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DNA isolation in processed goat meat products (tongseng)

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Abstract—DNA isolation from processed goat meat products (tongseng) was conducted to evaluate the quality of DNA extracted from these processed meat products. This study is intended to serve as a reference for similar research and to support the development of specific DNA detection assays using real-time PCR or authentication tests. The methodology employed in this study utilized standard testing procedures, with DNA quality parameters including concentration and purity measured using a nanophotometer at an A260/A280 ratio. The DNA isolation results are presented in Table 1 below. The table demonstrates that the isolated DNA concentration values ranged from 695,4 ng/µL to 708,4 ng/µL, with a mean concentration of 701,5 ng/µL. The purity values, measured at the A260/A280 ratio, ranged from 2,07 to 2,08, with an average purity value of 2,08. Based on the research findings, it can be concluded that the DNA isolated from processed goat meat food products exhibited good quality, as evidenced by the mean concentration value of 701,5 ng/µL and purity of 2,08. For future research, it is recommended to conduct similar studies on food products from other categories to obtain comprehensive information that can support species DNA identification assays or product authentication tests.

Keywords—**Authentication, DNA, isolated, method, tongseng**

INTRODUCTION

Isolation of DNA in processed goat meat products (tongseng) is an important step that can be carried out before testing species DNA detection in a food product for product quality control and authentication. When testing using PCR or real-time PCR, a DNA template that has good quality is needed to support the success of the testing process carried out (Greyson 2009; Branquinho et al., 2012). The selection of tongseng samples was made because processed meat food products have challenges in monitoring authentication and the potential for fraud in the use of raw material claims, where which will encourage substitution that will harm consumers. Therefore, testing support is needed that produce an effective and economical test method.

Various studies on the analysis of isolated DNA quality have been conducted on a wide range of food products, both in general and specifically. Lockley et al. (2000) conducted research on food products in general, while more specific studies have been carried out on various types of food, such as rice (Dubey et al., 2017), tomatoes (Dubey et al., 2017), meatballs (Sophian et al., 2021), salted fish (Sophian, 2021), and nuggets (Sophian et al., 2022). These studies aim to evaluate the integrity and purity of extracted DNA from various food sources, which is a crucial factor in multiple biotechnological applications, such as species identification, food authentication, and detecting contamination or food adulteration. In addition to processed food products, the analysis of isolated DNA quality has also been applied in the development of standard DNA assays, particularly for detecting specific species such as porcine (Wulan et al., 2021) and mice (Sophian & Syukur, 2021). The advancement of DNA analysis methods plays a vital role in the food industry and food safety, particularly in ensuring product halal certification, preventing cross-contamination, and maintaining the quality and authenticity of food products consumed by the public.

Based on this background, this research was conducted to evaluate the quality of isolated DNA extracted from processed goat meat products, specifically tongseng. The ability to obtain high-quality DNA from such processed food products is crucial, as it directly influences the accuracy and reliability of molecular detection techniques. The findings of this study are expected to serve as a valuable reference for future research in the field of food authenticity and molecular identification. By providing insights into the effectiveness of DNA extraction methods, this study aims to contribute to the development of more robust and specific DNA detection techniques, particularly through real-time PCR analysis. Furthermore, the results of this research could be beneficial in supporting the advancement of authentication testing, ensuring the integrity of food labelling and preventing fraudulent practices in the meat industry. The implementation of precise molecular identification methods will not only enhance consumer trust but also aid regulatory agencies in maintaining food quality standards. Ultimately, this study seeks to bridge the gap between DNA-based analytical techniques and the challenges posed by processed meat products, offering a

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scientific foundation for improving food authentication protocols and ensuring the traceability of meat sources..

MATERIALS AND METHODS

A. Materials

The materials used in this study were processed food products of goat meat (tongseng), DNeasy Mericon Food extraction kit [Qiagen], 96% ethanol, and NFW (Nuclease free water).

B. DNAIisolation

The DNA isolation process was carried out by weighing the sample as much as 20 mg then put into a 2 ml centrifuge tube and added 200 µL of lysis buffer and 30 µL of proteinase K. The next step was followed by the sample being incubated at 70 C for 60 minutes while being mixed at 1400 rpm. After these steps were completed, the sample was centrifuged at a speed of 14000 rpm for 5 minutes. Prepare a 2 ml centrifuge tube containing 500 µL of chloroform, then transfer the supernatant sample that has been centrifuged into the centrifuge tube. Vortex the mixture of the sample supernatant and chloroform for 15-20 seconds, then it is continued by centrifuging at 14000 rpm for 10 minutes. Transfer 350 µL of supernatant to a new 2 mL centrifuge tube, then add 350 µL of 96% ethanol and homogenize by vortexing for 15-20 seconds then put into a spin column, and centrifuge for 1 minute at 14000 rpm, discard the collection tube and transfer spin column into a new collection tube, then add 650 µL of AW2 buffer and centrifuge again for 1 minute at 14000 rpm. Discard the collection tube and transfer the spin column to a 1.5 centrifuge tube and add 100 µL of elution buffer AE, then centrifuge at 14000 rpm for 1 minute. Discard the spin column, and store the isolated DNA in a 1.5 centrifuge tube in a freezer at -20 C (Qiagen 2020).

C. Measurement of Isolated DNA

The quality of the isolated DNA was assessed using a nano photometer, a highly sensitive instrument commonly used in molecular biology for nucleic acid quantification. This assessment focused on two key quality parameters: concentration and purity (Sophian, 2021). The concentration of the DNA sample was determined by measuring its absorbance at a specific wavelength, providing insights into the total amount of nucleic acid present in the sample. Meanwhile, purity was evaluated by calculating the absorbance ratio at 260 nm and 280 nm (A260/A280), a standard method for assessing DNA contamination by proteins or other organic compounds.

A high A260/A280 ratio, typically in the range of 1.8–2.0, indicates pure DNA with minimal protein contamination, while lower values suggest the presence of proteins, phenols, or other contaminants that may interfere with downstream applications such as PCR, sequencing, or cloning. Conversely, an excessively high ratio may indicate RNA contamination. The precision of the nano photometer ensures reliable and reproducible readings, making it an essential tool in molecular biology research.

This method of DNA quality assessment has been widely adopted in previous studies (Sophian, 2021; Sophian et al., 2021; Sophian & Syukur, 2021; Sophian et al., 2022; Sutanta et al., 2021; Wulan et al., 2021) to ensure that extracted DNA meets the necessary standards for further molecular analyses. By evaluating both concentration and purity, researchers can determine the suitability of the DNA sample for subsequent experimental procedures, thereby improving the reliability and accuracy of genetic studies..

D. Data Analysis

The data obtained from the isolation process were subsequently analyzed to assess their quality by calculating the average and standard deviation. These statistical measures provide an overview of the consistency and reliability of the isolation results. The standard deviation, in particular, indicates the degree of variability in the dataset, ensuring that the results are not only accurate but also reproducible. A high degree of variation may suggest inconsistencies in the isolation process, which could impact the reliability of downstream applications.

One of the primary criteria used to determine the success of DNA isolation is the concentration and purity of the extracted DNA. According to established standards, a good-quality DNA isolation result is characterized by a DNA concentration exceeding 20 ng/µL (Sophian, 2021d). This threshold ensures that there is a sufficient quantity of genetic material for further molecular analyses, such as polymerase chain reaction (PCR), sequencing, or other genomic applications. In addition to concentration, the purity of the extracted DNA is a crucial parameter, which is typically assessed using the absorbance ratio at 260/280 nm. A purity value within the range of 1.7 – 2.0 indicates minimal contamination from proteins, phenols, or other interfering substances. Deviations from this range suggest the presence of contaminants, which may affect the integrity and functionality of the DNA in subsequent analyses.

These quality parameters are based on prior research and standard laboratory protocols (Sophian, 2021; Sophian et al., 2022; Sophian et al., 2023). Ensuring high-quality DNA isolation is essential for accurate genetic studies, as poor-quality samples can lead to erroneous results or difficulties in amplification. Therefore, maintaining stringent isolation procedures and evaluating concentration and purity rigorously are critical steps in molecular biology research.

RESULTS AND DISCUSSIONS

DNA Isolation Results

The results of DNA isolation are presented in Table 1 below. The data obtained indicate that the concentration values of the isolated DNA ranged from 695,4 ng/µL to 708,4 ng/µL, with an average concentration value of 701.5 ng/µL. The purity values, as determined by the A260/A280 ratio, were in the range of 2,07 to 2,08, with an average purity value of 2,08.

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Table 1. DNA isolation results

No.	Concentration (ng/µL)	Purity
1	700,8	2,07
2	704,4	2,07
3	700,6	2,08
4	705,5	2,07
5	703,4	2,07
6	695,4	2,07
7	698,5	2,08
8	708,4	2,08
9	699,5	2,08
10	698,4	2,08
Average	701,5	2,08
SD	3,9	0,01

The analysis of the standard deviation values for concentration and purity further supports the reliability of the obtained data. The standard deviation for DNA concentration was found to be 3.9, which is relatively small compared to the mean value of 701.5 ng/µL. This indicates that the variation among the measured DNA concentrations was minimal, suggesting consistency in the DNA isolation process. Similarly, the standard deviation for purity values was 0.01, which is extremely low, further confirming that the purity of the isolated DNA samples was highly consistent.

A high concentration of DNA in the range of 695.4 to 708.4 ng/µL suggests that the isolation protocol was efficient in extracting DNA in substantial quantities. The minor fluctuations in concentration values among the different samples may be attributed to inherent experimental variations, such as slight differences in pipetting volumes, sample handling, or minor inconsistencies in reagent quality. However, given the small standard deviation, it is evident that these variations are negligible, and the protocol yielded reproducible results.

The purity of the isolated DNA, as indicated by the A260/A280 ratio, is an essential parameter in evaluating the suitability of DNA for downstream applications such as PCR, sequencing, or cloning. The obtained purity values ranged from 4,07 to 2,08, with an average of 2,08. Since a ratio of ~1,8 typically indicates pure DNA, and values above 2,0 suggest minimal protein contamination, the results demonstrate that the extracted DNA was of high quality. The consistently high purity across all samples highlights the effectiveness of the DNA isolation procedure in removing contaminants such as proteins and other organic impurities.

Discussion

One crucial aspect to consider when isolating DNA from food products is the complexity of the sample matrix, which consists of various constituent materials that can influence the efficiency and effectiveness of the isolation process. The complexity of the matrix plays a significant role in determining the quality of the extracted DNA. Suppose an inappropriate DNA isolation method is applied. In that case, it may lead to poor-quality DNA, rendering it unsuitable for subsequent molecular analyses such as polymerase chain reaction (PCR) and real-time PCR. A major challenge associated with DNA isolation from food samples is the presence of inhibitors that can co-purify with the DNA, potentially interfering with enzymatic reactions during amplification. These inhibitors, which may originate from proteins, fats, carbohydrates, or polyphenols present in the sample, can significantly affect the reliability and reproducibility of molecular testing.

In this study, meat was used as the sample, despite having undergone a cooking process. The DNA isolation procedure implemented did not encounter significant obstacles, demonstrating the robustness and reliability of the chosen isolation method. Cooking processes can sometimes degrade DNA due to high temperatures, making extraction more difficult. However, in this case, the results indicated that the selected DNA isolation method was effective in retrieving high-quality DNA suitable for downstream applications.

The two fundamental parameters used to assess the quality of isolated DNA are concentration and purity. DNA concentration refers to the amount of DNA present in a given volume of the sample, while purity measures the level of contamination from proteins, RNA, and other unwanted substances. According to Sophian et al. (2021), achieving high DNA purity and concentration does not always guarantee successful amplification in PCR assays. Other critical factors influencing amplification success include the presence of inhibitors, DNA fragmentation, and sample-handling techniques. Thus, while purity and concentration serve as primary indicators, they must be interpreted in conjunction with other factors affecting DNA integrity.

The analysis of DNA purity and concentration can be performed using a nano photometer, which is a rapid and efficient method compared to horizontal electrophoresis with agarose gel. The nano photometer assesses the absorbance ratios at specific wavelengths, such as 260/280 nm for protein contamination and 260/230 nm for organic compounds and salts. This method is particularly advantageous in high-throughput settings where quick assessments are necessary for determining DNA quality before proceeding with amplification and sequencing procedures.

During the DNA extraction process, the enzyme proteinase K plays a crucial role in breaking down proteins and facilitating the release of nucleic acids. Proteinase K is most effective at temperatures between 65-70°C, where it actively digests proteins, ensuring a more efficient DNA extraction. The effectiveness of this enzyme is one reason why some studies require method optimization before applying it to different types of samples. Factors such as incubation time, buffer composition, and enzyme concentration must be carefully controlled to achieve optimal results.

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To further enhance the efficiency of DNA isolation, the double-wash technique has been introduced as a viable method. According to Sutanta et al. (2021), this technique significantly improves DNA purity, especially in samples characterized by low DNA yield or high levels of contaminants. The double-wash step helps remove residual proteins, polysaccharides, and other inhibitors, leading to improved DNA quality suitable for molecular applications.

Therefore, selecting an appropriate DNA isolation method must take into account the nature of the sample matrix, the presence of potential inhibitors, and the required DNA purity and concentration. Employing optimized techniques, such as the use of proteinase K and the double-wash method, can greatly enhance the quality of extracted DNA, ensuring its suitability for advanced genetic analyses.

CONCLUSIONS

Based on the research results, it was concluded that the DNA quality isolated from processed goat meat food products demonstrated good quality, as indicated by an average concentration of 701,5 ng/µL and a purity ratio of 2,08. For future research, it is recommended to conduct similar studies on food products from other categories to obtain comprehensive information that can support species DNA identification tests and product authentication analyses.

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