MALAYSIAN JOURNAL OF APPLIED SCIENCES 2025, VOL 10(2): 31-44
E-ISSN:0127-9246 (ONLINE)
http://dx.doi.org/10.37231/myjas.2025.10.2.476
https://journal.unisza.edu.my/myjas

ORIGINAL ARTICLE

Porcine DNA Authentication in Sugar Confectionery Products

Mohammad, A.A.[1], Sajali, N.[1*], Koh, C.C.[1], Desa, M.N.M.[2,3]

[1]Centre of Research for Innovation and Sustainable Development (CRISD), School of Engineering and Technology, University of Technology Sarawak, 96000 Sibu, Sarawak, Malaysia

[2]Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[*]Corresponding author: nurhayatie@uts.edu.my

Received: 28/08/2025, Accepted: 11/09/2025, Available Online: 31/10/2025

Abstract

Halal certification is vital for confirming the halal status of food products, particularly for Muslim consumers. Instances of halal fraud, including the use of counterfeit halal logos and the incorporation of non-halal ingredients in food products, negatively impact Muslim consumers. Gelatine, widely used in the food industry, varies in acceptability depending on its source, with porcine-derived gelatine strictly prohibited in Islam. Therefore, this research aims to identify the presence of porcine DNA in sugar confectionery products by targeting mitochondrial cytochrome b (*cytb*) via a real-time polymerase chain reaction (qPCR) assay and to compare the qPCR findings with the sources of gelatine and the presence of a halal logo. Nine food products (eight samples without a recognised JAKIM halal logo and one sample with a foreign halal logo not recognised by JAKIM) were purchased from a local supermarket in Sibu, Sarawak. DNA was extracted from the food products using the DNeasy Mericon Food kit, analysed with a spectrophotometer, and used as template DNA in the qPCR assay. Positive qPCR findings were validated through DNA sequencing and BLAST analysis. Of the nine products tested, eight contained detectable porcine DNA, including one product labelled with a foreign halal logo. Sequencing data confirmed *Sus scrofa* as the predominant species, with BLAST identities ranging from 86.52% to 100% against the NCBI database. In summary, these results highlight the risks posed by mislabelling and fraudulent halal logos, and emphasise the importance of rigorous certification, transparent labelling, and routine molecular verification to protect Muslim consumers and maintain trust in halal markets.

Keywords: food fraud; halal authentication; porcine DNA; real-time PCR; sugar confectionery products

Introduction

Gelatine is a polymeric, multifunctional ingredient produced through the controlled hydrolysis or heat-induced denaturation of collagen from animal skins, bones, and connective tissues (Alipal et al., 2021). Due to its gelling, foaming, and stabilising properties, it is extensively used in both food and pharmaceutical manufacturing. The primary commercial sources of gelatine include bovine and porcine skins, demineralised hooves, and bones (Rather et al., 2022). Religious dietary restrictions strongly influence the acceptability of gelatine. Muslims require gelatine to be free from pork and its derivatives, and bovine sources must be processed according to Islamic slaughtering practices, while Hindus avoid bovine-derived gelatine for religious reasons (Uddin et al., 2021).

From a consumption perspective, the global gelatine market continues to demonstrate strong growth potential. The market was valued at USD 7.05 billion in 2024 and is projected to reach USD 13.14 billion by 2030, representing a compound annual growth rate (CAGR) of 11.1% from 2025 to 2030**.** In 2024, Europe dominated the market, accounting for the largest revenue share of 38.3%, followed by the Asia-Pacific region, which represents the fastest-growing market. Among leading companies in the gelatine industry are GELITA AG, Rousselot, PB Leiner, Sterling Gelatin and Weishardt Holding SA (Grand View Research, 2025).

Gelatine consumption is primarily concentrated in the food sector (63%)**,** followed by medical applications (31%), and technical or other industrial uses (6%) (GMIA, 2019). Global demand continues to increase, particularly in the food industry, due to gelatine's multifunctional properties as a gelling, foaming, emulsifying, thickening, and water-binding agent. Within the food sector, it is widely used in confectionery, bakery products, and gelatine desserts to impart desirable texture, stability, and clarity. In confectionery, gelatine improves viscosity, foam stability, and moulding properties, while in bakery products, it helps delay staling by reducing starch retrogradation (Yu et al., 2019).

The global halal market has expanded significantly, driven by rising consumer demand for products that comply with Islamic law. In Malaysia, halal certification is managed primarily by the Department of Islamic Development Malaysia (JAKIM), which enforces stringent guidelines (Daud, Lee & Ismail, 2023). Halal logos displayed on product packaging enable consumers to readily evaluate compliance. However, the increasing variety of imported products bearing foreign halal logos, some of which are not recognised by JAKIM, has created opportunities for fraudulent labelling and the misuse of halal symbols. These practices undermine consumer trust and can lead to the consumption of non-compliant products by Muslim consumers. Furthermore, food fraud involving the use of non-halal ingredients, either deliberately or due to inadequate supply chain control, represents a serious infringement of religious rights (Mohamad, 2022).

Molecular biology techniques, particularly numerous identification methods that rely on DNA analysis, have been established. Among the techniques, species-specific PCR (Polymerase Chain Reaction) (Mohd Hafidz et al., 2020), PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) (Rahman et al., 2017), real-time PCR (qPCR) (Orbayinah et al., 2020), and DNA barcoding (Wu et al., 2019) have become valuable tools for food authentication. Among these, a probe-based TaqMan® real-time PCR is a highly robust assay known for its rapidity and sensitivity (Chen et al., 2020). TaqMan probes are oligonucleotides labelled with reporter and quencher dyes that specifically bind to target DNA sequences, minimising false positives (Kim, Lee and Lee, 2022). It has been extensively applied for identifying meat species in commercial meat products (Hossain et al., 2023).

Mitochondria are small, oval-shaped structures in cells that have their own DNA (mtDNA), separate from the nuclear DNA (nDNA) found in the cell's nucleus (Kowalczyk et al., 2021). Both types of DNA can be used as biomarkers in qPCR, but mtDNA is often preferred because it has several advantages. Compared to nDNA, mtDNA varies more between species, making it especially useful for detecting food adulteration and identifying species in processed foods (Cho et al., 2024). Its circular structure makes it more stable and resistant to degradation, even in highly

processed products like cheese, whey, or sausages (Nikzad et al., 2017; Ha et al., 2017). Another benefit is that mtDNA exists in many copies per cell, while nDNA only has two. This abundance makes mtDNA easier to detect with qPCR and increases sensitivity when working with very small amounts of DNA (Fazzini et al., 2018; Mohd Hafidz et al., 2020). Commonly targeted regions of mtDNA include cytochrome b, cytochrome oxidase I (COI), and the D-loop, which are widely used in species identification (Kowalczyk et al., 2021). Because of its stability, high copy number, and variability between species, mtDNA is considered one of the best targets for food authentication studies. For example, targeting the cytochrome b gene has been effective for detecting porcine DNA even in highly processed food matrices (Rahayu et al., 2020).

The origin of gelatine is often not declared on product labels, creating challenges for consumers, particularly Muslims, as mislabelled products containing porcine ingredients are strictly prohibited in Islam. This study takes a preliminary step toward protecting religious rights and consumer interests and contributes to the ongoing efforts in halal food authentication by detecting porcine DNA in gelatine-based products using a molecular approach with double-quench probe real-time PCR for enhanced sensitivity and specificity. The research focuses on products sold in local supermarkets in Sibu, Sarawak, which lack a recognised JAKIM halal logo or display unrecognised halal logos. By combining molecular detection, sequencing validation, and label analysis, this study seeks to uncover potential mislabelling practices, raise consumer awareness, and contribute to strengthening halal authentication systems.

Materials and Methods

Sample Collection

Nine sugar confectionery products were purchased from the local supermarket in Sibu, Sarawak. Porcine gelatine (Sigma-Aldrich, USA) was used as the positive control (PC). The samples were selected using purposive sampling, a non-probability sampling technique, based on criteria such as the absence of a recognised JAKIM halal logo and the presence of a foreign halal logo that JAKIM did not recognise. All the selected food products were declared to contain gelatine as one of the ingredients, except for M1. Sample M1 was labelled as “Mini Roll Marshmallow”; however, the ingredient list did not specify the presence of gelatine. The sample was initially included based on its product type (marshmallow), which typically contains gelatine. Table 1 lists the selected sugar confectionery products analysed in the present work.

Table 1. List of the sugar confectionery products tested.

No.	Codes	Products	Product type	Halal logo	Country of origin
1	G1	Gummy 1	Fruity gummy	N/A[a]	Japan
2	G2	Gummy 2	Gummy candy	Halal (Turkey)	Turkey
3	M1	Marshmallow 1	Mini roll marshmallow	N/A	Malaysia
4	M2	Marshmallow 2	Jelly filled marshmallow	N/A	Philippines
5	M3	Marshmallow 3	Grape filled marshmallow	N/A	Hong Kong, China
6	M4	Marshmallow 4	Peach filled marshmallow	N/A	China
7	M5	Marshmallow 5	3D marshmallow assorted flavour	N/A	China
8	M6	Marshmallow 6	Original flavoured marshmallow	N/A	China
9	M7	Marshmallow 7	Mixed fruits flavoured marshmallow	N/A	China
10	PC	Pork gelatine	Powder	N/A	USA[b]

[a]N/A, not available; [b]USA, United States of America

DNA Extraction

DNeasy Mericon Food Kit (Qiagen, Germany) was used to extract DNA from sugar confectionery products. The extraction method was carried out according to the manufacturer's instructions, with slight modifications. Two hundred milligrams of each food sample was weighed. Food Lysis Buffer (1 mL) was added to each of the food samples, while 5 mL of Food Lysis Buffer was added to the pork gelatine. Then, the food samples and pork gelatine were ground using a mortar and pestle, respectively. The step was performed separately for food samples and pork gelatine to break down the materials and ensure uniform homogenisation, thereby improving DNA extraction efficiency. Proteinase K (15-25 µL) was added, and the mixture was vortexed briefly to ensure complete distribution and moistening of the food samples.

The mixture was incubated at 60℃ overnight in a Thermo-shaker (Biosan, Latvia) with constant shaking (300 rpm). After incubation, the samples were cooled to room temperature (15-25℃) on ice to enhance inhibitor precipitation. The samples were centrifuged for 5 min at 2500 x g to obtain the clear supernatant. Then, 500 µL chloroform was pipetted into a new 1.5 mL microcentrifuge tube, and 700 µL of the clear supernatant was transferred to the microcentrifuge tube containing the chloroform. The mixture was then vortexed vigorously for 15 s and centrifuged at 14,000 x g for 15 min.

Following centrifugation, three distinct layers were formed, namely the aqueous phase layer containing DNA, the interphase layer, and the organic phase layer. Buffer PB (1 mL) was pipetted into a new 1.5 mL microcentrifuge tube, and 250 µL of the upper, aqueous phase was added to a microcentrifuge tube containing Buffer PB. The mixture was mixed thoroughly by vortexing. Then, 600 µL of the mixture was transferred into the QIAquick spin column and placed in a 2 mL collection tube. The QIAquick spin column was centrifuged at 17,900 x g for 1 min, and the flow-through was discarded. The step was repeated with the remaining sample, and the flow-through was discarded.

Then, 500 µL of Buffer AW2 was added to the QIAquick spin column. The QIAquick spin column was centrifuged at 17,900 x g for 1 min, and the flow-through was discarded. The centrifugation step was repeated to dry the membrane. The QIAquick spin column was transferred to a new 1.5 ml microcentrifuge tube. After that, 100 µL Buffer EB was added and pipetted directly onto the QIAquick membrane. The QIAquick membrane was incubated for 1 min at room temperature (15-25°C), and then centrifuged at 17,900 x g for 1 min to elute the DNA. The DNA was stored at -20°C until use.

Determination of DNA Purity and DNA Concentration

DNA concentration and purity were analysed using a spectrophotometer. A blank was prepared to calibrate the spectrophotometer before use. The choice of blank and diluent used to dilute DNA depends on the reagent used during the DNA elution step. Tris-Cl buffer (700 µL) was used as a blank. DNA was diluted 100-fold by adding 7 µL of the DNA sample to 693 µL of Tris-Cl buffer. The absorbance of the diluted DNA at 260 nm and 280 nm was measured and recorded. The purity of DNA was determined by using the formula shown in Equation 1, and the DNA concentration was calculated by using the formula shown in Equation 2 (Stephenson, 2016; Afnan Uda et al., 2019):

$$\text{DNA purity } (A_{260}/A_{280}) = \frac{A_{260}\ reading - A_{320}\ reading}{A_{280}\ reading - A_{320}\ reading} \quad (1)$$

where A_{260}, A_{280}, and A_{320} are the absorbance readings at 260 nm, 280 nm, and 320 nm, respectively.

$$\text{DNA concentration (µg/mL)} = (A_{260} - A_{320}) \times 50 \text{ µg/mL} \times \text{Dilution Factor} \quad (2)$$

where A_{260} is the absorbance at 260 nm, 50 µg/mL corresponds to an absorbance of 1.0 for pure double-stranded DNA, and the dilution factor is defined as the final volume divided by the aliquot DNA volume. The absorbance ratio of A_{260}/A_{280} between 1.7-2.0 indicates that the extracted DNA was pure (Wilson & Walker, 2005).

Porcine Species-Specific Primer and Probe Sequences

The primer pair and TaqMan probe sequences targeting the cytochrome b (*cytb*) mitochondrial region used in this study were adapted from Sajali et al. (2022). The TaqMan probe was designed by tagging 5-hexachlorofluoresceine (5-HEX) and 3-Iowa black FQ (3-IABkFQ) at the 5' and 3'-ends, respectively. These sequences were designed to amplify at 121 bp of the *Sus scrofa domesticus* (pork) mitochondrial *cytb*. The specificity of the chosen primers and probe sequences was verified using the Basic Local Alignment Search Tool (BLAST) software in the National Centre for Biotechnology Information (NCBI) website. The probes and the primer sequences were synthesised by Integrated DNA Technologies, Singapore.

Detection of Porcine DNA using Real-time PCR

The protocol for qPCR and thermal cycling conditions was based on GoTaq probe qPCR Master Mix product information (Promega, USA), with slight adjustments in terms of the total volume that was used in this study. Master Mix was prepared in a 1.5 mL tube containing 5 µL of GoTaq Probe qPCR Master Mix (1x), 0.25 µL of forward and reverse primer (0.25 µM each), 0.125 µL hydrolysis TaqMan probe (0.125 µM) and 2.375 µL nuclease-free water for each reaction. Then, the reaction mixture was mixed by a short spin in a centrifuge. The reaction mixture was transferred to each PCR tube. Lastly, 2 µL of DNA template was added to each respective PCR tube that contained the master mix, which makes up a final volume of 10 µL.

Porcine gelatine powder served as a positive control, while no template control (NTC) served as a negative control. Both positive control and negative control were included to ensure the validity of the experimental data. NTC was prepared by adding nuclease-free water instead of DNA into the reaction mixture. qPCR reactions were done in triplicate. The PCR tubes were capped and centrifuged briefly to ensure the reagent components remained at the bottom of the tube prior to being loaded into the DTprime Real-time thermal cycler (DNA technology, Russia). Thermal cycling conditions consisted of an initial GoTaq® DNA polymerase activation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. The real-time PCR data were analysed in triplicate and reported as mean Ct ± standard deviation.

Validation of Positive Samples through DNA Sequencing

Positive samples from the qPCR assay were prepared for DNA sequencing. The protocol for the detection of porcine DNA using real-time PCR, including thermal cycling conditions, was used to prepare the qPCR reaction, with the total reaction volume modified to 40 µL. Before DNA sequencing, the qPCR products were purified using the PrimeWay Gel extraction Kit/PCR Purification Kit (KIT-9050) (1st BASE, Malaysia). PCR product (100 µL) was transferred into a new 1.5 ml microcentrifuge tube. Then, 5 volumes of BD buffer (20 µL) were added to one volume of PCR product (4 µL). The PCR mixture was mixed by vortexing. The PrimeWay Gel/PCR Column was placed into the collection tube. The PCR mixture was transferred into the Primeway Gel/PCR Column. The PCR mixture was centrifuged at 11,000 x g for 30 s, and the flow-through was discarded.

Next, 750 µL of Wash Buffer was added to the PrimeWay Gel/ PCR Column, centrifuged at 11,000 x g for 30 s, and the flow-through was discarded. The centrifugation process was repeated at 18,000 x g for 3 min to dry the column. The PrimeWay Gel/PCR Column was placed into a new 1.5 mL microcentrifuge tube. After that, 40 µL of Elution Buffer was added to the centre of the PrimeWay Gel/PCR Column and the column was incubated for 1 min. The purified PCR product was centrifuged at 18,000 x g for 1 min to elute the purified DNA. The purified DNA was sent to Apical Scientific, Selangor, for sequencing analysis. The chromatograms were viewed using FinchTV software (Version 1.4.0), and the sequencing results were analysed using BLAST software on the NCBI website.

Results and Discussion

DNA was effectively extracted from all sugar confectionery products using the DNeasy Mericon Food Kit (Qiagen, Germany), employing the small fragment protocol designed for the recovery of total DNA from extensively processed food matrices. This protocol uses optimised binding conditions for column-based purification and is well suited for highly degraded DNA samples, typically yielding 100–200 bp fragments due to processing such as pasteurisation, thermal or high-pressure treatment, dehydration, irradiation, or pH alteration. The use of stringent column-binding conditions enhances the efficiency of recovering pure DNA from such fragmented materials. The DNeasy Mericon Food Kit was modified based on the cetyltrimethylammonium bromide (CTAB) extraction method, a widely used non-ionic detergent technique that enables efficient extraction of total nucleic acids from a wide range of tissue types (Qiagen, 2020).

Depending on the salt conditions, CTAB may form complexes with nucleic acids or with inhibitors, such as proteins, polysaccharides, and plant metabolites. The optimised protocols for the DNeasy Mericon Food Kit use CTAB in combination with Proteinase K to first digest compact tissue and subsequently precipitate proteins with simultaneous precipitation of other cellular and food-derived inhibitors. The inhibitors are precipitated by centrifugation, while the extracted DNA remains in solution. When chloroform is added to the samples, any remaining CTAB-protein, CTAB-debris, or CTAB polysaccharide complex that is not precipitated is removed along with other lipophilic inhibitors into the organic phase. Only the aqueous phase containing the DNA and significantly depleted inhibitors is processed further. The aqueous phase is then mixed with the binding buffer, which is pipetted into the QIAquick Spin Columns. After the elution step, the DNA obtained is quantified before it is ready for use in a downstream qPCR assay (Qiagen, 2020).

DNA purity is a key determinant of DNA quality and assessing both quality and suitability is essential before analytical procedures. The absorbance ratio at 260 nm to 280 nm (A_{260}/A_{280}) is commonly used to evaluate DNA purity. Ratios within the range of 1.7–2.0 indicate DNA free of protein or other contaminants and are generally considered pure (Piskata et al., 2019). Ratios below 1.7 suggest contamination with substances such as phenol, proteins, or other molecules that strongly absorb near 280 nm (Lucena-Aguilar et al., 2016). Residual impurities from extraction, such as chloroform, can also reduce this ratio, while incomplete lysis during extraction may lower DNA yield by preventing full disruption of cell membranes in food samples (Piskata et al., 2019). Conversely, A_{260}/A_{280} ratios above 2.0 may indicate RNA contamination, as RNA increases absorbance at 260 nm. In this study, 2 of 9 DNA samples (Marshmallow 1 and Marshmallow 3) showed ratios below 1.7, while no samples exceeded 2.0, suggesting the absence of RNA contamination. The A_{260}/A_{280} ratios for DNA extracts from sugar confectionery products are presented in Table 2.

In accordance with the Food Regulations 1985, all pre-packaged food products must comply with the labelling requirements stipulated under Regulation 11(1)(e)**,** which mandates that where a food consists of two or more ingredients (other than water, food additives, and added nutrients), each ingredient must be designated in descending order of proportion by weight**,** and,

wherever required, the proportion of each ingredient must also be declared (Food Act, 1983). This implies that the first ingredient listed contributes the largest proportion, while the last ingredient represents the smallest amount in the formulation, which ensures transparency, protects consumer rights, and facilitates informed purchasing decisions. Most sugar confectionery products contain low amounts of gelatine, which is typically listed towards the middle or end of the ingredient lists for marshmallow and gummy products. Standard formulations of marshmallows and gummies generally include sugar, corn syrup, water, gelatine, and flavourings, with gelatine added in small quantities as a gelling agent.

Table 2. Absorbance ratio (A_{260}/A_{280}) of sugar confectionery products and mean C_t values ± standard deviation for food samples detected by qPCR assay

No	Code	Products	Absorbance ratio (A_{260}/A_{280})	Mean Ct[a] values ± Standard deviation	Halal symbol
1	G1	Gummy 1	1.81 ± 0.19	29.08 ± 0.34	N/A[b]
2	G2	Gummy 2	1.80 ± 0.01	31.82 ± 0.74	Halal
3	M1	Marshmallow 1	1.53 ± 0.19	31.25 ± 2.95	N/A
4	M2	Marshmallow 2	1.87 ± 0.13	40.00 ± 0.00	N/A
5	M3	Marshmallow 3	1.59 ± 0.05	31.27 ± 2.62	N/A
6	M4	Marshmallow 4	1.87 ± 0.01	29.97 ± 3.96	N/A
7	M5	Marshmallow 5	1.77 ± 0.20	28.48 ± 1.38	N/A
8	M6	Marshmallow 6	1.81 ± 0.04	29.43 ± 0.37	N/A
9	M7	Marshmallow 7	1.79 ± 0.09	29.70 ± 0.20	N/A
10	PC	Pork gelatine	1.66 ± 0.42	29.75 ± 1.89	N/A
11	NTC	No-template control	-	40.00 ± 0.00	N/A

[a]Ct, cycle threshold; [b]N/A, not available

Based on ingredient labelling and formulation data, gelatine typically constitutes a small proportion of the total product weight in sugar confectionery. However, the gelatine content in marshmallows and gummies varies depending on the manufacturing process, as it is adjusted to achieve the desired texture and formulation. For instance, marshmallows generally contain up to 1-3% gelatine, which is sufficient to create a stable aerated foam structure (Alipal et al., 2021), whereas gummy candies typically contain higher levels, ranging from 5% to 10% (Hartel, Elbe, & Hofberger, 2018). In the present study, the concentration of gelatine in the tested food products was low; however, the purity of most DNA samples obtained was within the acceptable range, and the concentrations were adequate for qPCR analysis. Although some extracts yielded relatively low gelatine DNA concentrations, with the lowest being approximately 10 ng/µl, successful amplification was achieved due to the high sensitivity of the qPCR technique.

Porcine DNA in sugar confectionery products was successfully detected using qPCR targeting the *cytb* gene with a primer–probe sequence. The cycle threshold (Ct) in qPCR assay indicates the starting point of the exponential growth phase, representing the number of cycles required for the sample to produce enough DNA to be detected (North Dakota State University Veterinary Diagnostic Laboratory, 2022). Ct values obtained from amplification curves indicated the relative DNA concentration, where lower Ct values reflected higher amounts of target DNA and higher Ct values indicated lower amounts (Zilhadia, Izzah, & Betha, 2017; Ansor, Hariyanti, & Rahmi, 2023). In real-time PCR, amplification is confirmed by the progressive increase in fluorescence signals emitted by intercalating dyes or sequence-specific probes, eliminating the need for additional post-PCR processing (Khehra, Padda, & Zubair, 2025).

A total of 40 cycles of qPCR amplification were conducted, as illustrated by the representative amplification curve in Figure 1. The x-axis represents the number of PCR cycles, while the y-axis indicates the fluorescence intensity of the amplification reaction. A lower Ct value corresponds to a higher concentration of porcine DNA in the food samples. Positive and negative

controls were included to ensure the validity and reliability of the experimental data. Gelatine powder was used as the positive control, while a no-template control served as the negative control. The positive control (porcine gelatine powder) tested positive for porcine DNA with a mean Ct value ± standard deviation of 29.75 ± 1.89. The negative control showed a mean Ct value ± standard deviation of 40.00 ± 0.00, confirming the absence of porcine DNA. Since a lower Ct value indicates a higher concentration of porcine DNA, Sample M5 (28.48 ± 1.38) showed a slightly higher porcine DNA concentration compared to the control (29.75 ± 1.89). However, the difference was small and may not be statistically significant due to overlapping standard deviations.

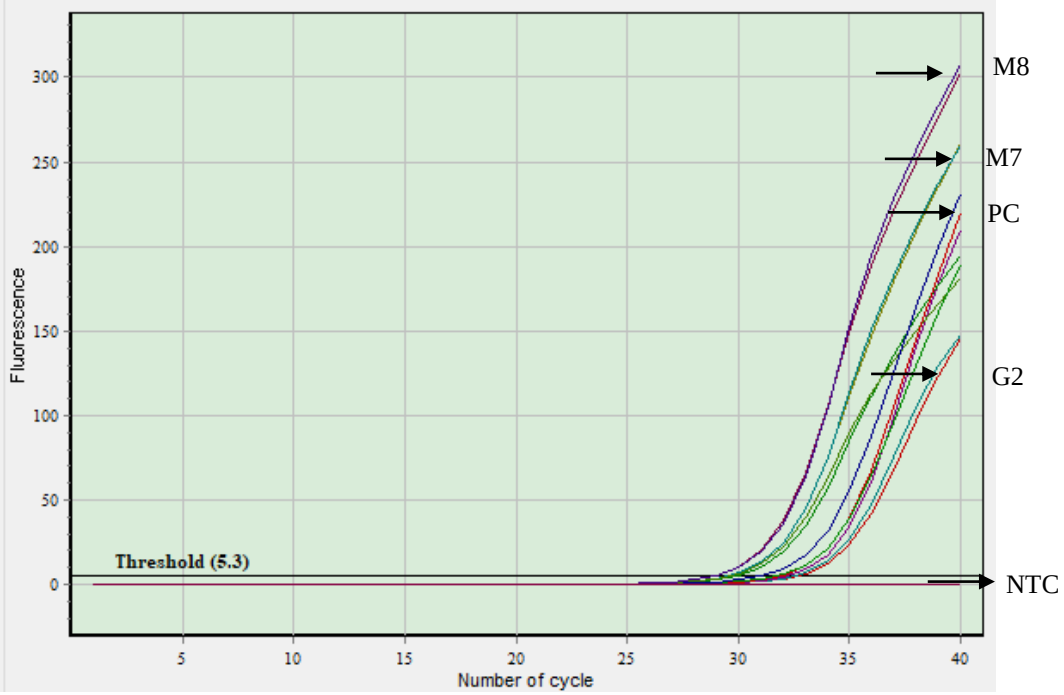

Figure 1. Representative of qPCR amplification curve for samples G2, M7, M8, NTC (no template control), and PC (positive control)

Based on the tested samples, most were imported from China, and none of these products disclosed any Halal status on their packaging. This finding is consistent with Ariffin et al., (2021), who identified the highest rates of mislabelling in Southeast Asia among products imported from China, Thailand, and Vietnam. In the present study, qPCR analysis confirmed the presence of porcine DNA in selected imported samples without Halal certification. Of the nine food samples tested, eight listed gelatines as an ingredient on their packaging, except for Sample M1 (Marshmallow 1).

However, none of the packaging, except for sample G1 (Gummy 1), specified the source or origin of the gelatine. Compared with the positive control, eight out of nine samples tested positive for porcine DNA, including Gummy 1, which explicitly declared pork gelatine as an ingredient but did not display a halal logo on the packaging. Only one sample, M2 (Marshmallow 2), had a mean Ct value ± standard deviation of 40.00 ± 0.00, indicating the absence of porcine DNA. Nevertheless, this finding still raises concerns among Muslim consumers. Although M2 tested negative for porcine DNA and contained no non-halal ingredients, the absence of a halal logo may reduce consumer confidence. Halal certification provides assurance that products have been thoroughly reviewed and verified as halal-compliant by certification bodies, which is particularly important for Muslim consumers (Azmi, 2023). Such ambiguity in labelling poses a challenge for consumers who rely on clear information to make informed choices.

A mislabelling issue was identified in sample G2, imported from Turkey. A positive result was detected with a mean Ct value ± SD of 31.82 ± 0.74, comparable to the positive control. The negative control showed no amplification, confirming the absence of cross-contamination during qPCR analysis. Despite carrying a halal logo, it was not recognised by JAKIM. This result raises significant concerns about product authenticity and labelling transparency. Such mislabelling may mislead Muslim consumers and undermine trust in halal certification. Figure 2 shows the halal logo displayed on G2 packaging, which JAKIM does not recognise as an authorised foreign halal certification body, while Figure 3 presents the Turkey halal logo officially recognised by JAKIM.

Figure 2. Halal logo on the packaging for sample G2.

Figure 3. Turkey halal logo approved by JAKIM (Department of Islamic Development Malaysia, 2025).

One of the samples, G1 (Gummy 1), which was labelled as containing pork gelatine as one of the main ingredients, was tested and confirmed positive when compared with the positive control. Porcine DNA was detected with a mean Ct value ± standard deviation of 29.08 ± 0.34. Thus, the labelling of sample G1 was accurate, as the presence of porcine was correctly indicated on the packaging label. Some manufacturers, however, prefer not to disclose the source of gelatine due to consumer perception issues, while others choose to provide clearer labelling and greater ingredient transparency to cater to a wider range of potential markets in line with dietary, religious, and cultural preferences (Mat & Mat, 2023).

Porcine gelatine is often preferred over bovine gelatine in the food industry due to its availability, higher gelling strength and cost-effectiveness (Jufri et al., 2023). Gel strength, also known as the “bloom value”, indicates the average molecular weight of gelatine and reflects its stiffness and strength, which together determine the overall quality of gelatine. The Bloom value typically ranges from 30 to 300 bloom, with values below 150 classified as low bloom, 150–220 as medium bloom, and 220–300 as high bloom. A higher bloom value, therefore, denotes greater gel strength and quality, and porcine gelatine has been reported to exhibit higher bloom strength than bovine gelatine within the pH range of 3–10 (Bhatia et al., 2023).

Consequently, the higher gel strength of porcine gelatine can improve the consistency and texture of food products such as marshmallows, jellies and gummy candies. Moreover, the gelling properties of porcine typically produce a smooth and uniform texture, which is desirable in many confectionery and processed food products. Pork is more widely consumed and more readily available than beef in many regions, accounting for over one-third of global meat production (Agarwal and Fulgoni, 2023). As a result, porcine gelatine is often more affordable and easier to source than bovine gelatine.

Four food samples (G1, G2, M4, and M5) that tested positive for porcine DNA, along with a positive control, were validated through DNA sequencing. The qPCR products were sent to Apical Scientific, Selangor, for sequencing analysis. DNA sequencing output was analysed using BLAST software on the NCBI website, and chromatograms were viewed with FinchTV software. Only forward sequences from the BLAST analysis were reported; however, the reverse primer sequence was cross-checked with the forward sequence, and both exhibit complementarity to one another. The DNA sequence of the samples, including the positive control, displayed a high percent identity with the target sequence in the NCBI database. According to the defined cut-off values, sequences with more than 30% identity are considered homologous and significant (Pearson, 2013).

In this study, the percent identity of BLAST analysis for positive food samples ranged from 86.52% to 100%, while the positive control showed 97.67% - 98.77%. Thus, the high percent identity confirmed that the positive food samples contained ingredients sourced from pigs (*Sus scrofa*), a common species detected in the samples. Table 3 presents the percent identity values of DNA sequencing for the four selected positive samples compared with the positive control.

Table 3. DNA sequencing analysis

Food samples	Common species	Percent identity (%)	
		Forward	Reverse
G1	*Sus scrofa*	98.73	95.18
G2	*Sus scrofa*	86.52	98.70
M4	*Sus scrofa*	97.50	100
M5	*Sus scrofa*	98.70	97.40
PC (control)	*Sus scrofa*	98.77	97.67

Conclusion

Halal authentication for sugar confectionery products is crucial in ensuring consumer acceptance, particularly among Muslim communities. With the growing global demand for halal food products, verifying the halal status of gelatine is a crucial step in meeting the needs of Muslim consumers, thereby enhancing both consumer confidence and the marketability of food products. The findings indicated that 8 out of 9 samples tested positive for porcine DNA, comprising 7 samples without a halal logo and 1 sample bearing an unrecognised JAKIM halal logo.

The molecular detection method applied in this study facilitated the extraction of high-quality and high-quantity DNA for halal authentication, through optimisation steps such as overnight incubation with constant shaking and increased volumes of proteinase K. These findings highlight the significance of accurate food labelling, particularly for the Muslim community in Sibu, Sarawak. Moreover, they raise awareness among both Muslim and non-Muslim consumers regarding halal certification and potential mislabelling issues in food products.

Acknowledgments

The authors would like to express their sincere gratitude to UTS/RESEARCH/1/2022/14 for their generous funding, which made this research possible. The authors would also like to acknowledge the collective support from the University of Technology Sarawak (UTS) community, whose resources and environment fostered an atmosphere conducive to productive research.

References

Afnan Uda, M. N., Parmin, N. A., Jambek, A. B., Hashim, U., Uda, M. N. A., & Shaharuddin, S. N. A. (2020). Evaluation and optimization of genomic DNA extraction from food sample for microfluidic purpose. *IOP Conference Series: Materials Science and Engineering, 743*, Article no. 012031.

Agarwal, S., & Fulgoni, V. L. (2023). Association of Pork (All Pork, Fresh Pork and Processed Pork) Consumption with Nutrient Intakes and Adequacy in US Children (Age 2–18 Years) and Adults (Age 19+ Years): NHANES 2011–2018 Analysis. *Nutrients*, *15*(10), Article no. 2293.

Alipal, J., Mohd Pu'ad, N.A.S., Lee, T.C., Nayan N.H.M., Sahari, N., Basri, H., Idris, M.I, and Abdullah, H.Z., (2021). A review of gelatin: Properties, sources, process, applications, and commercialisation. *Materials Today: Proceedings*, *42*(Part I), pp. 240–250.

Ansor, N. A., Hariyanti, & Rahmi, H. (2023). Narrative review: a study of pork DNA analysis methods in food gelatin. *Journal of Halal Science and Research, 4*(2), 120–129.

Ariffin, M. F. M., Riza, N. S. M., Hamid, M. F. A., Awae, F., & Nasir, B. M. (2021). Halal food crime in Malaysia: An analysis on illegal meat cartel issues. *Journal of Contemporary Issues in Business and Government*, *27*(2), 1407–1412.

Azmi, 2023. *Halal Symbol: More Than Just a Logo.* Retrieved from https://halalfoundation.org/the-importance-of-the-halal-symbol/

Bhatia, S., Al-Harrasi, A., Jawad, M., Shah, Y. A., Al-Azri, M. S., Ullah, S., Anwer, M.K., Aldawsari, M. F., Koca, E., & Aydemir, L.Y. (2023). A Comparative Study of the Properties of Gelatin (Porcine and Bovine)-Based Edible Films Loaded with Spearmint Essential Oil. *Biomimetics*, *8*, Article no. 172.

Chen, X., Lu, L., Xiong, X., Xiong, X., & Liu, Y. (2020). Development of a real-time PCR assay for the identification and quantification of bovine ingredients in processed meat products. *Scientific Reports, 10*, Article 2025.

Cho, Y.-W., Yoon, J., Song, S.-G., & Noh, Y.-W. (2024). Mitochondrial DNA as a target for analyzing the biodistribution of cell therapy products. *Scientific Reports*, 14, Article no. 7934.

Daud, A. H. M., Lee, U. H. M. S., & Ismail, A. (2023). The practice of halal certification: A case of Malaysia's halal meat-based industry. *International Journal of Academic Research in Business and Social Sciences. 13*(8), 1484 – 1497.

Department of Islamic Development Malaysia. (2025). *The recognised foreign halal certification bodies & authorities (As at 25th February, 2025).* Retrieved from Department of Islamic Development Malaysia website: https://myehalal.halal.gov.my/portal-halal/v1/pdf/cb/CBLIST-25FEBRUARY2025.pdf

Fazzini, F., Schöpf, B., Blatzer, M., Coassin, S., Hicks, A. A., Kronenberg, F., & Fendt, L. (2018). Plasmid-normalized quantification of relative mitochondrial DNA copy number. *Scientific Reports*, 8, Article no. 15347.

Food Act 1983 (Act 281) and Regulations. Kuala Lumpur: International Law Book Services.

Gelatin Manufacturers Institute of America. (2019). *Gelatin handbook*. Gelatin Manufacturers Institute of America.

Grand View Research, (2025). Gelatin Market (2025 - 2030). Retrieved from https://www.grandviewresearch.com/industry-analysis/gelatin-market-analysis

Ha, J., Kim, S., Lee, J., Lee, S., Lee, H., Choi, Y., Oh, H., & Yoon, Y. (2017). Identification of Pork Adulteration in Processed Meat Products Using the Developed Mitochondrial DNA-Based Primers*. Korean Journal for Food Science of Animal Resources*, 37(3), 464–468.

Hartel, R. W., Elbe, J. H., & Hofberger, R. (2018). *Confectionery Science and Technology*. Germany: Springer International Publishing.

Hossain, M. A. M., Zainal Abidin, S. A. S., Bujang, A., Taib, M. N., Sagadevan, S., Johan, M. R., & Ahmad Nizar, N. N. (2023). TaqMan multiplex qPCR for detecting animal species in meat and meat products: Development, recent advances, and future prospects. *Food Control*, *150*, 109761.

Jufri, N. W. S., Kareem, F., Ansari, M. A., Taib, S., Hong, S. P., & Ahmed, M. U. (2023). Label-free detection of porcine gelatin: A reliable immunosensor based on multi-walled carbon nanotubes and gold nano-urchins. *Food Chemistry Advances*, *3*(5), Article no. 100411.

Khehra, N., Padda, I. S., & Zubair, M. (2025). Polymerase chain reaction (PCR)*.* In StatPearls*.* StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK589663/

Kim, Y., Lee, H.-S., & Lee, K.-G. (2022). Detection of porcine DNA in Korean processed foods by real-time PCR. *Food Science and Biotechnology, 32*(1), 21–26.

Kowalczyk, M., Staniszewski, A., Kamiñska, K., Domaradzki, P., & Horecka, B. (2021). Advantages, Possibilities, and Limitations of Mitochondrial DNA Analysis in Molecular Identification. *Folia Biologica*, *69*(3), 101–111.

Lucena-Aguilar, G., Sánchez-López, A. M., Barberán-Aceituno, C., Carrillo-Ávila, J. A., López-Guerrero, J. A., & Aguilar-Quesada, R. (2016). DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. *Biopreservation and Biobanking*, *14*(4), 264–270.

Mat, S. A., & Mat, S. R. (2023). Customer Awareness for Gelatin-Based Marshmallow Snack: A Preliminary Reading. Universiti Sains Islam Malaysia. Retrieved from https://oarep.usim.edu.my/browse/author?value=Siti%20Alyani%20Mat

Mohamad, O. A. A. (2022). Islamic Literature Examining Food Fraud Regulations from A Systematic Review Approach. *American Journal of Science Education Research*, *1*(1), 1-7.

Mohd Hafidz, M. M., Makatar, W.-H., Adilan, H., & Nawawee, T. (2020). Detection of pork in processed meat products by species-specific PCR for halal verification: Food fraud cases in Hat Yai, Thailand. *Food Research*, *4*(S1), 244–249.

Nikzad, J., Shahhosseini, S., Tabarzad, M., Nafissi-Varcheh, N. & Torshabi, M. (2017). Simultaneous detection of bovine and porcine DNA in pharmaceutical gelatin capsules by duplex PCR assay for Halal authentication. *DARU Journal of Pharmaceutical Sciences*, *25*(1), Article no. 3.

North Dakota State University Veterinary Diagnostic Laboratory, (2022). *Understanding PCR Ct Values*. Retrieved from <https://www.vdl.ndsu.edu/understanding-pcr-ct-values/

Orbayinah, S., Hermawan, A., Sismindari, & Rohman, A. (2020). Detection of pork in meatballs using probe TaqMan real-time polymerase chain reaction. *Food Research, 4*(5), 1563–1568**.**

Pearson, W.R. (2013). An introduction to sequence similarity ("homology") searching. *Current Protocols in Bioinformatics*, *42*(1), Chapter 3, 3.1.1–3.1.8.

Piskata, Z., Servusova, E., Babak, V., Nesvadbova, M., & Borilova, G. (2019). The Quality of DNA Isolated from Processed Food and Feed via Different Extraction Procedures. *Molecules*, *24*(6), Article no. 1188.

Qiagen, (2020). *DNeasy® mericon® Food Handbook 2/2020*. Retrieved from https://www.qiagen.com/us/resources/resourcedetail?id=bd9cc2a8-aa71-4cb5-b6f7-97b3d7fc306d&lang=en

Rahayu, W. P., Dianti, A. R. W., Nurjanah, S., Pusparini, N., & Adhi, W. (2020). Detection of DNA pork in processed meat products with real-time polymerase chain reaction. *Food Research*, *4*(5), 1719–1725.

Rahman, M. M., Ali, M. E., Hamid, S. B. A., Bhassu, S., Mustafa, S., Hashim, U., Amin, M. A., & Razzak, M. A. (2017). Lab-on-a-chip PCR-RFLP assay for the detection of canine DNA in burger formulations. *Food Analytical Methods, 8*, 1598–1606.

Rather, J. A., Akhter, N., Ashraf, Q. S., Mir, S. A., Makroo, H. A., Majid, D., Barba, F. J., Mousavi Khaneghah, A., & 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*, 100945.

Sajali, N., Ting, S. M. L., Koh, C. C., Desa, M. N. M., Wong, S. C., & Abu Bakar, S. (2022). Meatball model of porcine DNA detection by TaqMan probe real-time PCR. *Food Research*, *6*(3), 136–144.

Stephenson, F. H. (2016). Nucleic acid quantification. In F. H. Stephenson (Ed.), *Calculations for Molecular Biology and Biotechnology* (3rd ed., pp. 97–129). United States: Academic Press.

Uddin, S. M. K., Hossain, M. A. 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*(Part A), 101422.

Wilson, K., & Walker, J. (2005). Principles and Techniques of Biochemistry and Molecular Biology. United Kingdom: Cambridge University Press.

Wu, Y., Yang, Y., Liu, M., Wang, B., Wang, Y., & Wang, H. (2019). Applying *COI* barcode to identify animal origin of food. *Journal of Food Science*, *84*(6), 1256–1265.

Yu, W., Xu, D., Li, D., Guo, L., Su, X., Zhang, Y., Wu, F. and Xu, X., (2019). Effect of pigskin-originated gelatin on properties of wheat flour dough and bread. *Food Hydrocolloids*, 94, pp. 183–190.

Zilhadia, Z., Izzah, A. N., & Betha, O.S. (2017). Comparison of SYBR Green and hydrolysis probe in analyzing porcine and bovine gelatin DNA using real time PCR. *Jurnal Sains Farmasi & Klinis*, *4*(1), 16-23.

How to cite this paper:
Mohammad, A.A., Sajali, N., Koh, C.C., Desa, M.N.M. (2025). Porcine DNA Authentication in Sugar Confectionery Products. *Malaysian Journal of Applied Sciences, 10(*2), 31-44.