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Short Communication: Analysis of purity and concentration of extracted DNA on salted fish processed food products

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Abstract. *Sophian A. 2021. Short Communication: Analysis of purity and concentration of extracted DNA on salted fish processed food products. Asian J Nat Prod Biochem 19: 21-24.* Analysis of purity and concentration of extracted DNA on salted fish processed food products was carried out in the microbiology and molecular biology testing laboratory of the Food and Drug Administration in Gorontalo. The purpose of this study was to analyze the extracted DNA on salted fish processed food products based on the concentration and purity values in the A260 / A230 and A260 / A280 wavelengths. The method used for purity and concentration analysis was the absorbance method using a nanophotometer. The samples used were 10 types of salted fish processed food products sampled from 5 (five) traditional markets in Gorontalo City. The sample was extracted using the spin column method with the Dneasy Mericon Food Kit (50) paint kit. 69514 (Qiagen). The research data showed that extracted sample concentration was in the range of 24,600 - 27,150 with an average of 25,745, while the purity value measured at A260 / A280 wavelength was obtained with a purity range between 1,668 - 1,768 with an average of 1,729. Based on the results of this study, it can be concluded that the results of DNA extraction carried out on salted fish and processed food products show a value that is in the category of good DNA extraction results.

Keywords: Concentration, DNA, fish, purity, salty

INTRODUCTION

Analysis of extracted DNA's concentration and purity is the first step and is an important part that must be done in carrying out DNA-based molecular testing. The success rate of extraction can be determined by the purity and concentration value produced. At this stage, extraction is carried out by separating DNA from other components such as fat and protein using chemical and enzymatic processes. This study corresponded to Corkill and Rapley (2008) that the extraction stage is the initial key to the molecular analysis process's success. WheAt this stage, the sample will pass through three main processes, namely the process of lysis, or cell wall destruction, separation of DNA from other components such as protein and fat, and purification processes.

According to Holme and Peck (1998), the lysis process in DNA extraction removes the genetic material in the cell membrane to be used in molecular analysis. This solution can be done with the help of chemical and enzymatic processes. The chemicals and remaining enzyme residues resulting from the lysis process will be purified in the extraction process to produce inhibitor-free DNA. It is necessary to analyze the purity and concentration of the extracted DNA. The extraction method used is a spin column extraction method or column centrifuge with the Dneasy Mericon Food Kit kit combined with a robotic extraction system. This extraction tool works automatically and reduces the role of humans in DNA extraction. The conventional extraction method takes several steps to add chemicals and requires an extended processing time (± 10 hours) (Andreas et al. 2000). Therefore, to save time and control the error rate in researchers, modifications are made to combine these two types of methods. The advantage of this system is that it has a more stable extraction yield compared to conventional methods. On the other hand, humans are critical factors contributing to errors in the DNA extraction process.

The purpose of this research is to provide preliminary information regarding DNA extraction techniques in processed salted fish samples. It can be used as preliminary research to develop authentication and identification tests of processed fish species traded in the market. It can also monitor the types of species used as raw materials for processed food based on salted fish by looking at whether the species used are categorized as protected or free species that can be traded.

MATERIALS AND METHODS

Materials

This study's materials were processed food products of salted fish, RNA-free water, and a Dneasy Mericon Food Kit (50) paint extraction kit. 69514 (Qiagen).

Sample preparation

The sample was weighed as much as 1 g, added 1 mL of Food Lysis Buffer, and 25 µl of proteinase K were

homogenized by vortex for 15 seconds. The sample was then incubated at 65°C for 60 minutes while in a shaker with a speed of 1100 rpm. The stage was continued by lowering the sample temperature by leaving it at room temperature for 30 seconds, then putting it in an ice block/freezer for 10 minutes. After cooling, the samples were centrifuged at a speed of 2500xg for 10 minutes. Samples undergoing the centrifuge process will then form 2 phases, carefully pipette 500 µl of chloroform into a new 2 mL tube and remove 700 µl of the clear layer without touching the precipitation at the bottom of the tube. And put it in a tube containing 700 µl of chloroform and vortex for 15 seconds, then centrifuge at 14000xg for 15 minutes. Take 350 µl of the clear layer. Put it in Qiacube, and use the standard method with 60 ul of EB buffer elution. The eluted DNA can be used directly for real-time PCR processing or stored at -20ºC or - 80ºC for long storage.

Qiacube setup

The initial stage started with inputting the Qiacube protocol for the Dneasy Mericon Food Kit kit. All systems are carried out automatically using a robotic system so that people's involvement is only during the initial lysis stage. The protocol used is to extract total DNA from raw or processed food material with standard methods. The sample was piped 350 µl into a 2 mL tube and then placed into the Qiacube. After that, proceed with arranging the kit, which will be used according to the protocol map. After all the results are appropriate, then the tool is run.

Purity and concentration analysis

Purity and concentration were analyzed using a nano photometer NP80 (IMPLEN). Method setting; Nucleic acid, dsDNA type, nano volume mode, 2 µL sample volume, nucleic acid factor 50.00, background correction 320 nm, air bubble recognition off, manual dilution factor 1.000.

DNA yield

After knowing the concentration and purity values, the next step is calculating the yield. DNA yield is the final DNA product calculated using the formula:

DNA yield (µg) = DNA concentration × total sample volume (mL)

Data analysis

Data analysis was carried out by comparing the purity and concentration values against DNA standards, where the purity at wavelength A260 / A280 was in the range of 1.7–2.1, while the concentration was greater than 20 ng / mL (Leninger 1975; Matlock 2015) or 2.0-2.5 (Eppedorf 2016).

RESULTS AND DISCUSSION

Data from concentration and purity analysis

The analysis of concentration and purity was carried out using a nano photometer, and the results were obtained (Table 1). It can be seen that the value of extracted sample concentration is in the range of 24.600 - 27.150 with an average of 25.745. The purity value was measured at A260 / A230 wavelength, and the results were obtained with a purity range between 0.814 - 0.874 with an average of 0.834. while purity values were measured at the wavelength A260 / A280, the results were obtained with a purity range between 1.668 - 1.768, with an average of 1.729.

According to Eppendorf (2016), the optimum purity value of DNA at the wavelength A260/A280 is in the range 1.8-1.9, while for RNA, it is in the range 1.9-2.0. This is different from the opinion expressed by Kirby (1990); Sambrook (1989) stated that the results of DNA extraction are good if the purity value is in the range of 1.8-2, the concentration is greater than 20 (ng / µl). When viewed from Table 1 above, the purity value that reads the wavelength A260/A280 shows a value below 2.00. Therefore, it can be concluded that the extracted DNA does not fall into a good DNA range. To conclude that a sample extracted can be tested using real-time PCR, the purity and concentration values that are the benchmark for isolation are good because of developments. Real-time PCR technology allows amplification to occur at even low concentrations depending on the sensitivity of a PCR device.

DNA yield

The research conducted obtained an average of 1357.50 with the lowest yield value of 1230.00 and the highest yield value of 1287.30. Other results are presented in Table 2.

The absorbance method is one method to perform yield analysis using a spectrophotometric instrument. The absorbance readings were carried out at a wavelength of 260 nm or (A260). This is because DNA at this wavelength absorbs light so that the resulting turbidity can be used to estimate the amount of DNA detected. Readings are carried out in the instrument's linear range (0.1 - 1.0).

Table 1. Extracted nano photometer data

Sample	Concentration ng/µL	Purity (A260/A280)
Sample 1	24.600	1.668
Sample 2	24.650	1.724
Sample 3	24.650	1.761
Sample 4	25.150	1.729
Sample 5	25.250	1.766
Sample 6	26.700	1.768
Sample 7	26.450	1.740
Sample 8	27.150	1.729
Sample 9	26.450	1.690
Sample 10	26.400	1.720
Average	25.745	1.729

Table 2. DNA yield

Sample	Concentration ng/µL	Total Dilution Volume (µl)	Yield
Sample 1	24.600	50	1230.00
Sample 2	24.650	50	1232.50
Sample 3	24.650	50	1232.50
Sample 4	25.150	50	1257.50
Sample 5	25.250	50	1262.50
Sample 6	26.700	50	1335.50
Sample 7	26.450	50	1322.50
Sample 8	27.150	50	1357.50
Sample 9	26.450	50	1322.50
Sample 10	26.400	50	1320.00
Average	25.745	50	1287.30

Discussion

In general, the DNA extraction process using the Phenol-Chloroform extraction system consists of three processes: cell lysis, purification, and precipitation. The lysis stage was carried out with proteinase K and Sodium Dodecyl Sulfate (SDS) enzymes. At this stage, SDS will lyse fats and proteins in the cell membrane so that the contents in the cell membrane come out. This process is carried out by heating at a temperature of 70°C while being shaken. This heating activates the proteinase K enzyme to carry out lysis (Renshaw 2015) actively. The Phenol-Chloroform extraction system uses phenol to bind proteins, fats, and carbohydrates, separated from other macromolecules. Phenol and Chloroform Isoamyl Alcohol-bounds proteins and polysaccharides will settle to the tube's bottom. DNA and water are in the top layer (Kado et al. 1981). To separate and purify the DNA is using a centrifuge column, where at the end of the washing process using alcohol, the remaining salt and phenol contained in the sample will come out and leave the DNA pellets. Sterile distilled water or nucleotide-free water can be used to pull the DNA pellets from the spin column.

This study's extraction technique combines conventional methods using a centrifuge and robotic techniques using Qiacube. The advantage of combining these methods is to produce a more stable DNA extraction. Table 1 shows the data from the DNA nanodrop reading results extracted. The DNA extraction system using a robotic system is the same as conventional extraction. The difference is in the use of robots to perform pipetting and centrifuge.

Analysis of purity and concentration is read using a nanophotometer by measuring the absorbance value at a wavelength of A260/A280. The A260/A280 wavelength is a standard method for detecting DNA concentration and purity value. In the salted fish samples analyzed, the sample matrix composed of fish meat and salt seeping into the meat also requires caution in carrying out DNA extraction to produce good DNA isolates for analysis at the next step, namely the amplification process.

Leninger (1975) stated that of the 5 nucleotide compositions that make up DNA or RNA, when reading the absorbance at wavelength A260/A280, they would show varying values, namely: guanine (1.15), adenine (4.50), cytosine (1.51), uracil (4.00) and thymine (1.47). The purity analysis results obtained from the absorbance readings are the average of these four or five nucleic acids' absorbance values. It is the basis for determining the purity value in general for DNA analysis in the range (of 1.8-2.0). For RNA, the range value will be greater than this value because one of the constituent components is uracil, which, when compared with DNA composed of thymine, uracil has a higher value, namely (4.00), so that if averaged, the purity value will be higher when paired with DNA.

Apart from all analyzes of the extracted DNA, the final stage of DNA analysis, of course, lies in the PCR itself, whether in further research it can detect the extracted DNA or not because the purity and concentration values shown are initial analyzes to assess the success of the DNA extraction process.

The results of DNA yield showed that the amount of DNA was quite large, namely around 1278.30. This value is considered very good compared to the DNA yield value required for DNA amplification using real-time PCR. Each PCR has different detection limit detection capabilities ranging from 0.01-2 p / µL (Perandin et al. 2004; Cnops et al. 2011; Kamau et al. 2013; Xu et al. 2015; Srisutham et al. 2017). Suggestions for further research should be continued until the confirmation test stage to prove that the results of DNA extraction are not good enough; the results can still be used.

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