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Halal authentication: Fourier transform infrared spectroscopy and multivariate calibration application for pork gelatin analysis in gummy candy

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Abstract

Gelatin is a substance obtained from the partial hydrolysis of collagen, which is derived from connective tissue, animal bones, and the skin of vertebrate animals, especially pork and cows. Therefore, this study aimed to find out whether the gelatin contained in gummy candy products in the Pasar Baru Bekasi is halal and not from pork. Fourier transform infrared (FTIR) spectrophotometer was combined with principal component analysis (PCA) and partial least square (PLS). The results of the score plot showed that the gelatin of pork and cows was found in different quadrants. Samples 3 and 4 were in quadrant 3, indicating similarities with bovine, while 1 and 2 were in their own quadrants, which shows that they were not obtained from pork or beef. The multivariate regression curve showed that the pattern of linear absorbance changes along with gelatin concentrations of pork and cows.

1. Introduction

Indonesia is a country where 85% of its population are Muslims, namely 207.2 million out of 237 million people. Previous studies revealed that there are halal and haram foods as well as drinks in the teachings of Islam. Therefore, food safety factors, such as halal authentication are a major concern for the Muslim community (Citrasari, 2015). Most of the non-halal or illegal goods in the market are pork derivatives, such as lard, gelatin, and pork-based products. A previous study also revealed that these products include dead flesh, blood, and meat slaughtered without mentioning the name of Allah (Mursyidi, 2013). Allah SWT said in the Qur'an Surat Al-Baqarah: 173, about the prohibition of the use of the element of porks, which means: "Surely Allah only forbids you from eating carrion, blood, porks, and animals that are slaughtered with other names than Allah". However, there is no sin for anyone who is in a forced state, does not want it, and does not exceed the limits. Surely Allah is merciful and merciful" (Q.S. AlBaqarah:173)

Pork or its derivatives, such as lard and gelatin are produced from pig bones or skin, and they are often compared to products derived from cows. Therefore, they are used as counterfeiting ingredients in food. Pork is often mixed with beef to obtain a bigger profit, while lard can be processed into higher-priced oils, such as cod liver oil (Rohman and Che Man, 2008; Riza *et al.*, 2022). In 2007, the world's gelatin production came from pork skin (44%), cowhide (28%), a mixture of pork and cow bones (27%), and other sources (1%). A previous study revealed that those derived from porks and cows are more in demand due to their higher quality (Shyni *et al.*, 2014)

Gelatin is one of the most common biopolymers obtained from the partial hydrolysis of animal collagen tissue. It also has unique properties and is widely used in pharmaceuticals, food, and cosmetics (Zilhadia *et al.*, 2018). Gelatin can act as a gelling, thickening, emulsifying, and stabilizing agent. Therefore, it is often used in food products, such as gummy candy, marshmallows, ice cream, and processed meats. Gelatin is also used in pharmaceutical fields as an ingredient for making hard and soft capsules, tablets, ointments for oral membrane mucosa, and gummy supplements (Nhari *et al.*, 2012).

Gummy candy is a soft-structured food, which was processed by the addition of hydrocolloid components, such as pectin, caragene, yeast, agar, gum, and gelatin used for texture modification to produce chewy and processed products (Badan Standardisasi National

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(BSN), 2008). The candy has a soft and chewy texture, and it can be eaten by chewing. It is made from a mixture of sucrose crystals, glucose syrup, and water, followed by the addition of a gelling agent that can form a soft gel and melt when chewed in the mouth. Gummy candy also consists of additional ingredients, such as flavor and color substances (Sandra *et al.*, 2015). Several studies have been carried out on the properties, applications, and structure of gelatin due to its wide usage, but those from different sources can be very similar in terms of their physical and chemical properties. This makes it difficult to distinguish the halal and haram types (Nemati *et al.*, 2004). Various analytical methods have been used to distinguish pork and cow gelatin, including realtime-PCR, Liquid Chromatography Mass spectrometry (LCMS), Enzyme-Linked Immunosorbent Assay (ELISA), chemical precipitation techniques, modified gold sensors NiO nanoparticles, and FTIR (Zilhadia *et al.*, 2018). Therefore, this study aims to find out whether the gelatin in gummy candy products on the new Bekasi market was halal and was not obtained from pork.

2. Materials and methods

2.1 Materials

The tools used in this study include Attenuated total reflection (ATR) - FTIR spectrophotometer (Agilent-USA), analytical balance (Ohaus-UK), gummy candy obtained from one of the traditional markets in Bekasi, beef gelatin (Sigma Aldrich-Germany) and pork gelatin (Sigma Aldrich-Germany).

2.2 *Sample*

Inclusion criteria were gummy candy that does not have a label/homemade, purchased at Pasar Baru Bekasi, and yellow in color. Meanwhile, those with a label, sweets, and not purchased at Pasar Baru Bekasi were excluded.

2.3 *Making gummy candy containing beef and pork gelatin*

A total of 3 g of pork and beef gelatin were weighed. The pork gelatin was prepared with concentrations of 0%, 10%, 20%, 30%, 50%, 70%, 90% and 100% (Table 1). It was then moistened with distilled water and stirred over a water bath at 60°C. Next, 17.5 g sucrose and 17.5 g glucose syrup were moistened with distilled water in a beaker glass. A total of 1 mL of citric acid and moistened gelatin were added slowly to a container containing glucose syrup and sucrose. The mixture was stirred and food coloring was added with stirring until homogeneous, as shown in Table 1. The mixture was poured into a molded container and allowed to stand for 1 hr until it became gummy (Schrieber and Gareis, 2007)

Table 1. Gummy concentration.

Gummy Candy Making		
Concentration (%)	Pork gelatin (g)	Cow Gelatin (g)
0	0	3
10	0.3	2.7
20	0.6	2.4
30	0.9	2.1
50	1.5	1.5
70	2.1	0.9
90	2.7	0.3
100	3	0

2.4 Sample testing with ATR-FTIR spectroscopy

The product obtained from the production process was cut thinly and evenly placed on the ATR diamond. The samples were scanned using ATR-FTIR spectroscopy at wave numbers 4000-700 cm^{-1} with a resolution rate of 4 nm (Hashim *et al.*, 2010)

2.5 Statistic analysis

PCA and PLS analysis was carried out by entering each of the absorbent data of beef, pork, and mixed gelatin as well as commercial gummy on gelatin-specific area wave numbers in Microsoft Excel. It was then changed to plot scores and graphs using the Minitab 17 software.

3. Results and discussion

The samples used include pure beef gummy gelatin candy, pure pork gummy gelatin candy, mixed gummy candy with concentration variations of 10%, 20%, 30%, 50%, 70% and 90%, and commercial gummy candy sold in Pasar Baru Bekasi. The formulation of gummy candy used was 17.5 g sucrose and 17.5 g glucose syrup as a sweetener, gelatin as a gummy base, 1 mL of citric acid as a salting agent, and food coloring (Schrieber and Gareis, 2007).

Analysis of ATR-FTIR of all samples was carried out to determine the spectrum produced on each of them using the number of waves and absorbents produced, as shown in Figures 1 and 2. The IR spectra result shows some characteristics in peptide chains (Table 2).

Results of PCA analysis show that when infrared light at a certain wavelength was absorbed by a molecule, it caused changes in vibration. Vibration in a molecule is affected by the energy transition that occurs in the infrared (Inayah, 2018). The value of the spectral peak was read and the results of wave numbers and absorbant values in the FTIR test were obtained. The value was read in the form of an image score plot to make it easier to distinguish between beef and pork

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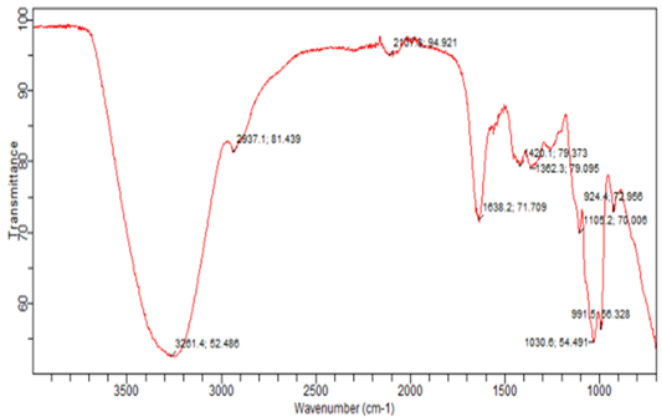

Figure 1. IR spectra of gummy from 100% cow gelatin.

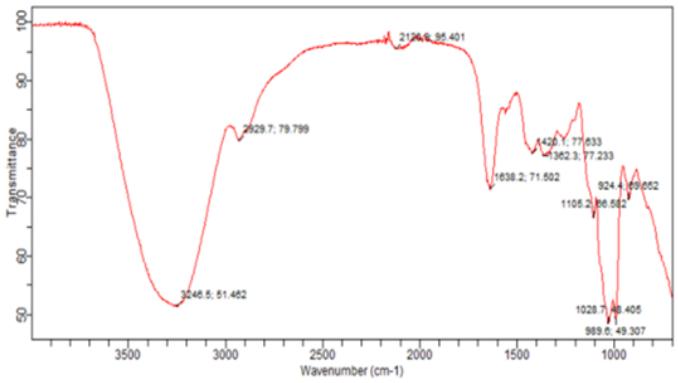

Figure 2. IR spectra of gummy from 100% pork gelatin.

Table 2. Characteristics of IR uptake in peptide chains.

Peptide chain	Wave number (cm^{-1})	Information
Amide A	3300	NH stretching
Amide B	3100	NH stretching
Measure I	1600-2690	C=O stretching
Amide II	1480-1575	CN stretching, NH bending
Amide III	1229-1301	CN stretching, NH bending
Amide IV	625-767	OCN bending
Amide V	640-800	Out-of-plane NH bending
Amide VI	537-606	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

Source: Kong and Yu (2007).

gelatin. The PCA value can show the points of the sample along with the FTIR wavelength variable that characterizes it in the graph. This shows that the Minitab can be used to differentiate both samples (Princess, 2013). Although the shape of the gelatin spectrum of cows and porks was very similar, the analysis through PCA clearly revealed the difference. PCA can differentiate both samples based on their absorption intensity in the FTIR test. The method can also be used as a variable input for regression and discriminant analysis.

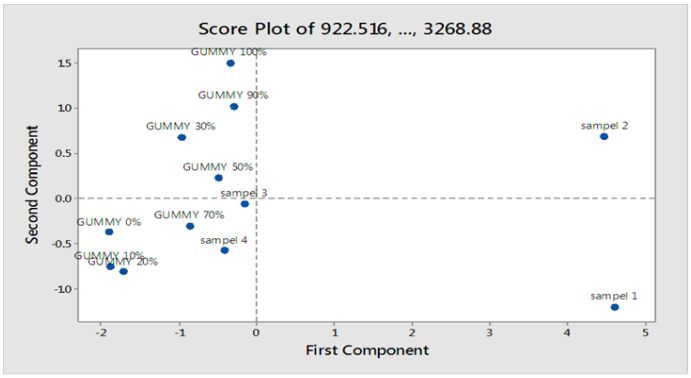

Figure 3. Score plot PCA pork gelatin, beef gelatin, mixture and sample. Quadrant 1: pork gelatin, quadrant 3: beef gelatin, quadrants 2 and 4: not pork and beef gelatin.

The PCA results are presented in the form of a plot score which can be seen in Figure 3. The image shows the existence of 4 quadrants of separators that can distinguish between pork and beef gelatin, as well as samples and mixtures. Samples 3 and 4 were in quadrant 3 with 0% gummy and mixed gummy of 10%, 20% and 70%. It was suspected that they have similar chemical and physical properties to cow gelatin. The 100% gummy pork gelatin and mixtures of 90%, 50%, and 30% were in quadrant 1. Samples 1 and 2 are in their own quadrants, 4 and 2, respectively. It was suspected that they were not pork or beef gelatin.

The results of the PCA analysis showed that each sample was grouped at different distances from each other. The gap indicates the degree of similarity, where the wider the gap, the less the similarity. In the score plot, pork and beef gelatin are in different quadrants, which shows that they can be distinguished in groups. The larger the presentation of pork gelatin, the higher the similarity of the gummy. This can be seen from the 90% gummy, which was adjacent. The 30% sample was located in the pork gelatin quadrant because the number of waves in the FTIR was similar to the number of pork gelatin waves. The 70% gummy found in the cow quadrant where the number of waves obtained is similar to the number of cow gelatin waves.

Multivariate calibration uses many variables to make predictions (Rohman and Che Man, 2008). The variables used in this study include absorbance on several FTIR wave numbers obtained from raw gelatin porks, cows and simulation samples. The multivariate calibration method was PLS (Partial Least Square), which uses predictor combinations rather than the original variant. Variables that show a high correlation with variable response were given an overburden because they are more effective for prediction (Rohman *et al.*, 2021). The leave-one-out validation was used to evaluate the results of linear regression calculations with PLS. The relationship between the actual values of pork gelatin concentration (Figure 4) and the concentration of bovine

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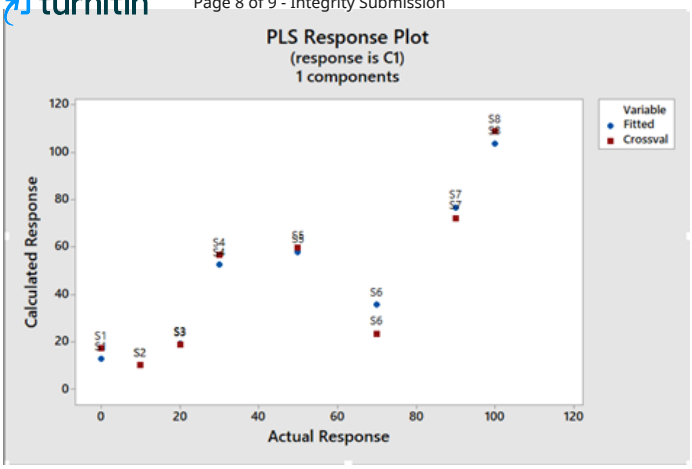

Figure 4. Linearity graphic of the concentration of pork gelatin to absorbance.

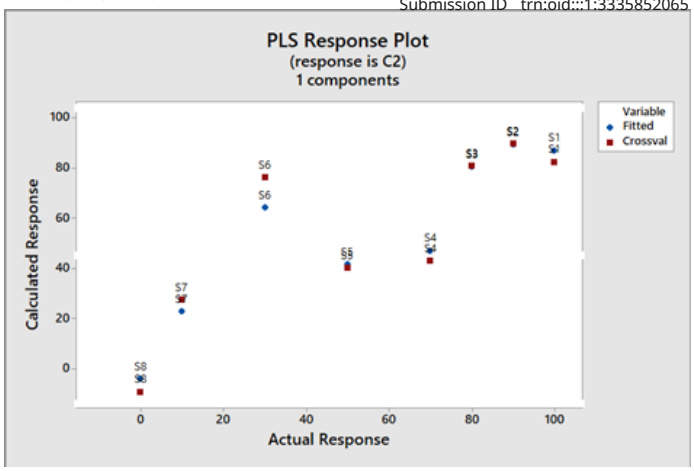

Figure 5. Linearity graphic of the concentration of bovine gelatin against absorbance.

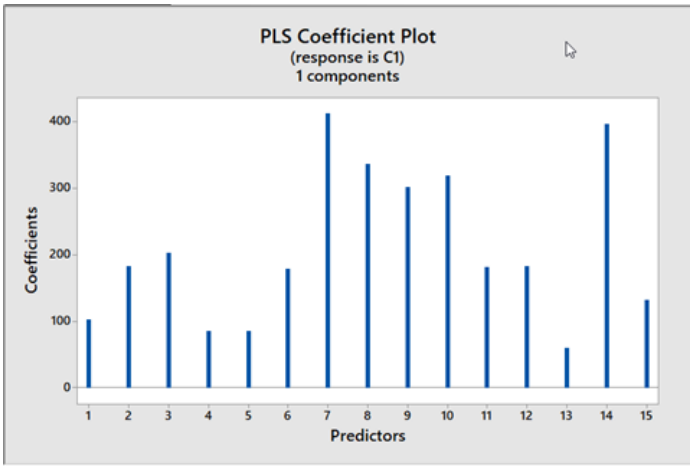

Figure 6. Absorbance plot of the coefficient on pork gelatin concentration.

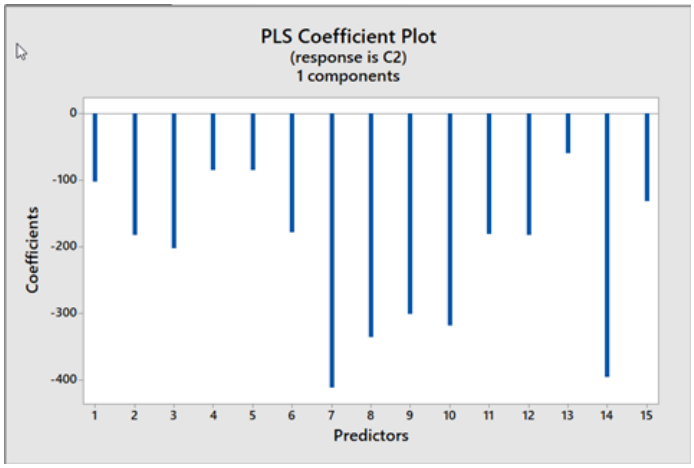

Figure 7. Absorbance plot of the coefficient on the concentration of bovine gelatin.

gelatin (Figure 5) with the predictable values of the PLS model showed that they are close to each other. The coefficient plot graph (Figures 6 and 7) reveals predictors 7 and 14, namely absorbance on wave numbers 1181.57 and 2940.87 cm^{-1} which have the highest weight in the regression equation either for pork or beef sample.

4. Conclusion

Based on the results, the FTIR spectrum in pork and cow gelatin was very similar, but both samples can be distinguished by combining FTIR analysis with PCA and PLS. The results of the score plot showed that the gelatin of porks and cows was found in different quadrants. Samples 3 and 4 were in quadrant 3, which shows that they have similarities with bovine gelatin. Meanwhile, 1 and 2 were in their quadrants indicating that they were not included in pork or cow gelatin. The four samples obtained from the new market did not contain pork gelatin.

Conflict of interest

The authors declare no conflict of interest.

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