DNA isolation in processed chicken meat products (nugget) using modified DNeasy Mericon Food kit (Qiagen)

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ABSTRACT

DNA isolation is one of the important steps in conducting PCR-based or real-time PCR-based molecular analysis. This study aims to test the quality of DNA isolation extracted from samples of processed food products from chicken meat (chicken nuggets). The novelty of this research lies in the DNA isolation technique modified from the manual kit which is used to produce good-quality DNA isolation results. The extraction method used was the modified extraction method and the standard extraction method of the DNeasy Mericon Food kit. Analysis of DNA quality was measured using a nanophotometer, where the analysis of DNA quality was analyzed based on the parameters of concentration values and purity values. Based on research data, it is known that the modified DNA extraction method produces a concentration value of DNA isolation with an average concentration value of 1295.1 ng/µL. Meanwhile, the value of the purity of the isolated DNA was 2.10 on average. while the standard DNA extraction method resulted in an average DNA isolation concentration value of 111.53 ng/µL. While the value purity of the isolated DNA was at an average value of 1.926. From the research data analyzed, it was found that the modified DNA isolation method has the advantage that the concentration of isolated DNA obtained is higher than the standard method, while the purity value of the DNA isolated from the standard method has better purity when compared to the modified method.

1. Introduction

The quality test of isolated DNA is one of the important steps in conducting PCR-based or real-time PCR-based molecular analysis. One way that can be done to produce high-quality DNA isolation results is to optimize the method of the extraction kit that will be used. The success of the optimization phase of the method will support the success of the analysis during testing. Optimization is done to determine the level of accuracy and effectiveness of a kit that will be used. Many studies have been conducted to analyze the quality or quality of DNA isolated from several processed food products such as processed soybean products (DiBernardo, DelGaudio, Galderisi, Cascino, & Cipollaro, 2007), salted fish, (Sophian, 2021a), crabs (Sophian, Purwaningsih, Muindar, Igirisa, & Amirullah, 2021), Tomatoes (Piskata, Servusova, Babak, Nesvadbova, & Borilova, 2019). There are many kits on the market for use in DNA extraction, making testers many options for using DNA isolation techniques to support the molecular testing process. However, an optimization stage must always be carried out to see the performance and toughness of a kit.

The challenge that arises in this research is how to get good DNA isolation results from the purity and quality concentration parameters of the isolated DNA because isolating DNA from fresh meat, it is certainly different from meat products that have been processed into food. Chicken nuggets are processed meat food products whose quality control can be carried out through species DNA detection parameters, so this research is supporting research to conduct surveillance testing for species DNA detection in chicken nugget products. the use of a modified extraction kit is expected to provide better DNA isolation results than the existing standard methods so it can be a novel aspect of this research.

The purpose of this research is to see the quality of isolated DNA extracted from samples of processed food products from chicken meat (chicken nuggets). This research is expected to be a source of information and reference for similar research so that it can contribute to the value of knowledge for researchers in the field of testing for authentication or detection of species DNA using PCR-based molecular techniques or real-time PCR.

2. Methodology

2.1. Materials

This study used ingredients consisting of 18 samples of chicken nuggets, NFW (Nuclease Free Water), 96% Ethanol (Merck), and DNeasy Mericon Food Extraction Kit [Qiagen].

2.2. Modified

The modifications carried out lie in the use of binding buffers which in the standard kit use PB buffer and elution buffer EB, but the modified method using 96% ethanol and elution buffer using Nuclease Free Water (NFW).

2.3. DNA extraction modified procedure

Weigh the sample weighing 20mg then put it in a 2mL centrifuge tube, add 700µL lysis buffer and 30µl proteinase K. Incubate in a thermoshaker at 70^{o}C for 60 minutes. After the incubation process is complete, then it is centrifuged at a speed of 14000rpm for 05 minutes. Pipette 350µl supernatant and put in a new 2ml centrifuge and add 350µl 96% ethanol. Homogenization by vortexing for 10 - 15 seconds, then transferring 700µl of sample suspension into a spin column and centrifuging at 14000rpm for 01 minute. Discard the collection tube and transfer the spin column to a new collection tube and add 600µl of wash buffer AW2 then centrifuge at 14000rpm for 02 minutes. Discard the collection tube and transfer the spin column into a 1.5mL centrifuge tube and add 100µl of NFW elution buffer and then centrifuge at 14000rpm for 01 minute. Discard the spin column and store the sample in a 1.5mL centrifuge tube. The next step is to analyze the quality of the DNA isolated by measuring using a nano photometer.

2.4. DNA extraction standard procedure

Standard extraction method made according to manual kit DNeasy Mericon Food kit (Qiagen) with a weighing sample weighing 20mg.

2.5. DNA quality analysis

Analysis of DNA quality was measured using a nanophotometer, where the analysis of DNA quality was analyzed based on the parameters of concentration value and purity value. The quality of the isolated DNA is good if the concentration value is greater than 20 ng/ul, while the purity value measured at ratio A_{260}/A_{280} is in the range of values of 1.7 - 2.1 (Sophian et al., 2021: Sophian, 2021c).

2.6. Data analysis

Interpretation of research data is interpreted using the average test of the concentration values and purity values obtained from measurements (Sophian & Abinawanto, 2022; Sophian et al., 2021; Sophian & Syukur, 2021; Sutanta, Wulan, Nabila, & Sophian, 2022; Wulan, Sutanta, & Sophian, 2021).

3. Result and discussion

3.1. Result DNA isolation using modified methods

The results showed that the DNA concentration was in the range of 1042.9 - 1598.4 with an average value of 1295.1. The purity value is in the range of 2.04 - 2.15 with an average value of 2.10. Complete data is presented in Table 1 below.

Table 1

DNA isolation results using modified methods

No	Concentration	Purity		
		A_{260}	A_{280}	A_{260}/A_{280}
1	1182.8	23.656	11.154	2.12
2	1187.8	23.757	11.320	2.10
3	1187.4	23.748	11.258	2.11
4	1570.2	31.404	14.888	2.11
5	1583.0	31.661	15.017	2.11
6	1598.4	31.967	15.139	2.11
7	1264.0	25.280	12.011	2.10
8	1268.8	25.376	12.059	2.10
9	1274.6	25.429	12.225	2.08
10	1147.6	22.951	10.820	2.12
11	1147.5	21.955	10.750	2.04
12	1139.8	22.796	10.775	2.12
13	1559.8	31.197	14.516	2.15
14	1456.8	29.136	13.832	2.11
15	1473.3	29.466	14.100	2.09
16	1042.9	20.859	9.9330	2.10
17	1055.5	21.110	10.061	2.10
18	1059.2	21.183	9.9220	2.13
Average	1295.1	25.840	12.272	2.10

The value of the purity was read using a nano photometer at the ratio A_{260}/A_{280}. From these data, it can be seen that the value of the variation on the sample is quite diverse where the value of the A_{260} ratio is in the range of 21.110 - 31.967 with an average value of 25.840 and the value of the A_{280} ratio is in the range of 9.922 - 15.139 with an average value of 12.272. The value of the A_{260}/A_{280} ratio is in the range of 2.04 - 2.13 with an average value of 2.10 (Eppendorf, 2016; Sambrook, Fritsch, & Miniatis, 1989; Qiagen, 2020).

3.2. Result DNA isolation using standard methods

The results of DNA isolation obtained DNA concentration values in the range of 110.50 - 112.65 with an average concentration value of 111.53. The purity value of isolated DNA is in the range of 1.911 - 1.943 with an average purity value of 1.926. Complete data is presented in Table 2 below.

Table 2

Data on DNA isolation results using the standard method

No	Concentration	Purity		
		A_{260}	A_{280}	A_{260}/A_{280}
1	112.65	2.190	1.103	1.932
2	112.25	2.183	1.111	1.914
3	112.10	2.183	1.105	1.926
4	111.90	2.180	1.103	1.928
5	112.15	2.179	1.107	1.915
6	112.00	2.181	1.103	1.928
7	112.15	2.180	1.104	1.922
8	112.25	2.181	1.096	1.935
9	111.90	2.176	1.090	1.943
10	111.45	2.165	1.090	1.932
11	111.10	2.164	1.097	1.924
12	110.50	2.150	1.080	1.939
13	110.90	2.151	1.088	1.920
14	111.00	2.158	1.088	1.930
15	110.75	2.149	1.093	1.911
16	111.10	2.155	1.083	1.932
17	110.80	2.150	1.092	1.914
18	110.60	2.149	1.088	1.922
Average	111.53	2.168	1.096	1.926

The purity value was read using a nano photometer at the A_{260}/A_{280} ratio. From these data, it can be seen that the value of the purity of the sample is quite diverse where the ratio value of A_{260} is in the range of 2.149 - 2.190 with an average of 2.168 and the value of the A_{280} ratio is in the range of 1.083 - 1.111 with an average of 1.096. The value of the A_{260}/A_{280} ratio is in the range of 2.04 - 2.13 with an average of 2.10.

3.3. Discussion

This research was conducted by modifying the maricon food kit extraction method by replacing the binding buffer PB with 96% ethanol and elution EB with Nuclease-Free Water (NFW). The results showed that the value of the concentration and purity of the isolated DNA was quite varied, but it can still be said to have good DNA quality when viewed from the data presented in Table 1. Several other studies on DNA analysis showed that the optimum value of DNA purity in the A_{260}/A_{280} ratio was in the range of 1.8 - 1.9, while RNA was in the range of 1.9 - 2.0

(Eppendorf, 2016), in the range of 1.8 - 2 (Kirby, 1990; Sambrook et al., 1989) which states that the results of DNA extraction. When viewed from Table 1 above, the purity value shows a value of 2.10 which indicates the presence of RNA contamination in the resulting DNA isolation (Sophian, 2021b; Sophian & Abinawanto, 2022; Sophian et al., 2021).

In carrying out DNA isolation, the lysis stage is the initial stage which plays an important role in supporting the success of the DNA isolation process. At this stage, the lysis process assisted by the proteinase K enzyme plays an important role. The proteinase K enzyme will only be active when incubation occurs at a temperature of 65 - 70^{o}C. Therefore, in several studies using this method, it is sometimes necessary to optimize the method before using it. Research conducted by Christensen, Bertram, Aaslyng, and Christensen (2012) shows that it is known that at a temperature of 65^{o}C, lysis will work well when incubated for 03 hours or more. If the incubation time is below 03 hours, the lysis process cannot take place completely.

The next stage after lysis is binding. In this method, this step is modified to produce a binding process with the help of alcohol in the form of 96% ethanol. The data generated shows that the binding results are going well, as evidenced by the detection of a very high concentration of isolated DNA in the range of 1042.9 - 1598.4 with an average concentration value of 1295.1. when viewed from the research of Sophian (2021d), isolated chicken nuggets showed a different concentration value from the research conducted. The value of the isolated DNA concentration can indicate the success of the DNA isolation technique used.

After passing the binding stage, the next stage is washing. This step was carried out 02 times to remove the remaining salt of alcohol and phenol. After passing through the washing, the sample was eluted using an elution buffer. In some kits, the elution buffer can be modified by using Nuclease-Free Water (NFW) as the DNA elution solution.

Based on the data presented in Table 2, the results of DNA isolation using the standard method have advantages in terms of the value of DNA purity that falls between the purity values of a good DNA standard, which is between 1.7 - 2.1, with the purity value of the sample DNA in the range of 1.911 - 1.943. with an average purity value of 1.926. This value is considered better than the purity value in the modified method, where the purity value is in the range of 2.04 - 2.15 with an average purity value of 2.10. However, if we look at the concentration value, the modified method has a much higher concentration value than the standard method. This is because in the standard method, the binding buffer. Using PB solution, while in the modified method, the binding buffer uses ethanol. The main role of ethanol is to remove the solvation shell surrounding the DNA, thereby allowing the DNA to precipitate in the form of pellets. In addition, ethanol helps promote DNA aggregation. Typically, about 70 percent ethanol solution is used during the DNA washing step. This allows the salt to dissolve while minimizing DNA solubility. The final 100 percent ethanol wash primarily used helps promote convenient evaporation of the ethanol from the DNA pellets, thereby preventing any residue. Ethanol is preferred over water because of its lower dielectric constant.

According to Khanuja, Shasany, Darokar, and Kuman (1999), any method used in analyzing the quality of isolated DNA will be influenced by the sample matrix used. Tests using DNA isolation techniques with an ethanol precipitation system are the most commonly used techniques in analyzing the quality of isolated DNA (Fregel, González, & Cabrera 2010). According to Clerget, Bourguignon-Igel, and Rederstorff (2015), in general, the DNA isolation protocol used will involve the addition of sodium acetate, sodium chloride, ammonium acetate, and lithium chloride with ethanol. The deposition process occurs because DNA is a polyanionic molecule due to the very large negatively charged phosphate group in the phosphodiester-linked backbone, which dissolves very well in polar solvents mainly because it has a high dielectric

constant such as water (Zumbo, 2013). Molecular water will mostly form a hydration shell in the area around the DNA to avoid the formation of ionic bonds with cations and prevent DNA deposition (Laage, Elsaesser, & Hynes, 2017). The DNA deposition process can occur due to the help of ethanol in the presence of high concentrations of chaotropic salts under high pH conditions (Poh & Gan, 2014). Since ethanol is a chemical that has a lower polarity than water, so when the ethanol concentration becomes 64% or higher, the DNA backbone phosphodiesters will be allowed to form ionic bonds with cations in solution to precipitate DNA.

4. Conclusions & recommendations

From the research data analyzed, it was found that the modified DNA isolation method has the advantage that the concentration of isolated DNA obtained is higher than the standard method, while the purity value of the DNA isolated from the standard method has better purity when compared to the modified method.

The recommendation suggested in this study is that it is necessary to test the methods that have been used on food products with complex matrices to ensure that the performance of the method can be recognized for its toughness before being used as an official method to be validated.

5. Conflict of interest

Authors state they have no conflict of interest and no affiliation or connection to or with any entity or organization, which may raise a question of bias in the discussion and conclusion of the manuscript.

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References

Christensen, L., Bertram, H. C., Aaslyng, M. D., & Christensen M. (2012). Protein denaturation and water-protein interactions as affected by low temperature long time treatment of porcine longissimus dorsi. *Journal of Meat Science, 88*(4), 718-722.

Clerget, G., Bourguignon-Igel, V., & Rederstorff, M. (2015). Alcoholic precipitation of small non-coding RNAs. *Methods in Molecular Biology (Clifton, N.J.), 1296*, 11-16. doi:10.1007/978-1-4939-2547- 6_2

DiBernardo, G., DelGaudio, S., Galderisi, U., Cascino, A., & Cipollaro, M. (2007). Comparative evaluation of different DNA extraction procedures from food samples. *Biotechnology Progress, 23*(2), 297-301. doi:10.1021/bp060182m

Eppendorf. (2016). *Nucleic acid photometry. Check of critical parameters*. Hamburg, Germany: Eppendorf AG.

Fregel, R., González, A., & Cabrera, V. M. (2010). Improved ethanol precipitation of DNA. *Electrophoresis, 31*(8), 1350-1352. doi:10.1002/elps.200900721

Khanuja, S. P. S., Shasany, A. K., Darokar, M. P., & Kuman, S. (1999). Rapid Isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant Molecular Biology Reporter, 17*(74), 1-7. doi:10.1023/A:1007528101452

Kirby, L. T. (1990). *DNA fingerprinting: An introduction*. New York, NY: Stockton Press.

Laage, D., Elsaesser, T., & Hynes, J. T. (2017). Water dynamics in the hydration shells of biomolecules. *Chemical Review, 117*(16), 10694-10725.

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 1188. doi:10.3390/molecules24061188

Poh, J.-J., & Gan, S. K.-E. (2014). Comparison of customized spin-column and salt-precipitation finger-prick blood DNA extraction. *Bioscience Reports, 34*(5), 629-634. doi:10.1042/BSR20140105

Qiagen. (2020). *DNeasy® mericon® food handbook. For extraction of total nucleic acids from a range of food sample types.* Retrieved February 10, 2022, from www.qiagen.com/shop

Sambrook, J., Fritsch, F., & Miniatis, T. (1989). *Molecular cloning laboratory manual* (3rd ed.). New York, NY: Cold Spring Harbor Laboratory Pr.

Sophian, A. (2021a). Short communication: Analysis of purity and concentration of extracted DNA on salted fish processed food products. *Asian Journal of Natural Product Biochemistry, 19*(1), 21-24. doi:10.13057/biofar/f190104

Sophian, A. (2021b). DNA isolation of chicken feathers from the base of the young feathers, the base of the old feathers, and the tip of the feathers. *Bioeduscience, 5*(2), 104-108. doi:10.22236/j.bes/526211

Sophian, A. (2021c). Detection of Species DNA in chicken meatball products using NGF genes as molecular markers. *BiosciED: Journal of Biological Science and Education, 2*(2), 47-51. doi:10.37304/bed.v2i2.3422

Sophian, A. (2021d). Species DNA detection using PGR gene genetic markers in chicken nuggets. *Indonesian Food Science & Technology Journal, 5*(1), 17-20.

Sophian, A., & Abinawanto. (2022). Study of Co1 and BIK BCL2 gene analysis in gorontalo local chicken. *Journal of Hunan University Natural Sciences, 49*(1), 220-227. doi:10.55463/issn.1674-2974.49.1.28

Sophian, A., & Syukur, A. (2021). Analysis of purity and concentration of isolated DNA in making raw DNA of rat species. *Eruditio: Indonesia Journal of Food and Drug Safety, 1*(2), 1-5. doi:10.54384/eruditio.v1i2.75

Sophian, A., Purwaningsih, R., Muindar, M., Igirisa, E. P. J., & Amirullah, M. L. (2021). Short communication: Analysis of purity and concentration of DNA extracted from intron patho gene spin extraction on crab processed food product samples. *Asian Journal of Tropical Biotechnology, 18*(1), 13-27.

Sutanta, M., Wulan, D. T., Nabila, Y., & Sophian, A. (2022). Application of double wash technique for species DNA isolation in soft capsule shell samples. *Eruditio: Indonesia Journal of Food and Drug Safety, 2*(1), 14-19.

Wulan, D. T., Sutanta, M., & Sophian, A. (2021). Short communication: Comparison of two commercial DNA extraction kit to obtain high quality porcine DNA. *Asian Journal of Tropical Biotechnology, 18*(2), 69-72.

Zumbo, P. (2013). *Ethanol precipitation. Weill cornell medical college publications*. Retrieved February 10, 2022, from http://physiology.med.cornell.edu/faculty/mason/lab/zumbo/files/ ETHANOL_PRECIPITATION.pdf