

## Analysis of Gelatin on Soft Candy using a Combination of Fourier Transform Infrared Spectroscopy (FTIR) with Chemometrics for Halal Authentication

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**Abstract:** The main ingredients of soft candies are gelatin made from the skin/bones of cows and pigs, i.e. bovine and porcine gelatin. This research aims to analyze the present bovine and porcine gelatin in soft candy using a fast and low-cost method. The method used is a combination of FTIR and chemometrics. The reference candy samples were made with formulation porcine gelatin concentrations of 15, 30, 45, 60, 75, 90 and 100%. As a control, we used a bovine gelatin reference candy. All the candy samples were measured with FTIR in the reflection mode in the wavenumber range from 4000 to 500  $\text{cm}^{-1}$ . Data analysis was carried out using the chemometric method with the Minitab 18 application. PLS calibration results in  $y = 0,99999x + 0,000396$  indicate a good correlation. The value of  $R^2 = 0,99999$  and the RMSEC of 0.03%. Internal validation with  $R^2 = 0,9999$  and RMSECV = 3.69% and external validation with  $R^2 = 0,9994$  with RMSEP = 1.28%. The PCA results show different quadrant classifications of bovine and porcine gelatin. Also, there are similarities between the market candy quadrant, bovine gelatin, and porcine gelatin. The fast method and low cost involved simple sample preparation, which were developed to detect and classify the presence of bovine and porcine gelatin in soft candy using FTIR and chemometrics.

**Keywords:** chemometric, FTIR, gelatin, soft candy

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## 1. Introduction

Candy is a popular sweet that children and parents alike enjoy. Various types of candy are available: hard candy, soft candy, chocolate candy, nougat, marshmallows, and so on. Soft candy is one of the most popular because it has taste variations and is often made in attractive shapes (Altınok et al., 2020; Barak et al., 2020; Gunes et al., 2022; Kim et al., 2019; Salamah et al., 2022). In addition, this candy has an elasticity that increases its popularity. Soft candy is made with gel-forming agents such as gelatin, carrageenan, or agar (Gómez-Guillén et al., 2011; Man et al., 2011; Pirsá & Hafezi, 2023; Suprayatmi et al., 2017; Tarahi et al., 2023; Wulandari et al., 2023).

Several studies relevant to this study, among others, according to Zilhadia et al. (2018), show the Fourier Transform Infrared Spectroscopy (FTIR) and principal component analysis (PCA) combination method could distinguish bovine and porcine gelatin extracted from gummy vitamin C. In trials of samples taken from the market (commercial), it was suspected that commercial vitamin C gummies contained gelatin sourced from cows. The similarities with Zilhadia's research were the analysis using the FTIR method and PCA data analysis. The difference with Zilhadia's research was the use of samples; in his research, he used gummy vitamin C, while this study used imported market candy from Korea and reference candy to validate the method. In addition to the research by Rahmawati et al. (2015), in their research, in the area  $3600\text{--}3200\text{ cm}^{-1}$ , there was O–H absorption, and in the absorption area  $1600\text{--}1200\text{ cm}^{-1}$ , there were N–H groups, indicating the presence of protein and peptide bonds. The results of this study indicated that FTIR and chemometric methods can be applied to detect porcine gelatin in soft candy samples.

According to the Indonesian Trade Promotion Center (2014), from 2012 to 2014, consumption of sweets increased by 5.34%, influenced by changing trends and exports for the sweet snack and candy industry. One of the many soft candies the public favors comes from South Korea. This increase was due to the Korean Wave in Indonesia. So, Korean products that enter Indonesia, one of which is soft candy, are popular. However, using gelatin in soft candies often worries the Muslim community because it often comes from bovine and porcine sources, specifically from bones or skin (Forooghi et al., 2023; Salamah et al., 2022; Samatra et al., 2022; Uddin et al., 2021; Wulandari et al., 2014). So, this study required a method to detect the function, compare candy containing bovine and porcine gelatin, and prove that combination chemometrics can be used in quantitative analysis and the classification of gelatin. One of the vibrational spectroscopies is FTIR, which relies on interactions between functional groups in the studied samples and electromagnetic radiation to produce vibrational energy levels (Alkhuder, 2022; Bunaciu et al., 2015; Fadlemoula et al., 2022; Fockaert et al., 2020; Rohman et al., 2020; Song et al., 2020). As quick, non-destructive and trustworthy procedures for authentication analysis in the food sector, FTIR spectroscopy has arisen, particularly when paired with attenuated total reflectance (ATR) and chemometric software (Cárdenas-Escudero et al., 2023; Melucci et al., 2019; Mendes & Duarte, 2021; Palumbo et al., 2022; Rodriguez-Saona & Allendorf, 2011). Based on FTIR spectral features, this approach can distinguish between real and contaminated food qualitatively and quantitatively (Fajriati et al., 2021; Joshi et al., 2023; Li et al., 2021; Luan et al., 2023; Sousa et al., 2018; Yan et al., 2023). So, this study used FTIR combined with chemometric.

The aim of this research was to predict whether the gelatin raw materials used in market soft candy products are haram or halal. Research on this theme was previously reported by Rahmawati et al. (2015) in her thesis manuscript with samples of soft candy brands from Indonesia. The novelty of this research was the different sample matrix because the market products used were soft candy produced in South Korea. Besides that, this research also used validation data, which is different from research that has been reported previously.

## 2. Materials and Methods

### 2.1. Materials and Instrument

The materials used in this research were market samples in the form of randomly obtained soft candy products produced in South Korea and marketed in Indonesia. The samples chosen did not have a halal label. In this research, a reference sample was also used, which was soft candy made by oneself with a composition as similar as possible to the market sample matrix, namely from sucrose, tartrazine, citric acid, porcine and bovine gelatin in certain proportions. The instrument used in this research was an FTIR spectrophotometer (One Perken Elmer USA spectrum) and the analysis was carried out with the Minitab 18 application chemometrics.

## 2.2. Methods

### 2.2.1. Reference Candy Making

This reference candy was intended to be used during calibration and validation. Pure porcine and bovine gelatin were diluted in distilled water and mixed in a water bath at 60°C. 500 mg of sucrose was diluted in 5 mL distilled water, as much as 1 mL citric acid and wet gelatin were added to the sucrose solution. Then, as shown in Table 1, tartrazine was remixed until homogeneous. After the solution became homogeneous, it was poured into the mold and left for 1 hour until it solidified into candy (Schrieber & Gareis, 2007). Tartrazine gave the candy an orange color. The citric acid provided an orange taste and is widely used in candies on the market. Citric acid also prevents sugar crystallization. Eight reference candies were produced: 100% bovine; 100% porcine; 90% porcine; 75% porcine; 60% porcine; 45% porcine; 30% porcine; and 15% porcine.

Table 1. Reference Candy Formulation

Concentration	Bovine gelatin (mg)	Porcine gelatin (mg)	Sucrose (mg)	Citric acid (mL)	Tartrazine (drops)
Porcine gelatin 15%	75	425	3,100	1	1
Porcine gelatin 30%	150	350	3,100	1	1
Porcine gelatin 45%	225	275	3,100	1	1
Porcine gelatin 60%	300	200	3,100	1	1
Porcine gelatin 75%	375	125	3,100	1	1
Porcine gelatin 90%	450	50	3,100	1	1
Porcine gelatin 100%	-	500	3,100	1	1
Bovine gelatin 100%	500	-	3,100	1	1

### 2.2.2. Isolation of Gelatin from Reference Candy and Market Candy from South Korea (Samples X, Y and Z)

The candy was cut into small pieces weighing 5 grams then dissolved using 5 mL of distilled water at 60°C. After the candy dissolved, 3 mL of the solution was taken and given an additional 12 mL of acetone at -20°C then incubated for 24 hours. The precipitate formed was removed and the supernatant was centrifuged for 25 minutes at 6000 rpm. The precipitate was then rinsed three times using 3 mL of acetone at -20°C. After that, the precipitate was weighed and diluted in a 1:1 ratio with distilled water at 60°C (Azira et al., 2012; Salamah et al., 2023).

### 2.2.3. Analysis of Gelatin in Candy Reference with FTIR

The precipitate that had undergone dissolution was included in the ATR, which measured 68 mm × 8 mm × 3 mm. Sample scanning used FTIR spectroscopy with 4000 to 500 cm<sup>-1</sup> wavenumbers with a resolution level of 4 nm (Hashim et al., 2010; Rohman & Salamah, 2018).

### 2.2.4. Data Analysis

PCA then analyzed the results with FTIR by inputting the absorption intensity data of porcine and bovine gelatin in the reference candy in the wavenumber of the special gelatin section into the Minitab version 18 software. Before analyzing samples using PCA, PLS (partial least squares) analysis was carried out first to calibrate and validate the method. Then, the validity of the calibration model was carried out based on the parameters. It was processed with PCA, which outputs a score plot. The score plot consisted of the number of PC1 (principal component 1) and PC2 (principal component 2) (Zilhadia et al., 2018).

## 3. Results and Discussion

Candy was made from two gelatin types: bovine and porcine. Organoleptically, as shown in Table 2, bovine gelatin is yellowish and has a distinctive odor, while porcine gelatin is bone white and has a distinctive odor that is more concentrated than bovine gelatin. This flavor is more pronounced when the gelatin is moistened.

Table 2. Comparison of Porcine and Bovine Gelatin

Porcine gelatin	Bovine gelatin
Bone white color	Yellowish color
Strong distinctive smell	Distinctive smell

In the following process, after the porcine and bovine gelatin were extracted, the analysis used FTIR, which produces almost the same spectrum pattern. The difference was from the low or high absorption in each spectrum.

Based on Figure 1, the results of the FTIR spectra of pure porcine gelatin and pure bovine gelatin were at frequencies of 4000–500  $\text{cm}^{-1}$ . Generally, gelatin comes from proteins whose structure has a carbon-hydrogen (C–H) functional group, a carbonyl group (C=O), a hydroxyl group (OH) and an amine group (NH). It can be seen that the frequency of 3435  $\text{cm}^{-1}$  in bovine gelatin and 3437  $\text{cm}^{-1}$  in porcine gelatin indicated the presence of hydroxyl stretching vibrations. Stretching OH had a band range at a frequency of 3100  $\text{cm}^{-1}$ –3500  $\text{cm}^{-1}$ . O–H groups were suspected to originate from water compounds when the candy was dissolved in distilled water.

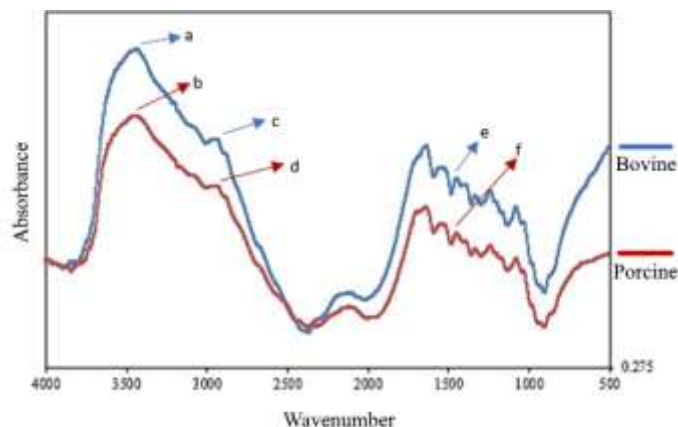


Figure 1. FTIR Spectrum of Pure Bovine Gelatin and Pure Porcine Gelatin with Descriptions a, b, c, d, e, and f in Table 3.

In addition, there were differences in the spectral intensities of bovine and porcine gelatin because the moisture content of porcine gelatin is higher than the humidity level of bovine gelatin, which results in the water content (O–H) in porcine gelatin being higher than that of bovine gelatin (Cebi et al., 2016). In the frequency number of 2931  $\text{cm}^{-1}$  in bovine and porcine gelatin, there was C–H stretching, which ranged from 2800 to 3000  $\text{cm}^{-1}$ . Furthermore, the carbonyl group (C=O) was 1680–1630  $\text{cm}^{-1}$ . In the figure, stretching C=O was found at a frequency of 1633  $\text{cm}^{-1}$ . The NH (amide) group did not have a clear peak in Figure 1 because it was covered by the peak of OH, which has a wide shape with strong absorbance.

Table 3. Estimation of Functional Groups in Pure Bovine and Porcine Gelatin

Name	Wavenumber of bovine and porcine gelatin ( $\text{cm}^{-1}$ )	Functional group	Vibration model
<i>a</i>	3435	O–H	Stretching
<i>b</i>	3437		
<i>c</i> and <i>d</i>	2931	C–H	Stretching
<i>e</i> and <i>f</i>	1633	C=O	Stretching

Optimization of wavenumbers for PLS calibration was carried out in the wavenumber range of 4000–500  $\text{cm}^{-1}$ . Furthermore, the optimized wavenumbers were obtained based on Table 4; the selected wavenumbers were 1660–1570  $\text{cm}^{-1}$  with a coefficient ( $R^2$ ) of 0.9999 and an RMSEC number of 0.03%. The correlation coefficient was a statistical measurement between two variables (Rohman et al., 2020). A correlation coefficient value that is close to 1 has perfect correlation strength. The predicted data variable (predicted value) approached the actual data variable (actual value). The parameters used to evaluate the model at the calibration stage were the RMSEC (Root Mean Square Error of Calibration) value. Root mean square error is the magnitude of the error rate in prediction results. Generally, there is no ideal value standard for RMSEC. The smaller the RMSEC value, or the closer it is to 0, the more accurate the prediction results (Rohman et al., 2020). The results of calculating the smallest RMSEC value obtained were at wave numbers 1600–1570  $\text{cm}^{-1}$ , namely 0.03. So, at this wave number, the predicted data results had the most accurate value.

Table 4. Wavenumber Optimization with PLS

Wavenumber ( $\text{cm}^{-1}$ )	$R^2$	Regression equation	RMSEC (%)
1001–924	0.9163	$y = 0.91639x + 5.382$	8.75
1170–1027	0.9639	$y = 0.9639x + 2.324$	7.45
1334–1236	0.9639	$y = 0.9639x + 5.382$	1.82
1521–1400	0.9999	$y = 0.9999x + 0.00127$	0.11
1660–1570	0.9999	$y = 0.9999x + 0.0004$	0.03

Internal validation was also done with the RMSECV (Root Mean Square Error of Cross Validation) parameter and the  $R^2$  value.

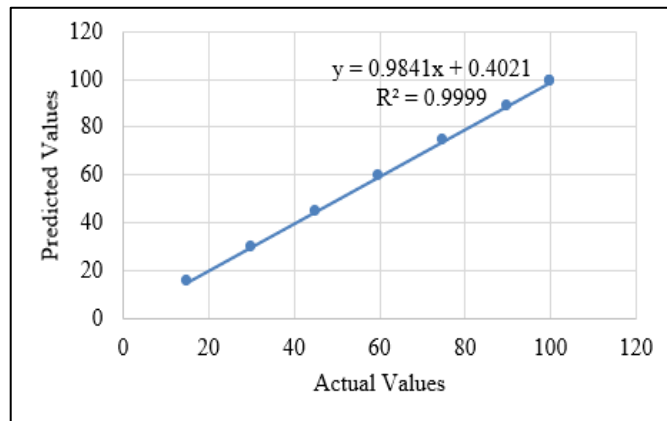


Figure 2. The Correlation Curve Between Actual and Predicted Values with the Internal Validation Model at Wavenumbers 1660–1570  $\text{cm}^{-1}$ .

Figure 2 shows that  $R^2$  had a value of 0.99999 with a linear regression equation  $y = 0.9841x + 0.4021$  and an RMSECV value of 3.69%. After that, as shown in Figure 3, external validation was carried out with the RMSEP and  $R^2$  value parameters. The equation  $y = 0.9996x + 1.0682$  was obtained with  $R^2 = 0.9994$  with an RMSEP value of 1.28%.

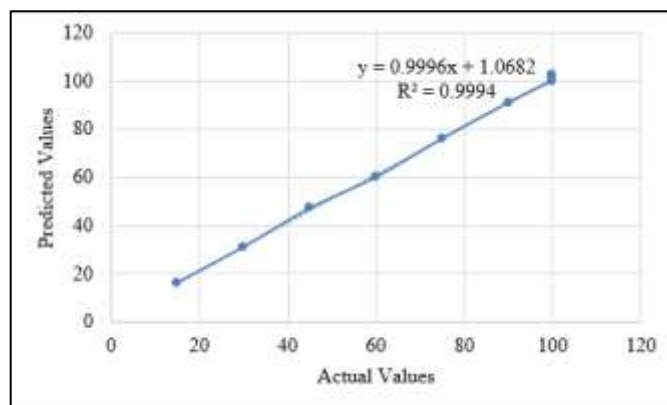


Figure 3. The Correlation Curve Between Actual and Predicted Values with the External Validation Model at Wavenumbers 1660–1570  $\text{cm}^{-1}$ .

Furthermore, the analysis results were processed using PCA chemometrics to be projected onto the plot and generated through the type of gelatin based on the variable profile of the gelatin component.

The results of the analysis showed separation between bovine gelatin, porcine gelatin and the three samples. Figure 4 shows the results of the PCA analysis in a score plot. In the figure, four dividing quadrants provide a difference between bovine and porcine gelatin, where only three are occupied by the plot.

The results of the PCA analysis showed the three samples were in separate quadrants at different distances. Sample X was in quadrant IV. Sample Y was in quadrant III, with the porcine gelatin standard. Moreover, the distance between the two was not far. Sample Z was in quadrant I and the same as the bovine gelatin standard, where the farther the oil distance, the greater the difference in sample characteristics (Miller & Miller, 2005); with porcine gelatin, the samples studied can be grouped based on their origin. The results show the combination chemometric method can be used to classify types of gelatin based on their origin.

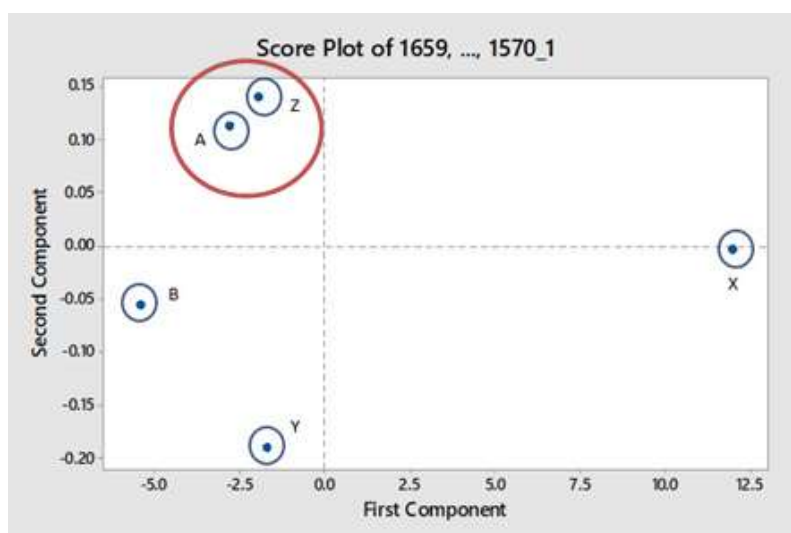


Figure 4. PCA Results Between 100% Bovine and Porcine Gelatin Reference Candy and Reference Candy, (A) Bovine Gelatin, (B) Porcine Gelatin, and (X, Y, Z) Gelatin from Market Candy.

The combination method of FTIR and chemometrics was reliable to identify porcine gelatin from soft candy. The method could detect functional groups in porcine and bovine gelatin in market soft candy and reference candy. From this study, it was predicted that the functional groups in pure porcine gelatin and pure bovine gelatin are C–H, O–H, C=O, and the possible presence of N–H groups. The results of this research were in accordance with research conducted by Zilhadia et al. (2018), showing that the functional groups read in the protein isolate spectra were free O–H, C–H, N–H, C=O, N–H, and C–N stretch. Also, distinguishing candies that contain porcine and bovine gelatin needs further research and data analysis. A similar spectrum for porcine and bovine gelatin makes combining FTIR with chemometrics necessary for better results. So, when combined with chemometrics, it was found there was a diverging quadrant classification between pure porcine gelatin and pure bovine gelatin. Also, there was a classification of sweets from South Korea in quadrants similar to pure porcine gelatin and pure bovine gelatin. However, some South Korean soft candies were not in a similar quadrant either.

#### 4. Conclusion

This research obtained an analytical method to differentiate soft candy from bovine or porcine gelatin, namely FTIR combined with chemometrics using PLS and PCA parameters. So, this research can be used to authenticate the halalness of candy products. The results of FTIR analysis of the extraction results of Korean market candy produced the functional groups that makeup gelatin protein, namely aliphatic C–H, O–H, C=O, and N–H. The results of FTIR analysis combined with PLS showed that the most optimum wave number produced a calibration curve of 1660–1570  $\text{cm}^{-1}$  with an  $R^2$  value of 0.9999 and an RMSEC number of 0.03%. The PCA results obtained by sample code Z were sourced from the isolation results of bovine gelatin reference candy. The sample code Y is thought to originate from porcine gelatin raw material because it is in the quadrant with the isolation results of porcine gelatin reference candy. Meanwhile, the research results could not identify the source of the gelatin used in the sample code X. But the method validation process with PLS should be replicated several times to ensure the method is valid.

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