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Determination of gelatin from marshmallows using a combination of fourier transform infrared (ATR-FTIR) spectroscopic and chemometrics for halal authentication

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ABSTRACT

Marshmallow is a foam that contains aerated sugar, which is stabilized with gelatin or egg albumin. In this research, a Fourier Transform Infra-Red (ATR-FTIR) spectroscopy combined with the Chemometrics methods is developed to distinguish the presence of porcine gelatin in marshmallows, which can then be used to identify halal products. This method provides fast and rapid testing of halal products. Marshmallows were made with varying concentrations of bovine and porcine gelatine as the reference. Commercial marshmallows were collected in the online marketplace by purposive sampling. Isolated gelatin was analyzed using the ATR-FTIR spectrophotometer, and data analysis was continued using PCA (Principal Component Analysis). The results showed that bovine gelatin absorbed at a wavenumber of 1638 cm^{-1}, while porcine gelatin produced a sharp absorption at 1697 and 1654 cm^{-1}. The results of PCA analysis show that 100% of bovine gelatin marshmallows (S100) have areas different from marshmallows containing a mixture of porcine gelatin. The PCA results of four samples (A1, A2, A3, and A4) show they are in the same area as 100% bovine gelatin marshmallows (S100). This shows that it is suspected that the four marshmallow samples tested did not contain porcine gelatin. The multivariate regression curve showed that the pattern of linear absorbance changes along with porcine gelatin concentrations with the highest coefficient equation is from wavenumber 1093.97 cm^{-1}.

Keywords: ATR-FTIR, Bovine gelatin, Chemometric, Marshmallow, Porcine gelatin

INTRODUCTION

Marshmallows are a foam that contains aerated sugar and is stabilized with gelatin and egg albumen. Marshmallows are a snack like candy, textured as soft, light, foam chewy in various shapes, aromas, flavors, and colors, so they are included in the confectionery product. Marshmallows are included in soft candy products, not jelly. One of the components that make up marshmallows is gelatin. Gelatin is a mixture of polypeptides. Its amphiphilic nature gives it foam-stabilizing properties. A typical gelatin polypeptide contains the amino acids alanine, glycine, proline, arginine, glutamic acid, and hydroxyproline. It is prepared from collagen isolated from animal bones and fish skins with a dilute acid. Gelatin is the most common protein biopolymer popularly obtained from partial hydrolysis of tissue animal collagen. Gelatin has unique properties, so its use is widespread in pharmaceuticals, food, and cosmetics (Zilhadia et al., 2018). Regarding its source, gelatin can come from mammals (cow skin, cow bones, pork skin) or fish (Rather et al., 2022). Amino acids composition of gelatine are different

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according to the resources. Asp, Gly, Arg, Met, Ser, His, and Ile were dominant in fish gelatine. Tyr, Phe, Val and Pro were dominant in porcine gelatine. Hyp was dominant in bovine gelatine (Sani et al., 2021). According to some religious dietary laws, eating gelatin prepared from animals is unacceptable. The pharmaceutical industry uses fish gelatin to make pill capsules, but it is scarcely used in food products because it is expensive (Furtado et al., 2022; Reza & Annissa, 2023).

Several methods can be used to differentiate bovine gelatin from porcine gelatin. Some analytical methods are used for screening PG and BG using physicochemical properties, including chemical precipitation, functional groups (FTIR spectroscopy), amino acid composition (liquid chromatography), detection and quantification of DNA (real-time polymerase chain reaction/RT-PCR), molecular weight distribution (electrophoresis), and protein (Enzyme-linked immunosorbent assay, ELISA). These methods are confirmed by identifying peptide markers specific for PG and BG using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and DNA-based method using polymerase chain reaction (Rohman et al., 2020).

In this research, a Fourier Transform Infra-Red (FTIR) spectroscopy method is developed to distinguish the presence of porcine gelatin content in marshmallows, which can then be used to identify halal. FTIR spectroscopy was chosen because it is a fast and non-destructive analytical technique, sensitive, and requires simple sample preparation, as well as the use of small amounts of chemical reagents and solvents (Rohman & Che Man, 2012; Zilhadia et al., 2018).

Pig-based derivatives in processed foods such as lard, gelatine, and pork meat can be detected using ATR-FTIR spectroscopy. This analysis can provide the best predictive and descriptive modeling by selecting and combining the optimal frequency region with isometric techniques (Siska et al., 2023). Cebi et al. used HCA and three-dimensional PCA chemometric techniques in two wave ranges, namely at 1722 – 1487 cm^{-1} and 1313 – 1124 cm^{-1} (Cebi et al., 2016). Hassan et al. have succeeded in distinguishing between fish, beef, and porcine gelatin using FTIR combined with chemometrics fuzzy autocatalytic set (c-FACS) using three dominant wavenumbers at 1470 – 1475 cm^{-1}, 1444 – 1450 cm^{-1} and 1496 – 1500 cm^{-1} respectively, which represent their unique signatures (Hassan et al., 2021). Differentiated cow and porcine gelatines from vitamin C-containing gummy products using a combination method of ATR-FTIR and PCA (Zilhadia et al., 2018).

RESEARCH METHOD

Materials

The samples used in this research were purchased randomly in the Jakarta, Indonesia market with 4 brands (coded A1, A2, A3, A4). Of these samples, 2 samples have a halal logo, and 2 samples do not have a halal logo. Other ingredients used were bovine gelatin (Sigma Aldrich), porcine gelatin (Sigma Aldrich), sucrose, glucose syrup, acetone (Merck), and aqua pro injection. The instruments used in this research were FTIR, centrifuge, refrigerator with a temperature of -20ºC, fume cupboard, and glass equipment, and data analysis was carried out using Minitab 21 software.

Methods

1. Reference Marshmallow Making

Table 1 shows that reference marshmallows containing pure porcine and bovine gelatine were diluted in 20 mL distilled water and mixed in a water bath at 60°C. 50 g of sucrose and 50 g of glucose syrup were diluted in 30 mL of distilled water at 105 – 115ºC. The gelatin solution is added to the sugar solution and stirred using a homogenizer until a fluffy mass is formed. The mass was molded and left at room temperature for 3 hours (Santoso et al., 2019; Sarofa et al., 2019).

2. Extraction of Gelatin from Reference and Market Marshmallow

The marshmallows were cut into small pieces weighing 2.5 grams and then dissolved using 2.55 mL of distilled water at 60°C. After the marshmallow had dissolved, 1.5 mL of the solution was taken, and an additional 6 mL of acetone was given at −20°C, then incubated for 24 hours. The precipitate formed was removed, and the supernatant was centrifuged for 20 minutes at 8000 rpm. The precipitate was then rinsed thrice 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 (Salamah et al., 2023).

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3. Analysis of Gelatin in Marshmallows Reference with FTIR

Gelatin (0.5 g) was diluted in heat at 60ºC, and the absorbance was read using FTIR at a wavelength of 4000 – 650 cm^{-1}.

Table 1. Reference Marshmallow Formulation.

Code	Bovine gelatine (g)	Porcine gelatine (g)	Sucrose (g)	Glucose Syrup (g)
S100	37.50	0	50	50
S80	30.00	7.50	50	50
S60	22.50	15.00	50	50
S50	18.75	18.75	50	50
S40	15.00	22.50	50	50
S20	7.50	30.00	50	50
S0	0	37.50	50	50

Data Analysis

PCA analysis was carried out by entering the absorption intensity data for each sample of bovine gelatin, porcine gelatin, standard series, and marshmallow at certain area wavenumbers of gelatin, and PLS analysis was carried out only from the standard series data using Minitab version 21 software.

RESULT AND DISCUSSION

Marshmallow is a sponge-shaped candy that mixes glucose and sucrose syrup to produce foam and gelatin (Hutabarat et al., 2024). Marshmallows are made with varying contents of bovine gelatin and porcine gelatin. Both marshmallow products produce the same organoleptic properties: white with a fluffy shape. We chose the marshmallow formulation, which consists of two sweeteners: sugar and corn syrup. The difference in the ratio of sucrose to corn syrup formulation of marshmallows gave firm or fluff texture differences (Boerner, 2021).

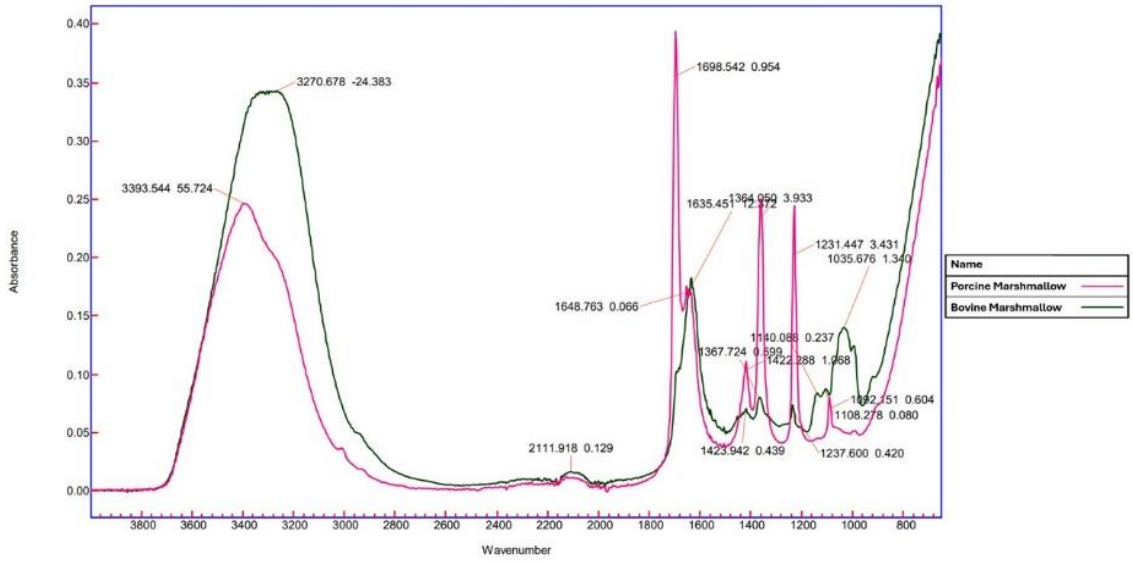

Figure 1. Overlay FTIR spectrum of porcine and bovine gelatin extracted from marshmallow.

The results of the FTIR spectrum analysis of beef and porcine gelatin show almost the same absorption patterns. The four spectral regions of bovine and porcine gelatin are 3600 – 2900 cm^{-1} (Amide A), 1656 – 1644 cm^{-1} (Amide I), 1560 – 1335 cm^{-1} (Amide II), and 1240 – 750 cm^{-1} (Amide III) (Hashim et al., 2010). As shown in Figure 1, the absorption results show that bovine gelatin and porcine gelatin have a spectrum of Amide A, Amide I, Amide II, and Amide III groups. The research results show that all samples have absorption at a wavelength of 3600 – 2900 cm^{-1}, which indicates the existence of N-H stretching bonds in the hydrogen bonds of the amide group. In bovine gelatin, this group is located at a wavenumber of 3278 cm^{-1}, while in porcine gelatin, this group is located at an

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absorption of 3390 cm^{-1}. Porcine gelatin exhibited a dominant band at Amide I (Jamalludin & Tukiran, 2018).

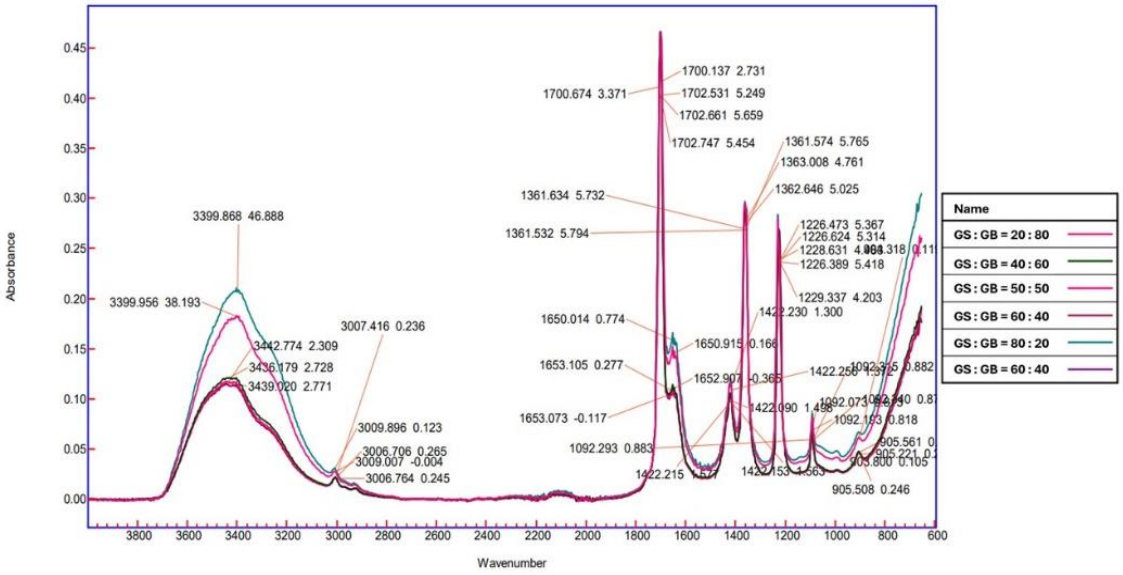

Figure 2. Overlay FTIR spectrum of marshmallow with variation bovine and porcine gelatine.

The presence of absorption at wave numbers 1656 – 1644 cm^{-1} indicates the presence of carbonyl C=O stretching bonds with contributions from NH and C-N stretching bonds, which are often referred to as the Amide I region. Based on Figure 2, bovine gelatin absorbed 1638 cm^{-1}, whereas Porcine gelatin produces sharp absorption at 1697 and 1654 cm^{-1} wavelengths. In the frequency range 1550 – 1520 cm^{-1}, absorption occurs, which shows that the Amide II group has an α helix structure (1550-1540 cm^{-1}) and a β sheet structure (1525 – 1520 cm^{-1}). The deformation of the N-H bond causes amide II vibration. Meanwhile, the frequency of 1500 – 1200 cm^{-1} represents CH_2 deformation. In this study, bovine gelatin absorbed at a wavelength of 1541 cm^{-1}, while porcine gelatin absorbed 1364 cm^{-1} (Hermanto et al., 2015).

Table 2. Wavelength of bovine and porcine gelatine from marshmallow sample.

Functional Group	Sample Wavelength	Intensity
Amide A (N-H bond)	3442.20 cm^{-1}	Medium
	3390.01 cm^{-1}	Medium
	3278.19 cm^{-1}	Medium
	3265.15 cm^{-1}	Medium
Amide I (C=O)	1654.94 cm^{-1}	Strong
	1638.16 cm^{-1}	Strong
	1636.30 cm^{-1}	Strong
Amide II (CH_2)	1541.25 cm^{-1}	Strong
	1421.98 cm^{-1}	Strong
	1364.21 cm^{-1}	Strong
	1362.34 cm^{-1}	Strong
Amide III	1231.89 cm^{-1}	Strong
	1093.97 cm^{-1}	Weak
	1023.15 cm^{-1}	Weak

Furthermore, the selected absorption results obtained from the FTIR tool (Table 2) were followed by analysis with PCA and PLS using Minitab version 21 software to see the grouping of each sample analyzed. The absorption results obtained from the FTIR tool were entered into Minitab version 21

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software to see sample grouping. The results shown in Figure 3 show that marshmallows containing 100% bovine gelatin have a different area than marshmallows containing 100% porcine gelatin.

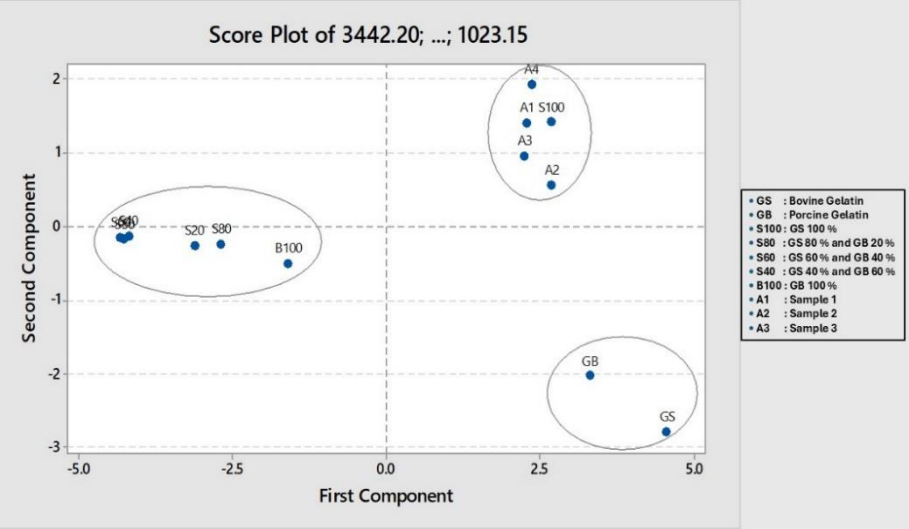

Figure 3. Analysis results using PCA on Minitab version 21 software.

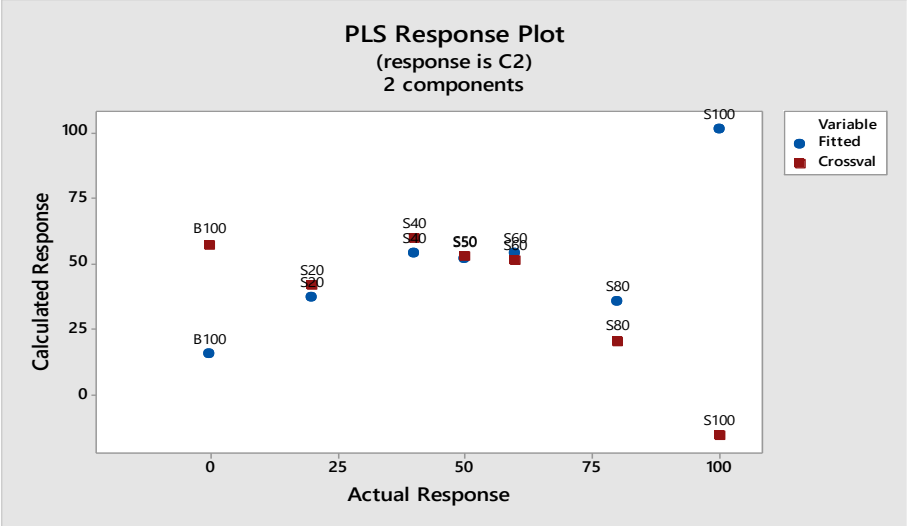

Figure 4. Analysis results using PLS on Minitab version 21 software.

The PCA score plot results in Figure 3 show that 100% of bovine gelatin marshmallows (S100) have a different area than marshmallows containing a mixture of porcine gelatin. For Bovine Gelatin (GS) and Porcine Gelatin (GB), the standard provides its area because the spectrum reading process does not go through an extraction process, giving a different spectrum compared to gelatin extracted from marshmallows.

All samples tested (A1, A2, A3, and A4) are in the same area as 100% bovine gelatin marshmallow (S100). This shows that it is suspected that the four marshmallow samples tested did not contain porcine gelatin and only contained bovine gelatin. The gelatin used comes from animal sources, such as halal, for consumption by Muslims. Likewise, porcine and bovine gelatin have different areas from pork and bovine gelatin extracted from marshmallows. This shows that the extraction process also influences the sample reading results.

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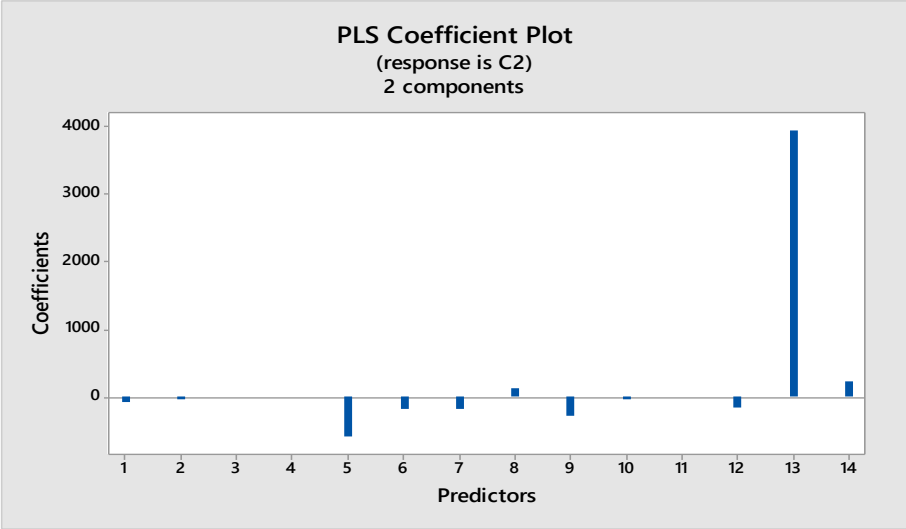

Figure 5. Absorbance plot of the coefficient on the concentration of bovine gelatin.

Multivariate calibration uses many variables to predict (Rohman & Man, 2008). The variables used in this study include absorbance on several FTIR wave numbers obtained from porcine gelatine, bovine gelatine, and a mixture of both gelatine in various concentrations in marshmallow form. 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., 2011). The leave-one-out validation was used to evaluate the results of linear regression calculations with PLS. The relationship between the actual values of porcine gelatin concentration (Figure 4) and the predictable values of the PLS model showed that they are close to each other except for marshmallow with 100% bovine gelatine. The coefficient plot graph (Figure 5) reveals predictor 13, namely absorbance on wavenumbers 1093.97 cm^{-1}, with the highest coefficient in the regression equation.

CONCLUSION

The difference between bovine gelatin and porcine gelatin in marshmallows is not directly visible via the FTIR spectrum. The FTIR method combined with PCA and PLS can classify bovine gelatin, porcine gelatin also, bovine gelatine, and porcine gelatin extracted from marshmallows. Commercial marshmallows are detected to contain gelatin sourced from cows.

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