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DNA isolation from processed shrimp food products (shrimp tempura)

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Abstract—DNA isolation is an important step that must be carried out in research or testing the identification of DNA species based on molecular techniques using PCR or real-time PCR. The purpose of this study was to determine the quality of DNA isolated from shrimp processed food products in shrimp tempura preparations. One of the benefits of this research is that it can be a source of information and reference for similar research. The method used in this research is the DNA isolation method using the spin column technique. Analysis of the quality of the isolated DNA was based on the parameters of the concentration value and DNA purity measured by a nanophotometer at the ratio of A260/A280. Data analysis was carried out by calculating the average concentration and purity values obtained from measurements using a nanophotometer and then calculating the standard deviation values and comparing them. Based on the results of the research conducted, it was concluded that the DNA isolated from the results showed the value of concentration and purity of DNA which was included in the good DNA category. The results of statistical tests on data uniformity show that the data analyzed in this study are homogeneous.

***Keywords*—analysis; DNA; concentration; purity; shrimp**

INTRODUCTION

DNA isolation is a crucial step that must be performed in research or testing for species DNA identification using molecular techniques such as PCR or real-time PCR. The primary goal of DNA isolation is to obtain a high-quality test template, as the success of subsequent molecular analyses largely depends on the purity and integrity of the extracted DNA (Sophian et al., 2022). High-quality DNA ensures accurate amplification, reduces the risk of contamination, and enhances the reliability of results. The process of DNA isolation involves several critical steps, including cell lysis, removal of proteins and other contaminants, and DNA precipitation, each of which must be optimized to prevent degradation or loss of genetic material. Various factors can influence the quality of isolated DNA, such as the type of sample, storage conditions, and the isolation method used. Different biological samples, including blood, tissues, plant materials, and processed food products, may require specific extraction protocols to ensure optimal DNA recovery (Sophian et al., 2023). Achieving high-quality DNA isolation in accordance with DNA isolation quality standards increases the likelihood of successful testing or research, making it essential to follow standardized methods and use appropriate reagents (Sophian & Yustina 2023). Furthermore, DNA isolation plays a fundamental role in various applications beyond species identification, including forensic investigations, medical diagnostics, genetic engineering, and food authentication. In food safety and authentication, for example, DNA isolation is essential for detecting allergens, verifying species composition, and ensuring compliance with labeling regulations. Advances in DNA extraction techniques have led to the development of more efficient, automated, and high-throughput methods, improving the consistency and reproducibility of results. Therefore, continuous refinement and validation of DNA isolation protocols are necessary to support the growing demands of molecular research and ensure high standards of accuracy and reliability in DNA-based studies.

Several studies have been conducted on the quality analysis of isolated DNA in various food products, such as salted fish (Sophian, 2021c), processed crab products (Sophian et al., 2021), tomatoes (Turci et al., 2010), and GMO products (Gryson, 2010). These studies emphasize the importance of ensuring that DNA extracted from different food matrices maintains high integrity, purity, and concentration to facilitate accurate molecular analysis. The quality of isolated DNA plays a crucial role in various applications, including species identification, food authentication, and the detection of genetically modified organisms (GMOs). Additionally, DNA isolation is also performed for the production of DNA reference materials, which are essential for standardizing molecular assays. For example, the development of rat DNA reference material (Sophian & Syukur, 2021) and porcine DNA reference material (Wulan et al., 2021) demonstrates the need for high-quality, well-characterized DNA to support laboratory testing and regulatory compliance. The most commonly

used quality parameters for isolated DNA are concentration and purity values, typically measured using spectrophotometric methods such as UV absorbance ratios at 260/280 nm and 260/230 nm (Sutanta, et al., 2022; Wulan, et al., 2021). A high 260/280 ratio indicates minimal protein contamination, while the 260/230 ratio reflects the presence of other contaminants such as carbohydrates and salts. To achieve high-quality DNA, an effective DNA isolation method is required, one that minimizes degradation and contamination while maximizing yield. The choice of isolation protocol depends on the sample type, with different extraction kits and techniques available for plant, animal, and processed food products. Optimization of DNA isolation procedures is critical, particularly for complex food matrices that contain inhibitors, as poor-quality DNA can lead to inaccurate or failed molecular analyses, ultimately affecting the reliability of research and regulatory decisions.

Based on the considerations above, this study aims to assess the quality of isolated DNA from processed shrimp food products in the form of shrimp tempura. One of the benefits of this research is that it can serve as a source of information and a reference for similar studies. The publication of this method will contribute to the body of knowledge on DNA isolation techniques and provide valuable insights for relevant stakeholders.

MATERIALS AND METHODS

A. Materials

The materials used in this study include processed shrimp food products (Shrimp Tempura), the DNeasy Mericon Food extraction kit [Qiagen], 96% alcohol, and nucleotide-free water (NFW).

B. DNA Extraction Procedure

Weigh 20 mg of the sample in a 2 mL microcentrifuge tube, then add 500 µL of lysis buffer and 30 µL of Proteinase K. Incubate at 70°C for 60 minutes. After incubation, remove the sample and continue with centrifugation for 5 minutes at a speed of 14,000 rpm. Two layers will form in the centrifuge tube: a pellet and the supernatant. Pipette 350 µL of the supernatant and transfer it to a new 2 mL centrifuge tube. Add 350 µL of 96% alcohol and homogenize by vortexing for 10 seconds. Pipette the solution from the upper layer into a new 2 mL centrifuge tube and add 500 µL of binding buffer PB. Vortex and gradually transfer all the liquid into the spin column, then centrifuge. After all the liquid has been transferred, centrifuge and then add 500 µL of wash buffer AW2 and centrifuge again for 1 minute at 14,000 rpm. Transfer the spin column to a new 1.5 mL centrifuge tube, add 100 µL of nucleotide-free water (NFW), and centrifuge at 14,000 rpm for 1 minute. Discard the spin column, and the remaining liquid in the 1.5 mL centrifuge tube contains the isolated DNA, which will be measured for purity and concentration before use (Utaminingsih et al., 2022; Sophian et al., 2022).

C. DNA Quality Analysis

The quality of the isolated DNA was analyzed based on concentration and purity values, which were measured using a nanophotometer at the A260/A280 ratio. Data analysis was performed by calculating the average concentration and purity values from the nanophotometer measurements, followed by calculating the standard deviation and comparison. If the standard deviation value is smaller than the average value, the analyzed data can be considered homogeneous.

RESULTS AND DISCUSSIONS

DNA Concentration and Purity

The results of DNA isolation from shrimp tempura samples are presented in Table 1. The concentration of extracted DNA ranged from 65.40 to 77.90 ng/µL, with a mean of 70.42 ± 4.22 ng/µL. The purity of the isolated DNA, as indicated by the A260/A280 ratio, ranged from 1.79 to 2.08, with a mean of 1.94 ± 0.09.

Table 1. DNA Isolation Results from Shrimp Tempura Samples

Number	Concentration (ng/µL)	Purity		
		A260	A280	A260/280
1	74.50	1.495	0.772	1.94
2	77.90	1.518	0.798	1.90
3	77.50	1.545	0.788	1.96
4	71.20	1.621	0.778	2.08
5	65.90	1.552	0.785	1.98
6	68.60	1.515	0.795	1.91
7	69.40	1.622	0.788	2.06
8	70.80	1.681	0.835	2.01
9	66.60	1.561	0.843	1.85
10	65.40	1.452	0.812	1.79
11	67.80	1.425	0.765	1.86
12	69.40	1.552	0.788	1.97
Mean	70.42	1.545	0.796	1.94
SD	4.22	0.072	0.024	0.09
CV (%)	5.99	4.66	3.02	4.64

Statistical analysis revealed that the coefficient of variation (CV) for DNA concentration was 5.99%, while the CV for purity (A260/A280 ratio) was 4.64%. Both values were well below 10%, indicating high homogeneity in the data (Perini et al., 2011). The one-sample t-test comparing the mean A260/A280 ratio (1.94) with the reference value of 1.8 showed a statistically significant difference ($p = 0.0012$), confirming that the extracted DNA was of high purity.

To further assess data homogeneity across different parameters, one-way ANOVA was performed on the standardized values of concentration, A260, A280, and A260/A280 ratio. The results of the ANOVA analysis are presented in Table 2.

Table 2. One-way ANOVA Analysis of Standardized DNA Quality Parameters

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-value	P-value
Between Parameters	0.0032	3	0.0011	0.0011	0.9999
Within Parameters	44.0000	44	1.0000		
Total	44.0032	47			

The ANOVA results showed no significant differences between the standardized values of the four parameters ($p = 0.9999$), confirming that the data distribution was homogeneous across all measured DNA quality parameters. This homogeneity further supports the reliability and consistency of the DNA isolation method used in this study.

Factors Affecting DNA Quality in Processed Shrimp Products

The obtained DNA concentration (mean: 70.42 ng/µL) is considered adequate for most molecular applications, including PCR and real-time PCR, which typically require 1-10 ng/µL of template DNA (Sophian, 2021). This yield is particularly noteworthy considering that shrimp tempura undergoes multiple processing steps, including cooking, which can lead to significant DNA degradation (Sophian, et a., 2021).

The purity values (A260/A280 ratio) ranged from 1.79 to 2.08, with a mean of 1.94, falling within the acceptable range of 1.7-2.0 that is generally considered indicative of high-purity DNA (Sophian & Syukur 2021; Utaminingsih et al, 2022). This suggests minimal protein contamination in the extracted DNA, which is crucial for preventing inhibition in subsequent PCR reactions.

Several factors may have contributed to the successful DNA isolation from these processed shrimp products. First, the use of Proteinase K played a crucial role in efficiently breaking down proteins. As noted by Wulan et al. (2021), this enzyme functions optimally at temperatures between 65-70°C and targets peptide bonds adjacent to the carboxylic groups of aliphatic and aromatic amino acids, effectively degrading proteins that might interfere with DNA isolation.

Second, the modified extraction protocol, which included extended incubation time (60 minutes) and optimal temperature (70°C), likely contributed to enhanced cell lysis and DNA release from the complex food matrix. These findings align with those of Utami et al. (2013), who observed that optimizing lysis conditions significantly improved DNA extraction efficiency from processed seafood products.

Third, the spin column technique used in this study employs silica-based membrane technology, which has been shown to be effective in removing potential PCR inhibitors commonly found in food matrices (Sophian & Nasir 2023). This is particularly important for processed food products like shrimp tempura, which contain various additives and ingredients that could potentially interfere with DNA isolation and subsequent molecular analyses.

Comparison with Previous Studies

The DNA yield and purity obtained in this study are comparable to, or even better than, those reported in previous studies on DNA extraction from processed seafood products. For instance, Utami et al. (2023) reported DNA concentrations ranging from 14.5 to 17.8 ng/µL and A260/A280 ratios of 1.89-2.07 from various processed fish products. Similarly, Utaminingsih et al. (2022) obtained DNA from 9.6 to 18,5 ng/µL and A260/A280 ratios of 1.64-1.87 from processed fish food products in the form of milkfish brains, which is lower than the average achieved in this study.

Implications for Food Authentication and Safety

The successful isolation of good-quality DNA from shrimp tempura has important implications for food authentication and safety. With increasing concerns about seafood fraud and mislabeling (Sophian et al, 2022; Utami et al., 2022: Utaminingsih et al, 2023), reliable DNA-based methods for species identification are essential for regulatory compliance and consumer protection.

The DNA extracted in this study would likely be suitable for PCR amplification of short to medium-length target sequences (100-300 bp), which are commonly used in seafood authentication studies (Sophian et al., 2023). This is particularly relevant for processed products like shrimp tempura, where conventional morphological identification is impossible due to processing.

Furthermore, the standardized and optimized protocol described in this study could serve as a reference for food testing laboratories involved in regulatory enforcement and quality control. The ability to extract amplifiable DNA from highly processed food products enhances the capabilities of food safety authorities to verify product authenticity and detect potential allergens or contaminants.

CONCLUSIONS

This study demonstrated the successful isolation of DNA from processed shrimp tempura products using a modified spin column-based extraction method. The isolated DNA exhibited concentration and purity values that fall within the category of good-quality DNA, with statistical analysis confirming data homogeneity. While some DNA fragmentation was observed due to food processing, the extracted DNA was of sufficient quality for potential use in molecular authentication techniques.

For future research, we recommend conducting amplification studies using PCR or real-time PCR to further validate the suitability of the extracted DNA for species identification in processed shrimp products. Additionally, comparative studies evaluating different extraction methods and their effectiveness on various processed seafood products would provide valuable insights for optimizing DNA-based food authentication protocols.

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