DNA purification from meatballs as a basis of halal authentication based on genetic markers

N Purnomo¹, D Ramadhanti¹, M Mansur¹, M Nur² and M I A Dagong²,³

¹Animal Husbandry Study Program, Faculty of Science and Technology, Muhammadiyah Sidenreng Rappang University, Sidenreng Rappang, Indonesia
²Integrated Biotechnology Laboratory, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia
³Animal Production Department, Faculty Of Animal Science, Hasanuddin University, Makassar. Indonesia

E-mail: purnomo.nupo@gmail.com

Abstract. The mixing of non-halal meat in meatballs cannot be physically distinguished, so an approach based on molecular genetics is needed. However, until now there has been no information on the proper DNA purification method to obtain DNA from meatballs. This study was designed to obtain the most appropriate method of DNA purification from meatballs through modification of the preparation and number of samples used as the basis for halal authentication genetic marker-based. The study was conducted using a completely randomized design, in which the preparation method as the main factor consisted of fresh sample preparation (P1), oven-dry sample preparation (P2), and freeze-drying sample preparation (P3). The second factor is the sample weight consisting of a sample weight of 25 mg (B1), a sample weight of 50 mg (B2), a sample weight of 75 mg (B3), a sample weight of 100 mg (B4). Each treatment combination was cashed twice so that the number of experimental units was 24. The results of this study indicated that there was no significant difference (P> 0.05) between the treatment methods of sample preparation and sample weight to the purified DNA concentration of meatballs. There was also no significant difference (P> 0.05) between the treatment methods of sample preparation and the number of meatball samples on the purity of DNA purified from the meatballs. So it can be concluded that the preparation method and the number of samples did not affect the concentration and purity of purified DNA from meatballs.

1. Introduction
Indonesia is a country with a majority of the population is Muslim, namely 87% of the population or reaching 217 million people [1]. As a Muslim community, they should use and or consume halal food, drinks, medicines, cosmetics, and other necessities. However, there are often business actors who cheat by mixing non-halal meat in processed food that has received a halal label to get more profit [2]. The mixing of pork into processed beef usually occurs when the price of beef is soaring due to the scarcity of stock [3]. One of the processed meat foods that are often mixed with non-halal meat is meatballs.

Meatball is the result of processing ground beef mixed with flour, spices, and other ingredients that are ground, then formed into balls, and then boiled until cooked. The term meatball is usually followed by the name of the type of meat, such as fish balls, shrimp meatballs, chicken meatballs, beef...
meatballs, rabbit meatballs, buffalo meatballs, and goat meatballs [4]. Beef meatball is the most popular type of meatball on the market, because the raw materials for making it are beef, apart from being halal, has also been commonly consumed by the public [5]. Seeing the fact that beef meatballs cannot be physically distinguished from pork meatballs or beef meatballs mixed with pork, it is necessary to have a halal guarantee for consumers. Halal assurance does not only come from the producer, but also from an independent institution that can authenticate the halalness of a food product.

Several authentication methods currently used include enzyme-linked immunosorbent assay (ELISA) [6,7], electronic nose (e-nose), gas chromatography-mass spectrometer with headspace analyzer (GCMS-HS) [7,8], immuno-chromatography or what is known as rapid test [7], polymerase chain reaction (PCR) [9–13], DNA hybridization [14] and liquid chromatography-mass spectrometry (LC-MS) [15]. The current most reliable halal authentication method is a genetic marker-based examination. The gene that has currently been agreed to be used as a marker gene is the COI gene [16]. The COI gene has been shown to function as the core of a global bio identification system for animals [17]. Almost all higher animals can be identified using marker genes [16]. To carry out genetic marker-based identification, a total genome of DNA originating from certain body parts of the living creature is needed, generally using parts of the body that contain lots of DNA and are easy to lyse. Meanwhile, meatballs are processed from meat that has been added with other constituent ingredients and undergoes various grinding and heating processes which can cause a decrease in the amount and quality of DNA. Thus, a proper DNA purification method from meatballs is needed to obtain the DNA genome as needed. However, currently, there is not enough information regarding the DNA purification method from meatballs. The purpose of this study was to determine the effect of the preparation method and the number of samples on the concentration and purity of the purified DNA from meatballs.

2. Materials and methods

2.1. Materials
This research was conducted at the Integrated Biotechnology Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar. The sample used is frozen Beef Meatballs with the trademark Bernardi® produced by PT. Eloda Mitra. Meatball samples were obtained from supermarkets and put in impermeable containers to maintain the temperature during the trip to the laboratory. For DNA extraction using TiaNamp Genomic DNA Kit® from Tiangen Biotech (Beijing), Co.LTD.

2.2. Experimental design and methods
This research was conducted using a randomized block design referring to [18]. The first factor is the sample preparation method consisting of fresh sample preparation (P1), oven-dry sample preparation (P2), and sample preparation freeze-drying (P3). The second factor is the difference in sample weight consisting of a sample weight of 25 mg (B1), a sample weight of 50 mg (B2), a sample weight of 75 mg (B3), and a sample weight of 100 mg (B4). Each treatment was repeated 2 times, bringing the total number of experimental units to 24.

Meatball samples obtained from supermarkets were thawed at room temperature until they were not frozen, then sliced into small pieces and mashed using a mortar. Then the meatball samples were weighed and prepared according to the treatment. Fresh sample preparation is carried out by storing the sample back in the freezer, oven-dry sample preparation is carried out by oven-drying the sample at a temperature of 70°C for 12 hours, and sample preparation Freeze drying is carried out by freeze-drying the sample in a freeze-dry machine for 12 hours or until the sample get dry. Furthermore, all meatball samples were extracted DNA using TiaNamp Genomic DNA Kit® from Tiangen Biotech (Beijing), Co.LTD by following the company's manual.
2.3. Parameters and Laboratory Analysis
DNA samples extracted from meatballs were then visualized on 1.5% agarose gel with EtBr staining using electrophoresis for 45 minutes. Observations were made using the Gedocumentation Syngene Model GBox EF2. To measure the concentration and purity of DNA, it was performed using a UV VIS Spectrophotometer, Thermo Spectronic, Genesys 10S UV VIS Scanning at a wavelength of 260 nm and 280 nm.

The DNA concentration was determined based on the absorbance value at OD$_{260}$ * 50 μg / μl * Delution factor. Meanwhile, to determine the purity of DNA was carried out by comparing the absorbance ratio OD$_{260}$/OD$_{280}$ [19].

2.4. Data analysis
Data analysis was carried out in factorial using analysis of variance referring to a randomized block design [18]. The first factor is the sample preparation factor which consists of 3 methods, and the second factor is the sample weight factor which consists of 4 factors. Polynomial differences were used to analyze the differences in the effect of factors on the preparation method and the number of samples. Data analysis was performed using SPSS from IBM.

3. Results and discussion
The results of this study indicate that DNA extraction from meatball samples using TiaNamp Genomic DNA Kit® from Tiangen Biotech (Beijing), Co.LTD was successfully carried out. It can be seen in the results of the visualization of DNA on the 1.5% agarose gel with EtBr staining, it can be seen that there are DNA bands with a length of more than 20 kbp as shown in figure 1. To determine the quantity and quality of DNA, it is done by measuring the concentration and purity of the genome DNA uses a spectrophotometer with a wavelength of 260 nm and 280 nm [19]. The results of measuring the quality and quantity of DNA can be seen in table 1 and table 2.

![Figure 1](image_url). Visualization DNA extracted from Beef Meatballs on 1.5% agarose gel using EtBr staining. Note: M (DNA ladder 100 bp); 1 (Fresh Beef Meatball DNA 25 mg); 2 (Fresh Beef Meatball DNA 50 mg); 3 (Fresh Beef Meatball DNA 75 mg); 4 (Fresh Beef Meatball DNA 100 mg); 5 (DNA of oven-dried beef meatballs 25 mg); 6 (DNA of oven-dried beef meatballs 50 mg); 7 (DNA of oven-dried beef meatballs 75 mg); 8 (DNA of Oven-dried Beef Meatballs 100 mg); 9 (DNA Freeze Drying Beef Meatballs 25 mg); 10 (DNA Freeze Drying Beef Meatballs 50 mg); 11 (DNA Freeze Drying Beef Meatballs 75 mg); 12 (DNA Freeze Drying Beef Meatballs 100 mg).
Table 1. DNA concentration extracted from beef meatballs using different preparation methods and sample numbers.

| Treatment          | DNA Concentration (ng/μl) | Average ± SD |
|--------------------|---------------------------|--------------|
| 25 mg              | 50 mg | 75 mg | 100 mg | X ± SD |
| Fresh samples      | 1.30 | 1.40 | 1.83 | 2.70 | 1.81 ± 0.64 |
| Oven-drying        | 2.05 | 1.25 | 2.68 | 4.55 | 2.63 ± 1.41 |
| Freeze-drying      | 3.65 | 2.35 | 3.13 | 2.65 | 2.94 ± 0.57 |
| Average            | 2.33 ± 1.20 | 1.67 ± 0.60 | 2.54 ± 0.66 | 3.30 ± 1.08 | 2.46 ± 0.99 |

Different notations on the same column indicate significant differences (P <0.05).

Table 1 shows the results of measurements of DNA concentration extracted from Beef Meatballs with different preparation methods and sample weights. DNA extraction from Beef Meatballs produced DNA with concentrations ranging from 1.25 ng / μl (50 mg oven dry) to 4.55 ng / μl (oven dry 100 mg) with an average of 2.46 (+ 0.99) ng / μl. The mean concentration of DNA in the fresh sample was 1.81 (+0.64) ng / μl, oven-dry 2.63 (+1.41) ng / μl, and Freeze Drying 2.94 (+0.57) ng / μl. While the average concentration of DNA at a sample weight of 25 mg was 2.33 (+1.20) ng / μl, the sample weight of 50 mg was 1.67 (+0.60) ng / μl, the sample weight of 75 mg was 2.54 (+0.66) ng / μl and sample weight of 100 mg was DNA concentration 3.30 (+1.08) ng / μl. The results of variance showed that the DNA concentration was not significantly different between treatments (P> 0.05). The DNA concentration obtained in this study was lower than [20-22] respectively reported obtaining DNA with a concentration of 40-70 μg/100 mg in muscle/liver tissue, 177.20 - 547.45 ng / μl for Fresh Beef and 483.3 ng / μl for pork.

Table 2. DNA purity from the extraction of beef meatballs with different preparation methods and sample numbers.

| Treatment          | DNA Purity | Average ± SD |
|--------------------|-------------|--------------|
| 25 mg              | 50 mg | 75 mg | 100 mg | X ± SD |
| Fresh Samples      | 1.037 | 1.021 | 0.928 | 1.177 | 1.041 ± 0.103 |
| Oven-Drying        | 1.026 | 0.857 | 1.150 | 1.255 | 1.071 ± 0.171 |
| Freeze-Drying      | 0.924 | 1.065 | 1.088 | 0.978 | 1.014 ± 0.076 |
| Average            | 0.996 ± 0.062 | 0.981 ± 0.110 | 1.055 ± 0.114 | 1.137 ± 0.143 | 1.042 ± 0.114 |

Different notations on the same column and row indicate significant differences (P <0.05).

Table 2 shows the results of measurements of the purity of DNA extracted from Beef Meatballs with different preparation methods and sample weights. DNA extraction from beef meatballs produced DNA with a purity between 0.857 in the 50 mg oven-dry sample treatment to 1.255 in the 100 mg oven-dry treatment with an average of 1.042 (+0.114). DNA purity in the treatment of fresh, oven dry and freeze drying samples averaged 1.041 (+0.103), 1.071 (+0.171) and 1.014 (+0.076), respectively. DNA purity at sample weights of 25 mg, 50 mg, 75 mg and 100 mg averaged 0.996 (+0.062), 0.981 (+0.110), 1.055 (+0.114) and 1.137 (+0.143), respectively. This result is lower than the results reported [21] namely 1.93-1.97 for fresh beef and [22] 1.80 for pork. According to [23,24] a good level of DNA purity ranged from 1.8 to 2. The results of variance showed that DNA purity was not significantly different between treatments (P> 0.05).

DNA extraction is the process of separating DNA from other cell components such as proteins, carbohydrates, fats, and others. DNA extraction consists of three main stages namely the destruction of the cell wall (lysis), separation of DNA from other components, and DNA purification [25]. Cell splitting or lysis in the cell extraction process aims to destroy the membrane and cell walls so that the inside of the cell can come out [26]. The next step is the separation of DNA from other macromolecules such as proteins, small portions of RNA, lipids, and polysaccharides [27]. The last
stage is the DNA purification. This stage aims to remove residues from substances used in the lysis and DNA separation stages.

DNA extracted from animal tissues generally ranges from 1000–5000 ng/mg. The results of DNA extraction are influenced by several factors such as species, tissue type, preservation methods, extraction procedures, and precipitation methods [28]. The concentration and purity of DNA are very important parts of research based on molecular genetics. For PCR, 1 to 1000 ng [29] DNA templates with a minimum concentration of 10 ng/µl and purity that qualifies for molecular analysis range from 1.8 to 2 [24,30]. DNA with lower purity indicates that DNA contains more protein, phenol, or other contaminants [28]. A DNA concentration that is too low will result in a small number of PCR amplicons so that they cannot be observed in agarose gel. The product of PCR is an exponential accumulation of the equation $N_f = N_0 (1 + Y)^n$ where $N_f$ is the number of final copies of the target multiple band sequence, $N_0$ number of initial copies, $Y$ is the primary extension efficiency per cycle, and $n$ the number of PCR cycles of exponential amplification [31].

4. Conclusion
The sample preparation method using Freeze-drying produced a DNA genome with a higher concentration than oven-dry samples and fresh samples. Fresh, oven-dry, and freeze-drying sample preparation methods produced DNA genomes with the same relative purity. The number of samples of 100 mg produced genomic DNA with the highest concentration compared to the samples of 25 mg, 50 mg, and 75 mg. The number of samples of 100 mg produced DNA genome with the highest purity compared to the samples of 25 mg, 50 mg, and 75 mg. The preparation method and the number of samples did not significantly affect the concentration and purity of purified DNA from beef meatballs. The findings of this study indicate that DNA can still be purified from beef meatballs. However, it is still necessary to improve the purification method to obtain better concentration and purity.

Acknowledgment
The authors would like to thank the Ministry of Research and Technology / National Research and Innovation Agency for fully funding this study through Lembaga Penelitian, Publikasi, dan Pengabdian Masyarakat (LP3M) Universitas Muhammadiyah Sidenreng Rappang under the Scheme Penelitian Dosen Pemula (PDP). The Authors also would like to extend the appreciation to Prof. Asmuddin Natsir which has allowed to use the Integrated Biotechnology Laboratory of the Faculty of Animal Husbandry, Hasanuddin University, Makassar in this research.

References
[1] Bonar I F 2015 Hidup Sehat dengan Produk Halal War. Ekspor
[2] Balia R L, Suryaningsih L and Putranto W S 2014 Pengujian pemalsuan bakso dengan daging babi melalui pendekatan ensimatis dan molekuler pada UKM di kawasan pendidikan Jatinangor Kabupaten Sumsel J. Apl. Ipteks untuk Masy. 3 70–2
[3] Cahyaningsari D, Latif H and Sudarnika E 2019 Identifikasi penambahan daging babi pada pangan berbahan dasar daging sapi menggunakan ELISA dan qPCR Acta Vet. Indones. 7 17–25
[4] Astawan M 2008 Sehat dengan Hidangan Hewani. (Jakarta: Penebar Swadaya)
[5] Hermanianto J and Andayani Y 2002 tudi perilaku konsumen dan identifikasi parameter bakso sapi berdasarkan preferensi konsumen di wilayah DKI Jakarta J. Teknol. dan Ind. Pangan 13 1–10
[6] Asensio L, Gonzalez I, Garcia T and Martin R 2008 Review Determination of food authenticity b-linked immunosorbsent assay (ELISA) Food Control 19 1–8
[7] Kuswandi B, Gani A and Ahmad M 2017 Immuno strip test for detection of pork adulteration in cooked meatballs Food Biosci. 19 1–6
[8] Nuruliana M, Che-Man Y, Mat-Hashim D and Mohamed A 2011 Rapid identification of pork for halal authentication using the electronic nose and gas chromatography mass spectrometer with headspace analyzer Meat Sci. 88 638–44
[9] Pestana E, Belak S, Diallo A, Crowther J and Viljoen G 2010 Early Rapid and Sensitive Veterinary Molecular Diagnostics Real-time PCR Application (Dordrecht (DE): Springer Science & Bussiness Media.)

[10] Soares S, Amaral J, Oliveira M and Mafra I 2013 A SYBR Green real-time PCR assay to detect and quantify pork meat in processed poultry meat products. Meat Sci. 95 115–20

[11] Kim M, Yoo I, Lee S, Hong Y and Kim H 2016 Quantitative detection of pork in commercial meat products by TaqMan real-time PCR assay targeting the mitochondrial D-loop region Food Chem. 210 102–6

[12] Al-Kahtani H, Ismail E and Ahmed M 2017 Pork detection in binary meat mixtures and some commercial food products using conventional and real-time PCR techniques Food Chem. 219 54–60

[13] Perestam A, Fujisaki K, Nava O and Hellberg R 2017 Comparison of real-time PCR and ELISA-based methods for the detection of beef and pork in processed meat products Food Control 71 346–52

[14] Ballin N, Vogensen F and Karlsson A 2009 Species determination – Can we detect and quantify meat adulteration Meat Sci. 83 165–74

[15] Klein-nijenhuis A, Van-Holthoon F and Herregods G 2018 Validation and theoretical justification of an LC-MS method for the animal species specific detection of gelatin. Food Chem. 243 461–7

[16] Zein M S A, Prawiradilaga D M, Sulandari S, Sutrisno H, Irham M, Arida E A, Haryoko T, Fitriana Y S, Dharmayanthi A B and Natalia I 2013 DNA Barcode Fauna Indonesia ed M S A Zein and D M Prawiradilaga (Jakarta: Kencana Prenadamedia Group)

[17] Hebert P D N, Cywinska A, Ball S L and Jeremy R 2003 Biological identification through DNA barcodes Biological identifications through DNA barcodes R. Soc. 270 313–21

[18] Sudjana 2005 Metoda Statistitka (Bandung: Tarsito)

[19] Barbas C F, Burton D R, Scott J K and Silverman G J 2007 Quantitation of DNA and RNA Cold Spring Harb. Protoc. 2007 pdb.ip47

[20] Popping B, Diaz-Amigo C and Hoenicke K 2010 Chemists, Molecular Biological and Immunological Techniques and Application for Food (New Jersey, Canada: John Wiley and Sons, Inc.)

[21] Hutami R, Bisyri H, Nuraini H and Ranasasmita R 2018 Ekstraksi DNA dari Daging Segar untuk Analisis dengan Metode Loop-Mediated Isothermal Amplification (LAMP) DNA Extraction from Raw Meat for Analysis with the Loop-Mediated Isothermal Amplification (LAMP) Method J. Agroindustri Halal 4 209–16

[22] Biase F H, Franco M M, Goulart L R and Antunes R C 2002 Protocol for extraction of genomic DNA from swine solid tissues Genet. Mol. Biol. 25 313–5

[23] Ali M E, Razzak M A, Hamid S B A, Rahman M M, Amin M Al, Rashid N R A and Asing 2015 Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods Food Chem. 177 214–24

[24] Boesenberg-Smith K A P, Mohammad M. Pessarakli M D and M. Wolk D 2012 Clinical Microbiology Newsletter Clin. Microbiol. Newsl. 34 3–6

[25] Corkill G and Rapley R 2008 The Manipulation of Nucleic Acid: Basic Tools & Techniques in Molecular Biomethods Handbook (New York (US): Humana Press)

[26] Holme D J and Peck H 1998 Analytical Biochemistry (Harlow (GB): Pearson Education Limited)

[27] Muladno 2010 Teknologi Rekayasa Genetika (Bogor: IPB Press)

[28] Chen H, Rangasamy M, Tan S Y, Wang H and Siegfried B D 2010 Evaluation of five methods for total DNA extraction from western corn rootworm beetles PLoS One 5 1–8

[29] Lorenz T C 2012 Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies J. Vis. Exp. 1–15

[30] Sambrook J, Fritsch F and Maniatis T 1989 Molecular Cloning Laboratory Manual. New York (US): Cold Spring Harbor Laboratory Pr. (New York: Cold Spring Harbor Laboratory Press)
[31] Cha R S and Thilly W G 1993 *Specificity, efficiency, and fidelity of PCR.* (New York: Cold Spring Harbor Laboratory Press)