

Identification of Gelatin source in Toothpaste products using Combination of Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy and Chemometrics

Anna P. Roswiem^{a,1,*}, Dewi N. Mustaqimah^b

^a Halal Research Center – Lembaga Penelitian Universitas YARSI, Jakarta, Indonesia

^b Faculty of Dentistry, Universitas YARSI, Jakarta, Indonesia

¹ annap_ros@yahoo.com Tel. +628 129 530 256

* corresponding author

ARTICLE INFO

Article history

Received February 6, 2020

Revised June 02, 2020

Accepted June 21, 2020

Keywords:

gelatin

toothpaste products

ATR-FTIR

chemometric

PCA and SIMCA

ABSTRACT

Toothpaste products traded within Indonesia are produced locally and internationally. Some of the toothpaste products may contain gelatin, a protein derived from bovine or porcine skin and bones. There are only few researchers studying gelatin in the toothpaste. Therefore, this study aimed to examine the presence of gelatin in toothpaste using the Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy method combined with chemometrics. The presence of gelatin in the sample was identified from the similarity of the infrared spectrum profile between the sample and the standard gelatin of cow and pig origins. The spectra data sets from ATR-FTIR were subject to Principal Component Analysis (PCA) and subsequent Soft Independent Modeling of Class Analogy (SIMCA) method to precisely classify the origin of the gelatin. The study showed that the ATR-FTIR method can be used to identify the presence of gelatin in toothpaste products. Gelatin was detected in two out of 42 samples. However, the combination of the ATR-FTIR technique with chemometry could not provide excellent illustration of the origin of gelatin in the toothpaste products. We presumed that the gelatin was only added in small amounts in these samples to serve as a stabilising or emulsifying agent.

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1. Introduction

Recently there has been a growing demand for halal products among Moslems. This demand includes not only foods and beverages, but also medicine, biological products, cosmetics and other consumer goods, such as shampoo and toothpaste. A study in Malaysia showed that dental material products which type of materials are: sponge, liquid, gel and paste may contain gelatin (Irfanita et al. 2017). Gelatin is a protein obtained from bone or skin of cows, pigs, or fish through hydrolysis. Gelatine that circulate and traded in the market can be produced by hydrolize protein from bone or skin of cow and pig (GMIA, 2012). Fish gelatin is obviously halal for Moslems and porcine gelatin is obviously haram. However, bovine gelatin could be halal or haram, depends on the implementation of Islamic methods during the slaughtering. Toothpaste products available in Indonesia may be sourced from other countries (imported) and within the country (produced locally). According to the article 4 of the Indonesian Law Number 33 of 2014 on Halal Product Assurance, products that enter, circulate, and traded in the territory of Indonesia must be certified

halal. The halal status of toothpaste products available in Indonesia is still doubtful, because there is no assurance from the manufacturer, which is usually reflected by a halal logo or halal certificate. Therefore, it is essential to trace the source of gelatin as one of questionable materials in order to ensure the halalness of a toothpaste product.

Various methods to identify the presence of gelatin in diverse products have been developed (Hafidz and Yaakob, 2011; Raraswati et al, 2014; Hermanto, 2015; Hashim et al, 2010; Demirhan et al, 2012; and Tarasa et al, 2005). One of them is using (ATR-FTIR) Spectroscopy. This method is applied to analyze gelatin in processed foods (Hashim, et al. 2010), capsule shells (Syafiqoh, 2014), and gelatin in tablet preparation of drug (Roswiem and Kesuma. 2018). The application of this method to authenticate the gelatin source in toothpaste products is still scarce. ATR-FTIR method can only identify the presence of gelatin in the samples. (Irfanita, et al. 2017). Combining this method with chemometrics allows researchers to investigate the source of gelatin (Rahmawati, et al. (2011; Hidayatullah (2014); for analysis of rat meat in meatballs beef (Guntarti, et al. 2017); for analysis of lard in crackers (Ernanto et al. 2016); and to investigate the source of animals hair (Rafi, et al. 2016). Therefore, we combined ATR-FTIR with chemometrics in this study to be able to authenticate gelatin source in toothpaste products.

2. Materials and methods

2.1. Samples

The samples of this study were local and imported toothpaste products, both in gel and paste forms.

2.2. Instruments

Nicolet™ iS™5 FTIR spectrometer with iD7 ATR accessory, OMNIC software (Thermo Scientific, USA), and incubator.

2.3. Procedures

2.3.1. Materials

Pure bovine and porcine gelatins were obtained from Sigma Aldrich (St. Louis, Mo USA). Commercial toothpastes were purchased from various retails, stores, markets and online shops in Jakarta, Indonesia.

2.3.2. Sample preparation

Standard gelatin was dissolved in deionized water and incubated at 50 °C for 10 minutes until clear solution was formed. The toothpaste samples were directly placed on the surface of ATR.

2.3.3. Preparation of homemade toothpaste

To ensure the presence of gelatin in a toothpaste, experimental tooth-paste samples containing bovine and porcine gelatin at concentrations of 5%, 10%, 15% and 20% were prepared. The paste was prepared by blending bovine or porcine powder, sodium bicarbonate and sodium chloride. The mixture was blended until it formed a paste-like texture (Irfanita et al, 2017).

2.3.4. Analysis of gelatin in samples with ATR-FTIR

Identification of gelatin in tooth-paste products was done qualitatively by comparing the infrared spectra data of toothpaste samples with the spectra data of pure bovine and porcine gelatin. All data were recorded within a range of 4000–400 cm⁻¹ with a 4 cm⁻¹ resolution and 32 scans (Rohman and Che Man, 2011).

2.3.5. Analysis of gelatin source

Several studies have reported FTIR spectroscopy in combination with a powerful chemometric technique, such as Principal Component Analysis (PCA) as a reliable analytical method to authenticate gelatin origins in certain products. PCA is a data projection method that is really helpful in classifying an object (Miller & Miller, 2005). After the PCA plot was generated, the next step was applying Soft Independent Modeling of Class Analogy (SIMCA) algorithm to distinguish between bovine gelatine and porcine gelatin in the toothpaste products (Branden and Hubert, 2005).

3. Results and discussion

3.1. Toothpaste Samples

The study involved 42 samples of toothpaste products manufactured in various countries. The majority of them were in the form of paste, the rest were gel (Table 1).

Table 1. The list of toothpaste samples and their countries of origin

No	Sample code	Toothpaste Type	Country of Origin	No	Sample code	Toothpaste Type	Country of Origin
1	P1	Paste	Indonesia	22	P22	Paste	Indonesia
2	P2	Paste	Indonesia	23	P23	Paste	Indonesia
3	P3	Paste	India	24	P24	Paste	Indonesia
4	P4	Paste	Singapore	25	P25	Gel	China
5	P5	Paste	Singapore	26	P26	Paste	Malaysia
6	P6	Paste	Uni Emirate Arab	27	P27	Pastel	Australia
7	P7	Paste	Indonesia	28	P28	Paste	Indonesia
8	P8	Paste	Uni Emirate Arab	29	P29	Paste	Indonesia
9	P9	Paste	Indonesia	30	P30	Paste	Thailand
10	P10	Paste	China	31	P31	Gel	China
11	P11	Paste	Thailand	32	P32	Paste	China
12	P12	Paste	Indonesia	33	P33	Paste	USA
13	P13	Paste	Indonesia	34	P34	Paste	Indonesia
14	P14	Gel	Indonesia	35	P35	Paste	Indonesia
15	P15	Gel	Spanyol	36	P36	Paste	Indonesia
16	P16	Gel	Indonesia	37	P37	Paste	Indonesia
17	P17	Gel	Indonesia	38	P38	Paste	Indonesia
18	P18	Paste	India	39	P39	Paste	Malaysia
19	P19	Paste	Indonesia	40	P40	Paste	Singapore
20	P20	Gel	Spanyol	41	P41	Paste	Thailand
21	P21	Paste	German	42	P42	Gel	Korea

3.2. Infrared Spectra of Pure Gelatin

Pure porcine and bovine gelatin were used as controls. Their infrared spectra were showed in Figure 1. According to our study, IR spectra of standard bovine and porcine gelatin, which served as standards, exhibited similar patterns. The spectra of both types of gelatin showed four same regions within a range of 3700–2400 cm⁻¹ (Amide A); 1700 – 1654 cm⁻¹ (Amide I); 1600 – 1500 cm⁻¹ (Amide II) and 1500–570 cm⁻¹ (Amide III) (Figure 1). This finding is slightly different with that of Irfanita et al (2017), which might be contributed by the difference in the resolution and the number of scans. Our study used 4 cm⁻¹ resolution and 32 scans, while Irfanita et al (2017) used 2 cm⁻¹ resolution and 16 scans.

The FTIR spectra also indicated that generally the bovine and porcine gelatin produced absorption peaks within nearly identical wavenumber, but when compared more thoroughly the peaks were relatively different. For example, the absorbance of porcine gelatin within Amide A range was comparatively higher than the absorbance of bovine gelatin. However, within the regions of Amide I and II (1700-1654 cm⁻¹ and 1600-1500 cm⁻¹) the absorption peaks of porcine gelatine were lower than those of bovine gelatin. Furthermore, within the range of Amide II there were two

absorption peaks and one absorption peak of amide group for porcine gelatin and bovine gelatin, respectively (Figure 1).

The absorbance within the range of 3290-3280 cm^{-1} is related to N-H stretching and intramolecular hydrogen bond of amino acids that comprise the gelatin. Parallel polarised absorption with N-H bonds showed hydrogen bond interaction within alpha helical structure of gelatin. Absorption peak may shift to a lower frequency when the strength of hydrogen bond rises (Hashim et al. 2010)

The Amide I band is generated by stretching vibration of $-\text{C}=\text{O}$ group (of peptide bond), with a little bit contribution of C-N bond stretching. Meanwhile, the Amide II band comes from a mixed vibration of N-H bending and C-N stretching in secondary amides (Vertogen and Theoparides, 2012). Therefore, according to Barth (2007), the bands within Amide I region are more widely used to determine a secondary structure of protein than the bands within Amide II region. Because there is only one amide group that contributes to the formation of secondary structure of protein. However, the bands within Amide III region can also be useful in determining protein secondary structure (Barth, 2007; Srivastara, et al. 2011).

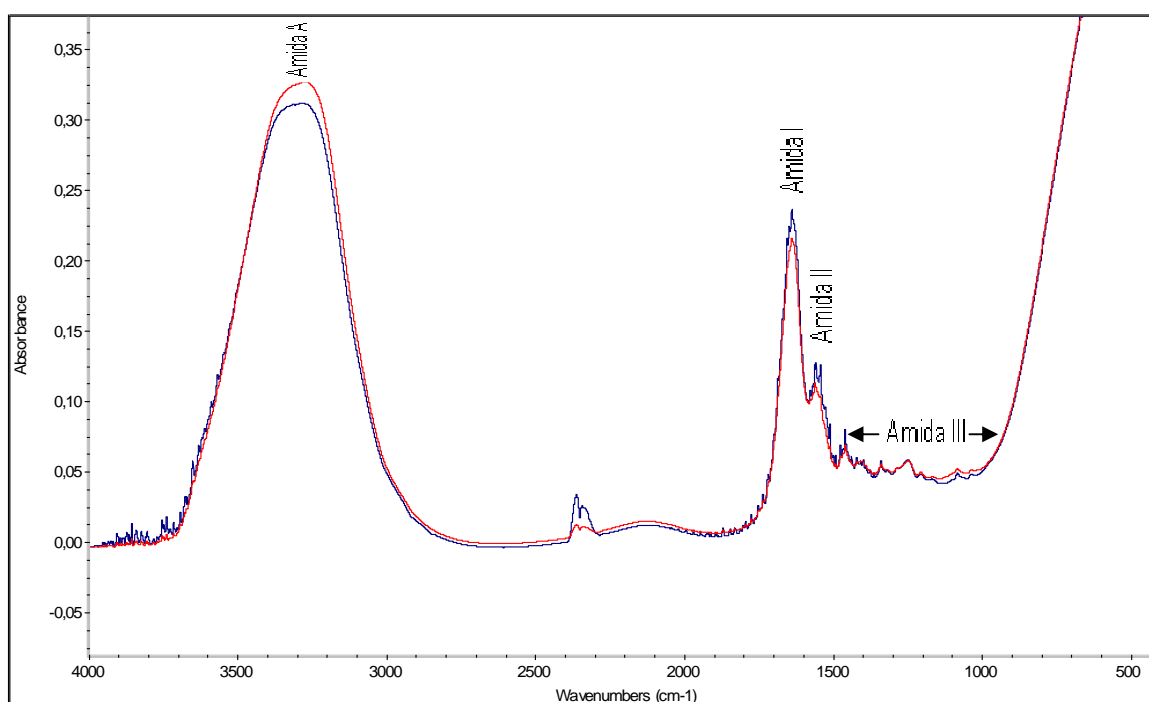


Fig 1. Infrared spectra of bovine and porcine gelatin

Note : Red = bovine gelatin at the concentration of 5%, Blue = porcine gelatin at the concentration of 5%

3.3. FTIR Spectra of homemade Toothpaste containing gelatin

Figure 2a and 2b showed IR spectra of homemade toothpaste containing gelatin at various concentration (5%, 10%, 15%, and 20%). It can be seen that the addition of gelatin into the toothpaste mixtures changed the intensity of absorption of Amide I and Amide II. The higher the gelatin concentration, the higher the intensity of absorption. Therefore, ATR-FTIR can serve as a reliable technique to analyse the presence of gelatin at various concentrations

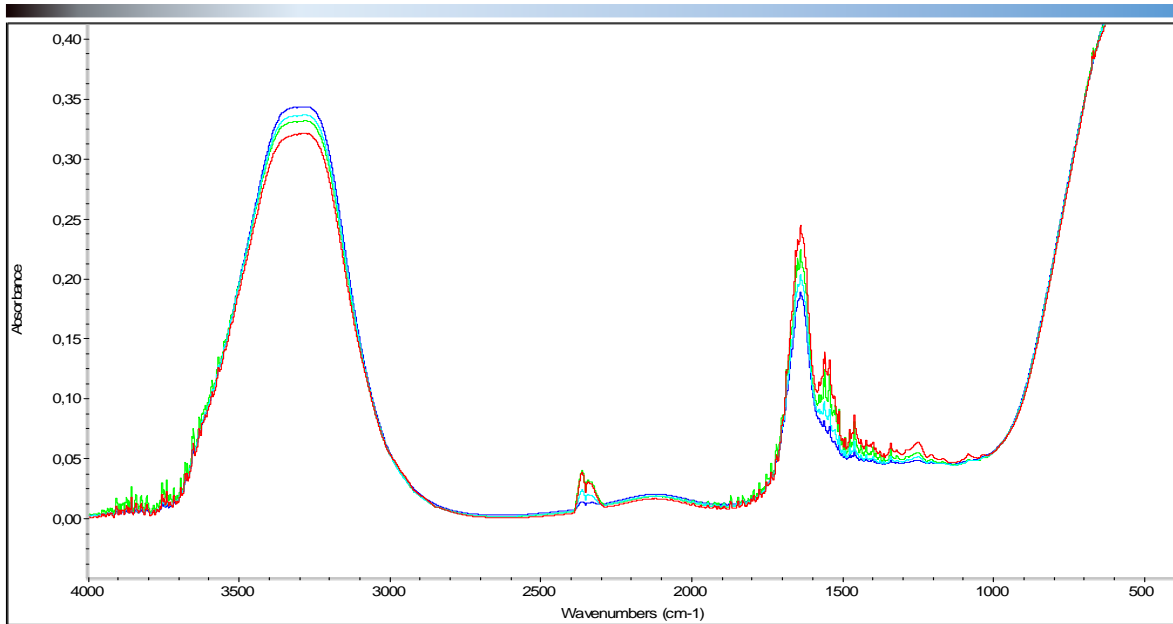


Fig 2a. FTIR spectra of homemade toothpaste containing added gelatin (5, 10, 15 and 20% concentration) within a range of 4000–570 cm^{-1}
Note : red : gelatine 20%; green : gelatin 15% light blue : gelatin 10%; dark blue : gelatin 5%

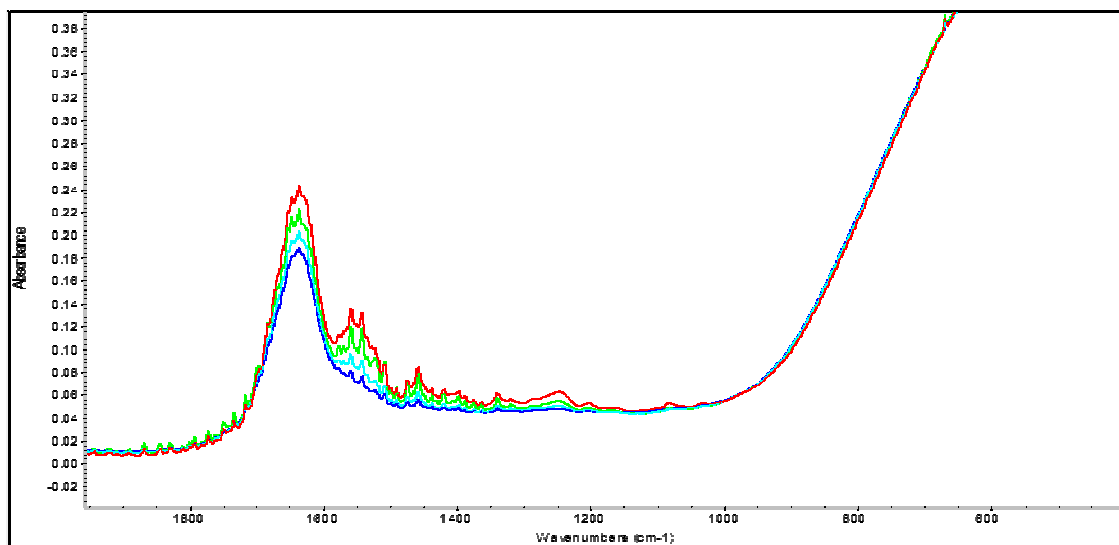


Fig 2b. FTIR spectra of homemade toothpaste containing added gelatin (5, 10, 15 and 20% concentration) within a range of 1800 – 570 cm^{-1}
Note : red : gelatine 20%; green : gelatin 15% light blue : gelatin 10%; dark blue : gelatin 5%

3.4. Detection of gelation in toothpaste samples using ATR-FTIR

The results of gelatin detection were depicted in Table 2 and Figure 2. Based on the similarity of spectra data between the samples and standard, also the library of OMNIC software, two out of 42 samples were detected with gelatin.

Table 2. Gelatin content in toothpaste samples

Sample	Texture	Gelatin Content *	Sample	Texture	Gelatin Content *
P1	Paste	-	P22	Paste	-
P2	Paste	-	P23	Gel	-
P3	Paste	-	P24	Gel	-
P4	Paste	-	P25	Gel	-
P5	Paste	-	P26	Gel	-
P6	Paste	-	P27	Gel	-
P7	Paste	-	P28	Paste	-
P8	Paste	-	P29	Paste	-
P9	Paste	+	P30	Paste	-
P10	Paste	-	P31	Gel	-
P11	Paste	-	P32	Paste	-
P12	Paste	-	P33	Paste	-
P13	Paste	-	P34	Paste	-
P14	Gel	-	P35	Paste	-
P15	Gel	-	P36	Paste	-
P16	Gel	-	P37	Paste	-
P17	Gel	-	P38	Paste	-
P18	Paste	-	P39	Paste	-
P19	Paste	-	P40	Paste	-
P20	Paste	-	P41	Paste	-
P21	Paste	+	P42	Gel	-

Note:* : + contains gelatin - does not contain gelatin

3.5. Sources Gelatin Contain in Toothpaste Samples

Figure 3 showed the infrared spectra of P9 and P21 compared with standard bovine and porcine gelatin within the range of 3700 – 3100 cm⁻¹ and 1700 – 1500 cm⁻¹. The spectra of both samples exhibited similar patterns with standard gelatin, which implied that these samples contain gelatin. To differentiate the source of gelatin, whether of bovine or porcine origin, IR spectra data were combined with chemometric techniques, using PCA followed by SIMCA. The results were shown on Figure 5 and 6.

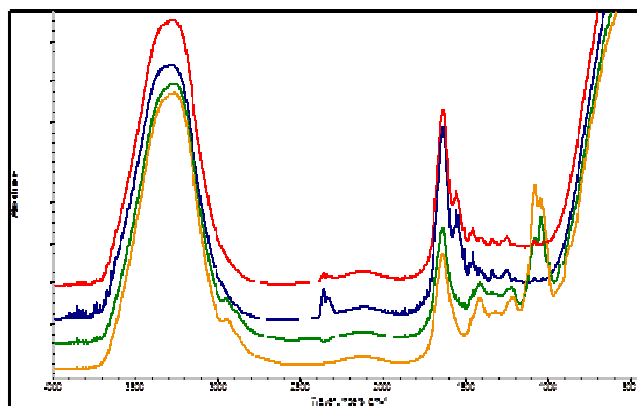


Fig 3. Spectra of toothpaste samples (P9 and P21) compared with standard gelatin of bovine and porcine origin. Note : Red = bovine gelatin 20%; blue = porcine gelatin 20%; green = sampel P9; yellow = sample P21

The IR spectra of P9 and P21 samples were slightly different with the spectra of standard gelatin within Amide III. Their absorbance was more intense then that of gelatin standard. However, the spectra images of P9 and P21 were similar to the spectra of samples used in Irfanita et al study (2017), namely BDM 05, BDM 14 dan BDM 16 (Figure 4).

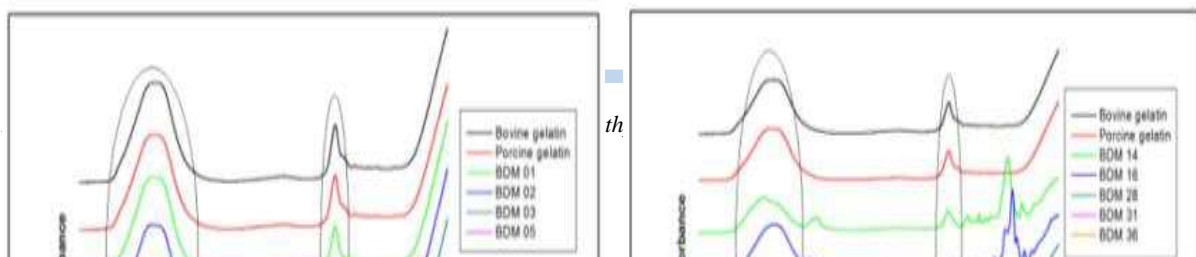


Fig 4. Infrared spectra of dental materials (Irfanita et al. 2017)

This may be due to the Si - O vibration of hydrate Silica which contained in P9 dan P21 samples (Table 3) within the range of 1200–800 cm⁻¹, with higher absorption peak.

Table 2. Ingredients of P9 and P21 toothpaste samples

Sample P9	Sample P21
Ingredients: Aqua, Disodium phosphate, Hydrated Silica, Sorbitol, Glycerine, Aroma, Steareth – 30, Amyloglucosidase, Sodium fluoride, Citric acid, Glucose oxidase, Potasium, Tocopheryl acetate, Sodium saccharine, etc.	Ingredients: Aqua, Hydrated silica, Sodium Lauryl Sulphate, Sorbitol, Cellulose, Gum, Aroma, Zink Citrate, Carrageenan, Sodium fluoride, Hydroxymethylcellulose, Limonena, Sodium Saccharine, Sodium citrate, etc

Based on OMNIC software, samples P9 and P21 were identified with gelatin. We suspected that the addition of gelatin into the P9 sample was due to the presence of unstable Tocopheryl acetate. Therefore, gelatin was needed as a **stabiliser**. Each of the kind of stabiliser is gelatin (Cole. 2000, Roswiem 2015). Other than that, we suspected that the addition of gelatin into the P9 sample was due to the presence of water and Steareth – 30 (polyethylene glycol ether derived from Stearic acid) compound. The Steareth–30 compound cannot dissolve in water. Therefore, addition gelatin into the P9 sample served as an **emulsifier**.

Similarly, the addition of gelatin into P21 sample might be stemmed from the presence of water and limonene (a terpene) which cannot dissolve in water. Therefore, in this case gelatin served as an emulsifier. One of the most common emulsifiers is gelatin. (Cole. 2000; Sahilah et al. 2012; Roswiem. 2015) The presence of gelatin which served as supporting material in the manufacturing process, such as an emulsifier or stabiliser, is mostly unreadable in the ingredient list of products on the packaging.

Our results showed that the ATR-FTIR is useful for detecting gelatin in samples, although it cannot distinguish the origins of the gelatin (pig or cow). According to Jaswir (2010), the combination of FTIR with chemometrics has been successfully applied to differentiate bovine gelatin from porcine gelatin. In this study, PCA was best performed on normalised spectral data of porcine and bovine gelatin standards within 570–1662 cm⁻¹ (Figure 5).

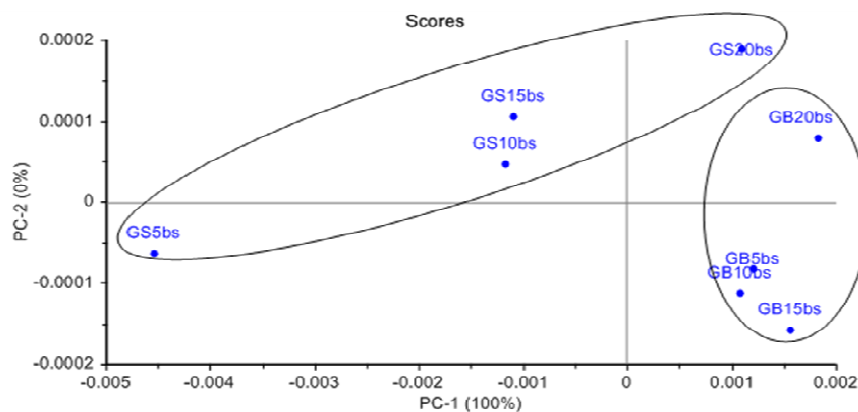


Fig 5. PCA pattern of normalised spectra of porcine and bovine gelatin at various concentration within 570 – 1662 cm^{-1}

Score plot of two main ingredients (Figure 5) was able to explain 100% of total variants (PC-1 = 100%, PC-2 = 0%). Figure 5 showed clustering pattern of standard gelatin which can be used to distinguish between porcine and bovine gelatin. The exception was made for 20% bovine gelatin which resembled porcine gelatin 20%.

The application of PCA plot above (Figure 4) was followed by SIMCA to predict samples P9 and P21 (Figure 6).

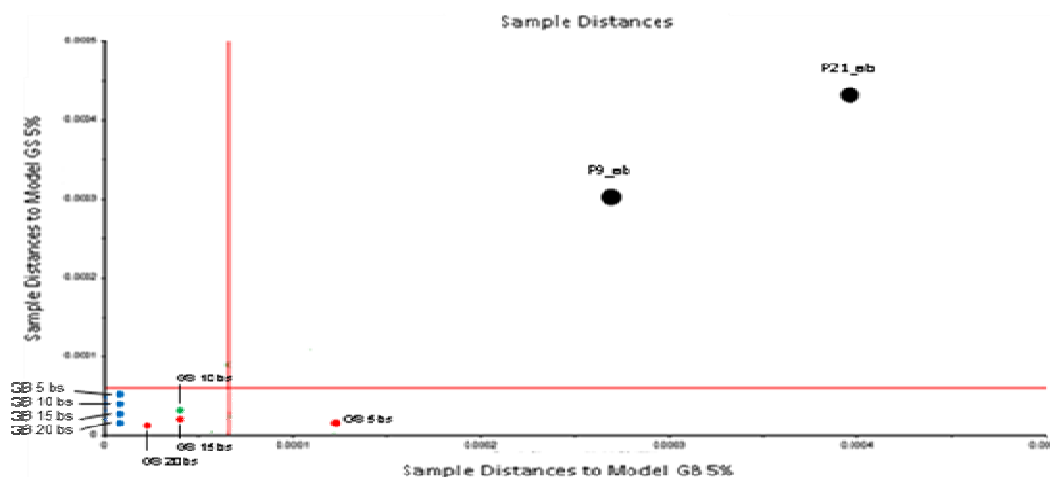


Fig 6. SIMCA used to predict the source of gelatin within samples

Note : Letter and blue circle : porcine gelatin (GB)
Letter and orange and green circle: bovine gelatin (GS)
P9 dan P21 : samples P9 and P21.

Figure 6 showed clustering plot for GS (yellow and green) dan GB (blue). X-axis was a model for GB and Y-axis is a model for GS with 5% significance level. In the plot, the data of P9 and P21 were spread in the curve away from clustering pattern with GB and GS. This may be due to the concentration of gelatin in both samples was less than 5%. This small concentration was attributed to the role of gelatin as a stabilising agent (P9) or emulsifier (P21) which only needed in small amount.

According to the discussion above, sample P9 and P21 contained gelatin which acted as a stabiliser or emulsifier. However, we were not able to distinguish the source of gelatin in the samples.

4. Conclusion

ATR-FTIR Spectroscopy technique can be used to detect the presence of gelatin in toothpaste products. Gelatin was found in two out of 42 toothpaste products. Nevertheless, ATR-FTIR Spectroscopy technique combined with chemometrics were not able to authenticate the source of gelatin within the samples. This may be due to the small amount of gelatin in the samples, considering the use of gelatin as a stabilising or emulsifying agent.

Acknowledgments

This research was funded by *Hibah Penelitian Internal Universitas YARSI*. We would like to thank *Yayasan YARSI* funding this study. We also thanks to Dedy Suseno for laboratory assistance

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