Detection of Pork Gelatin in Jelly Candy Using Fourier Transform Infrared (FTIR) and Polymerase Chain Reaction (PCR)

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Abstract. This study aims to identify the content of pork gelatin in jelly candy using Fourier Transform Infrared (FTIR) and Polymerase Chain Reaction (PCR) methods. This method provide information to the public in choosing halal and tested food products. By uses a stepwise cluster survey method to obtain a sample then the samples obtained were isolated in gelatin, analyzed using the FTIR spectrophotometer method, and continued with data analysis using PCA (Principal Component Analysis). In addition, DNA detection analysis of pork gelatin was carried out using the PCR method. The results of the study were FTIR spectrum at wavelengths of 1450 – 1300 cm^{-1}, 1543 cm^{-1}, and 2800-3000 cm^{-1}. The classification of gelatin sources in jelly candy with PCA resulted in the proportion value of Principal Component 1 (PC 1) of 39%, the value of the proportion of Principal Component 2 (PC 2) of 31%, the value of the proportion of Principal Component 3 (PC 3) of 14.5% and the cumulative value of PC 1, PC 2, and PC 3 is 84.5%. DNA amplification of jelly candy samples by PCR proved that all jelly candy samples A, B, C, D, and E did not contain pork.

Keywords: FTIR, PCR, Principal Component Analysis

1 Introduction

Halal food products in a country with a Muslim majority population is a major concern. Some of the ingredients used in food products such as gelatin, collagen, and fat are synthesized or extracted from mammals such as cattle, fish, and pigs. Products sourced from pork and its derivatives are prohibited for Muslims [1]. Meanwhile, the halal lifestyle is sweeping the world, not only in countries with Muslim-majority populations but also in countries with Muslim-minority populations. So the demand for halal products is quite large. Halal aspects are very broad, such as goods and/or services related to food, beverages, drugs, cosmetics, chemical products, biological products, genetically engineered products, as well as goods used, used, or utilized by the community [2].

Gelatin is a product in the form of hydrocolloids derived from the hydrolysis of animal or livestock collagen protein, which is hydrophilic which is found in many skins, bones, and animal tissues [3]. The use of gelatin as a gelling agent in jelly candy products needs to be considered because almost 80% of the gelatin produced comes from pork-skin. As explained by one of the gelatin supply companies, Gelatine Manufacturers of Europe (GME) that in 2018 almost 80% of gelatin produced came from pork skin, 15% came from split (thin layer on cowhide), while the remaining 5% derived from beef, fish, and pork bones [4].

The National Standardization Agency (2008) stated that jelly candy is a soft textured confectionery, which is processed with the addition of hydrocolloid components such as agar, gum, pectin, starch, carrageenan, gelatin, and others. which is used for texture modification to produce a chewy product [5]. One component of the hydrocolloid forming jelly candy as much as 23% of the use of gelatin in the food industry of 154,000 tons. Jelly candy is made by cooking sugar until it reaches the desired solid, then adding gelling ingredients (gelatin, agar, pectin, and carrageenan) then adding flavor and color, and finally printing [6].

The jelly candy in circulation uses a thickening agent in the form of gelatin, so it requires an analytical method that can ensure that the gelatin used is free from pork content. Gelatin contained in jelly candy can be identified using FTIR (Fourier Transform Infrared) spectroscopy and FTIR infrared spectrum data processing using chemometric methods with Principal Component Analysis (PCA) techniques. PCA is one of the chemometric methods for classifying the properties of a material or substance based on the similarities it has [7]. Through PCA modeling, it can provide an overview

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in classifying the profiles of bovine and pork gelatin [8], [9]. Furthermore, a confirmation method of porcine gelatin DNA detection analysis using Polymerase Chain Reaction (PCR) was used to support the results of this study. PCR is a technique for synthesis and amplification of DNA in vitro, through an enzymatic process using DNA polymerase enzymes and nucleotide primers that will hybridize with parts of DNA from two opposite directions [10]. The purpose of this study was to identify pork gelatin in jelly candy products in circulation using the Fourier Transform Infrared (FTIR) and Polymerase Chain Reaction (PCR) methods and to determine the source of gelatin used in jelly candy products.

Table 1. Percentage of transmittance at a wavelength of 1450-1300 cm^{-1}.

Gelatin Sample	Wavelength (cm^{-1})	Percentage transmittance (%)
Pig Control-1	1450 1384	31 31
Pig Control -2	1441 1384	31 31
Cow Control-1	1384	61
Cow Control-2	1384	31
Jelly candy B-1	1419 1383	53 52
Jelly candy B-2	1419 1383	53 52
Jelly candy C-1	1420 1376	65 65
Jelly candy E-1	1366 1341 1318	35 36 37
Jelly candy E-2	1378	49

2 Research Method

2.1 Research Materials

The ingredients used in the analysis of pork gelatin in jelly candy with FTIR were jelly candy purchased at a traditional market in East Surabaya (Indonesia), 10% acetic acid, saturated picric acid, KBr pellets, aquadest. Ingredients for DNA detection in pork gelatin include pork, jelly candy A, jelly candy B, jelly candy C, jelly candy D, jelly candy E proteinase-K 3 l+ TE buffer 750 l (TE pH 8 & SDS 1 % (v/v)), 20 l NaCl 5M, DNAZol 1 cc, phenol, chloroform, isopropanol, 70% ethanol, sterile distilled water. The materials used in DNA gelatin analysis were distilled water, DNA template, TE Buffer, dNTPs, MgCl2, Taq DNA polymerase, nuclease water, pork primer sequence with base sequence 5'-GCC TAA ATC TCC CCT CAA TGG TA-3' and 5'-ATG AAA GAG GCA AAT AGA TTT TCG-3', Ethidium Bromide, 2% agarose (0.4 gr + 20 ml TBE 0.5 x).

2.2 Research Tools

The tools used for the extraction of gelatin jelly candy in this study were analytical scales, glassware, heater, thermometer, scissors, mortar, magnetic stirrer, oven, filter paper. The tools used in gelatin analysis include Shimadzu Perkin Elmer Fourier Transform Infrared (FTIR) spectrophotometer, Shimadzu hand press, Microsoft Windows XP Professional operating system computer, and Minitab 16 software for chemometric analysis. The tools used in DNA detection in gelatin are Applied Biosystems GeneAmp PCR System 9700, Mupid-2plus electrophoresis machine, analytical balance, microtube, cold centrifuge, water bath, vortex, oven, Eppendorf tube, heater, and glassware.

2.3 Identification of Gelatin Using FTIR

Identification of gelatin using FTIR spectrophotometer was carried out by dissolving 2 mg of isolated gelatin mixed with 200 mg of KBr and then homogenized. Pellets were formed using a hand press (Shimadzu) with a pressure of 8 tons for 10 minutes. FTIR spectrum measurements were carried out at 4000-400 cm^{-1}. The results of the spectrum measurements will appear on a personal computer equipped with OPUS software version 4.2 [9].

2.4 Identification of Gelatin Using the FTIR Method

Identification of gelatin using FTIR spectrophotometer was carried out by dissolving 2 mg of isolated gelatin mixed with 200 mg of KBr and then homogenized. Pellets were formed using a hand press (Shimadzu) with a pressure of 8 tons for 10 minutes. FTIR spectrum measurements were carried out at 4000-400 cm^{-1}. The results of the spectrum measurements will appear on a personal computer equipped with OPUS software version 4.2 [9].

2.5 Identification of Pork Gelatin Using PCR

2.5.1 Extraction of DNA Gelatin in Jelly Candy

Extraction and isolation of gelatin DNA in DNA jelly candy was carried out first by separating the non-DNA components which consisted of several steps. 2.5 grams of jelly candy sample was dissolved in 5 ml of distilled water. It is then heated until the sample is completely dissolved. A total of 500 l of the sample was inserted into a 1.5 ml microtube by adding 3 l of proteinase-K and 750 l of TE buffer (Tris-EDTA pH 8 and SDS 1% (v/v). The microtube containing the sample was then incubated in a water bath at a temperature of 55 ^{o}C for 16 hours, then centrifuged at 3000 rpm for 10 minutes. The results of the centrifugation were then separated between the supernatant and the pellets formed.

The supernatant obtained was transferred to a new microtube containing 20 l of 5 M NaCl. The supernatant was incubated at 37 ºC for 1 hour. Next, it was centrifuged at 3000 rpm for 10 minutes and the supernatant was separated from the pellets formed. The volume of the supernatant was measured. The supernatant was transferred back to a new microtube and phenol was added according to the volume of the supernatant (1:1), the sample was vortexed until homogeneous. 0.2 cc of chloroform was added and then vortexed again. Samples were incubated for 24 hours. Samples were centrifuged at 6000 rpm for 15 minutes, separated between the supernatant and the formed pellets.

Volume measurements were carried out on the obtained supernatant. The supernatant was transferred back to a new microtube and added isopropanol according to the volume of the supernatant (1:1), then the sample was incubated for 30 minutes. The sample was centrifuged again at 1200 rpm for 10 minutes. The supernatant was separated from the formed pellet. The pellets were added with 0.5 cc of 70% ethanol to the pellets and then vortexed until homogeneous. Samples were incubated for 2 hours and then centrifuged at 12000 rpm for 10 minutes. The supernatant contained in the microtube was discarded, then the microtube was placed upside down for several minutes to dry. Add 50 l of sterile distilled water to the pellets obtained and stored at -20 ºC. DNA from each sample was then tested for purity and concentration using spectrophotometry.

2.5.2 Pork DNA Extract

The pork DNA extraction method is a modification that refers to research (Al-Kahtani, Ismail, and Asif Ahmed, 2017) which uses the DNA isolation method Kit High Pure PCR Template (Roche, Mannheim, Germany) . However, in this study, DNA extraction and isolation methods were used using a DNAzol reagent kit. A total of 0.05 grams of pork samples were dissolved in 500 ml of distilled water in a 15 ml centrifugation tube. Samples were incubated for 24 hours at room temperature. 1 cc of DNAZol was added to the sample and then vortexed until homogeneous. The samples were incubated again for 1 hour at room temperature. 0.2 cc of chloroform was added to the sample and then vortexed for 2 minutes. Samples were incubated at room temperature for 24 hours and then centrifuged at 6000 rpm for 15 minutes. Supernatant with pellets formed is separated [10].

The separated supernatant was then transferred to a new microtube and added isopropanol according to the volume of the supernatant (1:1). Samples were incubated for 30 minutes at room temperature. The sample was centrifuged again at 12000 rpm for 10 minutes. Then the supernatant was removed and 0.5 cc of 70% ethanol was added and vortexed until homogeneous. The sample was again incubated for 30 minutes and then centrifuged at 12000 rpm for 10 minutes. The supernatant contained in the sample was discarded, then the microtube was placed upside down for several minutes to dry. 50 μl of sterile distilled water was added to the pellet, then the pellet was stored at -20 ºC. DNA from each sample was tested for purity and concentration using spectrophotometry.

3 Result and Discussion

3.1 Peak of the Fourier Transform Infrared (FTIR) Spectrum of Gelatin

The results of gelatin isolation of jelly candy samples B, C, and E using the gelatin isolation method produced light yellow to dark yellow powdered gelatin solids [6]. The presence of yellow color in the isolated gelatin sample was due to the residual picric acid that was still present in the isolated gelatin. It is also found in research [11] that the use of picric acid in the extraction of gelatin in tablet drugs produces yellowish-colored powdered gelatin. The addition of picric acid which is a strong acid result in the presence of excess H+ ions, which indicates the presence of turbidity. While jelly candy samples A and jelly candy samples D due to differences in composition, the gelatin was not isolated, this could be due to the low temperature used for isolation. Heating is carried out to dissolve the gelatin at least 49 ºC or usually at a temperature of 60-70 ºC [12]. Therefore, due to the low isolation temperature, gelatin powder was not found in jelly candy A and jelly candy D. Jelly candies whose gelatin was isolated were then identified using FTIR.

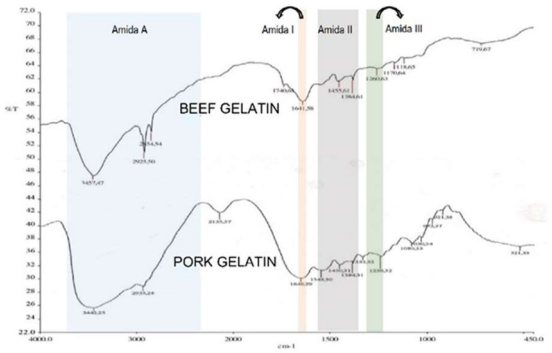

Fig. 1. Peak FTIR Spectrum of Cattle Gelatin Control and Pork Gelatin Control.

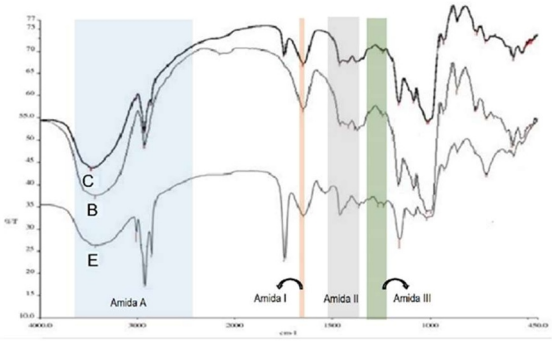

Fig. 2. Peak FTIR spectrum (a) gelatin jelly candy sample B, (b) gelatin jelly sample C, (c) gelatin jelly candy sample E.

Gelatin-like protein has a structure consisting of carbon, hydrogen, hydroxyl group (OH), carbonyl group (C=O), and amine group (NH) [9]. Figure 1. shows that

the spectrum peaks found in the bovine gelatin control are 11 and the spectrum peaks found in the pork gelatin control are 14. Figure 2. Shows the peak spectrum in the jelly candy sample found with a total number of varying peaks, in jelly candy sample B, jelly candy sample C, and jelly candy sample E have 23, 21, and 20 spectrum peaks, respectively. The number of peaks that vary is influenced by the vibration (vibration) of each molecule caused by the absorption of infrared light and will provide information in the form of the intensity of the infrared light absorbed, the more molecules that vibrate, the more diverse the peaks obtained. Infrared light passing through a compound causes vibrations between the molecules of the compound, resulting in a change in the dipole moment of a compound.

The typical wavelength of gelatin is divided into 4 parts, namely the absorption region of amide A at a wavelength of (3600-2300) cm^{-1}, amide I at a wavelength (1636-1661) cm^{-1}, amide II at a wavelength of (1560-1335). cm^{-1}, and amide III at a wavelength (1300-1200) cm^{-1} [9]. Figure 26. shows that the results of the isolation of gelatin from samples of jelly candy B, jelly candy C, and jelly candy E have all the typical absorption regions of gelatin in amide A, amide I, amide II, and amide III according to the study [9]. Figure 26. shows that the results of the isolation of gelatin from samples of jelly candy B, jelly candy C, and jelly candy E have all the typical absorption regions of gelatin in amide A, amide I, amide II, and amide III according to the study [13]. So the isolation of gelatin in jelly candy samples was successfully carried out. Each typical wavelength of gelatin and its functional groups in jelly candy samples can be seen in Table 13 and Table 14. Furthermore, the wavelengths used to distinguish the source of gelatin in jelly candy are in the region 1450–1300 cm-1, 1543 cm-1, and 2800-3000 cm-1. Because in the region of 1450-1300 cm-1, 1543 cm-1, and 2800-3000 cm-1, there are differences in the amino acid composition of bovine and pork gelatin, especially for glycine, proline, and arginine [14]. This is in accordance with the amino acid composition of bovine and pork gelatin (as listed in Table 2) which shows the difference in residue composition per 1000 total amino acid residues of glycine, proline, and arginine types in bovine skin gelatin and pork skin gelatin ranging from 64 to 131.

Next will be discussed the wavelength in each of the above regions. The first region that can distinguish the source of gelatin is at a wavelength of 1450-1300 cm^{-1}. All samples have a spectrum peak at a wavelength of 1450-1300 cm^{-1} which indicates the presence of the C-H functional group. At a wavelength of 1450-1300 cm^{-1} indicates the presence of C-H. The wavelength region of 1450-1300 cm^{-1} can distinguish the source of gelatin because in this region the difference in amino acid composition between pork gelatin and beef gelatin can be seen through the percent transmittance (number of frequencies of infrared radiation that is not absorbed) of each sample. The smaller the percentage of transmittance obtained by the sample, the greater the infrared light absorbed by the compound [15]. The small percentage of transmittance obtained by the sample also indicates more compounds contained in the sample. The following is Table 9. percentage transmittance at wavelength 1450-1300 cm^{-1}.

In table 1, the porcine control gelatin samples 1 and 2 have a transmittance percentage of 31, which is lower than the bovine control gelatin samples 1 and 2, which are between 31-61. This is because the amino acid content in porcine gelatin is greater than that of beef gelatin so that infrared rays are absorbed more and produce a lower percent transmittance in porcine control gelatin. The smaller the percent transmittance means that almost all of the transmitted frequencies are absorbed by the compound. The thicker a layer or the greater the concentration, the more atoms there will be, as well as the more frequent collisions of light particles with atoms so that it is more difficult for light to pass through, the lower the percentage of transmittance obtained. It is also supported by the statement that the difference in frequency at a wavelength of 1450-1300 cm-1 is indicated because the aliphatic amino acids of pork gelatin are greater than that of beef gelatin, where the amino acid composition between bovine gelatin and pork gelatin is relatively different, especially for glycine, proline and arginine residues [11].

However, the gelatin sample of jelly candy E-1 also found a small transmittance percentage between 35-37. This can be caused when the infrared ray is fired, there are still solvent compounds left in the gelatin sample of jelly candy E-1 which causes the compounds in jelly candy E-1 to be greater in addition to that the yellow color of gelatin isolated from jelly candy E is more concentrated than gelatin. On the other hand, the more Ag substrate coats the glass, the lower the transmittance percentage [5]. So the small percentage of transmittance obtained was due to the presence of residual solvent compounds in the gelatin jelly candy sample E-1 [16]. The second region that can distinguish the source of gelatin is at a wavelength of 1543 cm^{-1}. Only the control sample of pork gelatin showed absorption at a wavelength of 1543 cm^{-1}, while for jelly candy samples B, C, and E there was no absorption at a wavelength of 1543 cm^{-1}. This is because the absorption of jelly candy samples B, C, and E has shifted to 1459, 1461, 1508, 1542, and 1536 cm^{-1}.

This shift in the wave region is caused by the deformation of the N-H and C-H bonds in gelatin. The shift in wavenumber in amide II in the range of 1335-1560 cm^{-1} is due to the deformation of the N-H bond while the wavelength at 1500-1200 cm^{-1} represents the deformation of CH_2 [11]. Deformation of NH and CH bonds in gelatin is due to the triple helix structure of collagen which changes due to temperature and extraction time in the gelatin making process. The triple helix structure of collagen that has been heated or has been extracted into gelatin breaks down into an -helix structure. The α-helical structure of gelatin can decompose again into coil bonds (random) when exposed to high temperatures in the gelatin isolation process in jelly candy and cause deformation of NH. In the 1335-1560 cm^{-1} region, there are abnormalities in the molecular structure due to the transformation of -helices into random coil structures due to the extraction process which is characterized by the occurrence of N-H bending vibrations coupled with C-N stretching

vibrations [17]. Temperature and length of extraction time can cause triple helix changes as a result of the denaturation of collagen to gelatin [18]. Then the shift in wavelength in jelly candy samples B, C, and E 1459, 1461, 1508, 1542, and 1536 cm^{-1} was caused by the temperature and extraction time during gelatin manufacture. The third region that can distinguish the source of gelatin is the wavelength of 2800-3000 cm^{-1}. All gelatin jelly candy samples have different spectral peaks at a wavelength of 2800-3000 cm^{-1} can be seen in Table 2. The wavelength of 3000-2850 cm^{-1} is the absorption region of the C-H bond [12]. The following are the wavelengths and functional groups of the FTIR spectra at a wavelength of 2800-3000 cm-1 can be seen in Table 2.

Table 2. Wavelength and functional groups FTIR spectra at a wavelength of 2800-3000 cm^{-1}.

Sample	Wavelength (cm^{-1})	Bonding	Function Group
Beef gelatin control	2925 2854	C-H C-H	Alkana (strong) Alkana (bending)
Pork gelatin control	2935 2933	C-H C-H	Alkana Alkana
Jelly candy sample B	2929	C-H	Alkana
Jelly candy sample C	2926 2925 2854	C-H C-H C-H	Alkana Alkana Alkana
Jelly candy sample E	2925 2854	C-H C-H	Alkana (strong) Alkana (strong)

The results of the identification of wavelengths using FTIR can be concluded that the results of the isolation of jelly candy that have been studied are gelatin. Each jelly candy isolate has an absorption at a wavelength that can be used as a reference to continue the analysis using the Principal Component Analysis (PCA) method to determine the source of the gelatin used. Because the working principle of FTIR is to recognize the functional group of a compound from the infrared absorption carried out on the compound [9]. To find out more clearly the source of gelatin used in the jelly candy sample, further analysis was carried out at wavelengths of 1450-1300 cm^{-1}, 1543 cm^{-1}, and 2800-3000 cm^{-1} using the Principal Component Analysis (PCA) method on the control sample. cattle, control pigs, sample B, sample C, and sample E.

3.2 Analysis of PCA Data Processing Results

The results of the wavenumber and absorbance analysis of the FTIR were analyzed using the Principal Component Analysis method. Each sample analyzed using FTIR has almost similar absorption peaks [9]. However, if it is observed quantitatively, there is a difference in the height of the peak due to differences in the absorbance value in each sample. The absorbance value of each absorption in each sample can be seen in Table 1 and Table 2. The difference in absorbance values is analyzed by comparing the spectral peak's FTIR. Therefore, the PCA method is used to group the data, so that the differences in each sample can be specifically identified [15]. Absorbance analysis was carried out in the region of 1450-1300 cm^{-1}, 1543 cm^{-1}, and 2800-3000 cm^{-1} which will be processed by PCA using Minitab 16 software. The following are the wavelength regions 1450-1300 cm^{-1}, 1543 cm^{-1}, and 2800-3000 cm^{-1} can be seen in Figure 3.

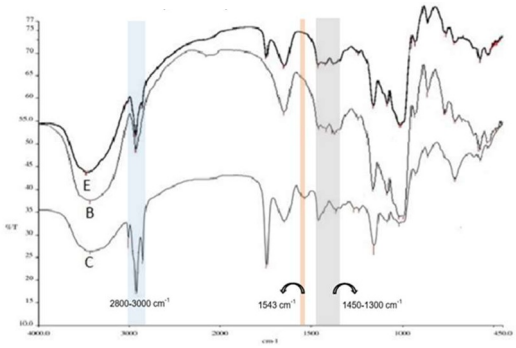

Fig. 3. The wavelength region is 1450 – 1300 cm^{-1}, 1543 cm^{1}, and 2800-3000 cm^{-1}.

The graphic results of the infrared spectrum pattern of absorption at wavelengths 1450-1300 cm-1, 1543 cm-1, and 2800-3000 cm-1 can show differences in amino acid composition in beef and pork gelatin, especially for glycine, proline, and arginine. Principal Component (PC) plays a role in reducing the dimensions of the original variable so that a new variable (PC) is obtained which is not correlated with each other but stores some of the information contained in the original variable [14]. One way to find out how many PCs should be taken to reduce a variable is by using a scree plot or eigenvalue. The method used to determine the number of principal components (PC) is by looking at the eigenvalues that are more than one or observing the scree plot by looking at the angled fault of the scree plot. The following is a scree plot of the peak of the functional group obtained from the analysis results, which can be seen in Figure 4.

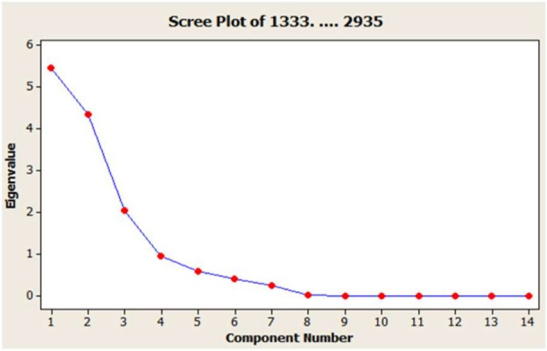

Fig. 4. Scree Plot Peak Functional Group

Table 3. Correlation Matrix Eigen Analysis Table

PC	Eigenvalues	Proportion	Cumulative
1	5.4545	0.390	0.390
2	4.3438	0.310	0.700
3	2.0297	0.145	0.845
4	0.9399	0.067	0.912
5	0.5800	0.041	0.953
6	0.3969	0.028	0.982
7	0.2523	0.018	1.000
8	0.0028	0.000	1.000
9	0.0000	0.000	1.000
10	0.0000	0.000	1.000
11	0.0000	0.000	1.000
12	0.0000	0.000	1.000
13	0.0000	0.000	1.000
14	0.0000	0.000	1.000

Based on Figure 4, the scree plot curve begins to slope or fracture at point 4. This shows that there are three main components that can be taken, namely PC1, PC2, PC3. In addition, taking the number of PCs can be seen in the eigenvalues in Table 11. Eigenvalues greater than 1 are PC1, PC2, PC3. So it can be concluded that the number of PCs formed is three PCs. From Table 3. it can be seen that the proportional value of PC1 is 39%, PC2 is 31%, and PC3 is 14.5% so that the cumulative diversity of the proportion values is 84.5%. This shows that the number of PCs selected can be used to distinguish the source of gelatin, the number of PCs selected is sufficient if it has a cumulative diversity of not less than 75% [19]. Furthermore, there is a score plot in the PCA analysis aimed at describing the data plot of the sample classification used. The following is a sample PCA score plot which can be seen in Figure 5. The score plot displayed is a 2-dimensional plot in which there are 4 quadrants. The x-coordinate is PC1/First Component and the y-coordinate is PC2/Second Component. In Figure 29 the area is divided into 4 quadrants, namely quadrant I, quadrant II, quadrant III, and quadrant IV.

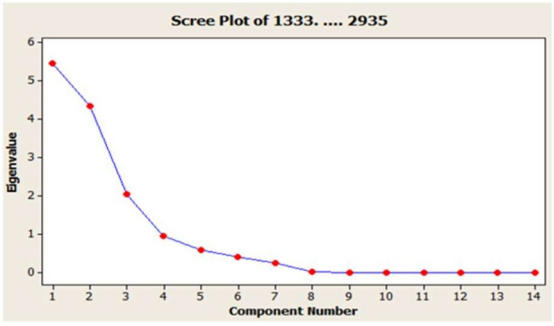

Fig. 5. *Score* Plot PCA Sample

Beef gelatin and gelatin isolated from jelly candy C and E are in quadrant II, pork gelatin is in quadrant III, and gelatin isolated from jelly candy B is in quadrant IV. Pork gelatin is in quadrant III, beef gelatin is in quadrant II, and gelatin extracted from gummy is in quadrant II and IV. The PCA plot scores obtained showed that none of the samples were in the pig quadrant [14]. The closer the distance between the points, the closer the relationship possessed by the analyzed samples [5]. So it can be concluded that the gelatin jelly candy sample B, sample C, and sample E are free from pork gelatin content.

3.3 Purity of DNA Isolate

The DNA from each sample and control analyzed yielded the purity and concentration as shown in Table 4. The results of the concentration and purity of DNA isolation in jelly candy samples can be seen in Table 4.

Table 4. Results of concentration and purity of DNA isolation on jelly candy samples.

Sample	Concentration (ng/µ*l*)	Purity
Bk	871.5	1.23
A	539.0	1.25
B	556.5	1.25
C	479.5	1.26
D	248.5	1.34
E	633.5	1.27

Note:
Bk = positive control pork
A-E = Sample jelly candy

The concentrations obtained ranged from 248.5 to 871,5 ng/µ*l*, the pork DNA isolate concentration was 871,5 ng/µ*l*; sample A was 539 ng/µ*l*; sample B was 556,6 ng/µ*l*; sample C was 479,5 ng/µ*l*; sample D was 248,5 ng/µ*l* ; and sample E was 633,5 ng/µ*l*. DNA with good quality generally has a concentration above 125 ng/µ*l* [7]. The results showed that the concentration obtained from DNA isolates had good quality because they were above 125 ng/µ*l*. With several levels above the DNA, the isolate can be continued for the PCR amplification process because PCR is quite sensitive and can detect levels up to 1 ng/ml [6].

The purity obtained from the control sample and the jelly candy sample ranged from 1.23 to 1.34, the purity of the pork DNA isolate was 1.23; sample A is 1.25; sample B is 1.25; sample C is 1.26; sample D is 1.34; and sample E is 1.27. The value of DNA purity obtained was below 1.8, indicating that the isolated DNA still contained contamination in the form of the protein [10]. In general, with the purity value obtained, DNA extracted from all samples can still be used for the amplification process.

3.4 Amplification Results with Polymerase Chain Reaction (PCR)

The next process is the identification of pork content in jelly candy samples using PCR. The following is a visualization of PCR results from positive control pigs, jelly candy samples A, jelly candy samples B, jelly candy samples C, jelly candy samples D, and jelly candy samples E can be seen in Figure 6.

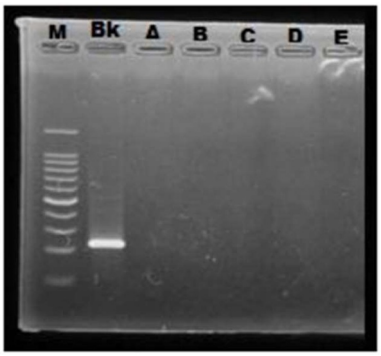

Fig. 6. Visualization of PCR results from each jelly candy, M = 100 bp marker; Bk = pork control; A-E = jelly candy sample.

The success of a PCR process depends on the primer used. The primer functions as a barrier for the target DNA fragment to be amplified and at the same time provides a hydroxy group (OH) at the 3' end which is needed for the existence of DNA [10]. This study used pork primer with nucleotide base sequence 5'-GCC TAA ATC TCC CCT CAA TGG TA-3' and 5'-ATG AAA GAG GCA AAT AGA TTT TCG-3'. Based on the results of PCR visualization using 2% agarose gel electrophoresis in Figure 6. Shows the results of PCR amplification of cty b from pork DNA as expected, which is about 212 bp, cty b gene amplicon from pork produces fragments of about 212 bp [14]. However, from the five samples of jelly candy that were tested, there was no indication that they contained/ contaminated pork DNA because there was no DNA band/band that appeared at 212 bp. The results of the gummy sample isolates did not show any DNA bands [10]. However, this does not show that the DNA isolation process was not successful because the isolation of pork DNA was successful. Meatball samples numbered 13 and pork DNA were amplified in the PCR results, while meatball samples numbered 1-12 were not amplified so it was concluded that samples numbered 1-12 did not contain pork. Based on the results of this PCR, it can be concluded that all of the jelly candy samples in circulation did not contain or were contaminated with pork or used beef gelatin.

4 Conclusion

There are 5 different brands of jelly candy products with a gelatin composition that are sold in the market. Two trademarks of jelly candy products that are labeled halal and 3 trademarks of jelly candy products that have not been labeled halal with gelatin composition. Identification of pork gelatin in jelly candy products was successfully carried out using a combination method of Fourier Transform Infrared (FTIR) and PCA, from samples of beef gelatin, pork gelatin samples, jelly candy samples B, jelly candy samples C, and jelly candy samples E. wavelengths 1450-1300 cm^{-1}, 1543 cm^{-1}, and 2800-3000 cm^{-1}. There are no jelly candy samples in the pork gelatin sample quadrant, so the gelatin used in the jelly candy samples does not contain pork gelatin, but contains beef gelatin. The results of the identification of FTIR and PCA were supported by the results of PCR research which showed that jelly candy products were detected negative for pork gelatin content because there was no DNA band that appeared at 212 bp.

We would like to thank the Faculty of Engineering, University of Pembangunan Nasional "Veteran" East Java, which has helped a lot in this research. So we can make this article.

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