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Detection of Pig DNA Contamination in Facial Creams Containing Collagen Using Conventional PCR

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Abstract

Background: Cosmetics are pharmaceutical preparations intended to support appearance or care for the body. Both local and imported facial whitening products are mostly marketed at varying prices, from cheap to expensive, making more people buy them. The supply of collagen extracted from mammals has recently been limited and is mostly obtained from the tendons and skin of cattle and pigs. The collagen content not listed on cosmetic packaging makes people worried about pork collagen being contaminated, therefore detection is needed to find out. This research aimed to detect whether there was pork contamination in facial cream products containing collagen with Cytochrome b (Cyt-b) primers using conventional PCR. **Method:** The research design is exploratory. The samples used were 5 facial cream samples and one positive control. Samples were subjected to DNA extraction, DNA concentration quantification, PCR, and DNA electrophoresis. **Result:** In the positive control, a DNA band of ~149 bp specifically targeting Cytochrome b was formed, while the facial cream sample did not form a DNA band. **Conclusion:** The five facial creams used as samples were not contaminated with pork contamination.

Keywords: cosmetics, collagen cream, cytochrome b, PCR

INTRODUCTION

Cosmetics are pharmaceutical preparations intended to support appearance or care for the body. Cosmetics are used to cleanse, beautify, increase attractiveness, or change a person's appearance. One cosmetic that is often used is cream/moisturizer [1]. The cream is a cosmetic that women often use. The cream is a semi-solid preparation with a thick form emulsion containing around 60% water and is used for external use [2]. Cream preparations have advantages, including a level of comfort in use and quite high aesthetic value. Facial creams usually contain antiaging and collagen, these ingredients have bioactivity which can prevent or improve signs of premature aging [2] Therefore, creams are now widely available on the market and are in demand among the public.

Collagen is one of the main components of the extracellular matrix of the dermis and articular cartilage which influences the formation of body tissue. Collagen is considered a product *nutraceutical* that is widely used as an ingredient or supplement in the food, pharmaceutical, and cosmetic industries because collagen promotes skin changes, such as reducing the formation of

wrinkles, increasing skin elasticity, increasing hydration, increasing the content, density, and synthesis of collagen, which are factors closely related to damage. skin related to aging [3].

The main structure of collagen consists of amino acids, mostly glycine (33%), proline, and hydroxyproline (22%). The secondary structure is formed from α chains of amino acids bundled into helices with three amino acids per turn, which untwist each other and form a tight tertiary structure. Basic collagen structure is quaternary structure associated with the superhelix. So far 29 types of collagen have been discovered including bovine and pig collagen [4].

The use of pork and its derivative products in cosmetic products including facial creams is considered haram in Islam. The halal of food products, drinks, cosmetics, medicines, and other consumer goods is something that is highly emphasized in Islamic Syariah. Every Muslim must pay attention and choose halal products [5]. The collagen content of some facial creams is not yet known, so a test is needed to see the content of the facial cream. One of the accurate tests to see the contents of facial cream is PCR.

PCR is a very sensitive method and can selectively amplify small amounts of target DNA present in a product using specific primers [6]. The resulting PCR product is then electrophoresed. The electrophoresis results were analyzed by visually comparing the band thickness. The optimal band in question is a band that is thick, single and matches the target size [7]. Pig DNA detection is now believed to be able to obtain accurate and fast results, because DNA acts as a reservoir or biological characterization of all living creatures, including pigs, according to the specific characteristics of molecular arrangements and connections [8]. Pig DNA detection can be done using specific genes. The cytochrome b gene is one of the genes in mitochondria that codes for proteins and is known to be a specific DNA marker for pigs [9]. Samples containing pork were identified by applying the restriction enzyme BseDI which will cut DNA from the cytochrome b gene [10]. Based on the above background, this research aims to detect pork elements in collagen using the Polymerase Chain Reaction (PCR) method using pig-specific primers, namely cytochrome b.

METHOD

This research was conducted in April 2024 at the Molecular Biology Laboratory, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang. The research design is descriptive experimental research. The samples for this study were 5 imported face creams whose contents were unknown and the control was pork. The tools used are *micropipette*, *tip*, *vortex mixer*, *centrifuge*, *microwave*, *water bath*, rotator DSR 2800 V, mold *gel agarose* or comb, BIO-RAD electrophoresis *Power Basic*, *power supply*, *UV transilluminator*, nanodrop spectrophotometer *Master GEN*, Biometra T *personal thermocycler*. The ingredients used are facial cream containing collagen in 5 samples and positive control pork (3 grams), lysis buffer 200 µl, phenol CIAA 1:1, Proteinase K 20 µl, *Ethanol* cold *96%* 1:1, *ethanol* 70% 500 µl, TE (Tris EDTA) 200 µl, PCR master mix 12,5 µl, ddH2O 6.5 µl, first *reverse* 2 µl, first *forward* 2 µl, DNA template 2 µl, gel *agarose* 2%, tris solution *acid* EDTA (TAE 1x), *fluorovue* 4 µl, dan marker 50 bp DNA ladder 5 µl.
The primers used for research are in the following table:

Table 1. Primers Cytochrome b [11]

Primary Name	Oligunucleotide	Ampli-con
Primer Forward	5'CTACGGGTCTGTTCCGTTGG3'	149 bp
First Reverse	5'ATGAAACATTGGAGTAGTCCTACTATTTAC3'	

DNA Extraction

DNA extraction was carried out by weighing 2gram samples of facial cream with collagen content and then placing them in a 1.5 ml conical tube, then adding 2 ml of lysis buffer to the conical tube and 20 µl (10 mg/ml) Proteinase K. After that vortexed and incubated at 55ºC for 60 minutes. CIAA phenol was added 1:1 and shaken for 15-20 minutes, centrifuged at 3000 rpm for 20 minutes at room temperature. The top layer was transferred into a 1.5 ml microtube. 96% cold ethanol is added in a 1:1 ratio and slowly homogenized until fine threads appear. The fine threads were transferred to another microtube and then washed with 500 µl of 70% ethanol by centrifuging at 12,000 rpm for 10 minutes at 4ºC 2-3 times. After that, the supernatant was discarded, and the pellet was air-dried. TE 200 µl was added to dissolve the DNA. Then continue measuring the concentration and purity of the DNA. DNA can be used for PCR or stored in the freezer.

DNA Quantification and Purity

2 µL of TE buffer was inserted into the Maestro nanopro as a blank. Then 2 µL of the isolated DNA was pipetted and put into the instrument, then the concentration and absorbance were measured to determine the purity of the DNA. The absorbance seen is with a wavelength of 260/280.

DNA Amplification (PCR)

PCR mixing was carried out by dissolving 12.5 µl Abclonal Master Mix, 6.5 µl ddH2O, 2 µl forward primer, 2 µl reverse primer, and 2 µl template DNA inserted into a special PCR microtube. The microtube is inserted into the thermocycler PCR device, then the temperature is set to the pre-denaturation stage at 95°C for 4 minutes, followed by the denaturation stage at 95°C for 30 seconds, then at the annealing stage at 63°C for 30 seconds and extended with temperature 72°C time 3 minutes. The final extension was carried out at a temperature of 72°C for 10 minutes and the cooling down stage was carried out at a temperature of 4°C for 10 minutes. The results of this amplification occurred in a total of 35 cycles.

DNA Electrophoresis

Electrophoresis was carried out by mixing 2 grams of agarose and 100 mL of 1x TAE buffer. The mixture was dissolved in the microwave until dissolved and 4 µL of DNA dye was added and then printed in a gel mold with a comb. Then 10 µL of PCR results were put into the chamber with a voltage of 75 volts for 60 minutes. The electrophoresis results were visualized using a UV transilluminator. The DNA band is compared with the marker to determine the length of the base.

Data Analysis

The research results were analyzed descriptively. The DNA band is compared with a marker to determine the base length of the sample. The target DNA length was 149 bp for the cytochrome b specific gene.

RESULTS AND DISCUSSION

DNA purity and concentration

The results of extracting facial cream containing collagen purity and concentration were tested using a Nano Pro MaestroGen MN-913A spectrophotometer. The results can be seen in Table 2.

Table 2. DNA Purity and Concentration

Sample Code	DNA concentration (ng/ul)	DNA purity (l260/280)
K1	34,76	1,916
K2	64,13	1,862
K3	90,93	1,851
K4	16,28	1,819
K5	82,76	1,892
K+	90,55	1,801

Based on Table 2, various DNA purity and concentrations were obtained. The results of measuring DNA purity in samples K+, K1, K2, K3, K4, and K5 showed pure DNA with results ranging from 1.801 – 1.916, which means the DNA is of good quality. The results of concentration measurements on K+, K1, K2, K3, K4, and K5 samples showed that the highest DNA concentration was 90.93 ng/ul, and the lowest DNA concentration was found, namely 16.28 ng/ul. The DNA purity and concentration values are still within the DNA purity and concentration range so that they can be used for gene amplification Cytochrome b.

Amplification of cytochrome b genes

DNA templates that have been tested for purity and concentration are followed by amplification of the CYT B gene using a tool thermocycler RS-232 with an estimated annealing temperature of 63 ˚C. The PCR results were then electrophoresed with an agarose concentration of 2% and visualized using a UV light UV transilluminator, with results can be seen in Figures 1 and 2.

Based on Figure 1, column M is a marker for the location of the base pair (bp) with a size of 50 kb. In Figure 1, the results of the amplification of the cytochrome b gene show that a DNA band was formed in the K+ sample. The concentration of DNA entering the agarose electrophoresis wells for K+ samples was 90.55 ng/ul, K1 samples were 34.76 ng/ul, K2 samples were 180.47 ng/ul, K3 samples were 90.93 ng/ul, K4 samples were 16.28 ng/ul and K5 samples were 82.76 ng/ul. The resulting DNA band is a single band that is parallel to the DNA marker which has a molecular length

of 149 bp. These results indicate that the cytochrome b gene was detected in the K+ sample (positive control).

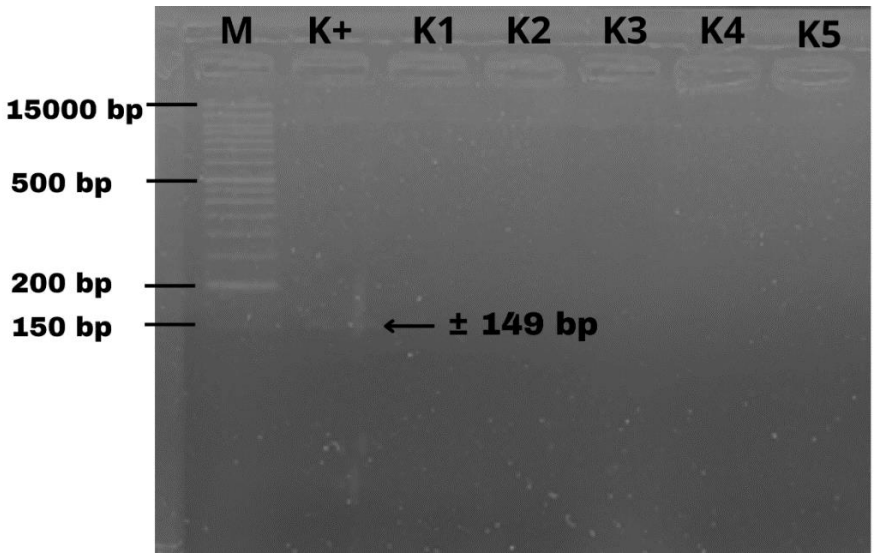

Figure 1. Results of 2% Agarose Gel Electrophoresis PCR Products

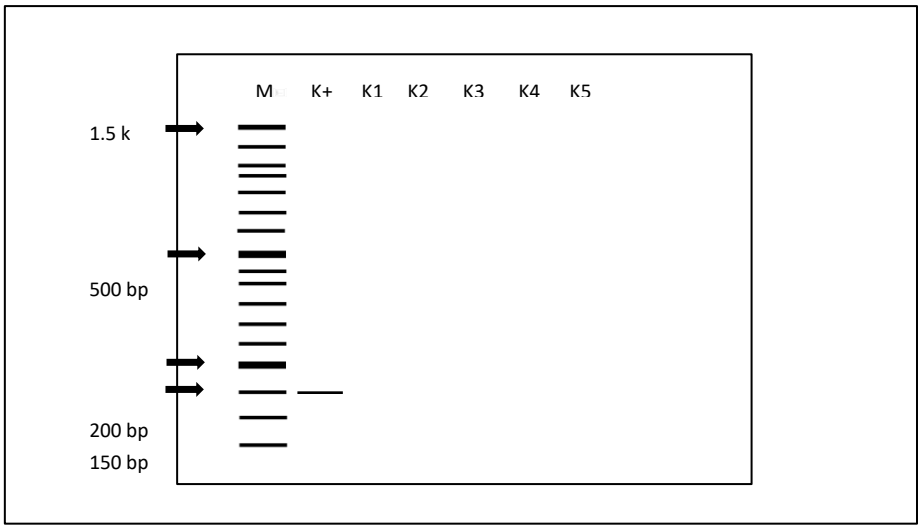

Figure 2. Representative diagram

Cytochrome b (Cyt-b) is an mtDNA that has a moderate mutation rate and a conserved nucleotide sequence position. Cytochrome B is a mitochondrial protein that functions as part of the electron transport chain and is the main subunit of the transmembrane cytochrome bc1 and b6f complex [12]. This research began with purchasing samples from the marketplace provided that the sample contained the most frequently purchased collagen. The number of samples obtained was 6 samples, of which 5 samples were cream containing collagen and 1 positive control sample was pork.

To determine the presence of pork contamination in facial cream, gene detection is carried out Cytochrome B uses Polymerase Chain Reaction (PCR). The initial step to detect the cytochrome B gene using PCR is to extract the sample to obtain a DNA template. Isolation was carried out on cream-shaped K1-K5 samples to separate pure DNA. Good DNA extraction is supported by the results of the quantity of DNA samples obtained. Next, the results of the sample DNA extraction are used as DNA templates in the Cytochrome b gene amplification stage.

Testing the DNA isolate is done by carrying out a quantitative test using a Maestro Nanopro spectrophotometer. The principle of Maestro nanopro is a spectrophotometer to pure DNA capable of absorbing ultraviolet light due to the presence of purine and pyrimidine bases. The test results are in the form of ratio values λ260/280 and DNA concentration values. DNA is said to be pure if the λ260/280 ratio value ranges from 1.8 – 2.00 [13], and the results of measuring the DNA concentration required for the PCR process range from 10 – 100 ng/ul [14]. Several things that play a major role in influencing the purity and concentration of the DNA produced are the extraction method used, DNA damage, and impurities/contaminants such as proteins or other solvents [15]. Contaminated samples can hinder the non-specific amplification process [16].

Based on Table 2, the DNA purity test results obtained were pure because the purity results were between 1.8-2.0. If the DNA purity value is below 1.8, this indicates that there is protein contamination or too much solvent was used, if the DNA purity value is above 2.0, it indicates that there is contamination with RNA or too little solvent was used [17]. The DNA concentration results show that the results are in the range of 10 – 100 ng/ul, with the lowest result being 16.28 of/ul in the K4 sample and the highest result being 180.47 of/ul in sample K2. High DNA concentration values in samples can be influenced by several factors, namely poor DNA isolation so that DNA samples that should be pure are mixed with impurities such as RNA and protein. Too high a concentration of template DNA can increase the possibility of mispriming, while concentrations that are too low can cause the possibility that the primer cannot find the target [18].

DNA template which is pure and has good concentration followed by amplification of the cytochrome b gene. In this study, amplification of the cytochrome b gene used temperature annealing 63˚C for 35 cycles. Stage annealing is one of the factors that influence the success of amplification is the temperature used because attaching the primer to the exposed DNA strand requires an optimal temperature. If the temperature is too high, it will cause amplification to fail because the primer will not attach. Conversely, if the temperature is too low, it will cause the primer to stick to the other side of the genome, resulting in the DNA formed having low specificity, so it is very important to find the optimum annealing temperature for the amplification process [19].

The PCR product was continued with DNA electrophoresis using a 2% agarose gel. Several things, such as the size of the DNA molecule, the concentration of the agarose gel, the composition of the electrophoresis buffer, and the voltage used, influence the electrophoretic migration of DNA through the agarose gel. Different concentrations of agarose gel can separate DNA fragments measuring 200 bp to 50 kb. The size of the gel pores used to separate DNA will be determined by the concentration of agarose used. The frequency of DNA will be further separated based on size as the

concentration of the gel matrix decreases. Since DNA is a negative molecule, the gel matrix will place it towards the positive pole. The DNA migration rate is influenced by the DNA molecular weight: the heavier the DNA molecule, the slower the migration rate, and the smaller the DNA molecule, the faster the DNA migration rate through the gel. Too much voltage can cause excessive heat, which can cause the gel to melt and interfere with the visualization of DNA bands [20].

The PCR products were visualized using a UV transilluminator. The principle of agarose electrophoresis is to separate, analyze, identify, and purify DNA fragments, nucleic acids or proteins based on differences in electric fields, charged molecules and particles will move toward electrodes that have opposite charges under the influence of the electric field. Excessive electrical voltage in gel electrophoresis must be taken into account because it can cause heat which can change the shape of DNA fragments or a smile effect. The smile effect can be caused by several things, such as the imperfect density of the agarose gel, the presence of impurities in the agarose gel, and changes in the direction of the electric field [21].

In this study, the results of separating DNA fragments using agarose gel electrophoresis with 75volt electrical voltage treatment for 60 minutes, perfect results were obtained, namely the formation of a DNA band in K+ (Positive Control) with a size of ±149 bp as measured by a location marker. basepair (markers). This indicates that the electric voltage and migration rate are correct in the electrophoresis process. In samples K1, K2, K3, K4, and K5 no DNA bands were found.

The DNA band that appears in K+ (pork) specifically targets the Cytochrome b gene because it is aligned with a molecule length of ±149 bp. This result is because K+ is real pork. Meanwhile, in samples K1, K2, K3, K4, and K5 no DNA bands were found, it can be concluded that samples K1, K2, K3, K4, and K5 did not contain pork collagen.

A limitation in this research is determining the appropriate annealing temperature for the sample so that the DNA bands obtained are not too clear. The DNA concentration is too small for the sample so it requires a large amount of DNA for PCR amplification. Sequencing to ensure the base length of the DNA sample.

CONCLUSION

Pork collagen was not found in the five facial cream samples. Imported face creams do not always contain pork collagen so they are safe for Muslims to use. Cosmetics users should pay attention to the ingredients first before using the product. Researchers can use DNA isolation procedures using kits so that the DNA concentration obtained can be higher.

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