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Review Article

Porcine-Derived Ingredients in Cosmetic Products and its Halal Authentication Method within Complex Matrices

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Abstract: Nowadays cosmetics are an important commodity and the market for halal cosmetics is seeing growth. Cosmetics that contain porcine-derived ingredients are typically the source of halal problems. Gelatin and collagen are porcine derivatives that are extensively used in cosmetics. Hence, verifying the presence of porcine derivatives in cosmetics by developing analytical methods is critical. Despite this urgency, determining porcine-derived components in cosmetics is challenging, since cosmetics are quite complex with variable matrix forms. Moreover, to the best of our knowledge, there are only a few papers on developing porcine derivatives analysis in cosmetic items. This mini-review objective is to depict the current understanding of determining porcine collagen and gelatin in cosmetic matrixes. The findings revealed that the LC-MS/MS method is superior for determining gelatin and collagen sources in complex matrixes due to its sensitivity and accuracy. PCR and ELISA methods have challenges with the marker degradation problem since the derivatives undergo extensive processing conditions, thus lowering the methods' specificity and sensitivity, especially in complex matrixes. The SDS-PAGE method applications are limited and the method is suitable for a relatively simple matrix. This review highlights findings that support future advancements in cosmetic analysis for halal authentication.

Keywords: Halal Authentication; Cosmetic Matrixes; Porcine; Analytical Challenges

1. INTRODUCTION

Cosmetics are essential products in modern life due to increasingly high beauty standards [1]. They promote well-being, maintain health, improve beauty, and boost self-esteem. The cosmetics sector has had substantial growth and is predicted to continue to grow in the years to come. According to the cosmetic market analysis, the size of the worldwide cosmetics business was assessed at USD 374.18 billion in 2023 and is expected to increase from USD 393.75 billion in 2024 to USD 758.05 billion by 2032 [2]. Additionally, this notable improvement in the cosmetics industry as a whole was followed by the expansion of the global halal cosmetics market, which was estimated to be worth USD 42.39 billion in 2023 and is projected to reach USD 47.76 billion and USD 115.03 billion in 2024 and 2032, respectively, as the Muslim population has a big market size with about 2.4 billion people globally. These increases are driven by Muslims' growing receptivity to more Islamic teachings and the general public's growing understanding of the advantages of cosmetics. As a result,

consumers' desire for transparency regarding the components in cosmetics has increased, particularly concerning the halal material authenticity of ingredients used [3], [4], [5], [6]. Moreover, halal issues regarding cosmetic products are typically due to the source of animal-derived cosmetic ingredients. This issue emerges since some widely used ingredients in cosmetics use non-halal animals, such as pigs, as their main sources. For instance, in the gelatin industry, pig skin is the main source, accounting for about 46% of global gelatin production. Bovine hides come in second at 29.4%, while both pig and cow bones come in third at 23.1% [7].

Despite the urgency of determining animal-derived sources in cosmetic products, there are a number of obstacles. Firstly, cosmetic products are highly complex with variable matrix forms, consisting of numerous and diverse substances with their particular properties, making cosmetics a challenging matrix to analyze [35],[36]. Furthermore, due to the near compositional similarities across various sources, it is highly challenging to confirm the origin of animal protein compounds like collagen and gelatine for halal purposes and to identify any adulteration. Moreover, the degradation of protein and DNA biomarkers might happen since the production process of gelatine and collagen engages high temperatures and pressures. Further cosmetics production process involving complex processing conditions couples the matrix factor difficulties [10], [11], [12].

Therefore, the development of analytical methods performing specific, sensitive, and reliable results is highly desirable. Several analytical techniques have been developed to identify components generated from porcine in complex matrixes, including cosmetics. These analytical methods are liquid chromatography-mass spectrometry tandem mass spectrometry or LC-MS/MS [13], [14], polymerase chain reaction [15], [16], enzyme-linked immunosorbent assay (ELISA) [17], [18], [19], [20], and SDS-PAGE with or without the combination of nucleic-acid based method for less complex matrixes such as gelatine capsule shell [21], [22]. Nonetheless, to the best of our knowledge, there are only a limited number of publications that focus on the development of porcine derivatives analysis in cosmetic products, differing from highly processed food that is more extensively studied. This mini-review discusses the presence of animal-derived ingredients in cosmetics and focuses on the analytical methods to determine the presence of porcine-derived ingredients determination in cosmetic products, especially gelatin and collagen ingredients. The review also highlights some key sample preparation related to the method. This review objective is to depict the method's ability to discriminate animal protein sources from various origins in complex cosmetic matrix samples thus facilitating further development toward cosmetic analysis in the context of halal authenticity.

2. MATERIALS AND METHODS

The literature search was carried out using Scopus, PubMed, ScienceDirect, Google Scholar, and Google Search to find English-language publications that mainly discussed different cosmetics ingredients or the development of certain analytical techniques in determining the sources of gelatin or collagen within various forms of cosmetic matrixes. The methods chosen were the renowned and widely used methods that have good specificity, sensitivity, and reliability for animal source determination in complex matrices.

Several reports on the determination of porcine-derived gelatin or collagen using other complex matrix samples besides cosmetics were also used, if from the literature search, there are no reports of the application in cosmetic matrixes, yet the method is known to be potential. Despite that, these reports were already performed using complex matrix samples, for instance highly processed food,

and could depict the method's ability to discriminate animal protein sources from various origins in complex cosmetic matrix samples. The discussion also involves advantages, challenges, and limitations regarding the respective method.

3. RESULTS AND DISCUSSION

3.1. Non-halal animal-derived ingredients in cosmetics

Porcine is one of the restricted animal sources used in cosmetic products. Porcine-derived ingredients, such as lard, glycerin, fatty acids, gelatin, and collagen, are commonly used in cosmetic products. These ingredients play many roles in the production of cosmetic products. Lard is frequently used in lipid-based cosmetic products including creams, lotion, and lipstick, whereas glycerin contributes as a humectant, denaturant, skin protectant, and lowering viscosity agent [23], [24]. Fatty acids and their derivatives are primarily used as surface active agents in cosmetic cleansers and as components of the emulsion stabilization system to lower interfacial surface tension [25]. Gelatin and collagen themselves have a variety of purposes in cosmetics, whether as active agents to provide skin protection and nutrition or as supporting components to meet specific formulation needs such as gelling and viscosity-enhancing agents, that present in various forms of cosmetic products [26], [27], [28].

Gelatin is made from the source protein collagen. Gelatin is a heterogenous blend of peptides that is produced by partially breaking polypeptide connections and eliminating cross-links between polypeptide chains. Collagen itself is the most prevalent structural protein in both vertebrates and invertebrates and composes about 30% of an animal's overall proteins [26]. Collagen is present in animal skin, tendons, cartilaginous tissues, and mammalian bones such as pigs, cows, donkeys, and horses. Furthermore, it is present in fowl including chickens, ducks, wilds, and turkeys as well as aquatic animals like tuna, sea cucumber, and jellyfish [29]. Gelatins and collagen found in the market are primarily derived from pigs as they are economical and also have superior physical and functional properties compared to other sources [30], [31], [32].

Different ideas for innovative cosmetics have emerged. The use of oral supplements to improve appearance is known as nutricosmetics. They are frequently called "oral cosmetics," "beauty pills," or "beauty from within." [33], [34], [35]. In the last few years, a variety of skincare products have used gelatin and collagen as excipients or as advantageous ingredients for beauty and appearance. Gelatin's special physicochemical properties, such as foaming, stabilizing, thickening, gelling, emulsifying, and binding, have led to its widespread application in pharmaceuticals and cosmetics [28]. Soft gelatin capsule is the most commonly utilized pharmaceutical form, behind tablets. Numerous dietary supplements are frequently encapsulated in gelatin capsules [21], [36]. Additionally, a wide range of cosmetic goods, such as bubbles, face creams, body lotions, shampoos, hair sprays, sunscreens, and bath salts, use gelatin as a gelling agent [37]. Furthermore, gelatin has also been utilized to protect the skin from UV radiation damage. The balanced lipids of the skin are maintained by gelatin through its antioxidant properties, which help to correct the damage to the skin's structure [27], [38]. The antioxidant enzymes that contribute to building up the body's defenses against oxidative stress are diminished when exposed to ultraviolet light. The study claims that employing gelatin hydrolysates will greatly boost the antioxidant enzymes' activity [27].

Collagen has been identified as a possible treatment for wrinkles and the aging process. One of the main indicators of the decline in skin quality with age is a reduction in collagen and elastin fibers,

which results in elasticity loss and wrinkle formation [26], [39]. Aging also causes the skin to produce less glycosaminoglycan (like hyaluronic acid), which makes the skin dry and loses its integrity since hyaluronic acid is the main molecule involved in skin moisture because of its unique capacity to hold water molecules [40], [41], [42]. A systematic review and meta-analysis were carried out by Pu et al. (2023) [43] to assess the impact of collagen supplements on skin aging. 26 RCTs were examined to assess how oral collagen supplements affected skin elasticity and hydration, two characteristics that characterize skin aging. Their study found that oral collagen supplements increased the moisture and elasticity of the skin, with noticeable improvements after eight weeks or longer of collagen supplementation. Another study by Sanz et al. (2016) [44] examining the influence of applied topically collagen on the process of aging found that approximately 75% of women treated with an applied topical product containing collagen proved anti-wrinkling effects and significant increases in the dermal density and elasticity of their skin when compared to those in the control group. In a similar vein, the study by Maia Campos et al. (2019) [45] revealed that healthy female participants who applied a topical product showed a substantial increase in skin elasticity and moisture.

Concomitantly with the advancement of technology, animal-derived-containing products have been extensively developed and are currently being utilized in the cosmetic and pharmaceutical industries. Consequently, the halal status of these products may be questioned. The acceptability of cosmetics made with animal-derived components usually depends on the type of animal from which the components are produced to meet religious restrictions. Halal itself denotes permissible things that are lawful by Islamic law, whereas non-halal or haram refers to those that are forbidden [6]. Generally speaking, a product is considered non-halal under Muslim law if it contains elements obtained from pigs or other animals that are not slaughtered in accordance with Islamic law [31], [46]. Muslims are severely forbidden from using or consuming any products that include non-halal components, even in trace amounts because halal products have a zero-tolerance policy [47]. Additionally, halal takes into account the entire process, from product manufacturing to distribution, rather than just the contents. In general, according to The Indonesia Council of Ulama and Malaysia Standard of Halal Pharmaceuticals, halal products need to adhere to a number of key principles, which are:

a. It should not include any animal parts or products that are not halal or that have not been killed in accordance with Islamic law.
b. Absent najs in accordance with Islamic law.
c. Demonstrate safety and effectiveness for humans with the recommended dosage, quality, and hygiene.
d. In accordance with Islamic law, was not made, processed, or prepared using machinery contaminated by najs. Halal requirements and Islamic law should be followed throughout the entire production process.
e. Don't include any human parts or derivatives, as this is prohibited by Islamic law.
f. Halal products are physically separated from any other products that do not meet the requirements listed in items a, b, c, d, e, or any other items that have been declared non-halal and najs by Islamic law during all processes, including preparation, processing, handling, packaging, storing, and distribution. Any potential cross-contamination must be prevented [6], [48], [49].

Halal certification can be viewed as an added benefit since the main criteria are the effectiveness, safety, and quality of all products [6]. Because of this, halal products are starting to become the new norm for safety and quality control, even among non-Muslim customers [24], [50]. The halal certification requires a proof document that shows the ingredients used or the final product does not contain any non-halal animal-derived ingredients. Thus, the analytical methods to determine this target analysis are highly in demand.

3.2. Analytical method for animal-derived identification in cosmetics

Various analytical techniques have been developed to identify components derived from porcine in complex matrixes, including cosmetics. These methods have different approaches and principles in determining the sources of gelatin and collagen with respective benefits and limitations. Liquid chromatography-mass spectrometry tandem mass spectrometry (LC-MS/MS) using specific peptide analysis for identification, polymerase chain reaction (PCR) exploring specific markers of the DNA, ELISA is dependent on antigen-antibody interaction, and SDS-PAGE relies on the molecular weight of protein. Table 1 compiles the benefits and limitations of respective analytical methods. Further respective descriptions of the methods are discussed below.

Table 1. List of some analytical methods for identifying porcine-derived components in complex matrixes along with each method's advantages and disadvantages

Methods	Advantages	Disadvantages	References
LC-MS/MS	• Precise mass measurement, superior sensitivity, and good resolution enable accurate identification compared to other techniques • Suitable for identifying complex and highly processed matrixes	• More expensive compared to other techniques • Time-consuming • Requiring specialized knowledge	[51], [54], [55]
PCR	• Easy to perform and frequently used for species identification • More affordable than the LC-MS/MS method	• Less sensitive and specific than LC-MS/MS especially in complex and highly processed matrices like cosmetics. • The quality of DNA isolates and DNA degradation can have an impact on the test result • The PCR approach is unable to identify the degree of contamination in a gelatin sample because DNA detection is not directly correlated with the amount of protein or peptide present in a sample.	[13], [54], [56], [57]
ELISA	• Easy to perform • Using low-cost reagents, therefore more affordable to perform than the LC-MS/MS method • Offer benefits over methods based on nucleic acid as proteins may have a primary structure that is more stable than DNA	• Less sensitive and specific than LC-MS/MS especially in complex and highly processed matrices like cosmetics • Heat processing may alter the original structure of the antigen's epitopes, making it more difficult for the antigen to be recognized by its particular antibody.	[13], [58], [59], [60], [61], [62]

continued Table 1..		• If certain antibodies are not readily available, it could take months to produce them	
SDS-PAGE	• Easy to perform • Inexpensive method	• Only suitable for relatively simple matrix	[21]

3.2.1. LC-MS/MS

In recent years, the liquid chromatography-mass spectrometry tandem mass spectrometry (LC-MS/MS) method using specific peptide analysis for identifying the animal sources of gelatin has garnered significant attention for development. Due to variations in amino acid sequence, mass spectrometry enables the identification of the animal sources of gelatin accurately [51]. As previously mentioned, collagen partially hydrolyzes to produce gelatin, with type I collagen being the most prevalent collagen in connective tissue among all 28 collagen types [52]. Because hydrolyzed type I collagen is the most common type of collagen, gelatins may contain peptides from this type of collagen. According to sequence alignment findings, the type I collagens of pig and cow, which are two of the most widely available gelatin sources, exhibit high similarity with 99% sequence similarity. Nonetheless, a small number of amino acid variations exist, allowing it possible to distinguish between the gelatin origin of porcine and bovine based on peptides derived from type I collagen [12], [53]. Table 2 tabulated several potential marker peptides for distinguishing between bovine and porcine gelatin.

Table 2. Several potential marker peptides to differentiate between porcine and bovine gelatin

Potential marker peptides	References
GRPGPPGPAGAR GEPGPTGVQGPPGPAGEEGK GSPGPAGPK QGPSGPSGER DLEVDTXLK GPNGEVGSAGPPGPPGLR GFPGSPGNVGPAGK GAPGPDGNNGAQGPPGPQGVQGGK GIPGEFGLPGPAGPR IGPPGPSGISGPPGPPGPAGK TGETGASGPPGFAGEK GPPGAVGNPGVNGAPGEAGR	[12]
GPPGESGAAGPAGPIGSR GSPGADGPAGAPGTPGPQGIAGQR	[63]
GEPGPAGSVGPAGAVGPR GPTGPAGVR GETGPAGPAGPVGPVGAR	[12], [63]

The determination of gelatines from various products through LC-MS/MS is conducted by extraction of gelatin from matrix samples, after which the extracted gelatin undergoes digestion with proteases like trypsin. The resulting peptides then are separated using liquid chromatography and subsequently sequenced through various databases available as part of the instrument to analyze the animal source of gelatin [54]. The comprehensive sample preparation procedure for gelatin extraction from cosmetic samples is presented in Figure 1.

In the sample preparation protocols for gelatin extraction (Figure 1), hexane is added to the sample to separate lipid contaminants which are commonly present in certain types of samples, such as cosmetics [13], [64]. Additionally, to examine the gelatin in the capsule shell, the sample is first washed with milli-Q water in order to remove the remaining residual contents of the capsule. The capsule shell is then dried, cut, and gone through further sample preparation [54], [60]. Unlike bottom-up proteomics protocols within meat samples which need reduction and alkylation steps, in order to break disulfide bonds and alkylate cysteine groups to prevent them from forming undesirable new disulfide bonds, in the gelatin-containing samples analysis, gelatin does not require reduction and alkylation steps, since gelatin proteins are already hydrolyzed during the production process [64], [65]. Subsequently, the protein digestion stage is performed by using trypsin enzyme in an ammonium bicarbonate solution at 37 - 40° C for 12 – 24 h [13], [14], [66], [67]. Various digestion methods have been developed to overcome this time-consuming conventional digestion process including the employment of ultrasound and microwave digestion methods thus speeding up the process from an overnight digestion to just a matter of minutes [68], [69].

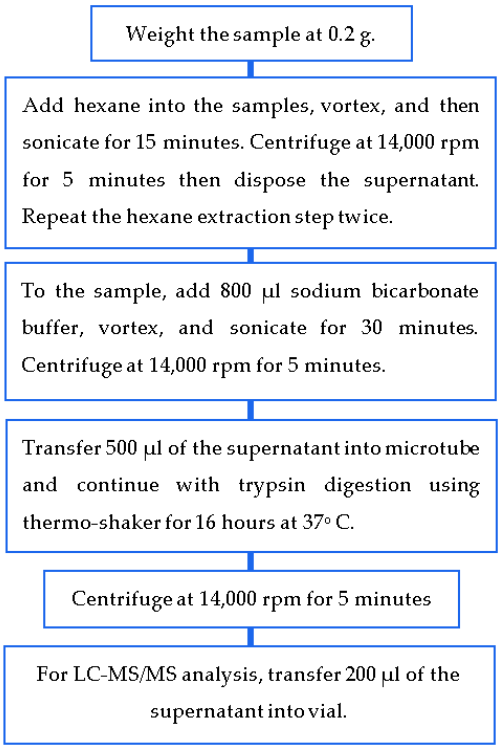

Figure 1. Sample preparation procedures for gelatin extraction from cosmetic samples using conventional digestion method [13]

Figure 2 illustrates the novel digestion approach procedures. However, these protocols need further optimization as the study demonstrated that compared to microwave and ultrasonic digestion, conventional digestion produced a higher detection rate [69]. Chia et al. (2020) [13] combined a UHPLC-fast separation with an SRM-based targeted method to develop a testing protocol on the utilization of triple quadrupole LC-MS/MS technology for detecting the presence of porcine gelatin in cosmetic and food confectionary samples. In order to evaluate the robustness sensitivity, and accuracy of the SRM-based LC-MS/MS method for identifying porcine gelatin, a number of tests were carried out using standard reference gelatin material as well as actual cosmetic and food samples. Firstly, the developed method was used to examine reference gelatin standards

derived from bovine and porcine sources. The results showed that no porcine markers were found in the bovine gelatin standard, while all 11 porcine peptide markers, that had been discovered in their previous study, were successfully identified in the porcine gelatin standard. To determine the positive identification of porcine gelatin, all SRM transition peaks must be correctly aligned, retention times must be matched, and the correct ion ratio between different ions for the same peptide marker. This application of the three levels of identification criteria guarantees reliable and accurate results, even at low detection limits.

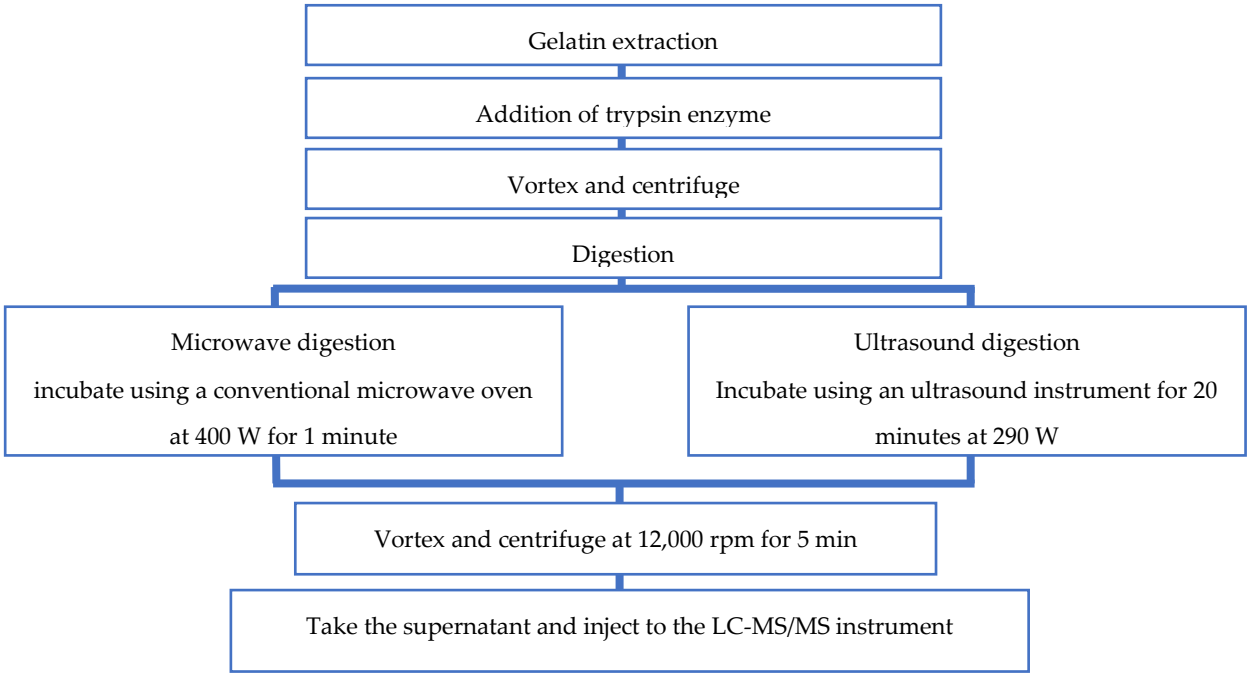

Figure 2. Microwave and ultrasound digestion protocols [69], [70], [71]

The method's performance under different matrix conditions was assessed by spiking porcine gelatin standard in hair moisturizer cream and facial gel samples to mimic different contamination levels (spiked solution concentrations of 0.01 %, 0.05 %, 0.1 %, 0.5 %, 1.0 %, and 5.0 %). As a result, even at the lowest concentration of 0.01% in the spiked sample, all 11 porcine peptide markers were detected. The method achieved a remarkable linear calibration curve, with an r^2 value exceeding 0.99 and the inter-day analysis of the samples demonstrated good reproducibility. Furthermore, a comparative study between the SRM-based LC-MS/MS method and the ELISA method was conducted on 27 different commercially available food and cosmetic products containing gelatin with the goal of examining the developed method's accuracy. For the majority of samples containing gelatin, the results indicated a positive correlation between the LC-MS/MS and ELISA methods. However, two cosmetic samples showed inconsistent results using the ELISA method and were confirmed to be negative for porcine peptides using the LC-MS/MS method. The LC-MS/MS method's feasibility for analyzing porcine gelatin in such challenging various cosmetic and food matrixes was confirmed by the experiment. According to the experiment's findings, LC-MS/MS demonstrated a sensitive, robust, and reliable method for identifying porcine derivatives in cosmetic and food products [13].

The presence of Hyp may influence the judgment findings of amino acid sequence, potentially resulting in inaccurate animal origin identification [54]. For instance, in bovine COL1A2 with the

marker peptide sequence $G^{308}AAGLP^{313}GVAGAPGLPGPR^{325}$, whereas the sequence $G^{310}AACLL^{315}GVAGAPGLPGPR^{327}$ is present in porcine COL1A2. In the process of determining the peptide sequence, if Pro313 hydroxylation (+16 mass) occurred, it could be mistaken with a similar mass of amino acids, such as Leu and Ile [51], [53]. Therefore, Jumhawan et al. (2019) [14] developed an LC-MS/MS method using the multiple reaction monitoring (MRM) method for the identification of porcine and bovine gelatins in pharmaceutical, cosmetic, and food products. With MRM, which is a modified form of SRM, several SRM transitions for the same or distinct analytes can be tracked in a single mass spectrometry run. MRM can recognize post-translational modifications (PTMs) and differentiate between very similar protein forms by monitoring several transitions in a single run. For protein identification in complex matrixes, MRM is therefore preferred [72]. Jumhawan et al. (2019) [14] study performed in silico MRM transitions prediction of bovine and porcine peptides using Skyline software. Skyline software can be used to identify the location and amount of proline hydroxylation in order to obtain all MRM transitions and to identify the variations in peptides caused by the hydroxylation of proline [54]. Table 3 shows the prediction of bovine and porcine marker peptides performed in the study. The prediction test indicates that the precursor and product ion mass-to-charge ratios (m/z) of porcine and bovine peptides are identical. Peptide sequences from porcine (GPPGSAGAPGK) and bovine (GPPGSAGSPGK) gelatins exhibit slight discrepancies, differ only by a single amino acid (the 8th amino acid), and unpredicted proline hydroxylation to gelatin occurrence may result in peptides with the same mass and identical MRM transitions for both species.

Table 3. In silico MRM transitions prediction of bovine and porcine marker peptides using Skyline software. Bold, underlined proline (P) signifies proline hydroxylation [14]

Peptide Marker	Sequence	Precursor (m/z)	Product (m/z)	
Bovine Specific Marker	GPPGSAGSPGK	456.2327++	y10	854.4367+
			y9	757.3839+
			y3	301.1870+
Porcine Specific Marker	G**P**PGSAGAPGK	456.2327++	y10	854.4367+
			y9	741.3890+
			y3	301.1870+

Moreover, Jumhawan et al. (2019) [14] study performed a method validation. Following sequence verification, 8 and 9 peptides were discovered to be specific for porcine and bovine gelatine, respectively. Every peptide marker performed good repeatability (RSD < 15%). The developed method can successfully identify adulteration levels in spiked bovine and porcine gelatin matrixes as low as 0.1%. Additionally, the method has shown success in determining porcine and bovine gelatin in the test of commercial goods (encompassing pharmaceutical capsules, personal care, and food products). In conclusion, the developed method provides a reliable strategy to identify and specify animal-based gelatins that enforce halal testing [14]. Overall, the LC-MS/MS method offers excellent resolution, high sensitivity in complex matrixes, and accurate mass measurement. Despite that, this method has several drawbacks such as not being widely available in laboratories, being time-consuming, high cost, and need for expertise [51], [54], [55].

3.2.2. Polymerase Chain Reaction

Because of its sensitivity and selectivity, the polymerase chain reaction (PCR) method is frequently used to identify species by identifying animal DNA present in the sample [54]. Since DNA

is unique and species-specific, enabling accurate identification of porcine-derived content, thus PCR is one of the established methods for identifying porcine-derived in different products. The use of PCR to identify the source of gelatin in powder and capsule shells has been the subject of numerous studies, whereas the use of PCR to determine gelatin and collagen in more complex cosmetic matrix samples has not yet been extensively performed. Table 4 compiled the use of PCR methods along with reported porcine primers for the analysis of gelatin in capsule shells.

Table 4. Several applications of PCR methods and primers for gelatin analysis in capsule shell samples

PCR Method	Applications	Porcine Primers	References
Conventional PCR	Identification of bovine and porcine gelatin in gelatin powders and gelatin capsule shells	Target gene: Cyt b 5′-GCCTAAATCTCCCCTCAATGGTA-3′ 5′-ATGAAAGAGGCAAATAGATTTTCG-3′	[70]
Real-time PCR	The identification of gelatin powders and capsule shells of bovine and porcine origin	Target gene: Cyt b 5′-CAACCTTGACTAGAGAGTAAAACC-3′ 5′-GGTATTGGGCTAGGAGTTGTT-3′	[71]
Multiplex PCR-RLFP	Differentiation of the gelatin sources of bovine, porcine, and fish in capsules	Target gene: Cyt b 5′-GGTAGTGACGAAAAATAACAATACAGGAC-3′ 5′-ATACGCTATTGGAGCTGGAATTACC-3′	[72]
Duplex PCR	Identification of bovine and porcine DNA in gelatin capsules	Target gene Cyt b 5′-ATGAAACATTGGAGTAGTCCTACTATTTACC-3′ 5′-CTACGAGGTCTGTTCCGATATAAGG-3′ Target gene: Cyt b 5′-GCCTAAATCTCCCCTCAATGGTA-3′ 5′-ATGAAAGAGGCAAATAGATTTTCG-3′	[73]

Zabidi et al. (2020) [16] developed methods for detecting porcine DNA in collagen cream cosmetic products by using conventional PCR with species-specific primer and electrophoresis gel. The study used a set of porcine-specific primers of 12SFW (5′-CCACCTAGAGGAGCCTGTTCT(AG)TAAT-3′) and 12SP (5′-GTTACGACTTGTCTCTTCGTGCA-3′) with the amplicon length of 387 bp. The study performed an optimization of primer annealing temperature by gradient the annealing step at 40 – 70^{o} C. Subsequently, PCR products were analyzed using electrophoresis gel in 1.0% (w/v) agarose gel. According to electrophoresis analysis, the annealing temperature of 44.4^{o} C exhibited the highest band intensity. Therefore, 44.4^{o} C was determined as the optimum annealing temperature and will be used for subsequent PCR assays. The analysis in cream sample products showed the presence of PCR amplicon, around 387 bp, in raw pork (positive control), piggy collagen cream, and hand cream which contains collagen from an unknown source. The study demonstrated that even though the cosmetic samples generally contained very small amounts of highly degraded porcine DNA, the developed method can detect and amplify the DNA.

Even though PCR is considered to be a selective and specific technique, the quality of the DNA used can affect the results [56]. Several components that may be found in cosmetic products including alcohol, metal ions, fats, pectin, detergents, and polysaccharides may interfere with PCR [73], [74], [75]. As a result, the DNA extraction method that is used should minimize these PCR inhibitors that

might be present in the samples [76]. There is a limited number of studies that have studied the efficiency of DNA extraction kits used in cosmetic sample preparation. Kim et al. (2018) [15] designed a real-time PCR method to identify porcine DNA in halal cosmetics and measured the real-time PCR detection limit to compare the effectiveness of different DNA extraction techniques. Figure 3 illustrates the workflows of RT-PCR to identify the presence of porcine DNA in cosmetics containing gelatin. A set of primers Sus NDH5 was developed in order to identify pig DNA in cosmetics (F: GCC TCA CTC ACA TTA ACC ACA CT and R: AGG GGA CTA GGC TGA GAG TGA A) with the amplicon size of 139 bp. Taqman probe (GGC GTA GGA TAY CCT CGT TTT TAC GT) was used in this experiment, labeled at the 5' and 3' ends with 6-carboxyfluorescein and black hole quencher 1, respectively. The specificity of the NDH5 primer was investigated by testing it with four bacterial strains nine vegetables, and eight different kinds of meat. The specificity result showed that, with the exception of the pig, no florescent signal was observed in non-target samples even after 40 cycles, proving the developed method is specific and facilitates the detection of porcine components in cosmetics. Additionally, tests conducted on 15 beauty goods revealed that none of the 15 samples examined using the developed rt-PCR contained any traces of pig DNA.

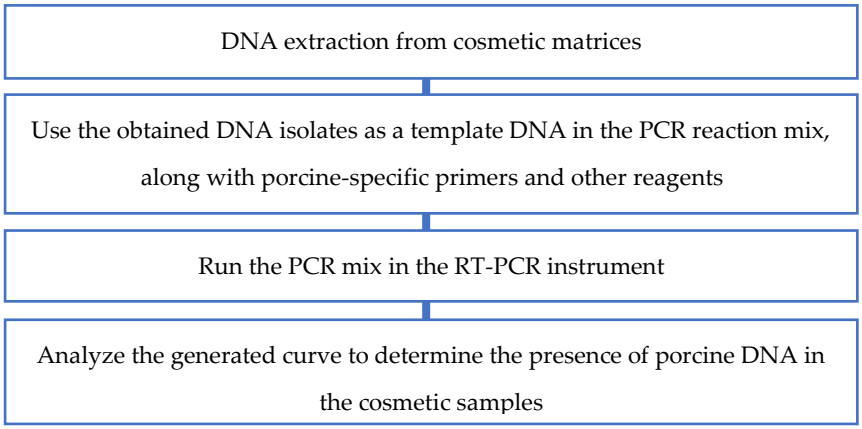

Figure 3. RT-PCR workflows of the source identification of gelatin in cosmetic matrices sample

Kim et al. (2018) [15] also conducted a test to examine how DNA extraction techniques affected real-time PCR amplification. Initially, cosmetic samples that will be spiked were confirmed with the developed method to detect the absence of porcine DNA. The confirmed cosmetic samples were then purposefully spiked with serially diluted porcine template DNA, then the DNA was extracted employing six distinct DNA extraction procedures (CTAB; Power Prep™ DNA extraction kit; QIAamp DNA stool mini kit; Wizard genomic DNA purification kit; TIANamp genomic DNA Kit; Nucleo spin food kit). Porcine DNA taken from the modified cosmetic samples was subjected to real-time PCR in order to investigate the trial's limit of detection. This LOD is then utilized to depict the performance of respective DNA extraction techniques. According to the findings, the rt-PCR method could identify the template DNA as low as 2.28×10^0 copies for the liquid-type mask pack using the Power PrepTM DNA extraction kit (Ct value of 38.51) and TIANamp Genomic DNA kit (Ct value of 38.71), which provide rt-PCR sensitivity 10–100 times higher than alternative extraction techniques. The rt-PCR limit of detection for the powder-type mask is 2.28×10^1 copies using the QIAamp DNA stool mini kit (Ct value of 37.63) and Power Prep™ DNA extraction kit (Ct value of 39.07). For both

kits, the rt-PCR limit of detection is 10–100 times greater than that of the alternative extraction techniques. Moreover, rt-PCR showed a limit of detection of 2.28 x 10^0 copies (Ct value of 39.07) when using the Power PrepTM DNA extraction kit on cream samples. This was 100–1000 times higher than the other extraction kits. Essential to note that hexane or chloroform treatment during the DNA extraction process results in fewer PCR inhibitors in cosmetic samples with high lipid content [77]. Hence, the Power Prep™ DNA extraction kit, which employs chloroform to extract DNA, may perform better yield in rt-PCR analysis. Therefore, this type of extraction kit can become a preferable option for isolating DNA within the gelatin component in cosmetic matrix samples. Additionally, Gina et al. (2024) [78] evaluated the effectiveness of two commercial DNA extraction kits to isolate DNA from gelatin powder, namely the DNeasy Mericon Food Kit (DM kit) and the Processed Food DNA Extraction (PF kit). DM kit was selected because it has been utilized to extract DNA from gelatin powder matrixes and drug capsules in several studies, whereas PF kit even though was developed for processed food products and has not been specifically reported for gelatin extraction, it may show effective in isolating DNA from gelatin matrixes. Nevertheless, the result showed that the PF kit produced a much higher DNA concentration than the DM kit, with 34.03 µL and 4.25 ng/µL, respectively. PF kit also gave better DNA purity levels in A260/280 and A260/230 ratios.

Unfortunately, because the experiment was conducted on cosmetic samples that had been spiked with serially diluted porcine DNA from meat samples, Kim et al. (2018) [15] experiment did not capture the comparison of the various extraction methods in terms of their performance to extract DNA from actual gelatin contained in cosmetics. Moreover, Gina et al.'s (2024) [78] experiment compared DNA extraction kits using gelatin powder rather than more complex cosmetic matrixes. Nonetheless, since the performance comparison of extraction methods on gelatin matrixes and cosmetics was limited to a small number of experiments, both studies provide an essential overview of DNA extraction methods' performance in highly complex samples and gelatin matrixes as well as depict the methods' potential ability to isolate DNA from gelatin contained in cosmetics. Additionally, both papers provide extraction kit choices that give favorable DNA isolate compared to other kits. Further experiment is needed to explore and compare how efficiently DNA extraction methods extract DNA from actual gelatin contained in cosmetics.

PCR method has several challenges as employing PCR to identify the origin of gelatin species has been proven to be quite challenging [13], [57]. DNA degradation is thought to be the primary factor influencing PCR success. This is a result of the high temperature and pressure involved during the processing of collagen into gelatin, leading to the almost complete destruction of any DNA present or DNA degradation into small fragments [10], [11], [13]. Furthermore, as the amount of protein or peptide in a sample is not directly correlated with the presence of DNA, the PCR method cannot be used to assess the degree of contamination in a gelatin sample [13].

3.3. ELISA

The other widely used technique is based on proteins and is known as ELISA (enzyme-linked immunosorbent assay). The basis of the ELISA analysis is the identification of specific antigens to proteins of the targeted species which is enabled by antigen-antibody interaction [13], [54]. Due to its sensitivity, specificity, and accuracy in identifying antigenic proteins, the ELISA method has been employed as a means of authenticity verification [58], [59]. The primary structure of proteins may be more stable than that of DNA, which can degrade under complicated processing circumstances and affect the identification result of the DNA-based gelatin authentication method [62]. Additionally,

the ELISA approach is a good alternative to other methods since it is simple to use, inexpensive reagents are available, and it can screen or quantify plenty of samples for the presence of target analytes [61]. Several studies have employed the ELISA method to detect gelatin in both raw and processed samples. Compared to several other formats of ELISA, indirect ELISA has been widely used for the determination of gelatin sources [18], [19], [20]. Figure 4 illustrates the indirect ELISA workflows for gelatin source differentiation. In indirect ELISA, a primary antibody that binds to the target protein is combined with a secondary antibody that is specific to the primary antibody. Before adding a primary antibody that will bind to the samples, the gelatin samples (antigen) on the microplate surface should be immobilized. Secondary antibodies that have been conjugate-labeled are then added to the primary antibodies in order to bind to them [79], [80].

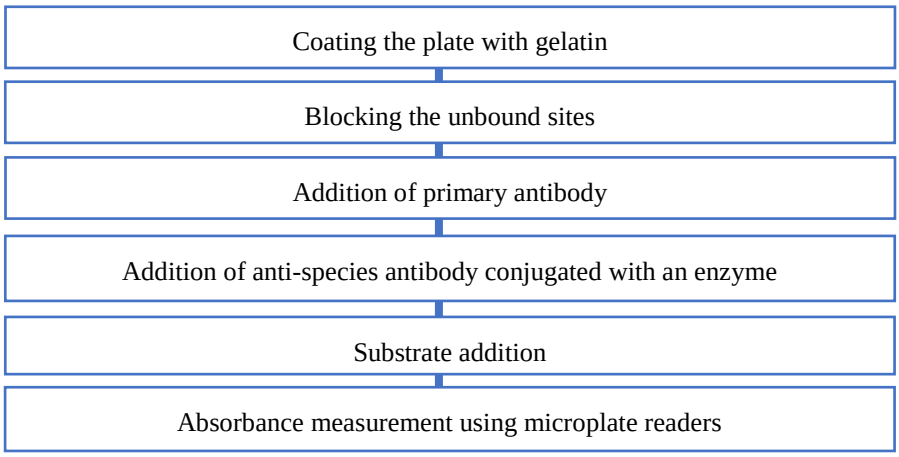

Figure 4. Indirect ELISA workflows for gelatin source differentiation

Using polyclonal anti-peptide antibodies, Venien & Levieux (2005) [20] created indirect ELISA formats to distinguish between raw bovine and porcine gelatines which can illustrate the ELISA method's ability to characterize the species origin of the gelatin. The two suggested sequences of bovine collagen, Peptide 1 (Glu-Phe-Asp-Ala-Lys-Gly-Gly-Gly-Pro-Gly) and Peptide 2 (Gly-Pro-Ala-Gly-Ala-Pro-Gly-Pro-Pro-Gly), were synthesized and used as the immunogens. Additionally, gelatins were also immunized to the rabbit, but because gelatin has very little immunogenicity, the molecule should undergo chemical alteration in order to produce antibodies in rabbits. Thus, tyrosylation was applied to the gelatins [81]. Then, at monthly intervals, rabbits (four rabbits for each antigen) received threefold immunization. Following each booster injection, the animals were bled seven to nine days later, and the sera were examined using indirect and competitive indirect ELISA to determine the antibody activity and specificity. The results showed that when used as coating antigens, the indirect ELISA performed using anti-peptide 2 antiserum enabled unambiguous discrimination among all of the bovine and porcine gelatines. On the other hand, when tested against collagen and gelatin, the anti-peptide 1 antibodies showed a low degree of reactivity. Furthermore, there was no discernible difference in the reactivity of bovine and porcine gelatins when utilizing a rabbit antiserum against bovine tyrosylated gelatins. This indirect ELISA could be used as a simple and rapid identification essay. Nevertheless, this format assay is unsuitable for identifying small quantities of bovine gelatin within porcine gelatin, as the results obtained from testing mixtures of bovine and porcine gelatin were relatively unsatisfactory. In contrast, the indirect competitive ELISA format that was designed exhibited a high sensitivity of 2 to 4 parts per 1000 for detecting bovine gelatines in pig gelatines that were bought from suppliers of laboratory chemicals. Additionally,

when industrial batches were examined, the sensitivity was 8 parts per 100 bovine gelatines in porcine gelatines.

Even though ELISA has several advantages, there are a number of obstacles to overcome. For instance, heat processing that denatures a target antigen may alter the original structure of its epitopes, making it more difficult for the antigen to be recognized by its particular antibody. According to a study by Doi et al. (2009), which used pAbs from rabbits (pAb1 and pAb2) and goats (pAb3) that were previously immunized using bovine gelatin as immunogen, the developed sandwich ELISAs reacted with porcine gelatin with a same or higher degree than that with bovine gelatin because the structures of the two gelatins are thought to be similar or their epitope areas are very similar. The developed method also has a limited specificity as it showed a low cross-reactivity to raw pork and cross-reacted with heated pork meat, goat, venison, boar, and rabbit meats. The high cross-reactivity with heated foods was most likely caused by the denatured collagen in meats and subsequently recognized by the antibodies used in this study.

Several approaches have been put out to get around the limitations. Among these is the discovery of thermostable proteins coupled with the production of particular antibodies directed against them. Since these thermostable proteins are present in both raw and highly processed samples, they can be utilized as target antigens for antibodies in the ELISA [82], [83], [84]. Additionally, the use of polyclonal antibodies is recommended over monoclonal antibodies for the identification of denatured proteins, especially in processed samples, due to their wider recognition of various epitopes and increased tolerance to minute changes [85]. For example, some processing methods may change or affect the accessibility of crucial epitopes, which could impair the immunoassay's capacity to identify antibodies [86], [87].

As far as we know, not much study has been performed to date examining the ability of ELISA to verify the origins of porcine gelatin in complex cosmetic samples. However, the ELISA approach has been performed for the detection and quantification of gelatines in relatively simple cosmetic matrixes, such as capsule shells, and various food matrix samples. Table 5 compiles several ELISA formats for the identification of gelatin in various matrixes. Therefore, these studies could have depicted the ability of the ELISA method to differentiate gelatin sources from various origins in complex cosmetic matrix samples. Overall, the use of the ELISA method for gelatin source identification is achievable although it is quite challenging.

Table 5. Some ELISA methods applied for the identification of gelatines in various matrix samples

ELISA Formats	Application	Remarks and Comments	References
Indirect Competitive ELISA	Determination of mammalian gelatin in capsules	All of the developed pAbs (pAb1 & pAb2), using porcine collagen sequences as the immunogens, showed cross-reactivity with more than 58% and 76% towards bovine gelatin from skin and bone, respectively. pAb1 showed cross-reactivity for more than 64% and 20% towards fish and chicken gelatins, respectively, while pAb2 exhibited <1% of cross-reactivity to both fish and chicken gelatin. Unfortunately, the study has limitations in the context of differentiating gelatin sources from porcine and bovine since all of the developed pAbs have cross-reactivity toward bovine, although pAb2 can discriminate mammalian from fish and chicken sources.	[88]

continued Table 5…

Competitive Indirect ELISA	Determination of porcine gelatin in edible bird's nest	Polyclonal antibodies (pAbs) from rabbits immunized with collagen amino acid sequences specific to pig species (pAb1, pAb2, and pAb3) were used to develop ELISA techniques. The results showed that pAb3 has superior selectivity among all of the developed pAbs. Moreover, pAb3 is sufficient for EBNs authentication from gelatin adulterants as pAb3 could identify porcine, bovine, and fish gelatines in EBN matrixes. However, the developed methods were not appropriate for detecting porcine gelatin for halal authentication since each pAb exhibited cross-reactivity with fish and bovine gelatines.	[18].
Competitive indirect ELISA	Determination of gelatin in confectionery products	The ELISA method is developed using porcine collagen immunogen to produce polyclonal antibodies. ELISA method performed low-cross reaction to fish and chicken gelatins but cross-reaction with bovine gelatin. The developed method is not appropriate for the identification of the presence of porcine gelatin as the developed pAbs showed cross-reactivity with bovine gelatin.	[19]
Sandwich ELISA	Determination of bovine and porcine gelatin in processed food	Novel sandwich ELISA methods were developed by using pAbs from rabbits (pAb1 and pAb2) and goats (pAb3) against bovine gelatin as an immunogen. Two sandwich ELISAs were performed by using pAb1-pAb2 and pAb3-pAb3. The cross-reactivity profiles of the two ELISAs were different. Both ELISAs showed positive responses toward boiled meat samples, although pAb3-pAb3 ELISA did not produce a positive response with boiled chicken. Furthermore, pAb3-pAb3 ELISA performed less cross-reactivity with boiled squid and various seafood and also produced weaker cross-reactivity with cooked meat. No negative nor false positives (except for gelatinized heated meat) were produced by the pAb3-pAb3 ELISA method. However, these developed methods mainly explored the ELISA's potency to differentiate bovine and porcine gelatin from fish gelatin in processed samples, as the results showed that both developed ELISA methods reacted with bovine and porcine gelatin. Therefore, the method has its limitations in the facet of halal authentication application.	[17]

3.3.1. Polypeptide Molecular Weight

Analytical techniques for the identification of the animal origin of gelatin are complex and expensive. Therefore, the development of simpler and less expensive methods to differentiate bovine and porcine gelatins is needed, especially in samples that do not have numerous and various components such as gelatin capsule shells in nutricosmetic or "oral cosmetic" products. Polyacrylamide gel electrophoresis (PAGE) is the most widely utilized technique for separating and assessing the molecular weight of proteins and peptides since it is simple to use and efficient [21]. Yap & Gam (2019) [22] developed a simple gel electrophoresis method to differentiate between gelatin capsules from cows and pigs using ammonium sulfate precipitation. In gelatin capsule identification, the sample preparation steps are relatively simple. Firstly, the content of the capsule is removed, then the capsule is cut and cleaned using blotter paper. Subsequently, the cut gelatin

capsule is weighed and dissolved. The result of the gel electrophoresis experiment showed the respective bands characteristic of porcine and bovine gelatins. Porcine gelatin has two bands at 110 kDa and 125 kDa, while bovine gelatin has two bands at greater molecular weights (125 kDa and 140 kDa). Through this band profile differences, bovine and porcine gelatin could be easily differentiated. A blind evaluation of the developed approach was also conducted by an authorized pharmaceutical organization, which used the developed gel electrophoresis method to examine 13 samples in total (8 capsule shells and 5 over-the-counter final products). The findings of the blind test demonstrated that the developed method could verify the source of gelatin as stated by the manufacturer for each sample.

The origin of gelatin can be identified by combining protein-based methods with nucleic acid-based methods. Malik et al. (2016) [21] demonstrated the use of these two approaches complement one another and aid in determining the origin of gelatin. They used polymerase chain reaction and electrophoresis to simultaneously identify and confirm the type of gelatin present in capsule shells. The results showed that pig gelatin had a broader molecular weight variation than bovine gelatin because, at optimal SDS-PAGE conditions, more bands were observed in porcine gelatin. Additionally, the results of the densitometry revealed that the densitometry profile of pig gelatin had 12 peaks, but the profile of bovine gelatin had just 4 major peaks. The densitometry profile analysis revealed that the porcine profile had peaks at < 100 kDa, whereas the bovine profile did not. Consequently, it is possible to distinguish between bovine and porcine gelatin using these peaks. Another specific difference was observed at the peak of 115 kDa of the porcine gelatin, whereas bovine gelatin does not have this peak.

Moreover, Malik et al. (2016) [21] employed principal component analysis (PCA) in conjunction with SDS-PAGE-densitometry to detect and confirm four gelatin capsule samples. PCA is a multivariate analysis that can find the connections between the samples and variables, capable of identifying patterns, grouping, similarities, and differences within the input data [89]. The result revealed that sample 1, was near the porcine gelatin reference and can be confirmed to contain porcine gelatin as labeled by producer. In contrast, samples 2 & 3, which are capsules containing gelatin from unidentified origins, were closer to the reference cow and were therefore likely to include bovine gelatin. Sample 4 exhibited a poor band pattern, and thus was unable to identify by SDS-PAGE-densitometry. Then, using universal oligonucleotide primers [forward (cyt b1): 5'-CCA TCC AAC ATC TCA GCA TGA AA-3' and reverse (cyt b2): 5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3'] and *BsaJI* as a restriction enzyme, a nucleic acid-based analysis (PCR-RLFP) was carried out to amplify the genomic DNAs of porcine and bovine. *BsaJ*I cut the 360 bp of target amplicon into two fragments of 228 and 131 bp for *Sus scrofa* and 316 and 44 bp fragments for *Bos taurus*. Using the nucleic acid-based method, it can be concluded that sample 1 was verified to contain porcine gelatin as labeled by the manufacturer as well as by the SDS-PAGE-densitometry coupled PCA result. Furthermore, the presence of bovine gelatin in samples 2 and 3—the same as in the protein-based method—was confirmed. After all, even while simultaneous approaches seem like a useful complement, it's crucial to maintain the simultaneous techniques at a low cost and have simple protocols.

4. CONCLUSION

In recent years, there has been a rise in the demand for cosmetics. Modern life has driven people to enhance their appearance. Along with such a phenomenon, customers are becoming more aware of embracing religious teaching, such as using halal products since the use of halal goods is highly advocated in Islam. Prohibited sources of animal-derived ingredients, such as porcine species mainly cause halal issues toward cosmetic products. Porcine-derived ingredients, such as gelatin and collagen, are extensively used in various forms of cosmetic products, whether as excipients or as beneficial components towards beauty and appearance since these ingredients offer economic advantages and superior properties among other animal protein sources. These advantages may result in the potency of adulteration practices and become a problem for the Muslim population since halal products have a zero-tolerance policy, meaning that haram ingredients are prohibited even in minuscule quantities. Therefore, the development of analytical methods performing specific, sensitive, and reliable results is highly desirable in order to support halal authentication regulations.

Various techniques have been performed for gelatin and collagen analysis in highly complex matrixes including liquid chromatography-tandem mass spectrometry (LC-MS/MS), polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and polypeptide molecular weight such as SDS-PAGE. These methods have different approaches and principles in determining the sources of gelatin and collagen, with respective benefits and limitations. Various forms of cosmetic products are present nowadays and only a limited number of experiments were conducted in examining porcine protein in cosmetic matrix samples. Therefore, further exploration, development, and optimization of the established method are needed. Further optimization in LC-MS/MS workflows such as the optimization of non-conventional digestion methods is needed to overcome the lengthy preparation problem in the LC-MS/MS method. The exploration of the DNA extraction method to isolate the DNA within actual gelatin contained in the cosmetic sample is also critical, to demonstrate the performance of the method in real-life samples in favor of establishing simple protocols and reducing time-consuming analysis. Further research on the ELISA capability to determine gelatin source identification in cosmetic matrix samples is also needed to give further understanding of the method's capability as an alternative technique for the expensive LC/MS-MS method since as far as we know there is no publication specifically examining gelatin sources from the cosmetic matrix samples using ELISA.

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References

[1] R. Honigman and D. J. Castle, "Aging and cosmetic enhancement," *Clinical interventions in aging*, vol. 1, no. 2, 2006, doi: 10.2147/ciia.2006.1.2.115.

[2] Anonim, "Cosmetics Market Size, Share & Industry Analysis, Global Trends, 2024-2032." Accessed: Oct. 13, 2024. [Online]. Available: https://www.fortunebusinessinsights.com/cosmetics-market-102614

[3] Anonim, "Halal Cosmetics Market Size, Share & Industry Impact Analysis, Global Trends, 2024 - 2032." Accessed: Oct. 13, 2024. [Online]. Available: https://www.fortunebusinessinsights.com/halal-cosmetics-market-106602

[4] V. Briliana and N. Mursito, "Exploring antecedents and consequences of Indonesian Muslim youths' attitude towards halal cosmetic products: A case study in Jakarta," *Asia Pacific Management Review*, vol. 22, no. 4, pp. 176–184, Dec. 2017, doi: 10.1016/j.apmrv.2017.07.012.

[5] A. Shamsuddin and F. M. Yusof, "Exploring the Issues and Challenges in Malaysian Cosmetic Halal: A Theoretical Framework," *NHJ*, vol. 1, no. 1, pp. 10–14, Aug. 2020, doi: 10.17977/um060.2020v1p010-014.

[6] N. A. A. Anuar, N. A. Tukiran, and M. A. Jamaludin, "Gelatin in Halal Pharmaceutical Products," *Malaysian Journal of Syariah and Law*, vol. 11, no. 1, pp. 64–78, 2023.

[7] A. Duconseille, T. Astruc, N. Quintana, F. Meersman, and V. Sante-Lhoutellier, "Gelatin structure and composition linked to hard capsule dissolution: A review," *Food Hydrocolloids*, vol. 43, pp. 360–376, Jan. 2015, doi: 10.1016/j.foodhyd.2014.06.006.

[8] M. Celeiro, C. Garcia-Jares, M. Llompart, and M. Lores, "Recent Advances in Sample Preparation for Cosmetics and Personal Care Products Analysis," *Molecules*, vol. 26, no. 16, p. 4900, Aug. 2021, doi: 10.3390/molecules26164900.

[9] Z. Zhong and G. Li, "Current trends in sample preparation for cosmetic analysis," *J of Separation Science*, vol. 40, no. 1, pp. 152–169, Jan. 2017, doi: 10.1002/jssc.201600367.

[10] A. S. Khayyira, V. M. Estepane, and A. Malik, "Rapid PCR–Based Detection Optimization of Porcine DNA in Gelatin Capsule Shell," *International Journal of Applied Pharmaceutics*, pp. 217–223, Nov. 2018, doi: 10.22159/ijap.2018v10i6.29346.

[11] Y. Yang, L. Li, H. Wang, M. Liu, and Y. Wu, "Development and verification of a quantitative real-time PCR method to identify and quantify gelatin derived from animal hide," *Journal of Food Science*, vol. 85, no. 9, pp. 2762–2772, Sep. 2020, doi: 10.1111/1750-3841.15362.

[12] X. Zhu *et al.*, "Determination of porcine derived components in gelatin and gelatin-containing foods by high performance liquid chromatography-tandem mass spectrometry," *Food Hydrocolloids*, vol. 134, p. 107978, Jan. 2023, doi: 10.1016/j.foodhyd.2022.107978.

[13] T. Chia, F. T. H. Boon, C. Cheah, C. T. Yang, and D. Ghosh, "Porcine Gelatin Peptide Detection in Cosmetic Products and Food Confectionery using a TSQ Altis Triple Quadrupole Mass Spectrometer." ThermoFisher, 2020. Accessed: Oct. 26, 2024. [Online]. Available: https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-90606-porcine-gelatin-peptide-detection-halal-testing-an90606-en.pdf

[14] U. Jumhawan, J. Xing, and Z. Zhan, "Targeted Proteomics Approach for Sensitive Detection of Bovine and Porcine Gelatins in Food, Pharmaceutical Capsules and Personal Care Products." Shimadzu, 2019.

[15] Y. S. Kim, H. K. Yu, B. Z. Lee, and K. W. Hong, "Effect of DNA extraction methods on the detection of porcine ingredients in halal cosmetics using real-time PCR," *Appl Biol Chem*, vol. 61, no. 5, Art. no. 5, Oct. 2018, doi: 10.1007/s13765-018-0389-x.

[16] A. R. Zabidi *et al.*, "Screening porcine DNA in collagen cream cosmetic products," *Food Res.*, vol. 4, no. S1, pp. 151–156, Feb. 2020, doi: 10.26656/fr.2017.4(S1).S05.

[17] H. Doi, E. Watanabe, H. Shibata, and S. Tanabe, "A Reliable Enzyme Linked Immunosorbent Assay for the Determination of Bovine and Porcine Gelatin in Processed Foods," *J. Agric. Food Chem.*, vol. 57, no. 5, pp. 1721–1726, Mar. 2009, doi: 10.1021/jf802733y.

[18] N. A. Tukiran, A. Ismail, S. Mustafa, and M. Hamid, "Determination of porcine gelatin in edible bird's nest by competitive indirect ELISA based on anti-peptide polyclonal antibody," *Food Control*, vol. 59, pp. 561–566, Jan. 2016, doi: 10.1016/j.foodcont.2015.06.039.

[19] N. A. Tukiran, A. Ismail, S. Mustafa, and M. Hamid, "Development of antipeptide enzyme-linked immunosorbent assay for determination of gelatin in confectionery products," *Int J of Food Sci Tech*, vol. 51, no. 1, pp. 54–60, Jan. 2016, doi: 10.1111/ijfs.12971.

[20] A. Venien and D. Levieux, "Differentiation of bovine from porcine gelatines using polyclonal anti-peptide antibodies in indirect and competitive indirect ELISA," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 39, no. 3, pp. 418–424, Sep. 2005, doi: 10.1016/j.jpba.2005.04.013.

[21] A. Malik *et al.*, "Simultaneous identification and verification of gelatin type in capsule shells by electrophoresis and polymerase chain reaction," *Journal of Pharmaceutical Investigation*, vol. 46, no. 5, pp. 475–485, Aug. 2016, doi: 10.1007/s40005-016-0245-0.

[22] B. K. Yap and L.-H. Gam, "Differentiation of bovine from porcine gelatin capsules using gel electrophoresis method," *Food Chemistry*, vol. 274, pp. 16–19, Feb. 2019, doi: 10.1016/j.foodchem.2018.08.111.

[23] J. Nikitakis and H. P. Breslawec, Eds., *International cosmetic ingredient dictionary and handbook*, Fifteenth edition. 2014. Washington, D.C.: Personal Care Products Council, 2013.

[24] E. Prihandiwati *et al.*, "A review of the developed methods for the analysis of lard and pork in food andpharmaceutical products for halal authentication," *Food Res.*, vol. 8, no. 3, pp. 14–27, May 2024, doi: 10.26656/fr.2017.8(3).095.

[25] G. R. Kelm and R. R. Wickett, "The Role of Fatty Acids in Cosmetic Technology," in *Fatty Acids*, Elsevier, 2017, pp. 385–404. doi: 10.1016/B978-0-12-809521-8.00012-X.

[26] D. Liu, M. Nikoo, G. Boran, P. Zhou, and J. M. Regenstein, "Collagen and Gelatin," *Annu. Rev. Food Sci. Technol.*, vol. 6, no. 1, pp. 527–557, Apr. 2015, doi: 10.1146/annurev-food-031414-111800.

[27] L. Sun, Y. Zhang, and Y. Zhuang, "Antiphotoaging effect and purification of an antioxidant peptide from tilapia (*Oreochromis niloticus*) gelatin peptides," *Journal of Functional Foods*, vol. 5, no. 1, pp. 154–162, Jan. 2013, doi: 10.1016/j.jff.2012.09.006.

[28] M. Usman *et al.*, "Valorization of animal by-products for gelatin extraction using conventional and green technologies: a comprehensive review," *Biomass Conv. Bioref.*, Jul. 2023, doi: 10.1007/s13399-023-04547-5.

[29] C. V. Toniciolli Rigueto *et al.*, "Gelatin films from wastes: A review of production, characterization, and application trends in food preservation and agriculture," *Food Research International*, vol. 162, p. 112114, Dec. 2022, doi: 10.1016/j.foodres.2022.112114.

[30] T. Ariffin *et al.*, "Capsule Shell of Pharmaceutical Products in Malaysia; The Sources and Halal Status," in *E-Journal on the 6th Integration of Knowledge (WCIK 2018).*, Kuala Lumpur: IIUM Repository, 2019.

[31] M. Hassan, D. Hussain, T. Kanwal, H.-M. Xiao, and S. Ghulam Musharraf, "Methods for detection and quantification of gelatin from different sources," *Food Chemistry*, vol. 438, p. 137970, Apr. 2024, doi: 10.1016/j.foodchem.2023.137970.

[32] M. Y. Samatra, N. Q. I. M. Noor, U. H. M. Razali, J. Bakar, and S. Md. Shaarani, "Bovidae-based gelatin: Extractions method, physicochemical and functional properties, applications, and future trends," *Comp Rev Food Sci Food Safe*, vol. 21, no. 4, pp. 3153–3176, Jul. 2022, doi: 10.1111/1541-4337.12967.

[33] T. P. Anunciato and P. A. Da Rocha Filho, "Carotenoids and polyphenols in nutricosmetics, nutraceuticals, and cosmeceuticals," *J of Cosmetic Dermatology*, vol. 11, no. 1, pp. 51–54, Mar. 2012, doi: 10.1111/j.1473-2165.2011.00600.x.

[34] C. Faria-Silva *et al.*, "Feeding the skin: A new trend in food and cosmetics convergence," *Trends in Food Science & Technology*, vol. 95, pp. 21–32, Jan. 2020, doi: 10.1016/j.tifs.2019.11.015.

[35] K. Pearson, "Nutraceuticals and skin health: key benefits and protective properties," *Journal of Aesthetic Nursing*, vol. 7, no. Sup1, pp. 35–40, Mar. 2018, doi: 10.12968/joan.2018.7.Sup1.35.

[36] A. Naharros-Molinero, M. Á. Caballo-González, F. J. de la Mata, and S. García-Gallego, "Shell Formulation in Soft Gelatin Capsules: Design and Characterization," *Advanced Healthcare Materials*, vol. 13, no. 1, p. 2302250, 2024, doi: 10.1002/adhm.202302250.

[37] M. A. Elgadir, M. E. S. Mirghani, and A. Adam, "Fish gelatin and its applications in selected pharmaceutical aspects as alternative source to pork gelatin." Accessed: Oct. 16, 2024. [Online]. Available: https://www.researchgate.net/publication/287311456_Fish_gelatin_and_its_applications_in_selected_pharmaceutical_aspects_as_alternative_source_to_pork_gelatin

[38] T. Chen and H. Hou, "Protective effect of gelatin polypeptides from Pacific cod (*Gadus macrocephalus*) against UV irradiation-induced damages by inhibiting inflammation and improving

transforming growth factor-β/Smad signaling pathway," *Journal of Photochemistry and Photobiology B: Biology*, vol. 162, pp. 633–640, Sep. 2016, doi: 10.1016/j.jphotobiol.2016.07.038.

[39] G. Cannarozzo *et al.*, "A New 675 nm Laser Device in the Treatment of Facial Aging: A Prospective Observational Study," *Photobiomodul Photomed Laser Surg*, vol. 39, no. 2, pp. 118–122, Feb. 2021, doi: 10.1089/photob.2020.4908.

[40] L. Baumann, "Skin ageing and its treatment," *J Pathol*, vol. 211, no. 2, pp. 241–251, Jan. 2007, doi: 10.1002/path.2098.

[41] S. P. Nistico, M. Silvestri, T. Zingoni, F. Tamburi, L. Bennardo, and G. Cannarozzo, "Combination of Fractional CO2 Laser and Rhodamine-Intense Pulsed Light in Facial Rejuvenation: A Randomized Controlled Trial," *Photobiomodul Photomed Laser Surg*, vol. 39, no. 2, pp. 113–117, Feb. 2021, doi: 10.1089/photob.2020.4876.

[42] D. J. Tobin, "Introduction to skin aging," *J Tissue Viability*, vol. 26, no. 1, pp. 37–46, Feb. 2017, doi: 10.1016/j.jtv.2016.03.002.

[43] S.-Y. Pu *et al.*, "Effects of Oral Collagen for Skin Anti-Aging: A Systematic Review and Meta-Analysis," *Nutrients*, vol. 15, no. 9, p. 2080, Apr. 2023, doi: 10.3390/nu15092080.

[44] M. T. Sanz *et al.*, "Biorevitalizing effect of a novel facial serum containing apple stem cell extract, pro-collagen lipopeptide, creatine, and urea on skin aging signs," *J Cosmet Dermatol*, vol. 15, no. 1, pp. 24–30, Mar. 2016, doi: 10.1111/jocd.12173.

[45] P. M. B. G. Maia Campos, M. O. Melo, and F. C. Siqueira César, "Topical application and oral supplementation of peptides in the improvement of skin viscoelasticity and density," *J Cosmet Dermatol*, vol. 18, no. 6, pp. 1693–1699, Dec. 2019, doi: 10.1111/jocd.12893.

[46] H. Elyasi, H. Rahimi, and A. Sepahvend, "Gelatin, Halal or Haram?," *Plant Biotechnology Persa*, vol. 2, no. 1, pp. 35–41, 2020.

[47] R. Akbarzadegan, H. Ahari, A. Sharifan, and A. A. Anvar, "Overview of the studies on authentication of gelatin using Fourier Transform Infrared spectroscopy coupled with chemometrics," *HHHM*, vol. 1, no. 2, Nov. 2020, doi: 10.30502/jhhhm.2021.244697.1021.

[48] J. Ab Latiff, "Halal Certification Procedure in Malaysia and Indonesia," *JKIHS*, vol. 5, no. 2, Nov. 2020, doi: 10.22373/petita.v5i2.102.

[49] Y. Herdiana, F. F. Sofian, S. Shamsuddin, and T. Rusdiana, "Towards halal pharmaceutical: Exploring alternatives to animal-based ingredients," *Heliyon*, vol. 10, no. 1, p. e23624, Jan. 2024, doi: 10.1016/j.heliyon.2023.e23624.

[50] T. Saha, T. Rifat, and S. Shimanto, "Prospects of Halal Pharmaceuticals," *Asian Journal of Ethnopharmacology and Medicinal Foods*, vol. 5, no. 2, pp. 17–23, 2019.

[51] G. Deng, S. Guo, F. Zaman, T. Li, and Y. Huang, "Recent advances in animal origin identification of gelatin-based products using liquid chromatography-mass spectrometry methods: A mini review," *Reviews in Analytical Chemistry*, vol. 39, no. 1, pp. 260–271, Jan. 2020, doi: 10.1515/revac-2020-0121.

[52] M. D. Shoulders and R. T. Raines, "Collagen Structure and Stability," *Annu. Rev. Biochem.*, vol. 78, no. 1, pp. 929–958, Jun. 2009, doi: 10.1146/annurev.biochem.77.032207.120833.

[53] G. Zhang *et al.*, "Mass spectrometric detection of marker peptides in tryptic digests of gelatin: A new method to differentiate between bovine and porcine gelatin," *Food Hydrocolloids*, vol. 23, no. 7, pp. 2001–2007, Oct. 2009, doi: 10.1016/j.foodhyd.2009.03.010.

[54] A. Rohman, A. Windarsih, Y. Erwanto, and Z. Zakaria, "Review on analytical methods for analysis of porcine gelatine in food and pharmaceutical products for halal authentication," *Trends in Food Science & Technology*, vol. 101, pp. 122–132, Jul. 2020, doi: 10.1016/j.tifs.2020.05.008.

[55] F. Rico *et al.*, "Meta-Analysis and Analytical Methods in Cosmetics Formulation: A Review," *Cosmetics*, vol. 11, no. 1, p. 1, Dec. 2023, doi: 10.3390/cosmetics11010001.

[56] N. Gryson, K. Messens, and K. Dewettinck, "Evaluation and optimisation of five different extraction methods for soy DNA in chocolate and biscuits. Extraction of DNA as a first step in GMO analysis," *J Sci Food Agric*, vol. 84, no. 11, pp. 1357–1363, Aug. 2004, doi: 10.1002/jsfa.1767.

[57] S. Soares, J. S. Amaral, M. B. P. P. Oliveira, and I. Mafra, "A SYBR Green real-time PCR assay to detect and quantify pork meat in processed poultry meat products," *Meat Science*, vol. 94, no. 1, pp. 115–120, May 2013, doi: 10.1016/j.meatsci.2012.12.012.

[58] L. Asensio, I. González, T. García, and R. Martín, "Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA)," *Food Control*, vol. 19, no. 1, pp. 1–8, Jan. 2008, doi: 10.1016/j.foodcont.2007.02.010.

[59] A. F. El Sheikha, N. F. K. Mokhtar, C. Amie, D. U. Lamasudin, N. M. Isa, and S. Mustafa, "Authentication technologies using DNA-based approaches for meats and halal meats determination," *Food Biotechnology*, vol. 31, no. 4, pp. 281–315, Oct. 2017, doi: 10.1080/08905436.2017.1369886.

[60] LSIH UB, *Webinar Aplikasi LC-MS/MS Untuk Analisis Gelatin*, (Oct. 28, 2022). Accessed: Nov. 09, 2024. [Online Video]. Available: https://www.youtube.com/watch?v=DZON1o7d-JQ

[61] R. M. H. R. Nhari, I. Hanish, N. F. K. Mokhtar, M. Hamid, and A. F. El Sheikha, "Authentication approach using enzyme-linked immunosorbent assay for detection of porcine substances," *Quality Assurance and Safety of Crops & Foods*, vol. 11, no. 5, pp. 449–457, Sep. 2019, doi: 10.3920/QAS2018.1415.

[62] F. Welker *et al.*, "Ancient proteins resolve the evolutionary history of Darwin's South American ungulates," *Nature*, vol. 522, no. 7554, pp. 81–84, Jun. 2015, doi: 10.1038/nature14249.

[63] H. H. Grundy *et al.*, "A mass spectrometry method for the determination of the species of origin of gelatine in foods and pharmaceutical products," *Food Chemistry*, vol. 190, pp. 276–284, Jan. 2016, doi: 10.1016/j.foodchem.2015.05.054.

[64] K. R. Dewi *et al.*, "Advances and key considerations of liquid chromatography–mass spectrometry for porcine authentication in halal analysis," *J Anal Sci Technol*, vol. 14, no. 1, p. 13, Feb. 2023, doi: 10.1186/s40543-023-00376-3.

[65] T. Müller and D. Winter, "Systematic Evaluation of Protein Reduction and Alkylation Reveals Massive Unspecific Side Effects by Iodine-containing Reagents," *Molecular & Cellular Proteomics*, vol. 16, no. 7, pp. 1173–1187, Jul. 2017, doi: 10.1074/mcp.M116.064048.

[66] B. Jannat *et al.*, "Distinguishing tissue origin of bovine gelatin in processed products using LC/MS technique in combination with chemometrics tools," *Food Chemistry*, vol. 319, p. 126302, Jul. 2020, doi: 10.1016/j.foodchem.2020.126302.

[67] U. Jumhawan, J. Xing, and Z. Zhan, "Detection and Differentiation of Bovine and Porcine Gelatins in Food and Pharmaceutical Products By LC/MS/MS Method." Shimadzu, 2017. [Online]. Available: https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/applications/application_note/10806/apo118005.pdf

[68] S. Cai *et al.*, "A quantitative strategy of ultrasound-assisted digestion combined UPLC-MS/MS for rapid identifying species-specific peptide markers in the application of food gelatin authentication," *LWT*, vol. 147, p. 111590, Jul. 2021, doi: 10.1016/j.lwt.2021.111590.

[69] K. R. Dewi *et al.*, "Application of LC-MS/MS Coupled with Various Digestion Methods for the Identification of Porcine Gelatin Markers in Confectionery Matrices," *Indonesian Journal of Halal Research*, vol. 5, no. 2, pp. 53–66, Aug. 2023, doi: 10.15575/ijhar.v5i2.21191.

[70] S. Cai *et al.*, "A quantitative strategy of ultrasound-assisted digestion combined UPLC-MS/MS for rapid identifying species-specific peptide markers in the application of food gelatin authentication," *LWT*, vol. 147, p. 111590, Jul. 2021, doi: 10.1016/j.lwt.2021.111590.

[71] W. Sun *et al.*, "Microwave-assisted Protein Preparation and Enzymatic Digestion in Proteomics," *Molecular & Cellular Proteomics*, vol. 5, no. 4, pp. 769–776, Apr. 2006, doi: 10.1074/mcp.T500022-MCP200.

[72] E. Gianazza and C. Banfi, "Post-translational quantitation by SRM/MRM: applications in cardiology," *Expert Review of Proteomics*, vol. 15, no. 6, pp. 477–502, Jun. 2018, doi: 10.1080/14789450.2018.1484283.

[73] T. Demeke and G. R. Jenkins, "Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits," *Anal Bioanal Chem*, vol. 396, no. 6, pp. 1977–1990, Mar. 2010, doi: 10.1007/s00216-009-3150-9.

[74] C. Schrader, A. Schielke, L. Ellerbroek, and R. Johne, "PCR inhibitors - occurrence, properties and removal," *J Appl Microbiol*, vol. 113, no. 5, pp. 1014–1026, Nov. 2012, doi: 10.1111/j.1365-2672.2012.05384.x.

[75] D. S. Smith and P. W. Maxwell, "Use of quantitative PCR to evaluate several methods for extracting DNA from corn flour and cornstarch," *Food Control*, vol. 18, no. 3, pp. 236–242, Mar. 2007, doi: 10.1016/j.foodcont.2005.10.001.

[76] D. S. Smith, P. W. Maxwell, and S. H. De Boer, "Comparison of Several Methods for the Extraction of DNA from Potatoes and Potato-Derived Products," *J. Agric. Food Chem.*, vol. 53, no. 26, pp. 9848–9859, Dec. 2005, doi: 10.1021/jf051201v.

[77] C. F. Terry, N. Harris, and H. C. Parkes, "Detection of Genetically Modified Crops and Their Derivatives: Critical Steps in Sample Preparation and Extraction," *Journal of AOAC INTERNATIONAL*, vol. 85, no. 3, pp. 768–774, May 2002, doi: 10.1093/jaoac/85.3.768.

[78] S. Gina, C. Rahmagiarti, I. M. Ummah, C. Sumantri, I. H. Suparto, and N. Darmawan, "Assessment of Commercial DNA Extraction Kits for Porcine Gelatin Detection Using RT-PCR and ddPCR," *sci. technol. indones.*, vol. 9, no. 3, pp. 605–612, Jun. 2024, doi: 10.26554/sti.2024.9.3.605-612.

[79] M. Alhajj, M. Zubair, and A. Farhana, "Enzyme Linked Immunosorbent Assay," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2024. Accessed: Nov. 01, 2024. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK555922/

[80] M. Garcia-Vaquero and A. Mirzapour-Kouhdasht, "A review on proteomic and genomic biomarkers for gelatin source authentication: Challenges and future outlook," *Heliyon*, vol. 9, no. 6, p. e16621, Jun. 2023, doi: 10.1016/j.heliyon.2023.e16621.

[81] M. Sela and R. Arnon, "Studies on the chemical basis of the antigenicity of proteins. 1. Antigenicity of polypeptidyl gelatins," *Biochem J*, vol. 75, no. 1, pp. 91–102, Apr. 1960, doi: 10.1042/bj0750091.

[82] X. Jiang, D. Fuller, Y.-H. P. Hsieh, and Q. Rao, "Monoclonal antibody-based ELISA for the quantification of porcine hemoglobin in meat products," *Food Chemistry*, vol. 250, pp. 170–179, Jun. 2018, doi: 10.1016/j.foodchem.2018.01.032.

[83] L. Liu, F.-C. Chen, J. L. Dorsey, and Y.-H. P. Hsieh, "Sensitive Monoclonal Antibody-based Sandwich ELISA for the Detection of Porcine Skeletal Muscle in Meat and Feed Products," *Journal of Food Science*, vol. 71, no. 1, pp. M1–M6, 2006, doi: 10.1111/j.1365-2621.2006.tb12393.x.

[84] J. Mandli, I. EL Fatimi, N. Seddaoui, and A. Amine, "Enzyme immunoassay (ELISA/immunosensor) for a sensitive detection of pork adulteration in meat," *Food Chemistry*, vol. 255, pp. 380–389, Jul. 2018, doi: 10.1016/j.foodchem.2018.01.184.

[85] A. Iqbal and N. Ateeq, "Effect of processing on the detectability of peanut protein by ELISA," *Food Chemistry*, vol. 141, no. 3, pp. 1651–1654, Dec. 2013, doi: 10.1016/j.foodchem.2013.04.102.

[86] E. A. E. Garber and J. Perry, "Detection of hazelnuts and almonds using commercial ELISA test kits," *Anal Bioanal Chem*, vol. 396, no. 5, pp. 1939–1945, Mar. 2010, doi: 10.1007/s00216-009-3424-2.

[87] B. Liu, D. Teng, X. Wang, Y. Yang, and J. Wang, "Expression of the soybean allergenic protein P34 in Escherichia coli and its indirect ELISA detection method," *Appl Microbiol Biotechnol*, vol. 94, no. 5, pp. 1337–1345, Jun. 2012, doi: 10.1007/s00253-012-4006-3.

[88] N. A. Tukiran, A. Ismail, S. Mustafa, and M. Hamid, "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)*, N. Muhammad Hashim, N. N. Md Shariff, S. F. Mahamood, H. M. Fathullah Harun, M. S. Shahruddin, and A. Bhari, Eds., Singapore: Springer Singapore, 2018, pp. 429–439. doi: 10.1007/978-981-10-7257-4_38.

[89] R. Garrido-Delgado, S. López-Vidal, L. Arce, and M. Valcárcel, "Differentiation and identification of white wine varieties by using electropherogram fingerprints obtained with CE," *J of Separation Science*, vol. 32, no. 21, pp. 3809–3816, Nov. 2009, doi: 10.1002/jssc.200900342.