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Concentration of isolated DNA face masks made of gelatin for halal authentication

Nazila Nur Hikmah[1], Any Guntarti[1,2], Nina Salamah[1,2*]
[1]*Faculty of Pharmacy, Universitas Ahmad Dahlan, Jl. Prof. Dr. Soepomo, S.H., Janturan, Umbulharjo, Yogyakarta, 55164, Indonesia*
[2]*Ahmad Dahlan Halal Center, Universitas Ahmad Dahlan, Jl. Prof. Dr. Soepomo, S.H., Janturan, Umbulharjo, Yogyakarta, 55164, Indonesia*
[*]*Corresponding author: nina.salamah@pharm.uad.ac.id*

ABSTRACT

The function of using a face mask is to remove dirt and dead skin cells found in the pores of the skin, open clogged pores and clean the remaining cosmetics that cannot remove with ordinary cleansers, repair and tighten the skin, provide nutrition, smooth, soften and maintain skin moisture. People are aware of halal-labeled cosmetics, one of which is face masks because some face masks contain gelatin. Some face masks on the market contain gelatin. Preparation of 5 reference samples with different concentrations. Preparation of face masks, DNA isolation, and checking the isolation results. The test results with the Nanodrop Spectrophotometer showed that the face mask DNA containing pig and cow gelatin extracted in this study ranged from 163.35 ng/µl in sample S6 to 83.550 ng/µl in sample S7. With the largest concentrations of 163.35 ng/µl and 83.550 ng/µl, while the highest purity value at the λ260/λ280 ratio was 1.41.

Keywords: DNA, Face mask, , Gelatin, NanoDrop spectrometer

INTRODUCTION

Indonesia is one of the countries with a Muslim majority. The large Muslim population in Indonesia affects the halal lifestyle, which becomes the basis for product selection (Nur, 2014). Therefore, the government issued a regulation, Law Number 33 of 2014, concerning the Halal Product Guarantee (UUJPH), which can protect consumers' selection of products. Halal status has become a global issue, including non-food products such as medicines and cosmetics (Widayat et al., 2019).

Halal cosmetics are unique because they adhere to Islamic teachings by avoiding ingredients derived from pigs and alcohol. They must also meet specific requirements in their production, storage, packaging, and distribution (Charity, 2017). Taking care of the skin involves various methods such as cleansing, refreshing, peeling, moisturizing, masks, and evaporation. Masks are crucial in removing dirt and dead skin cells from pores, unclogging them, and eliminating remnants of cosmetics that regular cleansers cannot remove (Sulastri & Chaerunisaa, 2018). They also help repair and tighten the skin, provide nutrients, and maintain moisture levels. These skincare practices are important for maintaining healthy and nourished skin (Fujiko, 2022). Currently, people are quite aware of the use of halal-labeled cosmetics, one of which is face masks, because some face masks contain gelatin

Some face masks on the market contain gelatin. Gelatin is a heterogeneous mixture of polypeptides produced by hydrolysis of collagen derived from animal connective tissue. Gelatin can obtain from animal skin and bones, such as pigs, cows, and fish (Rohman & Salamah, 2018). The application of gelatin in the food or non-food industry is very broad, such as in the processing of food products that produce chewy textures and in non-processing, one of which is into cosmetic ingredients such as masks because gelatin has transparent, tasteless, and solid physical properties so it is suitable for use as a basic ingredient in making mask (Jaya et al., 2022)

In this study, DNA isolation in face masks containing gelatin using a NanoDrop spectrometer (Rizko et al., 2020), Nanodrop spectrophotometer is a tool that has a principle by calculating the

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difference in UV light absorption where the double band of DNA can absorb UV light at 260 nm while contaminants in the form of proteins and phenols will absorb light with a wavelength of 280 nm. DNA purity can measure by the absorbance ratio to wavelengths of 260 nm and 280 nm; good DNA purity is 1.8-2.0 (Mollah et al., 2022).

Therefore, this study aims to isolate DNA in face masks containing gelatin for further development with real-time PCR methods for the analysis of pork gelatin (Setiaputri et al., 2020), especially related to the validation of real-time PCR analysis methods using specific primers and their application for halal authentication in cosmetic products, namely face masks containing gelatin (Widayat et al., 2019).

RESEARCH METHOD

Materials

The materials used in this study were bovine gelatin, porcine gelatin, PVA, propylene glycol (Merck), nipagin, 96% alcohol, ammonium bicarbonate buffer solution (Favorgren), trypsin (Favorgreen), and the tools used in this study are analytical scales, a favor-prep blood genomic DNA extraction mini kit, and a Nanodrop Spectrophotometer (Optima).

Methods

1. Reference Sample Preparation

   The modified face mask formula from (Mulia, 2021) is present in Table 1.

Table 1. Reference Face Mask Formulation (Jaya et al., 2022)

Materials	Unit	F1	F2	F3	F4	F5
Pork gelatin	g	2	5	8	10	-
Bovine gelatine	g	8	5	2	-	10
Propilen Glikol	g	2	2	2	2	2
PVA	g	2	2	2	2	2
Nipagin	mg	40	40	40	40	40
Etanol 96%	ml	2.5	2.5	2.5	2.5	2.5
Aquadest	ml	Add 100	Add 100	Add 100	Add 100	Add100

The preparation of gelatin masks with different concentrations is show in Table 1. Face mask samples is make from porcine and bovine gelatin with comparative concentrations of 100% porcine gelatin, porcine gelatin, and bovine gelatin ratio of 20:80, 50:50, 80:20, and 100% bovine gelatin. Gelatin in 4 ml of distilled water at 60oC in a beaker glass while stirring. While stirring, 2 g of PVA was developed with 8 mL of distilled water at 80^{o}C in a porcelain cup. PVA and gelatin were put into a mortar and mixed until homogeneous (Mixture A). A total of 2 g propylene glycol was mixed with mixture A and stirred until homogeneous (Mixture B). 40 mg of nipagin are dissolve with 2.5 ml of 96% ethanol in a beaker glass (mixture C). Mixture B was mixed into mixture C and crushed until homogeneous, then stirred, and each formula are add with distilled water until 20 ml (Setiawati & Sukmawati, 2018).

2. DNA isolation with favor-prep blood genomic DNA extraction mini kit

   The reference and market mask samples weighed 25 mg each and were put into a microcentrifuge tube. Then add 200 µl FATG1 buffer and mix well using a Micropestle. Next, add 20 µl Proteinase K and homogenize using a vortex device. Then, the sample was incubated at 600 until lysis (1-3 hours). Add 200 µl FATG2 Buffer to the sample mixture, mix well by pulse-vortexing, and incubate at 70°C for 10 minutes. Add 200 µl The procedure involves several steps for processing a sample mixture using ethanol. First, the sample mixture is mixed well by pulse-vortexing, and any droplets in the cap are removed by rotating the tube briefly.

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Then, the mixture, including the precipitate, is transferred to a FATG Mini Column. The column is then centrifuged at full speed for 1 minute and placed into a new Collection Tube. Next, 750 µl of Wash Buffer is added to the column, and the mixture is centrifuged at full speed for 1 minute, followed by discarding the flow-through. It is important to ensure that ethanol is added to the wash buffer when opened. Afterward, the column is centrifuged at full speed for 3 minutes to dry the column and remove any remaining liquid. Finally, 100 µl of preheated Elution Buffer or ddH_2O (pH 7.5-9.0) is added to the FATG Mini Column membrane, and the column is held for 3 minutes (Kamaliah, 2017).

3. DNA Isolation with NanoDrop Spectrometer

After making reference samples with different concentrations of 100% pig gelatin, pig gelatin, and cow gelatin ratio of 20:80, 50:50, 80:20, and 100% cow gelatin, after the reference sample was made, the reference sample and the market sample were extracted by DNA isolation with the favorprep blood genomic DNA extraction mini kit. Then the concentration was checked with a nanodrop spectrometer (Dayanti et al., 2019). As in the research that has been performed by (Kamaliah, 2017), the DNA extraction method using nanodrop spectrometer face mask DNA samples containing gelatin that have been isolated produce clear samples. The clear color indicates DNA at the bottom of the tube. The sample was then tested for purity and concentration quantitatively using NanoDrop.

Nanodrop spectrophotometry readings were taken to determine the purity of the extracted DNA. Nanodrop spectrophotometer is a tool that calculates the difference in UV light absorption where the DNA double band can absorb UV light at 260 nm. In comparison, contaminants in the form of proteins and phenols will absorb light with a wavelength of 280 nm. DNA purity can be measured by the absorbance ratio to wavelengths of 260 nm and 280 nm; good DNA purity is 1.8-2.0 (Muslish, 2021).

RESULT AND DISCUSSION

Different concentrations in the face mask reference samples were made to compare the mixing between reference samples, which were then isolated with the kit and tested with a Nanodrop spectro.

Table 2. DNA Spectrophotometer Results of Facial Masks Containing Bovine Gelatin and Pig Gelatin

No	Sample Code	Concentration (ng/µl)	Purity A260/A230
1	S1	0.0000	0.00
2	S2	0.4500	0.17
3	S3	0.0000	0.00
4	S4	2.2500	2.10
5	S5	2.5000	3.48
6	S6	163.35	1.41
7	S7	83.550	1.37
8	S8	3.200	1.34
9	S9	10.150	0.76
10	F1	9.7500	5.45
11	F2	3.5500	0.96
12	F3	0.0000	0.00
13	F4	0.6500	0.95
14	F5	1.1500	0.35

Description: S1-S9: Market mask samples; F1-F5: Reference mask samples

DNA isolation results are said to be pure if the absorbance ratio is at 1.8 - 2.0. The DNA purity value is calculated by dividing the absorbance value at a wavelength of 260 nm by the absorbance value

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at a wavelength of 280 nm. The light wavelength of 280 nm can be absorbed by contaminants in the form of protein or phenol. This causes DNA purity to be calculated by the absorbance value of 260 nm divided by the absorbance value of 280 nm with a purity value ranging from 1.8-2.0. The test results with the Nanodrop Spectrophotometer showed that the face mask DNA containing pig and cow gelatin extracted in this study had a range of 163.35 ng/µl in sample S6 to 83.550 ng/µl in sample S7 (Table 2). According to research conducted by (Muslish, 2021), a purity value below 1.8-2.0 indicates that the DNA is contaminated with proteins and polysaccharides, the absorbance value is less than 1.8. If DNA is contaminated with RNA, the absorbance value is more than 2.0. At the same time, some samples that have very low concentrations, including in the Nanodrop Spectrophotometer test, are samples with codes S1, S2, S3, S4, S5, S8, S9, F1, F2, F3, F4, and F5.

Several factors affect the Nanodrop Spectrophotometer results, including pipetting or transferring isolation samples from mini tubes to PCR tubes before the Nanodrop Spectrophotometer examination. The results obtained on the spectrophotometer are strongly influenced by the solvent components present in the stock DNA solution because DNA will read the concentration at a wavelength of 260/280. However, in this condition, the dissolved DNA impurities greatly affect the stability of the resulting DNA concentration. These impurities can be phenol compounds or contaminants that occur during DNA extraction. These contaminants can be carbohydrates or proteins. According to research conducted by (Ugroho et al., 2017), The concentration of template DNA required for PCR activities ranges from 10-100 ng/µl. If below this concentration, it can cause poor amplification of products. From the above table data obtained in testing with the NanoDrop Spectrometer, the codes that can be used for real-time PCR activities are S6 and S7. A real-time PCR test was conducted to determine the code sample's pig DNA content.

CONCLUSION

From the results of the research conducted, it is known that the quantitative test of DNA from face masks containing bovine and porcine gelatin has the largest concentration of 163.35 ng/µl and 83.550 ng/µl while the highest purity value at the λ260/λ280 ratio of 1.41. So, to continue with the real-time PCR development method, the samples that can be used are these two samples.

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