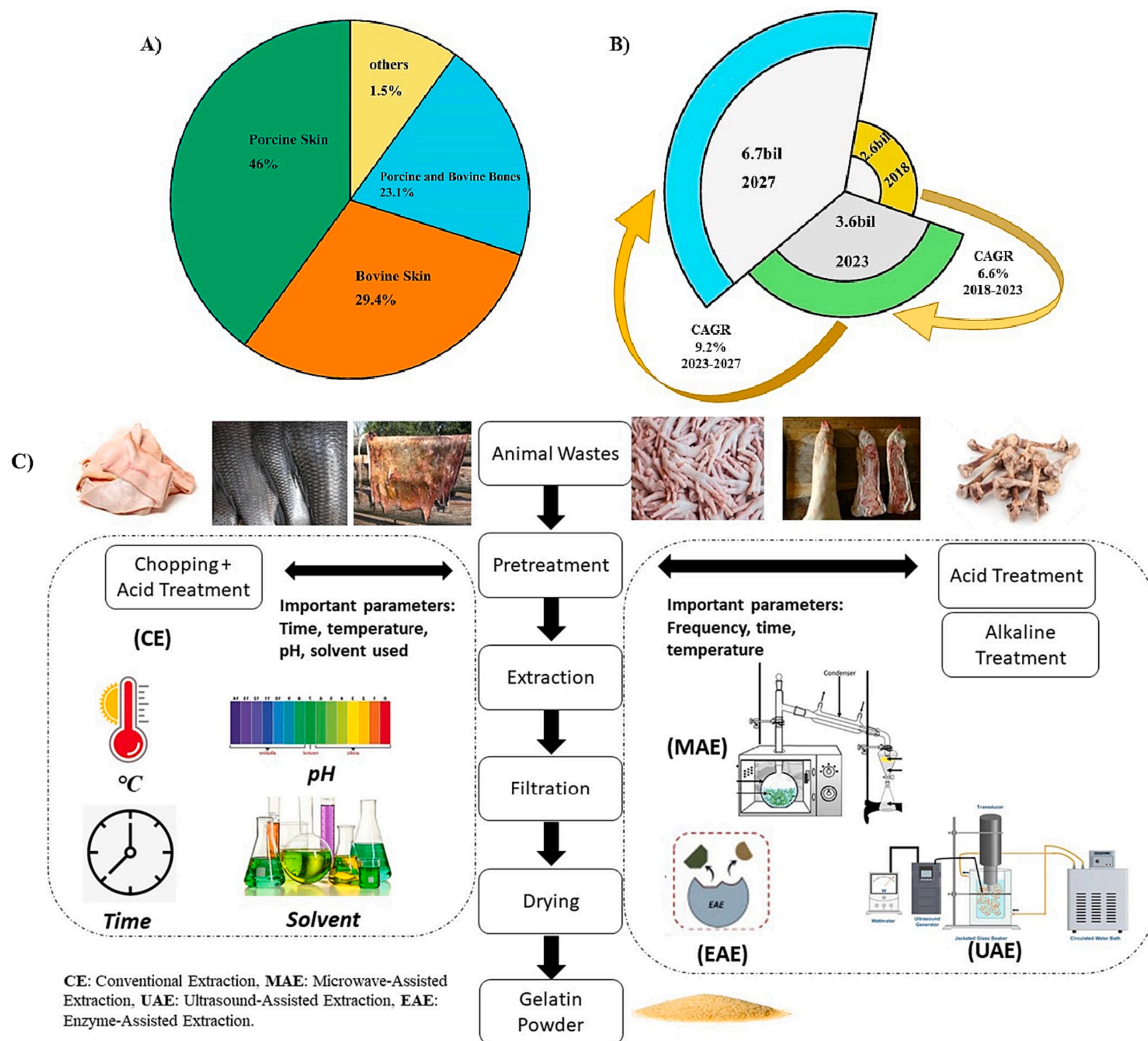

Fig. 1. (A) Commercial gelatin production from different sources, (B) its market size in respective years, and (C) gelatin extraction from collagen (Usman et al., 2023).

(Han et al., 2021). However, due to limitations in donkey husbandry and deer being not common livestock animals, the donkey and deer hide supply is insufficient to meet the increasing gelatin demand. Consequently, horse, porcine, and bovine hides are often added illegally as a DHG alternative. Therefore, the quality and efficiency of adulterated products cannot be guaranteed (Han et al., 2022). Moreover, after multiple processing steps during gelatin production, distinguishing deer and donkey hide gelatin from adulteration is extremely challenging (Ahsan et al., 2023).

Ensuring the gelatin origin for halal authentication and its adulteration is extremely challenging due to close similarities between the composition of different sources and the degradation of protein and DNA biomarkers during extraction, which is responsible for distinguishing one source from another (Zhu et al., 2023). DHG is mostly adulterated with horse and mule hide gelatin (Yang et al., 2023). Therefore, it is vital to establish sensitive, accurate, and high throughput methods for accurately identifying halal gelatin sources. Several modern and advanced analytical techniques are widely used to differentiate and

Table 1
Major authentication and quantification techniques for gelatin sources and their comparative features, advantages, and limitations.

Authentication Techniques	Main Features	Advantages	Disadvantages	Gelatin sources	References
Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE)	Sources based on molecular weight and polypeptide size distributions	• Simple Cheap Less sophisticated Needs a small sample amount	• Cannot differentiate gelatin sources with close species and in a complex mixture of processed food Not quantitative	Porcine and bovine	(Aina et al., 2013; Hermanto and Fatimah, 2013)
Enzyme-linked Immunosorbent assay (ELISA)	Detects gelatin sources based on antigen–antibody interaction, and the color change is observed visually or through spectroscopic techniques.	• Sensitive Fast Low-cost Used for qualitative and quantitative measurements	• Cannot detect gelatin in highly complex food mixtures Denaturing of protein-based biomarkers during food processing Cross-reactivity causes false positive results	Porcine and bovine	(Tukiran et al., 2016b; Venien et al., 2005a)
Polymerase Chain Reaction (PCR)	Identification of gelatin origin is done through species-specific DNA primers.	• Highly sensitive, specific, and reproducible Robust Biomarkers are highly stable (conventional PCR) Both qualitative and quantitative Both simplex and multiplex are possible (TaqMan PCR)	• Laborious Requires high expertise DNA extraction from highly processed food is challenging. DNA is damaged due to high temperature and pressure, leading to false resultsProbe designing, selection, and optimization are complicated (real-time RT-PCR)	• Porcine Bovine Fish Donkey hide	(Salamah et al., 2022; Zhang et al., 2019)
High-performance liquid chromatography coupled with principal component analysis (HPLC-PCA)	Distinguishes gelatin sources based on amino acid composition and classifies based on mathematical and statistical operations	• Requires a small sample amount Specific for the analytes of interests Rapid and sensitive	• Expensive Needs derivatization Cannot distinguish a gelatin mixture Requires statistical analysis (chemometric analysis)	• Porcine Bovine Fish	(Azilawati et al., 2015; Yuswan et al., 2021)
Liquid Chromatography coupled with mass spectrometry (LC-MS)	The digested peptides are separated through LC and are detected and mapped by mass spectrometry as biomarker peptides specific for each gelatin species	• High sensitivity, accuracy, and specificity Good resolution Rapid Can differentiate gelatin sources in a complex mixture Both qualitative and quantitative	• Requires pure samples and good technical expertise Laborious Expensive Hydroxylation of lysine and proline causes difficulties in the identification of biomarker peptides.	• Porcine Bovine Fish Donkey hide Deer hide Horse hide Mule hide	(Han et al., 2022; Sha et al., 2023)
Fourier transform infrared (FTIR)	Differentiate sources of gelatin-based on various functional groups present in the chemical composition of gelatin in terms of absorbance/transmittance.	• No extensive sample preparation Small sample amount Rapid Simple Routinely used Cheap	• Requires highly pure samples Needs chemometric tools for data interpretation Less sensitive Cannot differentiate gelatin in mixed matrices	• Porcine Bovine Fish	(Cebi et al., 2019; Irfanita et al., 2022)
Near-infrared spectroscopy (NIRS) and laser-induced breakdownspectroscopy (LIBS)	Screens gelatin samples at different wavelengths and emission results are interpreted to detect gelatin sources.	• Simple No sample preparation Cheap Not laborious	• Needs more validation of results Can only differentiate pure gelatin False identification due to other constituents.	• Porcine Bovine Fish	(Zhang et al., 2021)
Sensors	Interaction of analytes with the receptor at the sensor's surface produces signals based on optical or electroluminescence properties.	• Highly sensitive Portable Simple Cheap Handled easily It can be used routinely	• Compatibility of target material and sensor receptor is necessary Pure DNA is needed for biosensor development May detect one analyte at one time	• Porcine and bovine	(Adhikari et al., 2022; Widada et al., 2019)

quantify gelatin sources, such as gel electrophoresis, enzyme-linked immune sorbent assay (ELISA), polymerase chain reaction (PCR), spectroscopy, sensors, and liquid chromatography (LC) coupled with principal component analysis (PCA) and mass spectrometry (MS). The comparative features of these techniques are elaborated in Table 1 and an overview of gelatin sources, its applications, and existing and future authentication techniques are depicted in Fig. 2.

The current review discusses the potential of reported analytical techniques to detect and quantify different gelatin from different sources for halal authentication and adulteration in processed food and pharmaceutical samples. This review also emphasizes the challenges in accurate detection and future directions in this research field.

2. Techniques for gelatin identification and quantification

Accurate detection of gelatin-origin species is challenging due to the close similarities between their amino acid sequences. Identification of gelatin sources based on amino acid composition (Chromatographic methods) (Azilawati et al., 2015) and functional group (Fourier transform infrared spectroscopy) (Jariyah et al., 2021) can differentiate between pure forms. However, these techniques cannot differentiate gelatins in mixed food and pharmaceutical products since their chemical compositions (functional groups, amino acids) are similar. Therefore, other analytical techniques such as PCR (Salamah et al., 2022), LC-MS/MS (Yang et al., 2023), and ELISA (Tukiran et al., 2018) have been used to detect specific DNA markers, peptides, and proteins, respectively. Among these methods, FTIR has lower sensitivity and requires pure samples. Other techniques, such as gel electrophoresis (Azira et al., 2014) and FTIR (Jariyah et al., 2021), can specifically detect gelatin sources only. Meanwhile, ELISA (Tukiran et al., 2018), mass spectrometry (Cai et al., 2021), PCR (Zhang et al., 2019), and surface Plasmon resonance biosensors (Wardani et al., 2015) can also detect and quantify gelation sources in various food samples. Mass spectrometry is a sensitive technique; therefore, it is widely used to quantify gelatin adulteration in different sources (Cai et al., 2021). Different techniques used to detect and quantify gelatin are shown in Fig. 3 (A). Fig. 3 (B, C) demonstrates the number of publications on various techniques and literature available on different gelatin sources.

2.1. Gel electrophoresis

Electrophoresis is a simple separation technique for nucleic acids and proteins. Gel electrophoresis is widely used to simultaneously separate thousands of proteins (Maqsood et al., 2022). In two-dimensional gel electrophoresis, proteins are separated based on two different properties (isoelectric point and molecular weight) in two dimensions (Lee et al., 2020). The peptide composition in the gelatin varies based on processing techniques; therefore, the molecular weight varies accordingly. The molecular weight distribution of peptides/proteins in various species is a key separation factor for peptides in electrophoresis. The hydrolysis method used to convert the collagen into gelatin also contributes to the molecular weight distribution of peptides in gelatin. Authenticating gelatin sources using electrophoresis is simple, cheap, and rapid (Sharma et al., 2021).

Nur et al. (2012) conducted a trial study to differentiate the porcine and bovine gelatin in food and non-food samples by combining SDS-PAGE and PCA. Porcine skin gelatin samples showed 11 major polypeptide bands, while bovine skin samples showed only two. Azira et al. (2014) observed 16 prominent polypeptide biomarker bands of porcine gelatin with a molecular weight of 160–53 kDa, and bovine gelatin

Fig. 2. Different gelatin sources, applications, and existing and future authentication techniques.

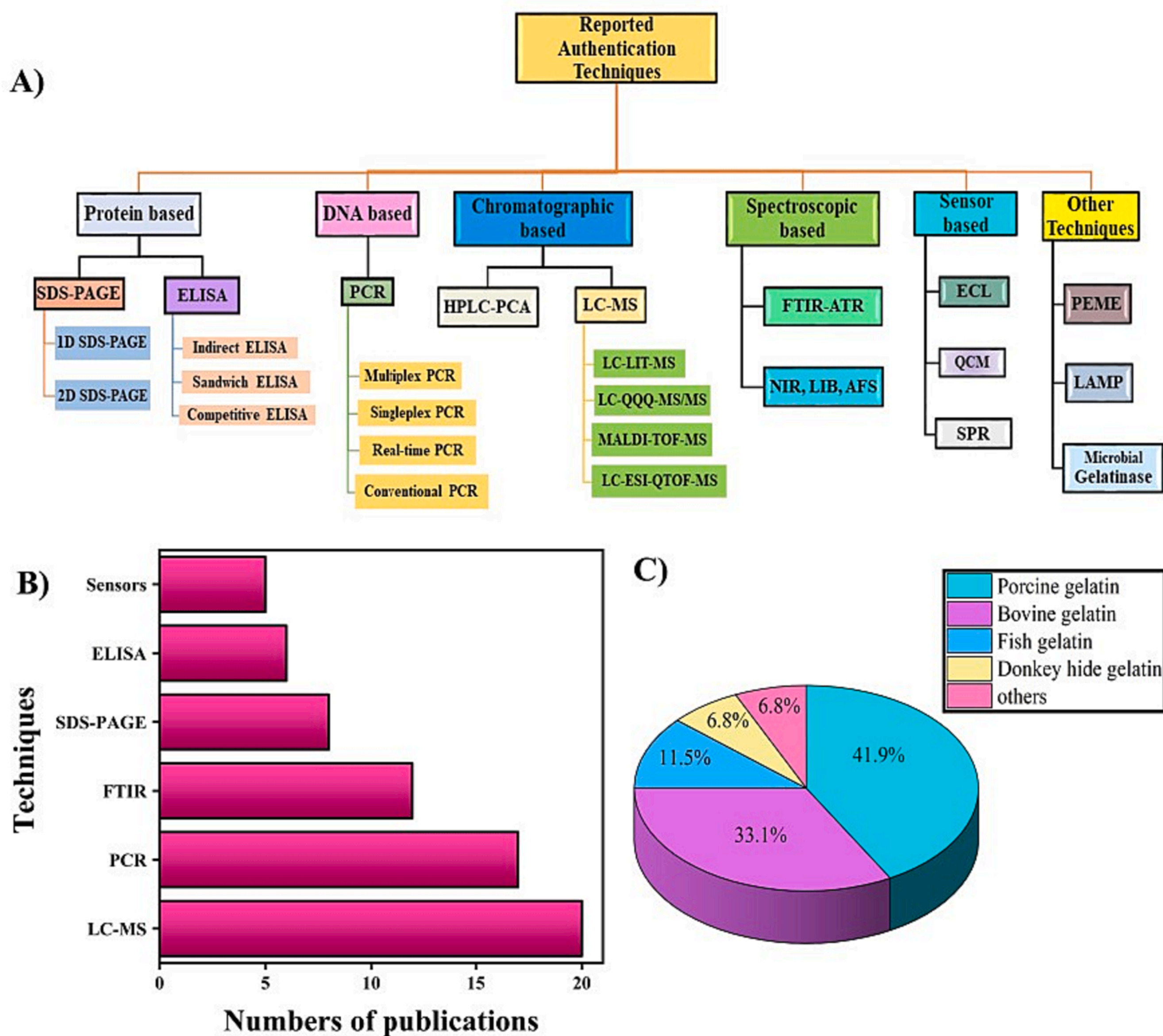

Fig. 3. (A) Flowchart of different techniques used for the authentication of gelatin sources, (B) numbers of publications on respective techniques, and (C) percentage of gelatin sources studied.

showed 2 dominant bands in the electrophoretic profile with a molecular weight of 135 and 110 kDa. Their developed method identified 5 % of porcine gelatin in the bovine gelatin mixture. However, the electrophoretic profile of real samples (homemade jellies) was undifferentiated. The electrophoretic pattern described by Hermanto and Fatimah (2013) using one-dimensional gel electrophoresis could only differentiate 2 fragments of porcine gelatin with a molecular weight below 28.6 and 36.2 kDa with a compromised resolution. To enhance the resolution, (Aina et al., 2013) used 2D-gel electrophoresis and identified polypeptide biomarkers of porcine skin gelatin in three commercial products, identifying 10 polypeptide biomarkers. They used 5 mixtures containing porcine and bovine gelatin for further validation with 1–5 % of porcine gelatin. 10 biomarker peptides were also identified with a detection limit of 1.0 % (w/w), suggesting that the obtained biomarkers can be used as a reference for gelatin detection in food products.

In another study, capsule shells were used as gelatin samples and gelatin sources were detected by SDS-PAGE-PCA (Malik et al., 2016). The hard translucent, soft, and hard red and blue capsules containing porcine, bovine, and unknown sources were studied as S1, S2, S3, and S4, respectively. According to the obtained protein bands, the densitometry profile showed 12 prominent peaks for porcine gelatin reference (PGR). 2, 7, and 9 peaks were obtained with a molecular weight of ± 135–100 and ± 200 kDa. In contrast, the bovine gelatin reference densitometry profile showed 4 dominant peaks with a molecular weight of 236, 222, 120, and 107 kDa, indicating that the bovine gelatin did not show a peak at 100 kDa, thus differentiating between porcine and bovine gelatins. The results illustrated that one sample contained porcine gelatin, and the other three contained bovine gelatins. Porcine and bovine gelatins in 13 double-blinded gelatin of hard and soft capsule supplements were differentiated. Distinctive bands at 110 and 140 kDa for porcine and bovine gelatin were differentiated in all 13 samples (Yap & Gam, 2019). Gel electrophoresis is a cheap and simple technique to detect gelatin from different sources; however, other protein fractions in food and non-food samples may produce false results. The gelatin in highly processed products goes through multiple steps, denaturing the protein at high temperatures; thus, the electrophoretic profiling may be uncertain for gelatin authentication. Therefore, limited studies on gelatin authentication have been published in the last few years using

this technique.

2.2. Enzyme-linked immune sorbent assay (ELISA)

ELISA is a well-known immunological detection technique for antigens or antibodies in different samples. This method has numerous applications in animal-origin gelatin detection in food and non-food products (Atefi et al., 2021). ELISA has four basic types: sandwich, competitive, direct, and indirect ELISA. Sandwich and indirect types are mostly used due to their high specificity and sensitivity toward antigens. Moreover, the detection of animal sources is based on antigens or antibody fixation at the specific surface sites of the enzyme. This technique can detect and quantify the analyte through the color intensity change after interactions (Uddin et al., 2021). Additionally, ELISA can authenticate various food products, such as peanut and soy proteins, in products containing meat, egg protein, and fish origin (Afzaal et al., 2022). Similarly, ELISA has extensive applications in identifying and quantifying gelatin origin in foods and pharmaceutical products (Mortas et al., 2022). It can simultaneously screen many samples without requiring sophisticated equipment, is easily available, and utilizes inexpensive reagents, yielding accurate and reliable results (Nhari et al., 2019).

Venien and Levieux (2005a) used indirect and competitive indirect ELISA to detect porcine and bovine sources of gelatin using polyclonal antibodies against tyrosylated. The competitive indirect ELISA detected and quantified bovine gelatin in the porcine gelatin mixture. However, tyrosylated gelatin samples containing porcine were more sensitive than bovine gelatin, and some antibodies showed lower specificity for various species. Venien and Levieux (2005b) described a modified method by adding antibodies against the putative collagen α1 in the bovine gelatin sequence to increase the specificity. Peptide two (Gly-Pro-Ala-Gly-Ala-Pro-Gly-Pro-Pro-Gly) showed higher reactivity and sensitivity toward bovine than porcine gelatin. However, the assay's sensitivity was influenced by gelatin's acid and alkaline treatments (gelatin degradation in processed samples), sources by-products such as skin, hide, and bone, and gelatin species origin, inducing false positive results.

Tukiran et al., (2016a) established another method using anti-peptide polyclonal antibodies generated from rabbits for porcine gelatin detection in edible bird's nests to increase the sensitivity of competitive indirect ELISA. The assay used a collagen protein amino acid sequence as α-2 (I) chain and α-1 (I) chain as antigens. The method used pAb1, pAb2, and pAb3 polyclonal antibodies from porcine gelatin. The pAb1 (14 peptide sequence), pAb2 (15 peptide sequence), and pAb3 (22 peptide sequence) as polyclonal antibodies were used against collagen α-2 (I) and α-1 (I), respectively. pAb3 demonstrated promising results with higher accuracy, sensitivity, specificity, and repeatability than pAb1 and pAb2.

The potential of ELISA was examined to analyze confectionary products ($n = 48$), such as premix powder, jelly, marshmallows, and gummies (Tukiran et al., 2016b). The proposed method used polyclonal antibodies against the peptide immunogen of the collagen α-2 (I) chain instead of monoclonal antibodies to recognize several epitopes in denatured proteins in processed food products. The cross-reactivity of the developed method was also checked with other gelatin sources, including chicken and fish, showing 1 % cross-reactivity. All 48 confectionery product samples showed no false positive results, demonstrating its promising efficiency. Pharmaceutical capsules were studied by Tukiran et al., (2016b) through a competitive indirect ELISA method based on anti-peptide pAb1 and pAb2. The first experiment showed that pAb1 possessed cross-reactivity for all studied samples, while pAb2 possessed < 1 % cross-reactivity to chicken and fish gelatins. To identify gelatin sources in commercial pharmaceutical capsule samples, pAb2 was selected since it could differentiate between porcine, bovine, and fish gelatins. The second method based on pAb2 demonstrated helpful results for mammalian gelatin source identification in both hard and soft shell capsules.

The ELISA method for gelatin detection possesses numerous advantages, such as cheap, rapid, better sensitivity, and high specificity. However, these methods also have several drawbacks, like none of the reported methods could detect fish gelatin and routinely analyze highly processed gelatins. These methods are unsuitable for repetitive analysis since biomarker epitopes are denatured and are less effective due to the lower specificity of antibodies, as different gelatin sources have close similarities in collagen sequences (Kuramata et al., 2022).

2.3. Polymerase chain reaction (PCR)

Limitations of the two protein-based methods grab the researchers' attention to develop DNA-based methods since DNA has higher stability under denaturing conditions (chemical treatment, pressure, and heat). DNA also exhibits remarkable sensitivity and gelatin quantification ability at the trace level (Böhme et al., 2019). PCR has been extensively used for the halal authentication of gelatin. It is a DNA-based technique that generates millions of DNA copies using oligonucleotides as primers (Kang et al., 2019). The PCR cycle includes denaturation, annealing, and extension steps (Kuslich et at., 2019). Numerous PCR techniques are developed to authenticate and detect gelatin species' origins in various food and non-food products. In conventional PCR, DNA fragments are amplified and obtained DNA is studied by gel electrophoresis. The electrophoretic profile separates DNA fragments based on their size, and these band fragments demonstrate the species' presence (Jäger et al., 2020). Another PCR-based method, restriction fragment length polymorphism analysis RFLP-PCR, has the advantages of low cost, adaptability to routine analysis, and simplicity. However, this method has fewer applications in processed food sample analyses since it requires the amplification of large DNA fragments, which is currently not possible in processed food samples due to thermal degradation (Rohman et al., 2020). The species-specific PCR, a targeted DNA sequence that is accurately amplified, is commonly used to identify different species. Real-time PCR (RT-PCR) can detect various species and directly monitor amplification products through sequence-specific DNA probes and fluorescent dyes. RT-PCR has high specificity, lower detection limits, and better sensitivity; thus, it has numerous applications in quantitative gelatin detection in processed food samples (Zhang et al., 2022).

RT-PCR is categorized into singleplex and multiplex, based on target amplifications per reaction. A single target gene sequence is amplified per reaction in singleplex real-time PCR. Designing a singleplex experiment is easier but costs more, requires many samples per reaction, and is laborious and time-consuming. In multiplex real-time PCR, multiple target gene sequences are amplified per reaction. Target sequences are detected using probes containing different dye labels. This method has the advantage of lower reagents and sample consumption and reduced time and cost of analysis. However, primer optimization is challenging (Chaudhary and Kumar, 2022). A schematic diagram of the PCR method for DNA detection in gelatin products is shown in Fig. 4.

Different studies have reported different PCR-based assays depending on target samples. Mohamad et al. (2018) detected porcine DNA in capsules, marshmallows, and candy products through molecular beacon-based real-time PCR and conventional PCR. Among marshmallows and candy samples, 17 were positive, and 86 were negative for porcine gelatin. The study showed that the chromosomal DNA of meat porcine repetitive element (MPRE) PCR assay was more sensitive, while mitochondrial DNA of cellbiohydrolase (CBH) PCR assay showed less sensitivity for porcine DNA in standard and capsule gelatin samples. However, conventional PCR was less sensitive, time-consuming, and was only suitable for qualitative analysis.

Therefore, real-time PCR can improve precision, sensitivity, automation, and analytical speed. Sultana et al. (2020) used a TaqMan probe through a single assay platform using a multiplex quantitative (qPCR) method to distinguish between porcine, fish, and bovine species. They studied approximately 35 processed food and dietary samples, suggesting that 2 out of 35 samples were positive for porcine species. DNA

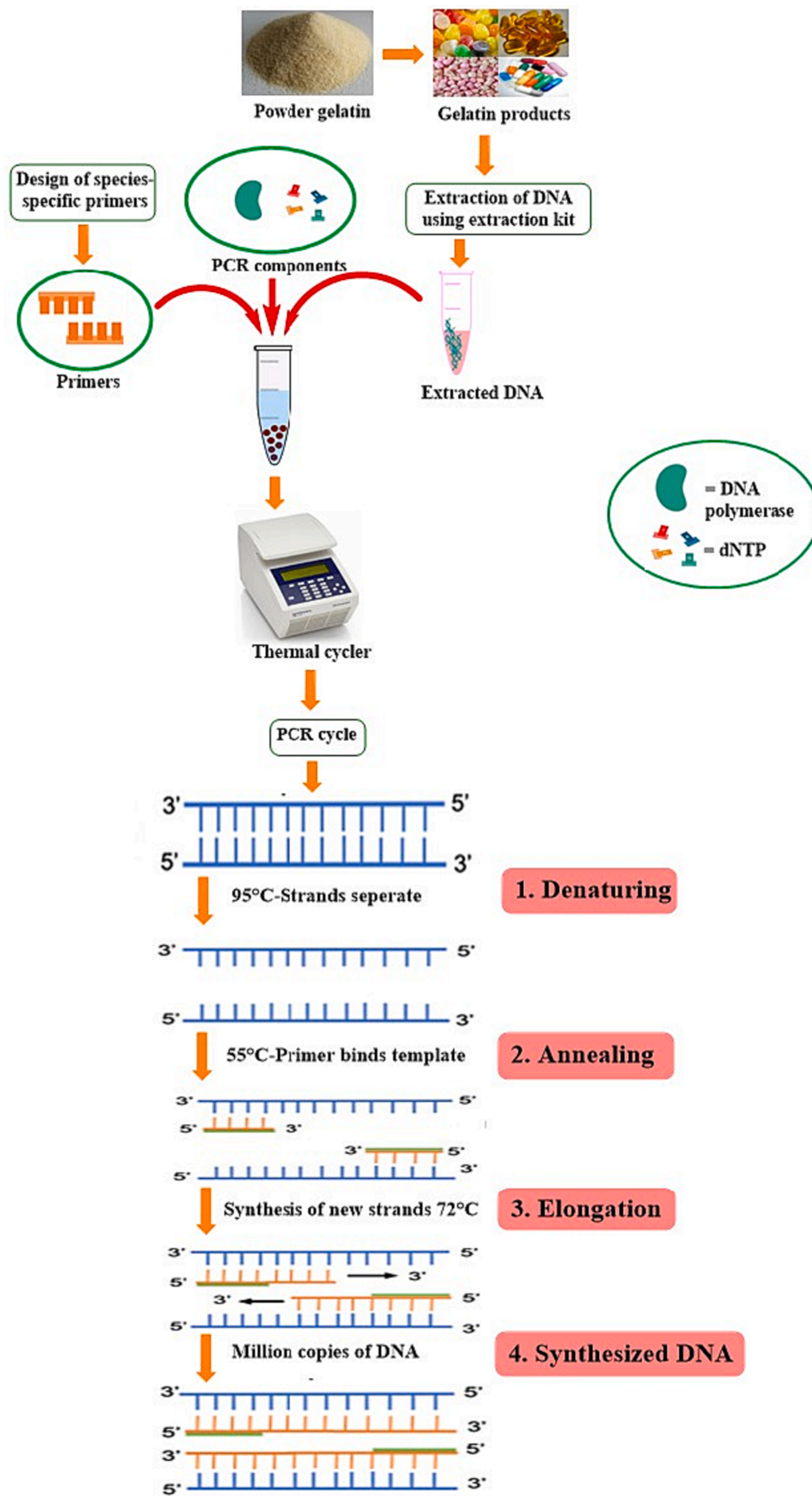

Fig. 4. Schematic diagram of PCR-based methods for DNA detection in gelatin products.

sequence analysis further confirmed the positive results of the two qPCR methods. The DNA sequencing analysis its 99–100 % similarity with Sus scrofa, a wild porcine species. They used multiplex qPCR for the first time to simultaneously detect these three species and used short amplicon lengths (106–166 bp) instead of previously long sizes (243–272 bp) due to their greater degradation tendency during food processing.

Detecting fish gelatin is also challenging due to the variety of fish species. Sultana et al. (2018) used a pair of universal fish primers to overcome the challenge of fish gelatin detection, screening 38 halal-branded confectionary products using multiplex PCR assay. Among 38 samples, 33 were positive for bovine, 2 for porcine, and 3 for eukaryotes. Zhang et al. (2019) identified the DHG through real-time PCR, analyzing donkey DNA with porcine, ox, and horse DNA as adulterants. Results showed that selected probes or primers could accurately detect donkey, horse, porcine, and ox DNA in gelatin samples with good reproducibility. Two of the four real samples contained donkey DNA, while the other two possessed donkey and horse DNA adulterants. Yayla et al. (2021) quantified porcine DNA in the porcine gelatin samples through a forensic method via real-time PCR. TübiGel was 10 times more sensitive for the porcine DNA in food samples than real-time PCR of Biotecon, the commercially available kit for DNA detection. The TübiGel method detected 6 positive samples from 10, demonstrating a better response for porcine DNA than the real-time PCR of Biotecon.

A recent study by Salamah et al. (2022) studied 30 samples of gelatin powder, pastilles, marshmallows, and jelly powder via a species-specific singleplex PCR. No products contained porcine gelatin but bovine gelatin. However, the singleplex PCR assay could not detect multiple species simultaneously, and the analysis cost was also high. The method also needed further validation (Kamandi et al., 2022). Salamah et al. (2022) identified bovine gelatin in gummy candy products via RT-PCR. Four samples purchased from markets were tested, and all four were positive for bovine gelatin with an efficiency value (E) of 99.62 %. The PCR-based techniques have been used by many researchers due to their higher sensitivity and stability for DNA than proteins. However, DNA extraction is challenging due to its low content and interaction with other gelatin residues, is time-consuming, and RT-PCR equipment is also expensive (Mortas et al., 2022).

2.4. High performance liquid chromatography (HPLC)

Chromatographic techniques have shown remarkable identification potential for chemical components with close structures in food items since this authentication is crucial in food authentication research (Peng et al., 2022). The most common chromatographic techniques for biomolecule separation include gas chromatography (GC) and high-performance liquid chromatography (HPLC). GC is only reliable for volatile and semi-volatile chemical components (Wang et al., 2020). However, no study has been reported for gelatin authentication using GC. The compound separation by liquid chromatography (LC) is based on three basic chemical features: polarity, size of molecules, and electrical charge. LC is a reliable analytical tool for the separation and purification of numerous chemical components, such as proteins, peptides, amino acids, carbohydrates, chiral compounds, vitamins, phenolic compounds, and pigments (Aydoğan, 2019; Wang et al., 2020). Gelatin sources have been typically identified based on amino acid compositions through LC with UV–Vis and fluorescent detectors. Different derivatizing agents are used to detect amino acids by LC-based methods. Although various modes are available, reverse phase (RP) HPLC is a reliable separation tool since it can rapidly separate various amino acids, facilitating gelatin detection from different sources (Ademola et al., 2018; Rohman et al., 2020).

2.4.1. High performance liquid Chromatography-Principal component analysis (HPLC-PCA)

PCA is a statistical and mathematical operation widely used with chromatographic tools to differentiate variables in analogous profiles. PCA coupled with chromatographic methods is also reported to authenticate and detect gelatin sources. The method can distinguish gelatin sources based on amino acid sequence. Both porcine and bovine gelatin sources were distinguished in capsule shells and candies samples through amino acids profiling by HPLC-PCA (Raraswati et al., 2013; Widyaninggar et al., 2012). However, derivatizing agents, such as *ortho*-paraldehyde in 2–2-mercaptoethanol, showed less specificity, and the derivatization method was time-consuming. Therefore, Azilawati et al. (2015) used 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate as a highly reactive porcine, bovine, and fish gelatin amino acid analysis agent. The correlation and pattern results described that seven samples contained bovine gelatin, and five samples contained porcine gelatin.

Hydroxyproline is a major constituent of collagen, and its concentration varies in different sources (Alpoim-Moreira et al., 2022). Yuswan et al. (2021) successfully differentiated 59 gelatin-based samples by monitoring hydroxyproline as a signature amino acid. The variability in hydroxyproline was the main gelatin differentiation factor in multiple samples. The method benefitted the gelatin classification; however, further validation was required to differentiate porcine and bovine gelatins. The preliminary information in these methods was insufficient for accurate identification. Despite this, the chromatographic-chemometric tools are not described for gelatin identification in the mixture of numerous gelatin sources, and there is also the possibility of amino acid transformation in Maillard reaction during food processing, preventing their application in gelatin source authentication.

2.4.2. High performance liquid chromatography -mass spectrometry (HPLC-MS)

Amino acid compositions in different gelatin species are similar, making its differentiation challenging. In liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, gelatins are extracted from different products, including capsule shells, jellies, and marshmallows, and then subjected to trypsin digestion. The digested peptides are separated by LC, detected by MS, and sequenced using different databases (Fig. 5). The gelatin quantification and adulteration are further investigated using various protocols and mathematical models (Deng et al., 2020). Due to its high resolution and accuracy, specific peptides are easily and authentically analyzed in gelatin-containing products through LC-MS/MS (Tukiran et al., 2019).

Sha et al. (2014) established an HPLC-ion trap/orbitrap high-resolution mass spectrometry-based method to identify 18 marker peptides of porcine and bovine gelatin. They used ^{16}O-^{18}O exchange labeling with trypsin, enabling highly accurate and sensitive quantification of gelatin in the mixture. However, the method followed complicated sample preparation and handling of sophisticated equipment and, thus, was not practically applicable. Later, Flaudrops et al. (2015) established a simple sample preparation method using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) to differentiate bovine and porcine gelatins. The detection limit for porcine gelatin mixture with bovine gelatin was 20 %. However, the method was unsuitable for trace detection of gelatin due to less sensitivity.

Sha et al. (2020) detected 17 peptide markers in pure bovine gelatin, and 7 were further quantified with good linearity, reproducibility, and specificity. Subsequently, two peptides with *m/z* values of 1076.01þ and 824.91þ were used to quantify bovine gelatin in mixed edible films and food additives. However, the gelatin quantification through this method is challenging due to the food matrix influence in highly processed food samples. Sha et al. (2020) studied the effect of the extraction process on gelatin identification using three types of porcine gelatin at different temperatures. After extraction from porcine skin, the obtained gelatins were digested into peptides and then studied by LC-MS/MS. 97, 88, and 58 characteristic marker peptides were identified from the porcine gelatin extracted in the first, second, and third stages. All samples contained 46 common characteristic marker peptides. Guo et al. (2020)

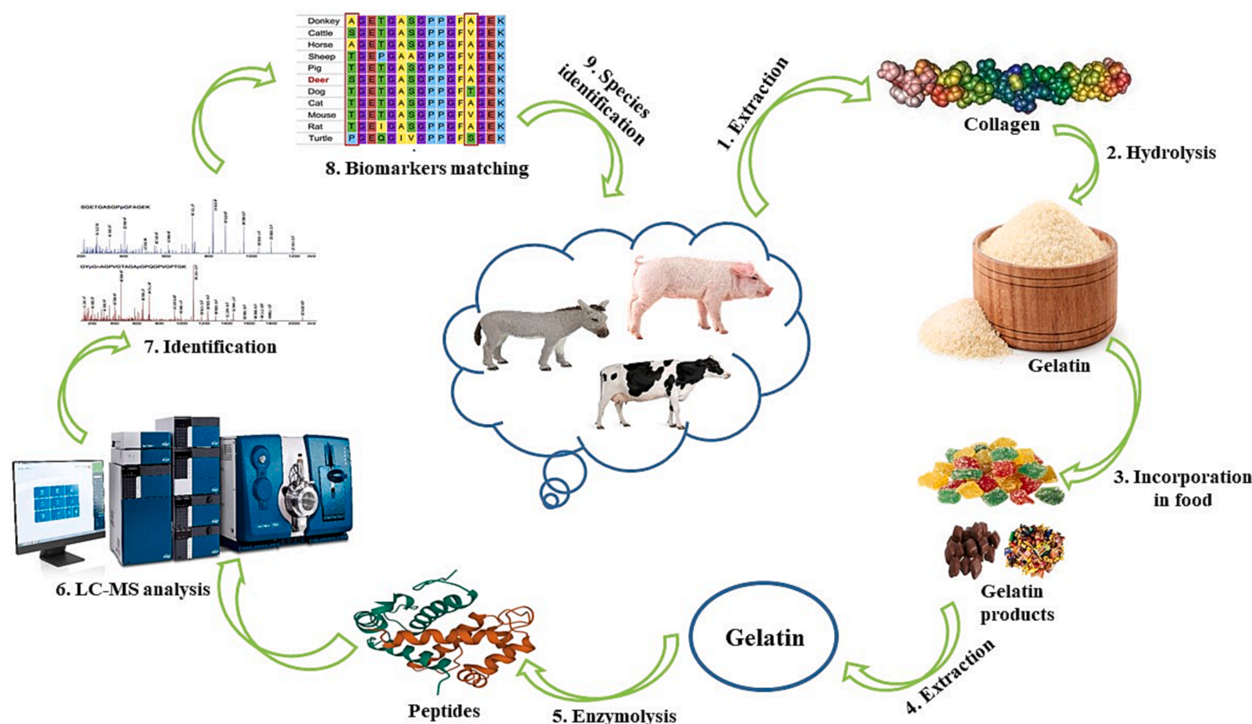

Fig. 5. Schematic representation of LC-MS methods for peptide markers identification.

used an LC-MS/MS quantitation method and screened three equine family species peptides: SGQPGTVGPAGVR, GASGPAGVR, and GATG-PAGVR. The E1 biomarker peptide was only detected in DHG; however, E2 and E3 were detected in mule gelatin and absent in donkey and horse hide. The developed also detected hybrid and horse hide gelatins at a very low percentage of 0.10 % and 0.05 %, respectively. A robust and rapid 10-minute MRM (multiple reaction monitoring) method was developed by Yang et al. (2023) to differentiate porcine, bovine, donkey, horse, and mule hide gelatins. DHG marker peptides were detected in commercial samples and Chinese patent medicines. Cai et al. (2021) also reported a similar study combining LC-MS/MS and bioinformatics to check whether fewer species-specific peptides can distinguish DHG from other homologous species, identifying 2 specific peptides. Both peptides could not distinguish DHG from gelatin from horse and mules' skin. Therefore, more peptide biomarkers were discovered to distinguish DHG from horse and mule hide gelatins.

Han et al. (2022) proposed a new strategy based on response-boosting MS signals to quantify gelatin peptide markers in donkey hide. To enhance detection sensitivity and absolute quantification, amino acid residues (valine, glycine, and alanine) were employed for real samples. The detection limit, linearity, precision, repeatability, and accuracy (recovery) of species-specific peptides were 0.02 ~ 0.98 ng/mL, $r^2 > 0.997$, RSD (relative standard deviation) < 8.5 %, <8.9 %, and 89.4 %~106.5 %, respectively. A protein database was also used to identify species' peptide markers to authenticate their origin. The donkey and horse species matched well; however, the donkey had no specific horse marker peptides. Wu et al. (2022) developed a database-independent strategy by comparing different animal tryptic peptides (donkey, horse, bovine, and porcine) using LC-QTOF-MS/MS. 14 specific peptide markers were identified, including 4, 1, 3, and 6 biomarker peptides of porcine, donkey, horse, and bovine species, respectively. A quantitative method using LC-QQQ-MS/MS in MRM mode was established to validate donkey-specific markers. Results revealed that 57 samples contained 110 adulterants, suggesting that the obtained markers were specific for quantitative and qualitative analysis of Ejiao-based products.

Similarly, Zhang et al. (2022) verified gelatins from porcine, horses, donkeys, and bovine using LC-MS/MS. Approximately 12 species-specific marker peptides were identified in DHG. Moreover, the commercial DHG items and homemade mixed gelatin products were investigated to check the method's applicability. Results illustrated that 9 samples containing DHG were identified according to product labels, while others were adulterated with horse gelatin. Liu et al. (2019) used untargeted and targeted mass spectrometry-based methods to identify specific DHG peptides. A comprehensive study was done on the peptidomics profile of tryptic peptides using mathematics set theory. Among all peptides, 2 were identified as DHG-specific, revealing that both were specific enough to differentiate DHG from other animals' gelatin.

Deerhorn gelatin (DCG), an expensive food gelatin throughout Asia, has endured adulterants due to DHG addition. No previously reported methods and data were available to distinguish the peptide biomarkers of different animal tissues of the same species origin (DHG and DCG). Han et al. (2021) established an effective and robust method to differentiate DHG and DCG by a label-free technique using nano-LC-MS/MS. 20 peptides were selected as potential biomarkers, and 4 were validated. The optimized method was applied to 5 commercial DHG and DCH samples, and results showed that DCG-C1, DCG-C2, and DCG-C3 were adulterated 97.7 %, 104.7 %, and 96.4 %, while DCG-C4 and DCG-C5 were adulterated 50.8 % and 38.8 % with DHG, respectively. The developed strategy was highly accurate, rapid, and selective for DHG and DCG differentiation and could be helpful for the quality control and depth authentication of adulterants in food samples.

The tryptic and peptic digestion of gelatin requires a long time. Cai et al. (2021) reduced gelatin's tryptic and peptic digestion time from more than 12 h to 5 min using an ultrasound-assisted digestion approach, followed by ultra-performance liquid chromatography (UPLC) MS/MS analysis. They distinguished the animal origin of gelatins within 20 min in a single run, screening 25 commercial samples from different sources, including 10 donkey hide, 5 deer hide, 5 bovine hide, and 5 porcine hide samples. Results revealed that 2 deer hide samples were adulterated with 900 g/kg and 265 g/kg of bovine-hide gelatin, and among 3 DHG samples, 2 were adulterated with 66 g/kg and 381 g/kg horse hide gelatin, and 1 was adulterated with 786 g/kg of bovine hide gelatin. Zhu et al. (2023) used 11 peptide biomarkers to

identify porcine, bovine, and donkey gelatins, establishing an MRM method for the most sensitive peptides. Porcine gelatin was well identified in the mixtures using selected biomarker peptides.

Currently, fish gelatin demand is increasing, and its adulteration authentication is performed by LC-MS/MS. Sha et al. (2023) identified gelatin in 7 commercial cyprinid fishes, including Wuchang bream, bighead, silver, crucian, common, black, and grass carp. Theoretical peptides of mammalian collagen, porcine, and bovine were compared

Table 2
LC-MS detected marker peptides of different gelatin sources.

Peptide Sequence	*m/z*	Transitions	CE (eV)	Ion source/Temp (°C)	Technique	Ref
Donkey						
HGN*RGEPGPVGSVGP*VGAVRGPSGPQGVRGDK	806.1	645.5 → 659.8	NA	ESI/450	LC-LTQ-Orbitrap-MS/MS	(Cai et al., 2021)
GPTGEPGK	371.6	371.6 → 487.2				
GPTGEPGKPGDK	570.2	570.4 → 698.3	NA	ESI/500	LC-Q-Trap-MS/MS	(Zhang et al., 2022)
GATGPAGVR	393.2	393.2 → 499.3	20	ESI/300	LC-QQQ-MS/MS	(Guo et al., 2020)
AGETGASGPP*GFAGEK	724.8	724.8 → 875.5	31	ESI/500	LC-Q-Trap-MS/MS	(Han et al., 2022)
GYP*GDAGPVGAVGAP*GPHGPVGPTGK	768.0	768.0 → 286.0	44			
HGDRGEP*GPVGSVGPVGAVGPR	691.0	691.0 → 809.4	34	ESI/ 500	LC-Q-Trap-MS/MS	(Cai et al., 2021)
GEAGPAGPAGPIGPVGAR	765.9	NA	32	ESI/350	LC-QQQ-MS/MS	(Zhu et al., 2023)
GPSGPQGVR	427.7		21			
GPAGPTGPVGK	469.2		21			
SGQPGTVGPAGVR	519.8		25			
Porcine						
HGN*RGEPGPAGSVGPAGAVGPRGPSGPQGIRGEK	799.1	639.7 → 567.7	NA	ESI/450	LC-LTQ-Orbitrap-MS/MS	(Cai et al., 2021)
GPTGDPGK	364.6	364.6 → 574.2				
GEPGPTGVQGPPGPAGEEGK	924.5	747.9 → 832.6	NA	ESI/350	LC-MSD-Iontrap-MS/MS	(Cheng et al., 2014)
GETGPAGPAGPVGPVGAR	773.9	NA	34	ESI/350	LC-QQQ-MS/MS	(Zhu et al., 2023)
GPTGPAGVR	406.2		19			
IGPPGPSGISGPPGPPGPAGK	897.5		43			
TGETGASGPPGFAGEK	731.8		28			
TGETGASGPP*GFAGEK	739.9	739.9 → 875.4	31	ESI/500	LC-Q-Trap-MS/MS	(Han et al., 2022)
GYP*GNPGPAGAAGAP*GPQGAVGPAGK	736.0	736.0 → 851.1	21			
GYP*GDAGPVGAVGAP*GPHGPVGPTG	770.4	770.4 → 286.0	45			
TGETGASGPP*GFAGEK	739.9	739.9 → 818.5	30	ESI/ 500	LC-Q-Trap-MS/MS	(Cai et al., 2021)
GYP*GNPGPAGAAGAHP*GPQGAVGPAGK	736.0	736.0 → 526.4	27			
AGVMGPP*GSR	473.0	473.0 → 586.1	26	ESI/300	LC-QQQ-MS/MS	(Guo et al., 2018)
GETGPAGPAGPVGPVGAR	774.3	774.3 → 977.8	30			
GEP*GPTGVQGPP*GPAGEEGK	926.0	926.0 → 833.1	35			
Bovine						
HGN*RGEP*GPAGAVGPAGAVGPRGPSGP*QGIRGDK	791.6	791.9 → 974.5	NA	ESI/450	LC-LTQ-Orbitrap-MS/MS	(Cai et al., 2021)
GPSGDPGK	357.6	357.6 → 301.1				
GEAGPSGPAGPTGAR	641.8	548.3 → 632.4	NA	ESI/350	LC-MSD-Iontrap-MS/MS	(Cheng et al., 2014)
GEAGPSGPAGPTGAR	641.3	641.3 → 726.4	NA	ESI/500	LC-Q-Trap-MS/MS	(Zhang et al., 2022)
GEAGPSGPAGPTGAR	641.3	NA	29	ESI/350	LC-QQQ-MS/MS	(Zhu et al., 2023)
GETGPAGPAGPIGPVGAR	780.9		39			
SGETGASGPPGFVGEK	738.8		28			
SGETGASGPP*GFVGEK	746.9	746.9 → 903.5	31	ESI/500	LC-Q-Trap-MS/MS	(Han et al., 2022)
GYP*GDAGPVGAAGAP*GPQG-PVGPVGK	755.0	755.0 → 617.5	21			
SGETGASGPP*GFVGEK	746.9	746.9 → 846.5	29	ESI/ 500	LC-Q-Trap-MS/MS	(Cai et al., 2021)
GYP*GDAGPVGAAGAP*GPQGPVGPVGK	755.0	755.0 → 553.4	26			
Fish (seven cyprinid fish)						
GAAGP*PGATGF*PGAAGR	722.3	NA	NA	NA	LC-Q-Orbitrap-MS/MS	(Sha et al., 2023)
G*PPGPMGPPGLAGPPGE*PGR	913.9					
GP*PGPMGPPGLAGPPGEPGR	905.9					
GA*PGPSGPPGPAGANGDK	760.3					
GDSGP*PGLTGF*PGAAGR	773.3					
GESGPAGPSGFAGP*PGADGQTGQR	1085.9					
GYTGLDGR	419.7					
Horse						
HGHRGEP*GPVGSVGPVGAVGPR	698.3	698.3 → 809.4	38	ESI/ 500	LC-Q-Trap-MS/MS	(Caiet al., 2021)
GASGPAGVR	386.2	386.2 → 402.2	NA	ESI/500	LC-Q-Trap-MS/MS	Zhang et al., 2022)
HGHRGEP*GPVGSVGPVGAVGPRGP*SGPQGVRGDK	811.6	649.7 → 670.8	NA	ESI/450	LC-LTQ-Orbitrap-MS/MS	Cai et al., 2021)
GPSGEPGK	364.6	364.6 → 487.2				
Deer						
HGN*RGEP*GPAGAVGPAGAVGPRGPSGP*QGIRGDK	791.6	791.9 → 974.5	NA	ESI/450	LC-LTQ-Orbitrap-MS/MS	(Cai et al., 2021)
GPTGDPGK	364.6	364.6 → 574.2				
SGETGASGPPGFAGEK	724.9	724.9 → 802.4	31	ESI/ 500	LC-Q-Trap-MS/MS	(Cai et al., 2021)
SGETGASGPP*GFAGEK	732.9	732.9 → 818.4	30			
GYP*GNAGPVGTAGAP*GPQGPVGPTGK	765.2	765.2 → 554.4	25			
GYP*GDAGPVGTAGAP*GPQGPVGPTGK	65.5	765.5 → 554.4	29			
GYP*GDAGPVGTAGAP*GPEGPVGPTGK	765.8	765.8 → 554.9	25			
GEVGPAGPDGFAGPAGAAGQAGAK	670.9	670.9 → 531.4	25			

P*: Hydroxyproline, **N*:** Deamidation, **CE:** Collision energy, **eV:** Electron volt, ***m/z*:** Mass to charge ratio, **ESI:** Electrospray ionization, **LC:** Liquid chromatography, **LTQ:** Linear trap quadrupole, **MSD:** Mass selective detection, **MS/MS:** Tandem Mass spectrometry, **QQQ:** Triple quadrupole, **Q-Trap:** Quadrupole linear ion trap.

with commercial fish samples, suggesting that silver, grass, and crucian carp collagen contained unique peptides. Results suggested that 7 common characteristics peptides were identified among 7 cyprinid fish gelatin, while in crucian, grass, and silver carp, 42, 44, and 36 unique characteristics peptides were detected, respectively. Therefore, using unique characteristics peptides in fish gelatin improved the identification by comparing with mammalian species.

Gelatin obtained from other marine species, such as cannonball jellyfish, yellowfin tuna, and *Rhizostoma pulmo,* is beneficial due to their biological potency (Domenico et al., 2019; Sol et al., 2022; Nurilmala et al., 2020). Gelatin peptides obtained from these marine species possess great antioxidant properties. However, studying the biomarkers peptide sequences of gelatin obtained from marine species other than fish using mass spectrometry would diversify this research field in the future (Nurilmala et al., 2022). Some peptide biomarkers from different gelatin sources detected by LC-MS are listed in Table 2.

In contrast, LC-MS methods for gelatin detection are specific to certain gelatin types without considering the variations in gelatin sources and food production processes. The LC-MS method may produce false positive matching of peptides due to the sequence of highly repetitive motifs within collagen and gelatin molecules incorporated due to hydroxylation sites and their relative abundance.

2.5. Spectroscopic methods

2.5.1. Infrared spectroscopy coupled with chemometric tools

Infrared (IR) spectroscopy is commonly used to confirm adulteration and halal authentication in various food and non-food products and determine the fat content in lard or porcine in chocolate (Abidin et al., 2023), the lard content in the mixture of cow, chicken, and lamb, and lard contents in crackers (Siddiqui et al., 2023). In IR, samples are identified based on peak differences in the spectrum (Guerrero-Pérez et al., 2020). Attenuated total reflectance (ATR) is a powerful analytical tool in which a sample is contacted with the ATR element, and spectrums are recorded based on that contact (Tamara et al., 2023). FTIR and ATR have been used to determine collagens' physiochemical, morphological, chemical, and intercross-linking properties. IR is fast, environmentally friendly, requires little or no sample preparation, and can be used routinely for halal authentication. However, due to complex peaks, researchers combined FTIR data with PCA and cluster analysis (CA) to differentiate gelatin origins.

Hashim et al. (2010) distinguished between bovine and porcine gelatin through FTIR-ATR, indicating that both are similar within the 650–4000 cm^{-1} spectral range. The major differences were observed between the N—H group area (3280–3290 cm^{-1}) and the hydrogen-bonded area (1200–1660 cm^{-1}). The amide bond with specific spectral characteristics was a discriminative region for gelatin origins identification with different spectral intensities. This method rapidly distinguished porcine and bovine gelatin; however, the high purity requirements for this method can be challenging. Therefore, porcine, bovine, and fish gelatin mixture was distinguished through FTIR spectra in the mid-IR region (650–4000 cm^{-1}) (Cebi et al., 2016). The peaks of amide-I (1600–1700 cm^{-1}) and amide-II (1520–1565 cm^{-1}) were differentiated and used as variables in PCA and hierarchical cluster analysis, successfully distinguishing gelatin origins.

Cebi et al. (2019), Irfanita et al. (2022), and Jariyah et al. (2021) reported similar studies to identify porcine and bovine gelatin in various commercial products using FTIR-ATR combined with PCA. They used amide I and II peaks at 1300–1450 cm^{-1}, 1543 cm^{-1}, and 2800–3000 cm^{-1} for PCA analysis. Similarly, Cebi et al. (2019) used 20 commercial food samples (gummy candies) in their study. Irfanita et al. (2022) found that real dental sample BDM 01 contained porcine and bovine DNA content, and BDM 14 had only bovine DNA. They identified 3 samples from porcine sources; the rest were from bovine gelatins. Jariyah et al. (2021) demonstrated that none of the 5 jelly candies contained porcine gelatins. The same method was applied by Hassan et al. (2021) using an autocatalytic set of chemometric tools. The results revealed that porcine, bovine, and fish gelatin showed characteristic peaks at 1470–1475 cm^{-1}, 1444–1450 cm^{-1}, and 1496–1500 cm^{-1}, respectively. Although FTIR spectroscopic methods are widely used to rapidly differentiate gelatin sources, the sensitivity in highly processed foods and non-food samples is compromised, which may cause false identifications.

2.5.2. Other spectroscopic techniques

Some other spectroscopic methods have also been used to explore gelatin properties. Near-infrared spectroscopy (NIRS) is applied to quantify the Bloom value, pH, moisture, and viscosity of bovine and porcine gelatins and edible gelatin adulteration (Zhang et al., 2018). Fluorescence spectroscopy can monitor the composition changes in porcine gelatin with aging (Duconseille et al., 2016). Laser-induced breakdown spectroscopy (LIBS) is used to identify adulteration in porcine gelatin (Zhang et al., 2019). The advantage of a data fusion strategy for multiple spectroscopic data is improved classification and accuracy. Zhang et al. (2021) studied data fusion of fluorescence spectroscopy, NIRS, and LIBS to identify different gelatin origins, including porcine and bone, bovine and bone, and fish skin. They used fused data from each spectroscopic method and applied them to the random forest model (RFM) to classify 5 gelatin origins. Results revealed that data fusion improved the discrimination accuracy of gelatin origins. Moreover, NIRS and fluorescence spectroscopy showed better results for gelatin source classification than LIBS due to remarkable molecular structure and composition information. However, the obtained data must be repeatable for gelatin authentication via spectroscopic methods to get reliable information, which is challenging in highly processed foods (Hameed et al., 2018).

2.6. Sensing methods

Numerous methods have been developed to detect gelatin sources based on different working principles. However, each method has its drawbacks, such as low specificity, low sensitivity, time consumption, high cost, the requirement of an expert person, and sophisticated instrumentations. Since gelatin is a processed protein and its authentication can be challenging; therefore, these methods cannot accurately detect the trace level of porcine gelatin. In addition, on-site detection of gelatin is difficult using these methods. Hence, a highly specific, sensitive, cheap, rapid, robust, and portable method is required to compensate for all these limitations. Different types of biosensors can overcome all these challenges. Recently, researchers have focused on developing novel sensors and biosensors to investigate different analytes in food and non-food items (Adhikari et al., 2022). Biosensors are based on recognizing biologically sensitive components like nucleic acids and enzymes. The detector quantifies and detects specific signals for each analyte. Nanomaterials are widely used as sensors, including metals, metal oxides, quantum dots, metal–organic frameworks, and carbon nanotubes (Mei et al., 2022). Multiple carbon nanostructured materials (CNSMs) based sensors are currently being used to detect target proteins with higher sensitivity (Joshi et al., 2021). The synthesis of CNSMs requires fewer reagents and solutions, reducing the method's cost. Since nanostructured carbon materials have a high surface area, remarkable catalytical activity, exceptional porosity, and high electrochemical conductivity, they have gained tremendous attention in bio-sensing research (Eissa et al., 2021).

Electrochemiluminescence (ECL) biosensors have been widely used since they are versatile, stable, and cheaper than previously developed methods such as chromatography, mass spectrometry, PCR, and ELISA (Cai et al., 2021). An ECL biosensor was employed by Adhikari et al. (2022) for gelatin detection using a carbon nanofiber fabricated screen-printed electrode (CNF-SPE) modified with carbon nano-horns (CNHs) and nafion (NAF). They immobilized anti-gelatin as bio-recognition with 0.1 % bovine serum albumin (BSA) and applied it for porcine gelatin detection in food samples. The developed biosensor showed remarkable

linearity and reproducibility; however, the selectivity and specificity were compromised in real samples.

The quartz crystal microbalance (QCM) acts as a mass sensor, the target analyte is attached to the QCM sensor surface, and the increase in mass is measured. QCM shows rapid response and high sensitivity, is easy to use, and is stable for long-term operations. QCM-based sensors have multiple applications in environmental analysis, protein identification, biomolecule interaction, and clinical targets (Alanazi et al., 2023). Muharramah et al. (2020) designed a QCM sensor using polyaniline and nickel nanoparticles and applied it to determine porcine and bovine gelatin in homemade ice cream samples. The real sample results were compared with standard results. The frequency shifted to a positive value for the samples containing porcine gelatin, while the frequency was shifted to a negative value for bovine gelatin. The frequency shift value increased proportionally with analyte concentration. A similar study was reported by Pradini et al. (2018). They used a modified QCM sensor to detect porcine and bovine gelatin in hard shell capsules. The standard porcine and bovine gelatins were detected in demineralized water and then validated in real samples. The designed sensors are cost-effective with simple and easy setups as compared to other techniques. However, the matrix interference in real samples may induce false results.

Aptamers biosensors employ a small strand of oligonucleotides, such as single-stranded DNA or RNA, attached to target proteins possessing high affinity or specific ligands. Widada et al. (2019) conducted authenticated porcine gelatin using a graphene oxide aptamer biosensor. Surface plasma resonance (SPR) based biosensors are rapid, sensitive, and reliable for biomolecule detection in food items. Wardani et al. (2015) reported an SPR biosensor to differentiate and quantify porcine and bovine gelatins, which showed promising results for gelatin quantification from various sources. However, further validation is needed for better and more reliable identification of gelatin in complex food mixtures. The sensors based methods have a lot of potential in this research area since they are sensitive, simple, easy to operate, inexpensive, and portable. Table 3 shows the comprehensive features of reported studies for gelatin detection in different sources using sensor-based methods.

2.7. Other techniques

In pulsed electro-membrane extraction (PEME), targeted analytes move across a selectively supported liquid membrane under an electrical field and are extracted by the acceptor solution in a hollow fiber membrane. PEME has potential applications in amino acid selective extraction (Eie et al., 2021). Rezazadeh et al. (2015) used PEME to detect gelatin in different sources by derivatizing amino acids and analyzing them by HPLC. The proposed method could differentiate porcine and bovine gelatin sources through asparagine and glutamine amino acids. Similarly, loop-mediated isothermal amplification (LAMP) is another DNA-based detection method, simpler than PCR. LAMP has advantages over PCR since it can recognize 6 specific regions of targeted DNA, enhancing detection sensitivity and specificity (Soroka et al., 2021). Many studies have reported LAMP for porcine DNA identification in meat and other food samples (Girish et al., 2020). Tasrip et al. (2021) employed the LAMP method to rapidly detect porcine DNA gelatin in processed foods. They used LAMP-specific porcine primers for gelatin samples, successfully detecting the porcine DNA with a lower detection limit and no cross-reactivity with 14 other animals' DNAs, indicating its promising specificity. In addition, they detected porcine DNA in 5 out of 32 samples through an easy, rapid, sensitive, and reliable method. However, the method requires more assessments and validation for more samples. The LAMP method has also been used with portable molecular diagnostic devices to detect DNA in DHG with a 10——4 ng/μL detection limit. Using the smartphone as a portable detector device was the study's strength, along with the rapid response (30 min) and reduced cost (Sheu et al., 2021).

Shahimi et al. (2021) utilized microbial resources to identify porcine gelatin using bacterial enzymes. Gelatinase enzyme was extracted from bacterial strains and applied to porcine, bovine, and fish gelatins. The study revealed that gel EA1-9 of E is a novel protein enzyme (gelatinase) that could hydrolyze porcine gelatin. Since it is only a preliminary study, there is a further need to evaluate gelatinase specificity towards porcine gelatin. Developing a robust and specific dipstick (flow device) using the protein-based method is the future target of this research.

3. Challenges and future perspectives

Existing analytical techniques possess multifarious advantages; nonetheless, they face numerous challenges and drawbacks for accurate gelatin identification and quantification in processed food samples. Protein denaturation during the gelatin processing at high temperatures, DNA extraction from gelatin samples, and close similarity of marker peptides are key challenges in gelatin authentication. The existing problems can be addressed with innovative portable approaches that offer faster, cheaper, sensitive, and easier operations. Some future validation initiatives employ double gene-targeted multiplex PCR to detect animal species. The designed short-length biomarkers (73, 90, 106, 120, 138, and 146 bp) are more stable toward high temperatures, thus overcoming the drawback of denatured DNA (Khalil et al., 2021). Therefore, double gene targeting can commercially be a new pathway for gelatin origin identification.

Recombinase polymerase amplification (RPA) has gained sufficient attention in species authentication. RPA is widely applied to detect adulteration in animal-derived ingredients, such as porcine, duck, and horse meat (Kissenkötter et al., 2020; Zhou et al., 2023). The advanced isothermal DNA amplification methods are superior to traditional molecular methods since RPA can be used onsite with portable equipment, enabling faster and simpler detection (Lin et al., 2021; Zhao et al., 2022), demonstrating enough detection potential for gelatin in food products.

Table 3
Comprehensive features of reported studies to detect gelatin sources using various sensor-based methods.

Types of Sensor	Materials used	Sources detected	Detection range	Real samples	Limitations	Ref
Electro-chemiluminiscence	Carbon nano-horns (CNHs) and nafion (NAF) on carbon nanofiber	Porcine gelatin	1 pg mL^{-1}	Not studied	No real samples were studied to verify the potential of the sensor.	(Adhikari et al., 2022)
Quartz Crystal Microbalance (QCM)	Polyaniline and nickel nanoparticles on gold electrode	Porcine and bovine gelatin	Not given	Homemade Ice cream	Not qualitative Interference may cause false results	(Muharramah et al., 2020)
Electrochemical	Polyaniline and nickel nanoparticles on gold electrode	Porcine and bovine gelatin	Not given	Hard capsule shells		(Pradini et al., 2018)
Surface Plasmon resonance (SPR)	Gold-coated BK7 prism	Porcine and bovine gelatin	Porcine 0.66 % (w/w) bovine 0.38 % (w/w)	Homemade jellies	Validation is needed in highly processed food.	(Wardani et al., 2015)

Moreover, nano-based microarrays and biosensors are emerging tools for gelatin source authentication. The attractive features of nanomaterials provide a new platform to engineer materials with DNA-specific recognition sites using electrochemistry and spectroscopic tools. Nanomaterials are fused with specific probe DNA in biosensors, thus widening their application prospects. Single and multiplex platforms are reported for real DNA targets of microbial species and porcine (Sultana et al., 2023) without concerning porcine DNA for gelatin authentication. Similarly, combining nanomaterials with biotechnology can lead to new and remarkable pathways for gelatin detection with enhanced efficiency and sensitivity. In the future, using gold nanoparticles in PCR may detect degraded and inferior-quality DNA with a low amount in processed food samples under extreme temperatures (Khalil et al., 2019).

Currently, lateral-flow immunoassay combined with nanomaterials (nano-ELISA) is becoming a vital research direction for halal food authentication, including porcine residue detection in meat products (Hendrickson et al., 2021; Hendrickson et al., 2023). Nano-ELISA possesses higher stability and sensitivity, low cost, faster analysis time, and requires small sample amounts compared to conventional ELISA. Therefore, it can be a promising alternative detection method for porcine gelatin in processed food samples. One attractive feature of nano-ELISA is its integration with smartphones. The test strips (small, disposable, and narrow paper strips or other materials coated with nanomaterials) can potentially make it a domestic practice. Several challenges still exist to developing high-performance and stable nanomaterials for nano-ELISA (Kua et al., 2022; Wu et al., 2019). Additionally, for gelatin authentication, Raman scattering, chemical imaging, fluorescent spectroscopy, and hyperspectral imaging approaches can be explored. Online spectra and imaging libraries would also benefit this field.

4. Conclusion

Gelatin is extensively used in foods, cosmetics, and pharmaceuticals and is obtained from the collagen of various animals (porcine, bovine, fish, donkey, and horse). The acceptability of various gelatin sources is limited from a religious perspective. Donkey and deer hide gelatins suffer from adulteration; thus, authenticating and quantifying gelatin in different products is urgently required. Several analytical techniques have been used for gelatin analysis, including SDS-PAGE, ELISA, PCR, LC-PCA, LC-MS, FTIR, and numerous sensors with variable efficiencies. Each method has a different working principle for gelatin detection. These reported methods show good sensitivity and specificity for various gelatin sources in different samples, with certain limitations and challenges that must be addressed in future studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data is provided in the manuscript

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References

Abedinia, A., Nafchi, A. M., Sharifi, M., Ghalambor, P., Oladzadabbasabadi, N., Ariffin, F., & Huda, N. (2020). Poultry gelatin: Characteristics, developments, challenges, and future outlooks as a sustainable alternative for mammalian gelatin. *Trends in Food Science & Technology, 104*, 14–26.

Abidin, S. A. S. Z., Nizar, M., Nizar, N. N. A., Shukor, M. S. A., Zainal, R., & Ab Mutalib, S. R. (2023). Halal concerns on lard in food products and its detection methods. Halalpshere, 3, 79–90. Adhikari, J., Rizwan, M., & Ahmed, M. U. (2022). Development of a label-free electrochemiluminescence biosensor for the sensitive detection of porcine gelatin using carbon nanostructured materials. *Sensors & Diagnostics, 1*, 968–976.

Afzaal, M., Saeed, F., Hussain, M., Shahid, F., Siddeeg, A., & Al-Farga, A. (2022). Proteomics as a promising biomarker in food authentication, quality and safety: A review. *Food Science & Nutrition, 10*, 2333–2346.

Ahsan, S., Liaqat, A., Khaliq, A., Iqbal, R., Chughtai, M. F. J., Mehmood, T., & Sameed, N. (2023). *Current trends and prospects of transforming animal waste into food* (pp. 469–503). In Climate Changes Mitigation and Sustainable Bioenergy Harvest Through Animal Waste: Sustainable Environmental Implications of Animal Waste. Springer.

AI-Temimi, W. K. A., Kalaf, A., Imran, R. A., Alsamir, M. A., & Zulfiqar. (2021). Enzymatic and thermal extraction of gelatin from fish scales and study its sensory properties. *International Journal of Pharmaceutical Research, 13*, 1043–1054.

Aina, M., Amin, I., Hafidz, R. M. R., & Yaakob, C. (2013). Identification of polypeptide biomarkers of porcine skin gelatin by two-dimensional electrophoresis. *International Food Research Journal, 20*, 1395–1399.

Alanazi, N., Almutairi, M., & Alodhayb, A. N. (2023). A review of quartz crystal microbalance for chemical and biological sensing applications. *Sensing and Imaging, 24*, 10.

Alipal, J., Pu'Ad, N. M., Lee, T., Nayan, N., Sahari, N., Basri, H., & Abdullah, H. (2021). A review of gelatin: Properties, sources, process, applications, and commercialisation. *Materials Today: Proceedings, 42*, 240–250.

Alpoim-Moreira, J., Fernandes, C., Rebordão, M. R., Costa, A. L., Bliebernicht, M., Nunes, T., & Ferreira-Dias, G. (2022). Collagen type III as a possible blood biomarker of fibrosis in equine endometrium. *Animals, 12*, 1854.

Atefi, M., Bagheri, V., Mahmoudzadeh, M., Fard, M. N., & R.. (2021). Gelatin: Overview of identification methods. *Human, Health and Halal Metrics, 2*, 25–34.

Aydoğan, C. (2019). Nanoscale separations based on LC and CE for food analysis: A review. *Trends in Analytical Chemistry, 121*, Article 115693.

Azilawati, M., Hashim, D., Jamilah, B., & Amin, I. (2015). RP-HPLC method using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate incorporated with normalization technique in principal component analysis to differentiate the bovine, porcine and fish gelatins. *Food Chemistry, 172*, 368–376.

Azira, T. N., Man, Y. C., Hafidz, R. R. M., Aina, M., & Amin, I. (2014). Use of principal component analysis for differentiation of gelatine sources based on polypeptide molecular weights. *Food Chemistry, 151*, 286–292.

Bahar, A., & Kusumawati, N. (2021). Comparison of the physico-chemical properties of type-B halal gelatin from bovine and goat skin material. *IOP Conference Series: Earth and Environmental Science, 709,* 012033, IOP Publishing.

Böhme, K., Calo-Mata, P., Barros-Velázquez, J., & Ortea, I. (2019). Review of recent DNA-based methods for main food-authentication topics. *Journal of Agricultural and Food Chemistry, 67*, 3854–3864.

Cai, S., Zhao, K.-X., Jiang, M.-T., Han, S.-Y., Zheng, Y.-F., Liu, X., & Liu, R. (2021). Collagen derived species-specific peptides for distinguishing donkey-hide gelatin (Asini Corii Colla). *Chinese Herbal Medicines, 13*, 261–266.

Cebi, N., Dogan, C. E., Mese, A. E., Ozdemir, D., Arıcı, M., & Sagdic, O. (2019). A rapid ATR-FTIR spectroscopic method for classification of gelatin gummy candies in relation to the gelatin source. *Food Chemistry, 277*, 373–381.

Cebi, N., Durak, M. Z., Toker, O. S., Sagdic, O., & Arici, M. (2016). An evaluation of Fourier transforms infrared spectroscopy method for the classification and discrimination of bovine, porcine and fish gelatins. *Food Chemistry, 190*, 1109–1115.

Chaudhary, P., & Kumar, Y. (2022). Recent advances in multiplex molecular techniques for meat species identification. *Journal of Food Composition and Analysis, 110*, Article 104581.

Cheng, X.-L., Wei, F., Chen, J., Li, M.-H., Zhang, L., Zhao, Y.-Y., & Lin, R.-C. (2014). Using the doubly charged selected ion coupled with MS/MS fragments monitoring (DCSI-MS/MS) mode for the identification of gelatin species. *Journal of Analytical Methods in Chemistry, 2014*, Article 764397.

Deng, G., Guo, S., Zaman, F., Li, T., & Huang, Y. (2020). Recent advances in animal origin identification of gelatin-based products using liquid chromatography-mass spectrometry methods: A mini review. *Reviews in Analytical Chemistry, 39*, 260–271.

Duconseille, A., Andueza, D., Picard, F., Santé-Lhoutellier, V., & Astruc, T. (2016). Molecular changes in gelatin aging observed by NIR and fluorescence spectroscopy. *Food Hydrocolloids, 61*, 496–503.

Eie, L. V., Rye, T. K., Hansen, F., Halvorsen, T. G., & Pedersen-Bjergaard, S. (2021). Electromembrane extraction of peptides and amino acids–status and perspectives. *Bioanalysis, 13*, 277–289.

Eissa, S., Al-Kattan, K., & Zourob, M. (2021). Combination of carbon nanofiber-based electrochemical biosensor and cotton fiber: A device for the detection of the Middle-East respiratory syndrome coronavirus. *ACS Omega, 6*, 32072–32080.

Flaudrops, C., Armstrong, N., Raoult, D., & Chabriere, E. (2015). Determination of the animal origin of meat and gelatin by MALDI-TOF-MS. *Journal of Food Composition and Analysis, 41*, 104–112.

Girish, P. S., Barbuddhe, S. B., Kumari, A., Rawool, D. B., Karabasanavar, N. S., Muthukumar, M., & Vaithiyanathan, S. (2020). Rapid detection of pork using alkaline lysis-loop mediated isothermal amplification (AL-LAMP) technique. *Food Control, 110*, Article 107015.

Guerrero-Pérez, M. O., & Patience, G. S. (2020). Experimental methods in chemical engineering: Fourier transform infrared spectroscopy—FTIR. *The Canadian Journal of Chemical Engineering, 98*, 25–33.

Guo, S., Deng, G., Duan, X., Zhou, X., & Huang, Y. (2020). Marker peptide combination for source identification of gelatins obtained from equidae hides by LC–MS/MS detection. *Polymer Testing, 88*, Article 106576.
Guo, S., Xu, X., Zhou, X., & Huang, Y. (2018). A rapid and simple UPLC-MS/MS method using collagen marker peptides for identification of porcine gelatin. *Royal Society of Chemistry advances, 8*, 3768–3773.
Hameed, A. M., Asiyanbi, H. T., Idris, M., Fadzillah, N., & Mirghani, M. E. S. (2018). A review of gelatin source authentication methods. *Tropical Life Science Research, 29*, 213–227.
Han, S., Yan, Z., Huang, X., Cai, S., Zhao, M., Zheng, Y., & Hou, R. (2022). Response boosting-based approach for absolute quantification of gelatin peptides using LC-MS/MS. *Food Chemistry, 390*, Article 133111.
Han, S., Zhao, K., Cai, S., Jiang, M., Huang, X., Chen, S., & Liu, R. (2021). Discovery of peptide biomarkers by label-free peptidomics for discrimination of horn gelatin and hide gelatin from *Cervus nippon Temminck*. *Food Chemistry, 363*, Article 130347.
Hashim, D., Man, Y. C., Norakasha, R., Shuhaimi, M., Salmah, Y., & Syahariza, Z. (2010). Potential use of Fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatins. *Food Chemistry, 118*, 856–860.
Hassan, N., Ahmad, T., Zain, N. M., & Awang, S. R. (2021). Identification of bovine, porcine and fish gelatin signatures using chemometrics fuzzy graph method. *Scientific Reports, 11*, 9793.
Hendrickson, O. D., Zvereva, E. A., Dzantiev, B. B., & Zherdev, A. V. (2021). Sensitive lateral flow immunoassay for the detection of pork additives in raw and cooked meat products. *Food Chemistry, 359*, Article 129927.
Hendrickson, O. D., Zvereva, E. A., Pridvorova, S. M., Dzantiev, B. B., & Zherdev, A. V. (2023). The use of Au@Pt nanozyme to perform ultrasensitive immunochromatographic detection of banned pork additives in meat products. *Food Control, 154*, Article 110013.
Hermanto, S., & Fatimah, W. (2013). Differentiation of bovine and porcine gelatin based on spectroscopic and electrophoretic analysis. *Journal of Food and Pharmaceutical Sciences, 1*, 6.
Irfanita, N., Lestari, W., Mirghani, M. E. S., Jaswir, I., Octavianti, F., & Haris, M. S. (2022). Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy coupled with principal component analysis and polymerase chain reaction (PCR) assay for the detection of porcine and bovine gelatins in dental materials. *Tropical Life Sciences Research, 33*, 133.
Jäger, R., & Weiher, H. (2020). Polymerase chain reaction. *An introduction to molecular biotechnology: Fundamentals, methods and applications, Journal of Clinical Microbiology, 30*, 2826–2829.
Jariyah, J., Yulistiani, R., Afdilah, S. W., & Mas'udah, K. W. (2021). Detection of pork gelatin in jelly candy using Fourier transform infrared (FTIR) and polymerase chain reaction (PCR). *Edition Diffusion Press Sciences, 328*, 01006.
Joshi, P., Mishra, R., & Narayan, R. J. (2021). Biosensing applications of carbon-based materials. *Current Opinion in Biomedical Engineering, 18*, Article 100274.
Kamandi, N., Ghobadi Dana, M., & Ghavami, M. (2022). Molecular identification of gelatin origin in pastilles and jelly products collected from Tehran markets. *Journal of Food Biosciences and Technology, 12*, 11–18.
Kang, T. S. (2019). Basic principles for developing real-time PCR methods used in food analysis: A review. *Trends in Food Science & Technology, 91*, 574–585.
Khalil, I., Hashem, A., Nath, A. R., Julkapli, N. M., Yehye, W. A., & Basirun, W. J. (2021). DNA/nano based advanced genetic detection tools for authentication of species: Strategies, prospects and limitations. *Molecular and Cellular Probes, 59*, Article 101758.
Khalil, I., Yehye, W. A., Julkapli, N. M., Rahmati, S., Sina, A. A. I., Basirun, W. J., & Johan, M. R. (2019). Graphene oxide and gold nanoparticle based dual platform with short DNA probe for the PCR free DNA biosensing using surface-enhanced Raman scattering. *Biosensors and Bioelectronics, 131*, 214–223.
Kissenkötter, J., Böhlken-Fascher, S., Forrest, M. S., Piepenburg, O., Czerny, C.-P., & Abd El Wahed, A. (2020). Recombinase polymerase amplification assays for the identification of pork and horsemeat. *Food Chemistry, 322*, Article 126759.
Kua, J. M., Azizi, M. M. F., Abdul Talib, M. A., & Lau, H. Y. (2022). Adoption of analytical technologies for verification of authenticity of halal foods: A review. *Food Additives & Contaminants: Part A, 39*, 1906–1932.
Kuramata, H., Hashiba, M., Kai, Y., Nishizawa, K., Inoue, T., Kikuchi-Ueda, T., & Oshikane, H. (2022). Animal species identification utilising DNAs extracted from traditionally manufactured gelatin (Wanikawa). *Heritage Science, 10*, 183.
Kuslich, C. D., Chui, B., & Yamashiro, C. T. (2019). Overview of PCR. *Current Protocols Essential Laboratory Techniques, 18*, 27.
Lee, P. Y., Saraygord-Afshari, N., & Low, T. Y. (2020). The evolution of two-dimensional gel electrophoresis-from proteomics to emerging alternative applications. *Journal of Chromatography A, 1615*, Article 460763.
León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V. M., Vargas-Torres, A., Zeugolis, D. I., & Aguirre-Álvarez, G. (2019). Hydrolyzed collagen—sources and applications. *Molecules, 24*, 4031.
Lin, L., Zheng, Y., Huang, H., Zhuang, F., Chen, H., Zha, G., & Wei, H. (2021). A visual method to detect meat adulteration by recombinase polymerase amplification combined with lateral flow dipstick. *Food Chemistry, 354*, Article 129526.
Liu, R., Huang, Y., Xu, H., Zheng, Y., Liu, Y., Han, S., & Duan, J.-A. (2019). A strategy for identifying species-specific peptide biomarkers in deer-hide gelatin using untargeted and targeted mass spectrometry approaches. *Analytica Chimical Acta, 1092*, 32–41.
Malik, A., Sutantyo, M. L., Hapsari, I., Sinurat, A. V., Purwati, E. M., Jufri, M., & Suryadi, H. (2016). Simultaneous identification and verification of gelatin type in capsule shells by electrophoresis and polymerase chain reaction. *Journal of Pharmaceutical Investigation, 46*, 475–485.
Maqsood, S., Amjad, S., Hayat, M. T., & Maqsood, N. (2022). Use and advancement of analytical and instrumentation systems: Two-dimensional gel electrophoresis, electrospray ionization, matrix assisted laser. *National Journal of Biological Sciences, 4*, 22–34.
Mei, Y., He, C., Zeng, W., Luo, Y., Liu, C., Yang, M., & Huang, Q. (2022). Electrochemical biosensors for foodborne pathogens detection based on carbon nanomaterials: Recent advances and challenges. *Food and Bioprocess Technology, 15*, 498–513.
Mohamad, N. A., Mustafa, S., Khairil Mokhtar, N. F., & El Sheikha, A. F. (2018). Molecular beacon-based real-time PCR method for detection of porcine DNA in gelatin and gelatin capsules. *Journal of the Science of Food and Agriculture, 98*, 4570–4577.
Mortas, M., Awad, N., & Ayvaz, H. (2022). Adulteration detection technologies used for halal/kosher food products: An overview. *Discover Food, 2*, 15.
Muharramah, A., Permata, L., Juwono, H., Sugiarso, R., & Kurniawan, F. (2020). Detection of gelatin in ice cream using QCM sensor. *IOP Conference Series: Earth and Environmental Science, 493*, Article 012028.
Nhari, R. R., Hanish, I., Mokhtar, N. K., Hamid, M., & El Sheikha, A. (2019). Authentication approach using enzyme-linked immunosorbent assay for detection of porcine substances. *Quality Assurance and Safety of Crops & Foods, 11*, 449–457.
Noor, N. Q. I. M., Razali, R. S., Ismail, N. K., Ramli, R. A., Razali, U. H. M., Bahauddin, A. R., & Shaarani, S. M. (2021). Application of green technology in gelatin extraction: A review. *Processes, 9*, 2227.
Nurilmala, M., Hizbullah, H. H., Karnia, E., Kusumaningtyas, E., & Ochiai, Y. (2020). Characterization and antioxidant activity of collagen, gelatin, and the derived peptides from yellowfin tuna (Thunnus albacares) skin. *Marine Drugs, 18*, 98.
Nurilmala, M., Suryamarevita, H., Husein Hizbullah, H., Jacoeb, A. M., & Ochiai, Y. (2022). Fish skin as a biomaterial for halal collagen and gelatin. *Saudi Journal of Biological Sciences, 29*, 1100–1110.
Peng, J., Ma, L., Kwok, L.-Y., Zhang, W., & Sun, T. (2022). Untargeted metabolic footprinting reveals key differences between fermented brown milk and fermented milk metabolomes. *Journal of Dairy Science, 105*, 2771–2790.
Pradini, D., Juwono, H., Madurani, K. A., & Kurniawan, F. (2018). A preliminary study of identification halal gelatin using quartz crystal microbalance (QCM) sensor. *Malaysian Journal of Fundamental and Applied Sciences, 14*, 325–330.
Ranasinghe, R. A. S. N., Wijesekara, W. L. I., Perera, P. R. D., Senanayake, S. A., Pathmalal, M. M., & Marapana, R. A. U. J. (2022). Functional and bioactive properties of gelatin extracted from aquatic bioresources: A review. *Food Reviews International, 38*, 812–855.
Raraswati, M. A., Triyana, K., & Rohman, A. (2013). Differentiation of bovine and porcine gelatins in soft candy based on amino acid profiles and chemometrics. *Journal of Food and Pharmaceutical Sciences, 2*, 28–34.
Rather, J. A., Akhter, N., Ashraf, Q. S., Mir, S. A., Makroo, H. A., Majid, D., & Dar, B. N. (2022). A comprehensive review on gelatin: Understanding impact of the sources, extraction methods, and modifications on potential packaging applications. *Food Packaging and Shelf Life, 34*, Article 100945.
Rezazadeh, M., Yamini, Y., Seidi, S., & Aghaei, A. (2015). Pulsed electromembrane extraction for analysis of derivatized amino acids: A powerful technique for determination of animal source of gelatin samples. *Talanta, 136*, 190–197.
Rigueto, C. V. T., Rosseto, M., Alessandretti, I., de Oliveira, R., Wohlmuth, D. A. R., Menezes, J. F., & Pizzutti, I. R. (2022). Gelatin films from wastes: A review of production, characterization, and application trends in food preservation and agriculture. *Food Research International, 162*, Article 112114.
Rohman, A., Windarsih, A., Erwanto, Y., & Zakaria, Z. (2020). Review on analytical methods for analysis of porcine gelatine in food and pharmaceutical products for halal authentication. *Trends in Food Science & Technology, 101*, 122–132.
Salamah, N., Erwanto, Y., Martono, S., & Rohman, A. (2022). Employment of real-time polymerase chain reaction for the identification of bovine gelatin in gummy candy. *Indonesian Journal of Pharmacy, 33*, 448–454.
Sha, X.-M., Jiang, W.-L., Hu, Z.-Z., Zhang, L.-J., Xie, Z.-H., Lu, L., & Tu, Z.-C. (2023). Traceability and identification of fish gelatin from seven cyprinid fishes by high performance liquid chromatography and high-resolution mass spectrometry. *Food Chemistry, 400*, Article 133961.
Sha, X.-M., Tu, Z.-C., Wang, H., Huang, T., Duan, D.-L., He, N., … Xiao, H. (2014). Gelatin quantification by oxygen-18 labeling and liquid chromatography–high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry, 62*, 11840–11853.
Sha, X.-M., Wang, G.-Y., Li, X., Zhang, L.-Z., & Tu, Z.-C. (2020). Identification and quantification of gelatin by a high-resolution mass spectrometry-based label-free method. *Food Hydrocolloids, 101*, Article 105476.
Shahimi, S., Lamri, M. F., Mutalib, S. A., Khalid, R. M., Tab, M. M., & Khairuddin, F. (2021). Gene expression of microbial gelatinase activity for porcine gelatine identification. *Food Chemistry, 355*, Article 129586.
Sharma, N., Sharma, R., Rajput, Y. S., Mann, B., Singh, R., & Gandhi, K. (2021). Separation methods for milk proteins on polyacrylamide gel electrophoresis: Critical analysis and options for better resolution. *International Dairy Journal, 114*, Article 104920.
Sheu, S.-C., Huang, J.-Y., Lien, Y.-Y., & Lee, M.-S. (2020). Specific, sensitive and rapid authentication of donkey-hide gelatine (Colla corii asini) in processed food using an isothermal nucleic acid amplification assay. *Journal of Food Science and Technology, 57*, 2877–2883.
Sheu, S., Huang, C., & Chen, J. (2021). Portable molecular diagnostics device for identification of *Asini Corii Colla* by loop-mediated isothermal amplification. *Inventions, 6*, 51.
Siddiqui, M. A., Md Khir, M., Ullah, Z., Hasan, M. A., Saboor, A., & Magsi, S. A. (2023). Infrared spectroscopy-based chemometric analysis for lard differentiation in meat samples. *Computers, Materials & Continua, 75*, 2860–2871.
Soroka, M., Wasowicz, B., & Rymaszewska, A. (2021). Loop-mediated isothermal amplification (LAMP): The better sibling of PCR? *Cells, 10*, 1931.

Sultana, S., Azlan, A., Desa, M. N. M., & Mahyudin, N. A. (2023). Multiplex platforms in biosensor based analytical approaches: Opportunities and challenges for the speciation of animal species in food chain. *Food Control, 149*, Article 109727.
Sultana, S., Hossain, M. M., Azlan, A., Johan, M. R., Chowdhury, Z. Z., & Ali, M. E. (2020). TaqMan probe based multiplex quantitative PCR assay for determination of bovine, porcine and fish DNA in gelatin admixture, food products and dietary supplements. *Food Chemistry, 325*, Article 126756.
Sultana, S., Hossain, M. M., Zaidul, I., & Ali, M. E. (2018). Multiplex PCR to discriminate bovine, porcine, and fish DNA in gelatin and confectionery products. *Food Science and Technology, 92*, 169–176.
Tasrip, N. A., Mohd Desa, M. N., Khairil Mokhtar, N. F., Sajali, N., Mohd Hashim, A., Ali, M., & Kqueen, C. Y. (2021). Rapid porcine detection in gelatin-based highly processed products using loop mediated isothermal amplification. *Journal of Food Science and Technology, 58*, 4504–4513.
Tukiran, N. A., Anuar, N. A. A., & Jamaludin, M. A. (2023). Gelatin in halal pharmaceutical products. *Malaysian Journal of Syariah and Law, 11*, 64–78.
Tukiran, N. A., Ismail, A., Mustafa, S., & Hamid, M. (2016a). Determination of porcine gelatin in edible bird's nest by competitive indirect ELISA based on anti-peptide polyclonal antibody. *Food Control, 59*, 561–566.
Tukiran, N. A., Ismail, A., Mustafa, S., & Hamid, M. (2016b). Development of antipeptide enzyme-linked immunosorbent assay for determination of gelatin in confectionery products. *International Journal of Food Science & Technology, 51*, 54–60.
Tukiran, N. A., Ismail, A., Mustafa, S., & Hamid, M. (2018). Indirect competitive enzyme-linked immunosorbent Assay (ELISA) for the determination of mammalian gelatin in pharmaceutical capsules. *In proceedings of the 3rd International Halal Conference (INHAC 2016)*, 429-439, Springer Singapore.
Uddin, S. M. K., Hossain, M. M., Sagadevan, S., Al Amin, M., & Johan, M. R. (2021). Halal and kosher gelatin: Applications as well as detection approaches with challenges and prospects. *Food Bioscience, 44*, Article 101422.
Usman, M., Ishaq, A., Regenstein, J. M., Sahar, A., Aadil, R. M., Sameen, A., & Alam, A. (2023). Valorization of animal by-products for gelatin extraction using conventional and green technologies: A comprehensive review. *Biomass Conversion and Biorefinery, 13*, 1–13.
Venien, A., & Levieux, D. (2005a). Differentiation of bovine from porcine gelatines using polyclonal anti-peptide antibodies in indirect and competitive indirect ELISA. *Journal of Pharmaceutical and Biomedical Analysis, 39*, 418–424.
Venien, A., & Levieux, D. (2005b). Differentiation of gelatins using pol'yclonal antibodies raised against tyrosylated bovine and porcine gelatins. *Journal of Immunoassay and Immunochemistry, 26*, 215–229.
Wang, S., Chen, H., & Sun, B. (2020). Recent progress in food flavor analysis using gas chromatography–ion mobility spectrometry (GC–IMS). *Food Chemistry, 315*, Article 126158.
Wardani, D. P., Arifin, M., Suharyadi, E., & Abraha, K. (2015). Quantitative detection of bovine and porcine gelatin difference using surface plasmon resonance based biosensor. *Optical Sensors, 9506*, 187–194.
Widada, H., Rohman, A., Jenie, R., & Sismindari, S. (2019). Application of graphene oxide on aptamer-based biosensor development for authentication of gelatin. *International Journal of Applied Pharmaceutics, 11*, 254–258.
Widyaninggar, A., Triyana, K., & Rohman, A. (2012). Differentiation between porcine and bovine gelatin in capsule shells based on amino acid profiles and principal component analysis. *Indonesian Journal of Pharmacy, 23*, 104–109.
Wu, L., Li, G., Xu, X., Zhu, L., Huang, R., & Chen, X. (2019). Application of nano-ELISA in food analysis: Recent advances and challenges. *Trends in Analytical Chemistry, 113*, 140–156.
Wu, W.-J., Li, L.-F., Fung, H.-Y., Cheng, H.-Y., Kong, H.-Y., Wong, T.-L., & Huo, C.-Y. (2022). Qualitative and quantitative qnalysis of ejiao-related animal gelatins through peptide markers using LC-QTOF-MS/MS and scheduled multiple reaction monitoring (MRM) by LC-QQQ-MS/MS. *Molecules, 27*, 4643.
Yang, P., Bi, Q., Li, Y., Liao, J., Ding, Y., Huang, D., & Zhang, J. (2023). Identification of five gelatins based on marker peptides from type i collagen by mass spectrum in multiple reaction monitoring mode. *Journal of Agricultural and Food Chemistry, 71*, 5851–5860.
Yap, B. K., & Gam, L.-H. (2019). Differentiation of bovine from porcine gelatin capsules using gel electrophoresis method. *Food Chemistry, 274*, 16–19.
Yuswan, M. H., Jalil, N. H. A., Mohamad, H., Keso, S., Mohamad, N. A., Yusoff, T. S. T. M., & Desa, M. N. M. (2021). Hydroxyproline determination for initial detection of halal-critical food ingredients (gelatin and collagen). *Food Chemistry, 337*, Article 127762.
Zhang, H., Liu, Z., Zhang, J., Zhang, L., Wang, S., Wang, L., & Hu, J. (2021). Identification of edible gelatin origins by data fusion of NIRS, fluorescence spectroscopy, and LIBS. *Food Analytical Methods, 14*, 525–536.
Zhang, H., Sun, H., Wang, L., Wang, S., Zhang, W., & Hu, J. (2018). Near infrared spectroscopy based on supervised pattern recognition methods for rapid identification of adulterated edible gelatin. *Journal of Spectroscopy, 2018*, 1–8.
Zhang, H., Wang, S., Li, D., Zhang, Y., Hu, J., & Wang, L. (2019). Edible gelatin diagnosis using laser-induced breakdown spectroscopy and partial least square assisted support vector machine. *Sensors, 19*, 4225.
Zhang, J., Lu, Y., Zheng, S., Ma, Z., Wu, M., Zhang, Y., & Cao, H. (2023). Identification of donkey-hide gelatin and donkey-bone gelatin based on marker peptides. *Food Science and Technology, 182*, Article 114881.
Zhang, J., Wu, M., Ma, Z., Zhang, Y., & Cao, H. (2022). Species-specific identification of donkey-hide gelatin and its adulterants using marker peptides. *PLoS One, 17*, Article 273021.
Zhao, G., Wang, J., Yao, C., Xie, P., Li, X., Xu, Z., & Shen, X. (2022). Alkaline lysis-recombinase polymerase amplification combined with CRISPR/Cas12a assay for the ultrafast visual identification of pork in meat products. *Food Chemistry, 383*, Article 132318.
Zhou, C., Wang, J., Xiang, J., Fu, Q., Sun, X., Liu, L., & Wang, J. (2023). Rapid detection of duck ingredient in adulterated foods by isothermal recombinase polymerase amplification assays. *Food Chemistry: Molecular Sciences, 6*, Article 100162.
Zhu, X., Gu, S., Guo, D., Huang, X., Chen, N., Niu, B., & Deng, X. (2023). Determination of porcine derived components in gelatin and gelatin-containing foods by high performance liquid chromatography-tandem mass spectrometry. *Food Hydrocolloids, 134*, Article 107978.