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Narrative review: a study of pork DNA analysis methods in food gelatin

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ABSTRACT

Gelatin is a substance resulting from the partial hydrolysis of collagen consisting of animal cartilage, skin, and bones from water-soluble polypeptides. The problem formulation in this study is what DNA analysis methods can be used to detect pig DNA in food gelatin. This study aimed to collect, review, and conclude information regarding the most widely used DNA analysis method to detect pig DNA in food gelatin. From the results of the literature study, it can be concluded that the most widely used extraction method in the analysis of porcine DNA in food gelatin is the DNeasy® Mericon Food kit extraction method for good purity results, there is the foodproof® III Kit method, the universal Wizard KIT Promega®, the DNeasy® Mericon Food kit, and the Column based FavorPrep™ Food DNA Extraction Kit. The pig DNA analysis method is widely used in conventional PCR. Sensitivity, fast testing time, and gh acporcine gelatin sample was identified by the appearance of a band on the electrophoresis results.
Keywords: Food Gelatin, Extraction Method, DNA Analysis, Journal Review.

INTRODUCTION

In Law Number 33 of 2014 Concerning Halal Product Guarantees. According to Article 1, "products" include items and/or services linked to beverages, pharmaceuticals, food, cosmetics, biological products, genetically altered products, and chemical products, as well as goods used, employed, or eaten by the general public, particularly food gelatin. Article 4 of the legislation says that items entering, circulating, and trading on Indonesian territory must be halal certified.

Gelatin is a substance resulting from the partial hydrolysis of collagen consisting of animal cartilage, skin, and bones from water-soluble polypeptides (Milovanovic & Hayes, 2018). Gelatin makes capsule shells, tablet coatings, emulsifiers, gel formers, thickeners, suspenders, and film formers (Aisyah, Huda, Azhar, & Fazilah, 2014). In the food industry, gelatin is widely used as a raw material for gummies, soft candy, ice cream, marshmallows, and jellies. In various industrial sectors, gelatin is widely used in industrial sectors such as cosmetics, pharmaceuticals, photography, medical products, and food.

Gelatin-based food is a food ingredient whose halal status is still doubtful. The source of extracted gelatin is circulating in the market, generally from cowhide, pork skin, beef bones, and pork bones (Sarbon, Badii, & Howell, 2013). The results obtained in the extraction process are called DNA isolates. DNA isolation methods, namely the CTAB method, SDS method, kit method, modification method, and the DNAZole method. In various DNA analysis tests, the initial stage that is carried out is DNA isolation. The DNA isolation stage results largely determine the next process's results (Nurhayati & Darmawati, 2017).

Determining gelatin derived from pigs can use DNA Polymerase Chain Reaction analysis(Amqizal et al., 2017). Polymerase Chain Reaction is a method of replication or DNA fragment amplification enzymatically without using organisms or in vitro, involving several cycles (repetitive stages). At each stage repeated, there is a duplication of the target number of double-stranded DNA up to millions of DNA fragment copies (Nurhayati & Darmawati, 2017).

The Polymerase Chain Reaction method is a DNA-based method, one type of detection method commonly used to determine the content of pigs and their derivatives. DNA-based RT-PCR analysis (Real Time Polymerase Chain Reaction) can be made in processed products. This method was obtained to determine the gelatin mixture of cattle and pigs at a contamination level of 1% (Demirhan, Ulca, & Senyuva, 2012) & (Cai, Gu, Scanlan, Ramatlapeng, & Lively, 2012).

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Tests using the Polymerase Chain Reaction have been carried out by many previous researchers, in the research of Cai et al. (2012), namely to determine and calculate the amount of bovine and porcine gelatin in the gelatin mixture using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) method and in research by Fadhlurrahman et al. (2015) namely detecting pork gelatin in soft candy using the PCR-RFLP method as one of the halal foods. Based on this description, a literature study was carried out to further examine the study of pig DNA analysis methods in food gelatin. This aims to see what analytica detect pig DNA in food gelatin.

RESEARCH METHOD

Research design

The method used in this research is the literature study method. The keywords used in English are (extraction) or (isolation) and (DNA) and (analysis) or (detection) and (porcine) and (gelatin) and (food). Then, it is continued to study or examine the articles so that a narrative journal will be produced.

Time and Place of Research

This journal literature study was carried out in November 2021 – February 2022. The research was conducted online (in a network).

Population and Sample

1. Population

The research population is research journals published in 2011-2021. Sources of research data were obtained by search engines on Google Scholar, Science Direct, and Pubmed using both English and Indonesian, so the results obtained were 1,440 journals. Then, the topic and title relatedness were selected to become 74 journals, the final journal which can be analyzed according to the problem formulation and objectives and what has been reviewed according to inclusion into 11 journals.

2. Sample

The research sample is a scientific journal that meets the inclusion criteria and is not included in the exclusion criteria and the search for articles using the PRISMA diagram of the flow of literature compilation.

Method

1. Data Collection

   Techniques for collecting data are as follows: Looking for journal papers relevant to the next journal review. Choose articles that closely fit the criteriarnals examined. The collected journal articles will be assessed based on the differences arising from different journals, and then the outcomes of numerous journals that are suited to the study goals will be concluded.

   The data collected was in the form of primary literature. Primary literature was obtained from Google Scholar, Science Direct, and Pubmed. Data were selected based on predetermined inclusion and exclusion criteria.

2. Inclusion criteria

   Inclusion criteria are criteria that define research participants as research samples that fulfill the standards for inclusion as a sample. (Notoatmodjo, 2012). Timeframes other than 2011-2021 are excluded from this study, journals using Indonesian and English, and journals indexed by online databases such as Google Scholar, Science Direct, and Pubmed. Journals that have free full-text and accessible journals. Journals that use gelatin in food. The DNA extraction and analysis method used is a polymerase chain reaction.

3. Exclusion criteria

   Exclusion criteria are factors that determine whether research participants may represent the sample because they do not fulfill the standards of a research sample (Notoatmodjo, 2012). Exclusion criteria in this study are timeframe other than 2011-2021. Journals that use other languages besides Indonesian and English. Journals that are not free full text and not indexed by online databases such as Google Scholar, Science Direct, and Pubmed. Review and book cannot be used as data.

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4. Data analysis

Finding results search data will be analyzed using the PRISMA diagram of the review preparation flow.

RESULTS AND DISCUSSION

This study, 11 journal articles were used, consisting of 2 Indonesian-language journals and 9 English-language journals. The collected journal articlcomprised 10 extraction methods and 5 DNA analysis methods. Based on the results of the collection of articles that have been selected, the results of the methods from each journal, along with the analysis for DNA, are obtained.

Table 1. The DNA extraction of article review.

No	Sample	Extraction Methods	Conc (ng/µL)	Purity	Agarose Gel	Reference
1.	(7) Soft candies, (2) marshmallows, (2) gums	*DNeasy® Mericon Food Kit* (QIAGEN)	<0.0005 0.0238 <0.0005 0.0484 0.0164 0.0174 0.0208 <0.0005 0.0226 <0.0005 <0.0005	-	-	(Colmenero, Martínez, Roca, & García-Vázquez, 2016)
2.	(76) Candy and (27) marshmallow	*Wizard® SV Genomic DNA Purification System* (Promega, Madison, USA)	-	-	-	(Omar et al., 2018)
3.	(43) *samples of the soft and fruity chew confectionery (gum drops), marshmallows/cakes, jelly, and Turkish delight*	*Kit sure food® prep animal X* (CONGEN, R-Biopharm, jerman)	-	-	-	(Demirhan et al., 2012)
4.	*Soft candy (jelly candy, Chewing gum, marshmallow)*	Modification based on research. Erwanto *et al*., (2014), Ilhak & Arslan (2007)	13.35 19.53 23.63 33.98 36.84 59.45 74.81 89.36 141.67 178.36	1.76 1.45 1.57 1.46 1.48 1.49 1.66 1.27 1.5 1.15	359 bp	(Fadhlurrahman, Wardani, & Widyastuti, 2016)
5.	(15) *Soft gel candy*	Universal *Wizard Kit Promega®*	1.579 0.887	1.700 1.821	±149bp	(Rachmawati,

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No	Sample	Extraction Methods	Conc (ng/µL)	Purity	Agarose Gel	Reference
			0.770 0.611 2.097 3.480 1.101 2.185 1.686 2.425 3.688 0.930 2.675 0.939 1.278	1.840 1.964 1.808 1.910 2.045 1.930 2.020 1.977 1.950 2.117 1.879 1.851 2.025		Rokhim, Munir, & Agustina, 2018)
6.	Gummy candies, Candies, Chewing Gum	Foodproof® Sample Preparation Kit III (Biotecon Diagnostics)	47±14.2 81.3±11.9 48.3±5.3	1.8±0.09 1.8±0.07 1.8±0.09	-	(Yayla & Ekinci Doğan, 2021)
		TübiGel Method	116±29.3 87.2±4.2 39.2±4.7	1.2± 0.05 1.2±0.12 1.5±0.13		
7.	(2) Marshmallow, (2) Jelly	DNeasy® Mericon Food Kit (Qiagen, Hilden, Jerman)	-	-	212 bp	(Shabani et al., 2015)
8.	(12) Jelly, (9) marshmallow, (6) candy, (2) chewing gum, (1) gummy pizza	Milligan Method	-	-	134 bp 290 bp	(Amqizal et al., 2017)
9.	(14) Gummy, (10) marshmallow, (9) candy and (5) pastilles	DNeasy® Mericon Food kit	8-34 12-26 4-24 7-37.1	1.7-2.00 1.7-2.2 1.7-1.9 1.8-2.00	87 bp	(Sultana et al., 2018)
10.	(26) jelly, marshmallow, chewing gum, candy, and cake	DNeasy® Mericon Food kit	Jelly (Beef gelatin) $31x10^{-6}$, cake (Beef gelatin) $13x10^{-7}$, Candies (No gelatin labeled)	-	134 bp 290 bp	(Al-Kahtani, Ismail, & Asif Ahmed, 2017)

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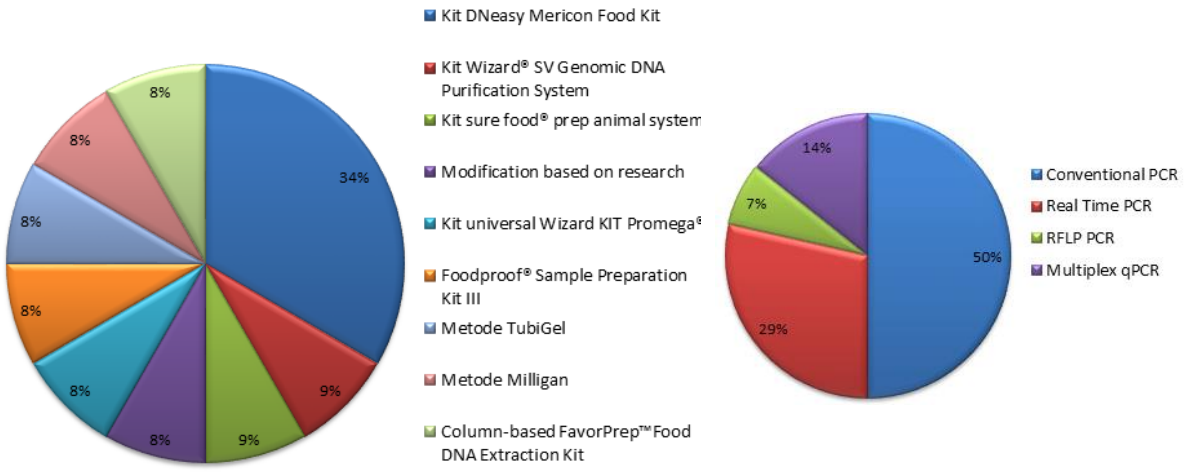
No	Sample	Extraction Methods	Conc (ng/µL)	Purity	Agarose Gel	Reference
			$42x10^{-8}$			
11.	(10) *Gummy sweets*, (10) *Candy and pastilles*, (3) *jellies* and *puddings*	Column-based FavorPrep™Food DNA Extraction Kit	18-34 4-24 8-25	1.7-1.9 1.7-1.9 1.8-1.9	-	(Sultana et al., 2020)

DNA extraction is a series of processes to separate DNA from other cell components(Nurhayati & Darmawati, 2017). Various molecular biology analyses require deoxyribonucleic acid extraction results with good quality stages and good purity. The DNA extraction results must be free from various contaminants, namely RNA (ribonucleic acid) and proteins that can interfere with the ongoing process of the polymerase chain reaction. Each method of isolation or extraction of deoxyribonucleic acid (DNA) has certain modifications, and the same principles are used to damage the inhibitors in the source of deoxyribonucleic acid (Muladno, 2002).

Table 1 shows various DNA extraction methods used in food gelatin, namely Dneasy Mericon Food Kit, Wizard SV Genomic DNA Purification System, Sure Food Pref Animal X Kit, Universal Wizard Kit Promega, Foodproof ®sample Preparation Kit III (Biotecon Diagnostics), Sure Food PREP Advanced (R-Biopharm), Tubigel Method, Column-based FavorPrepTM Food DNA Extraction Kit, Milligan Method and modification method based on previous research.

The results of the percentage of journal article reviews that are widely used in research can be seen in Figure 1. The extraction method that is often used is the DNeasy® Mericon Food Kit method has a percentage of 34%, while the DNA analysis method often used is the conventional PCR method with a percentage of 50%.



(A) (B)

Figure 1. Percentage of articles with (A) DNA extraction and (B) DNA analysis methods.

The extraction method results are the different concentrations and purity of DNA (Table 1). The DNA generally has a good concentration, is >125 ng/µl. Small values can be affected by the repeated purification process on phenol: chloroform: isoamyl alcohol (P: C: I) (Fadhlurrahman et al., 2016).

In a study by (Griffin & Griffin, 2021), nucleotides' maximum absorption of light was used at a wavelength of 260 nm. Whereas at a wavelength of 280 nm, maximum absorption by proteins. The purity value of DNA is obtained by calculating the absorbance value of λ260/λ280 nm, and for the calculation results, the value of DNA purity ranges from 1.8 to 2.0 (Lucena-Aguilar et al., 2016).

Molecular Molecular analysis of DNA results with a range of 1.8-2.0 has been noted to meet the desired requirements (Sambrook & Russell, 2001). The purity value of DNA is below 1.8, indicating that the extracted DNA still contains contaminants, namely protein, and the purity value of DNA above

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2.0 indicates that the extracted DNA still contains RNA contaminants. According to Widayat et al. (Widayat, Winarni Agustini, Suzery, Ni'matullah Al-Baarri, & Rahmi Putri, 2019), apart from protein and RNA, kit components or reagents are carried along in the extraction process, such as alcohol, chloroform, and phenol, which can affect the total DNA purity results.

It can be concluded that the results of the research by (Sultana et al., 2018), (Rachmawati et al., 2018), (Sultana et al., 2020), and (Yayla & Ekinci Doğan, 2021) have a purity value that matches the value of DNA quality provisions. In the study by (Colmenero et al., 2016), (Omar et al., 2018), (Demirhan et al., 2012), (Shabani et al., 2015), (Amqizal et al., 2017) and (Al-Kahtani et al., 2017) there is no purity value. DNA purity values below 1.8 can potentially disrupt the running process of PCR (Rachmawati et al., 2018).

The non-optimal results of DNA extraction are contamination by secondary metabolites, debris, and phenols. For example, in the lysis stage in tissues or cells, several physical methods such as grinding samples exist. Cell membrane destabilization occurs due to lysis (destruction) chemically using detergents that can dissolve lipids in cell membranes (Rachmawati et al., 2018).

Table 2 shows various PCR (polymerase chain reaction) techniques for DNA analysis in pigs. DNA analysis in the journal articles reviewed were PCR amplification, real-time PCR, conventional PCR, RFLP PCR, and multiplex PCR. The main difference between conventional polymerase chain reaction and RT-PCR is that real-time polymerase chain reaction adds the potential for quantitative analysis. In contrast, conventional polymerase chain reaction is usually qualitative and only gets negative and positive results.

Table 2. The DNA analysis methods of article review.

No	Sample	DNA Analysis Methods	Agarose Gel	CT/CQ	LOD	Reference
1.	(7) Soft candies, (2) marshmallows, (2) gums	PCR	118 bp	-	-	(Colmenero *et al*. 2016)
2.	(76) Candy and (27) marshmallow	PCR	74 bp	-	-	(Omar *et al*., 2018)
3.	(43) *samples of the soft and fruity chew confectionery (gum drops), marshmallows/ cakes, jelly, and Turkish delight*	Real-Time PCR	-	Ct 30.04 and 43.00 (Gum drop Germany) Ct 36.3 (Marshmallows Turkey)	1%	(Demirhan *et al*. 2011)
4.	*Soft candy (jelly candy, Chewing gum, marshmallow)*	PCR RFLP	359 bp 228 bp-131 bp	-	-	(Fadhlurrahman *et al*. 2015)
5.	(15) *Soft gel candy*	PCR	±149bp	-	-	(Rachmawati*et al*. 2018)
6.	Gummy candies,	Real-Time PCR	-	27.91± 0.12 23.21± 0.12 33.16± 0.15	0.01% (TübiGel)	(Yayla and Dogan 2021)

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No	Sample	DNA Analysis Methods	Agarose Gel	CT/CQ	LOD	Reference
	Candies			31.80± 0.26 34.39± 0.16 33.35± 0.32 33.53± 0.03 32.97± 0.17 34.87± 0.21 33.32± 0.11 32.41± 0.31 31.85± 0.56	0.1% (Biotecon)	
7.	(2) Marshmallow, (2) Jelly	PCR	212 bp	-	0.1%	(Shabani *et al*. 2015)
8.	(12) Jelly, (9) marshmallow, (6) candy, (2) chewing gum, (1) gummy pizza	PCR and Real-Time PCR	134 bp 290 bp	20.540 to 34.157	-	(Amqizal *et al*. 2017)
9.	(14) Gummy, (10) marshmallow, (9) candy and (5) pastilles	Multiplex PCR	87 bp	-	0.001 ng	(Sultana *et al*. 2018)
10.	(26) jelly, marshmallow, chewing gum, candy, and cake	PCR and Real-Time PCR	134 bp 290 bp	35.92 40.14 41.71	≤0.0001 ng/µL	(Al-Kahtani *et al*. 2016)
11.	(10) *Gummy sweets*, (10) *Candy and pastilles*, (3) *jellies* and *puddings*	Multiplex qPCR	-	30.12 ± 0.22 (Yupi Gummy Pizza) 28± 0.2 (Jelly Bean)	0.15 ng/µL	(Sultana *et al*. 2020)

One method that can increase the sensitivity and specificity of PCR results is the RFLP (Restriction Fragment Length Polymorphism) method (Fadhlurrahman et al., 2015). The RFLP method utilizes restriction enzymes, which will differentiate between pig DNA and porcine homologous DNA based on the restriction sites of the enzymes used. With the multiplex PCR method, it is possible to Amplify two or more target sequences (multi-targets) with a single PCR reaction to conserve tools, money, time, and reagents.

In this study, the real-time polymerase chain reaction DNA amplification analysis was performed to investigate the growth in the curve and the cycle threshold (ct) value on the amplification curve. The cycle threshold is the number of times the sample's repeated stages (cycles) begin to be read, signaling the start of the exponential growth phase. The higher the ct value, the less target DNA there is; conversely, the lower the ct value, the more target DNA there is. (Zilhadia, Izzah, & Betha, 2017).

In the research by Demirhan et al. 2011, Yayla and Dogan 2021, Amqizal et al. 2017, Al-Kahtani et al. 2016, Sultana et al. In 2020, Ct/Cq results were available. The Ct (cycle threshold) value of vertebrates and the Ct (cycle threshold) value of pigs to determine the concentration of pig DNA. The

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validation check of the Quantification Kit and Progenus Easyfast™ Pig protocol from EPC has a cycle threshold value (Ct) of pigs and cycle threshold (Ct) of vertebrates, which is 30.00. In contrast, in the validation check of NTC, it has a cycle threshold (Ct) value of pigs and cycle threshold (Ct) of pigs and cycle threshold (Ct) of pigs. Ct) vertebrates, namely above the number 38.00.

is more precise and can identify even minute amounts of extremely degraded DNA. (Al-Kahtani et al., 2017) demonstrated this by utilizing RT-PCR to detect pig deoxyribonucleic acid (DNA) in gelatin and gelatin-containing food items at 0.0001 ng/L. Sensitivity, testing time, and accuracy are all factors to consider when selecting the real-time polymerase chain reaction technique for species identification testing.

The results of electrophoretic data can be seen in Table 1. The deoxyribonucleic acid (DNA) ladder is a standard DNA of known size (250-10000 bp) used in determining fragment size by comparing the mobility of the DNA fragments that have been obtained. Bovine and swine gelatin samples were detected by the appearance of two bands obtained from the results of the electrophoresis process.

With a PCR amplification result of 359 bp using primer cyt b and the creation of two DNA fragments measuring 228 bp and 131 bp using the BseD1 enzyme, the PCR-RFLP approach was able to identify between pig DNA and bovine DNA (Fadhlurrahman et al., 2016). The primers Pork-F and Pork-R were employed in this investigation to enhance the internal segment 149 bp of the mitochondrial cytochrome b (cyt b) gene (Rachmawati et al., 2018). (Omar et al., 2018) generated a unique pig-specific primer to amplify the 74 bp amplicon of the mitochondrial genome's D-loop region (accession number: FM244467.1).

DNA fragments with a length of 212 bp were amplified by the method described in the study (Shabani et al., 2015), which showed that DNA degradation could not present a major obstacle to the PCR technique. In the study by Amqizal et al. (Amqizal et al., 2017), four products were positive for pork contamination, as indicated by the amplification of 134 bp and 290 bp fragments. All the biomarkers used were stable and detectable in all conditions due to the shorter length aspect where the pork amplicon was only 87 bp (Sultana et al., 2020).

Suppose a negatively charged deoxyribonucleic acid (DNA) molecule is given an electric field at both ends of the gel. In that case, the negatively charged DNA will move from the negative pole (cathode) to the positive pole (anode). The movement of DNA in the gel depends on the size or weight of the DNA molecule, the concentration of agarose, the conformation of the DNA, the strength of the electrophoretic buffer, and the electrical voltage used. Deoxyribose nucleic acid (DNA) can be visualized because ethidium bromide (EtBr) dye can be trapped between pairs of DNA bases, which, when irradiated with UV radiation, can emit light until it can be detected.

According to Muladno (Muladno, 2002), the quality of deoxyribonucleic acid can be estimated by looking at the fluorescence intensity emitted by EtBr (ethidium bromide) compared to standard deoxyribonucleic acid. In the pig genome, the DNA band is thinner, and there are many smears; for bovine genomic DNA bands, it is thicker, andhase few smears. Deoxyribonucleic acid is fragmented in the mechanical treatment process so that DNA fragments with a smaller molecular weight move faster away from the well, which is assumed to be a smear.

The detection limit is the smallest limit test parameter owned by a tool or instrument to measure a certain amount of analyte. The relative detection limit is the capacity to recognize the target species with the lowest ratio in the meat combination, and the absolute detection limit is the ability to distinguish the target species with the fewest templates (Li, Li, Liu, Wei, & Wang, 2021). LOD values for detecting pigs in the simplex assay and multiplex PCR (data not provided) are comparable to more costly and newly published real-time PCR-based approaches (Al-Kahtani et al., 2017). This technique identifies and quantifies 0.15 ng/L DNA of a target species in pure and mixed gelatin samples (Sultana et al., 2020).

CONCLUSION

The results of the literature study collected after reviewing 11 journals, according to the inclusion and exclusion criteria, the DNeasy® Mericon Food kit extraction technique is the most extensively used in the analysis of pig DNA in food gelatin, and the Foodproof Kit method produces high purity findings. ® III, the Wizard KIT Promega® universal kit, the DNeasy® Mericon Food kit, and the column based FavorPrepTMFood DNA Extraction Kit. The pig DNA analysis method that is widely used is

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conventional PCR. Sensitivity, fast testing time, and high accuracy are considerations in selecting the polymerase chain reaction method for species identification testing. The porcine gelatin sample was identified by the appearance of a band on the electrophoresis results.

REFERENCES

Aisyah, N., Huda, N., Azhar, M., & Fazilah, A. (2014). Poultry as an alternative source of gelatin. *Health and the Environment Journal*, *5*(1), 37–49.

Al-Kahtani, H. A., Ismail, E. A., & Asif Ahmed, M. (2017). Pork detection in binary meat mixtures and some commercial food products using conventional and real-time PCR techniques. *Food Chemistry*, *219*, 54–60. https://doi.org/10.1016/j.foodchem.2016.09.108

Amqizal, A., Ibrahem, H., Al-Kahtani, H. A., Ismail, E. A., Hayat, K., & Jaswir, I. (2017). Identification and verification of porcine DNA in commercial gelatin and gelatin containing processed foods. *Food Control*, *78*, 297–303. https://doi.org/10.1016/j.foodcont.2017.02.024

Cai, H., Gu, X., Scanlan, M. S., Ramatlapeng, D. H., & Lively, C. R. (2012). Real-time PCR assays for detection and quantitation of porcine and bovine DNA in gelatin mixtures and gelatin capsules. *Journal of Food Composition and Analysis*, *25*(1), 83–87. https://doi.org/10.1016/j.jfca.2011.06.008

Colmenero, M. M., Martínez, J. L., Roca, A., & García-Vázquez, E. (2016). Authentication of commercial candy ingredients using DNA PCR-cloning methodology. *Journal of the Science of Food and Agriculture*, *96*(3), 859–867. https://doi.org/10.1002/jsfa.7158

Demirhan, Y., Ulca, P., & Senyuva, H. Z. (2012). Detection of porcine DNA in gelatine and gelatine-containing processed food products-Halal/Kosher authentication. *Meat Science*, *90*(3), 686–689. https://doi.org/10.1016/j.meatsci.2011.10.014

Fadhlurrahman, Wardani, A. K., & Widyastuti, E. (2016). Deteksi gelatin Babi pada soft candy menggunakan metode PCR-RFLP sebagai salah satu pembuktian kehalalan pangan. *Jurnal Teknologi Pertanian*, *16*(2), 81–88.

Griffin, H. G., & Griffin, A. (2021). Nucleic acid quantitation. *PCR Technology*, 196–196. https://doi.org/10.1201/b12376-35

Li, J., Li, J., Liu, R., Wei, Y., & Wang, S. (2021). Identification of eleven meat species in foodstuff by a hexaplex real-time PCR with melting curve analysis. *Food Control*, *121*(March). https://doi.org/10.1016/j.foodcont.2020.107599

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. https://doi.org/10.1089/bio.2015.0064

Milovanovic, I., & Hayes, M. (2018). Marine gelatine from rest raw materials. *Applied Sciences (Switzerland)*, *8*(12), 1–20. https://doi.org/10.3390/app8122407

Muladno. (2002). *Seputar teknologi rekayasa genetika*. Pusataka Wirausaha Muda.

Notoatmodjo, S. (2012). *Metodologi penelitian kesehatan*. Rineka Cipta.

Nurhayati, B., & Darmawati, S. (2017). *Biologi sel dan molekuler*. Kementerian Kesehatan Republik Indonesia, Badan Pengembangan dan Pemberdayaan Sumber Daya Manusia Kesehatan.

Omar, S., Hasan, M., Abu-Romman, S., Ramadan, H., Qatatsheh, A. A., & Al-Dmoor, H. (2018). Design and validation of short-amplicon length PCR assay for the detection of porcine gelatin in commercial candy and marshmallow products. *Current Research in Nutrition and Food Science*, *6*(3), 742–747. https://doi.org/10.12944/CRNFSJ.6.3.16

Rachmawati, Y., Rokhim, S., Munir, M., & Agustina, E. (2018). Deteksi kontaminan fragmen DNA pengkode Cyt b Babi pada sampel softgell candy tak berlabel halal. *Indonesia Journal of Halal*, *1*(1), 25. https://doi.org/10.14710/halal.v1i1.3115

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual. Third Edition. In *United States: Cold Spring Harbor Laboratory Press.*

Sarbon, N. M., Badii, F., & Howell, N. K. (2013). Preparation and characterisation of chicken skin gelatin as an alternative to mammalian gelatin. *Food Hydrocolloids*, *30*(1), 143–151. https://doi.org/10.1016/j.foodhyd.2012.05.009

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Shabani, H., Mehdizadeh, M., Mousavi, S. M., Dezfouli, E. A., Solgi, T., Khodaverdi, M., Rabiei, M., Rastegar, H., & Alebouyeh, M. (2015). Halal authenticity of gelatin using species-specific PCR. *Food Chemistry*, *184*, 203–206. https://doi.org/10.1016/j.foodchem.2015.02.140

Sultana, S., Hossain, M. A. M., Azlan, A., Johan, M. R., Chowdhury, Z. Z., & Ali, M. E. (2020). TaqMan probe based multiplex quantitative PCR assay for determination of bovine, porcine and fish DNA in gelatin admixture, food products and dietary supplements. *Food Chemistry*, *325*, 126756. https://doi.org/10.1016/j.foodchem.2020.126756

Sultana, S., Hossain, M. A. M., Zaidul, I. S. M., & Ali, M. E. (2018). Multiplex PCR to discriminate bovine, porcine, and fish DNA in gelatin and confectionery products. *Lwt*, *92*(June), 169–176. https://doi.org/10.1016/j.lwt.2018.02.019

Widayat, W., Winarni Agustini, T., Suzery, M., Ni'matullah Al-Baarri, A., & Rahmi Putri, S. (2019). Real Time-Polymerase Chain Reaction (RT-PCR) sebagai alat deteksi DNA Babi dalam beberapa produk non-pangan. *Indonesia Journal of Halal*, *2*(1), 26. https://doi.org/10.14710/halal.v2i1.5361

Yayla, M. E. A., & Ekinci Doğan, C. (2021). Development of a new and sensitive method for the detection of pork adulteration in gelatin and other highly processed food products. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *38*(6), 881–891. https://doi.org/10.1080/19440049.2021.1902574

Zilhadia, Z., Izzah, A. N., & Betha, O. S. (2017). Perbandingan metode SYBR Green dan hydrolysis probe dalam analisis DNA gelatin Sapi dan gelatin Babi menggunakan real time polymerase chain reaction. *Jurnal Sains Farmasi & Klinis*, *4*(1), 16. https://doi.org/10.29208/jsfk.2017.4.1.194