

Combination of Fourier Transform Infrared (FTIR) with chemometrics for halal authentication of face mask products made from gelatin

Salamah Nina^{1,2*}, *Adawiyah Rabiatul*¹, *Guntarti Any*¹, and *Susanti Hari*¹

¹Department Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia

²Ahmad Dahlan Halal Center, Universitas Ahmad Dahlan, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia

Abstract. Porcine gelatin is often used to replace bovine gelatin because its production costs are more affordable, so there are concerns about contamination with haram ingredients such as porcine, along with the boom in face mask products circulating in Indonesia. This research was conducted to determine the origin of gelatin in facial mask products using the differences in the functional groups of bovine and porcine gelatin. Reference face mask samples were made with a concentration variation ratio of pure bovine gelatin and pure porcine gelatin of 1:0; 0.75:0.25; 0.50:0.50; 0.25:0.75; and 0:1. The gelatin contained in reference face mask samples and face mask samples circulating on the market was isolated with acetone, which was then carried out by a vortexing process and centrifuged to obtain the supernatant. The supernatant obtained was then analyzed using the Fourier transform infrared (FTIR) method to determine the absorbance value and wave number. Next, it was analyzed further using chemometrics. The results of the FTIR analysis showed the compound composition of gelatin consisting of groups O-H, C-N, C=O, C-H, and C-O. The number of waves in the 1235–1077 cm⁻¹ optimization result was used for the PLS and PCA analyses. PCA analyses showed that one gelatin mask product was in a square with gelatin masks used as porcine gelatin references, while the other two samples were outside the bovine and porcine gelatin quadrants. The conclusion of this study was that of the three samples of facial masks studied, one came from porcine gelatin, and the other two were of unknown gelatin origin.

1 Introduction

According to data from the World Population Review in 2021, Indonesia is the country with the largest Muslim population in the world with a total Muslim population estimated at around 231 million people or the equivalent of 87.2% of the total Indonesian population.

* Corresponding author: nina.salamah@pharm.uad.ac.id

With the growing number of Muslims, the halal industry has grown rapidly. Halal means 'permitted' under Islamic law and, on the contrary, haram means 'forbidden' [1]. Halal has become a part of a Muslim's life. Halal aspects are very broad, such as food, beverages, medicines, cosmetics, and others [2,3]. This research was also created as a result of researchers observations of economic practices that occur in the field, especially in industry amidst the trend of halal industry issues and the implementation of the international Sustainable Development Goals (SDGs) project.

A halal certification is necessary for a product to enter the halal market. If at the time of the audit it is declared free of non-halal material, then a halal certificate will be issued. This means that the manufacturer has given a guarantee that the product is safe for the consumer . Some cosmetic products, such as lotions, creams, and facial masks, use porcine derivatives such as fatty acids, glycerin, and collagen in the production process. Most facial masks, especially those that remove comedones can be assured to contain gelatine. The application of gelatin is very commonly found in the cosmetic industry as a gel-forming ingredient in many cosmetics including facial masks, facial creams, body lotions, shampoos, hair spray, sunscreen, and bath soaps [1,4,5]. This is because gelatine has a unique and beneficial function. Gelatin generally functions as a gel-forming, thickening, and stabilizing agent. In addition, gelatine can act as a glue agent, emulsifier, and texture coating [6]. The commonly used gelatine comes from animals like cattle and porcine, because it is easy to obtain and is of better quality. Porcine gelatin is often used to replace cattle gelatin because it has similar functions and more affordable production costs [7].

The use of porcine gelatin would pose problems for Indonesians, whose majority are Muslims, as it is linked to a religious ban on halal and illegal. If the cosmetic product used contains components of porcine derivatives in any quantity, it will be illegal for Muslims to consume or use it [8,9]. O mankind, eat of the good that is in the earth, and do not follow the steps of the devil, for he is your clear enemy. For that it is necessary to do a study to find out the validity of cosmetic raw materials in particular gelatin based on the source of the face mask product. The same research has been carried out by [10] using the FTIR spectroscopy method and multivariate analysis proved to be able to analyze the presence of porcine fat and its derivatives in lipstick products. So this research was done with similar methods but with different samples using facial mask products.

2 Material and Methods

Materials: Three brand samples of facial masks sold at Shopee, acetone (Merck), bovine gelatine (Sigma Alderich), porcine gelatin (Sigma Alderich), aquades (Brataco), charcoal powder (Food Grade), and sodium benzoate (Food Grade).

2.1 Preparation and Isolation of samples

Preparation: All ingredients are weighed according to the formulation in Table 1. Then mixed and mixed to homogeneous.

Table 1. Formulation of facial masks reference [11]

Materials	Formula Component (g)					Function
	A	B	C	D	E	
<i>Charcoal powder</i>	3	3	3	3	3	Active substances
Bovine gelatin	1	0.75	0.50	0.25	-	Bases
Porcine gelatin	-	0.25	0.50	0.75	1	Nases
Sodium benzoate	0.1125	0.1125	0.1125	0.1125	0.1125	Preservative

Isolation: The gelatin mask weighs 3 grams and then dissolves in a beaker glass containing 3 mL of aquades that are heated to 60°C and mixed until dissolved. Then the mixture is transferred into the reaction tube and added 12 mL of acetone at a temperature of -20°C. The mixture then divortex for 5 minutes and is stored in the freezer for 24 hours at -20°C. Formed two phases, then the deposit phase is collected and placed in separate containers. All deposits are then washed three times using 3 mL of acetone at -20°C. Then all deposits that have been washed are moved into a porcelain cup to be dried in the oven at 105°C [12].

2.2 Data Analizes

Data analysis is done by PLS and PCA with the Minitab 21 application. The result of the spectrum is then converted into the form of data tables of the relationship between the absorption of each sample with the number of waves in the measurement range 4000 - 400 cm⁻¹. (internal dan eksternal). The three have the same way of finding true values and predictive values in the Minitab 21 application. The difference lies in the calculation of the RMSE.

The first step is to select a range of waves to be optimized. The number of waves selected is the one that contains the group of functions studied. Next, enter the wave count and absorption data into the Minitab 21 application. Then select the Stat menu, select Regression, select Partial Least Squares so that the PLS window will appear. In the Responses column filled with the bound variable (y), the Model column is filled in with the predictor (x), the Categorical predictors column has filled the number of PCs to be counted or left empty. Next click Options, select Leave-One-Out, click OK. The result will appear between the predicted value and the actual value [13].

Once the result is obtained, the next step is to calculate the determination coefficient (R²), the regression equation, and the RMSE value in a Microsoft Excel application. Open the Microsoft Excel app, then select the "Data" menu, select "Date Analysis", select "Regression". Enter (x) and (y) in the columns "Input X Range" and "Insert Y Range". Switch to "Standardized Residuals". Click "OK" and the R² value will appear and intercept. The calculation of the RMSE value is obtained with the square of the deduction of the actual value by the predictive value summed and divided by the amount of data [14].

The PCA analysis is then performed on the Minitab 21 application by incorporating the previously optimized wave count and absorption. Then click the "Start" menu, select "Multivariate", select "Principal Component" and a PCA window will appear. On the Variables column filled with the range of wave numbers used. Type of Matrix selected on Correlation. Then click "Graphs", check all, and the result will appear in the form of score-plot curves, eigenvalue, proportion, and cumulative values.

3 Results and Discussion

FTIR Spectroscopy Results: The results of the scanning of the spectrum of bovine gelatin and porcine gelatin from the reference facial masks performed at the range of wave numbers 4000-400 cm⁻¹ can be seen in Figure 1 Both spectrum if observed visually have quite identical patterns but if seen quantitatively of each absorption will be different.

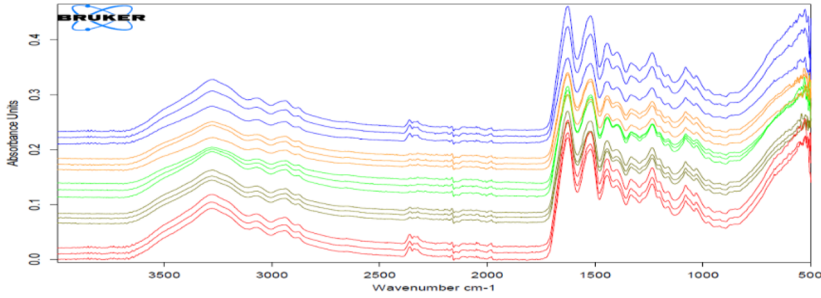


Fig. 1. IR spectrum of reference gelatin with different concentration comparisons of bovine and porcine in sequence 1:0 (blue) ; 0.75:0.25 (yellow) ; 0.5:0.5 (green) ; 0.25:0.75 (chocolate) ; 0:1 (red) each 3 replications

After the scanning process using the FTIR spectroscopic photometer, IR spectrum data was obtained and several peaks were detected at the number of waves as in Table 2.

Table 2. Functional groups of bovine gelatin and porcine gelatin of 100% reference masks

Reference [15]	Wave Number (cm ⁻¹)		Intensity	Peak Shape	Functional Group
	Bovine Gelatin	Porcine Gelatin			
3200-3400	3277	3278	Medium	Wide	O-H
1000-1350	1332	1332	Medium	Wide	C-N
1630-1680	1626	1626	Strong	Sharp	C=O
1375-1465	1445	1445	Medium	Wide	C-H alifatik
1000-1300	1079-1234	1079-1234	Medium	Wide	C-O

The peaks appearing at the wave numbers 3277 cm⁻¹ and 3278 cm⁻¹ indicated the presence of the absorption tape from the OH function group [15]. The next peak was observed at the 1332 cm⁻¹ wave number with medium to strong intensity interpreting the existence of the C-N function group [15]. In gelatin structures such as Figure 1., the carbonyl group (C=O) is one of the most easily recognizable bands. Carbonyl bands are usually the most intense bands in a spectrum and appear in the area 1680-1630 cm⁻¹ [13]. The same peak is also seen in the IR spectrum of bovine and porcine gelatin reference at a wave number of 1626 cm⁻¹. The next peak can be seen at the wave count of 1445 cm⁻¹ with a medium intensity that is the absorption tape of the alifatic C-H function group [15]. The next peak detected in the IR spectrum is at the number of waves 1079-1234 cm⁻¹ which is the vibration of the carboxylic acid group [15].

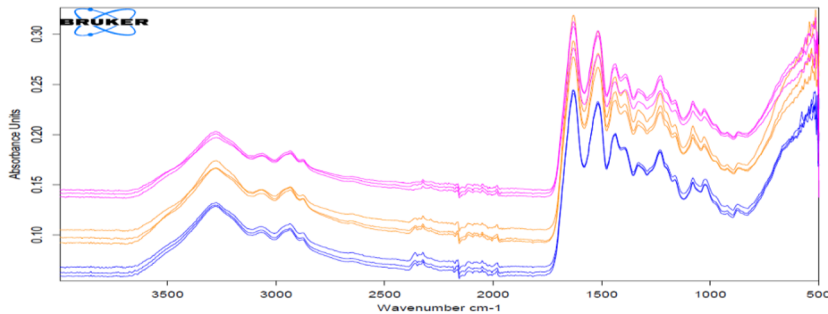


Fig. 2. Gelatin IR spectrum of facial mask samples code ACN (pink) ; CHR code (yellow) ; DU code (blue) respectively 3 replications

The face mask used as a sample is a product sold at Shopee with its main content of gelatin. The samples of facial masks used come from 3 different brands with ACN, CHR, and DU codes. The gelatin isolation result is powdered and then read with FTIR instruments in the 4000 - 400 cm^{-1} wave range. All spectrum samples of facial masks showed identical results and only had differences in absorption as in Figure 2. The similarities and differences are also visible if all spectrum of reference gelatin and gelatin samples are combined like in Figure 3. Based on all the IR spectrum obtained, the visually visible peaks that emerge from the reference gelatin and gelatin samples are very difficult to distinguish. So, there is a need for a combination of analysis methods using chemometrics, i.e. PLS to reduce and validate data and PCA to group the data in a plot score quadrant to see clear differences.

PLS Calibration and Validation Models: PLS regression provides the advantage of forming a PLS regression component that can describe the correlation between the variables x and y . Each component on the PLS-regression is obtained by maximizing the kovarians between the y variables with any linear function possible from the x variable. In analysis with FTIR spectroscopy, PLS is often used to extract information from a complex spectrum with overlapping peaks, the presence of impurities, and noise from the FTIR spectral photometer instrument [16].

The first step to analyze the data with the PLS is to optimize the wave count data to determine the number of waves to be used to make the calibration and validation models. The wave number used in the optimization phase is the number that has a functional cluster characteristic of the compound or the fingerprint area. The optimization of the wavelength determination is done to obtain the optimum performance of the calibrating model and to provide a good correlation between the actual values of the reference bull gelatin and porcine reference, as well as the gelatin contained in the face mask product sample to the predictive value using FTIR spectroscopy as seen from the determination coefficient value (R^2) that is close to 1. According to the International Conference on Harmonization in [17] when the value of the determinance factor (R^2) is closer and closer to 1, then the relationship between the predicted value and the actual value is better. After obtaining the value R^2 that is nearer, the next step is to calculate the RMSEC (Root Mean Standard Error of Valibration) value, then to see the values that are used on the model of the Erroration, it is better to see that the value is lower and lower [17]. The optimization of the number of waves on can be seen in Table 3.

Table 3. Wave number optimization for PLS multivariate calibration

Wave Number (cm^{-1})	Coefficient of Determination (R^2)	RMSEC	Regression
1627-1518	0.9675	6.7135	$y = 0.9675x + 1.6227$
1522-1444	0.9879	4.0958	$y = 0.9879x + 0.6037$
1500-1400	0.9839	3.3152	$y = 0.9839x + 0.6394$
1444-1233	0.9663	6.8369	$y = 0.9663x + 1.6827$
1235-1077	0.9992	1.0406	$y = 0.9992x + 0.0386$
1235-1000	0.9916	3.3464	$y = 0.9916x + 0.4180$

The number of waves used for multivariate calibration with PLS is optimized to obtain the best prediction model. The optimization results are compiled as in Table 3., which shows the best results with the highest determination coefficient value (R^2) and the lowest RMSEC value. The number of waves is at 1235-1077 cm^{-1} with the value of R^2 being 0.9992 and the RMSEC value is 1.04%. Therefore, the number of the waves used for calibration and validation in this study is 1235-1077 cm^{-1} . The curve in Figure 3A. shows the relationship between the axis x (actual value) and the axis y (prediction value) of the calibrations model at the wave count of 1235-1077 cm^{-1} .

The calibration results show that the free variable (axis x) can explain 99.92% of the bound variable (axis y). The obtained RMSEC value is low at 1.04%, which means that the modeling used is also acceptable. To evaluate the future prediction model, use cross validation method with leaves one out technique on Minitab 21. This technique will extract some data and create a new model with the remaining data. This validation process is called internal validation. If the parameter RMSECV (Root Mean Square Error of Cross Validation) is low and the value of the determination coefficient (R^2) is close to 1, the model is said to be valid [18]. Curves like in Figure 3B. show the relationship between the actual value and the predicted value using internal validation.

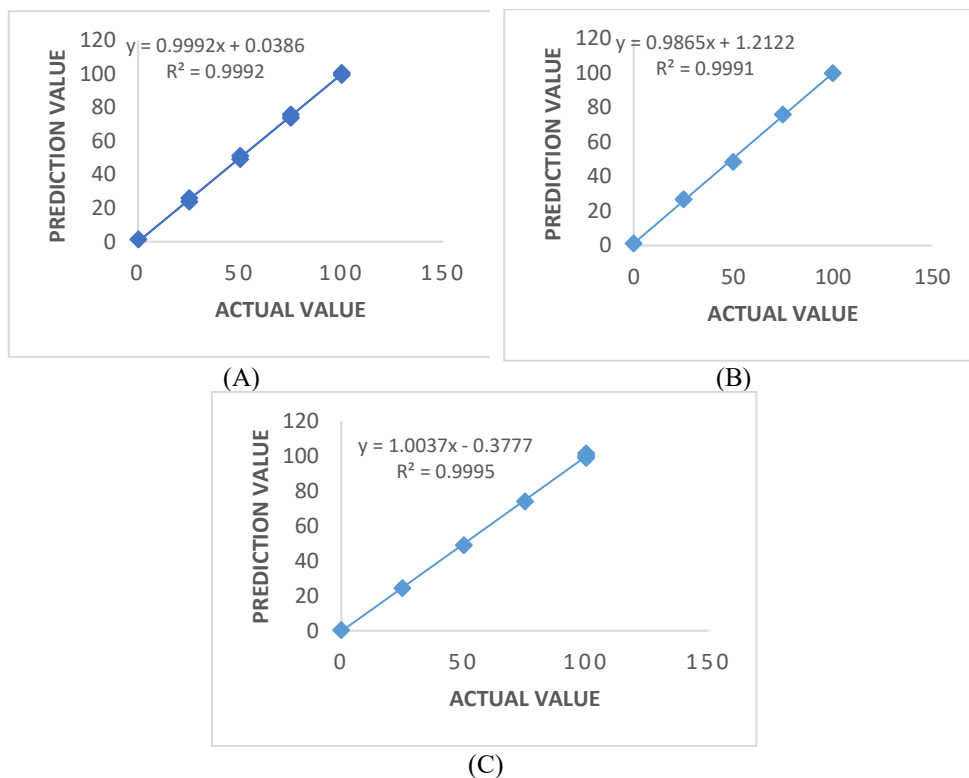


Fig. 3. Curve relationship between axis x (actual value) and axis y (prediction value), A. RMSEC (calibration model at wave number 1235-1077 cm^{-1}); B. RMSECV (internal validation); C. RMSEP (external validation) model

From this curve the equation $y = 0.9865x + 1.2122$ is obtained with $R^2 = 0.9991$ and the value of RMSECV = 0.78%. The smaller the RMSECV, the better the predictive ability of the built model. After that, an external validation process of model regression was performed resulting in correlation curves with equations $y = 1.0037x - 0.3777$ and $R^2 = 0.9995$ as well as the root error of the predicted average square (RMSEP) = 0,94%, as seen in Figure 3C. In other words, with these R^2 and RMSEP values, then the model can project data accurately [18].

The value of the determination coefficient (R^2) acts as an accuracy parameter, with a value of R^2 approaching 1 indicating that the relationship between the actual value and the predicted value is well formed and the accurate value is also increased. On the other hand, the smaller values of RMSEC, RMSECV, and RMSEP indicate that the errors of the models created are also decreasing.

Grouping Data with PCA: Principal Component Analysis (PCA) is basically a technique of multivariate data reduction, when there is a correlation between variables. Objects or samples with almost identical main components (PCs) have identical physico-chemical properties, so PCA can be used for clustering. Therefore, PCA is often referred to as a latent variable because of its ability as a grouping technique. PCA is a technique to reduce the amount of data, when there is a correlation between the data. However, it should be noted that PCA is not a useful technique if the variables are not correlated [19].

The chemometric analysis using PCA is done by preparing the absorbance data in Microsoft Excel which is a combination of the bull and porcine gelatin absorbances reference on all series of rates, as well as 3 (three) samples of facial mask products. The absorption data used are only those that are in the range of wave numbers previously obtained during optimization at 1235-1077 cm⁻¹.

According to the results of PCA analysis, eigenvalue, proportion, and cumulative data are obtained for each PC (main component). The variation of each PC as well as the influence of each variable is indicated by the eigenvalue, whereas the proportion shows the contribution of the individual PC to the variable variation. In this study, the value of the eigenvalue is greater than 1, which indicates that there is influence and colleration between the variables. This study uses PC1, PC2, PC3, and PC4, each representing 99.8% of the variable.

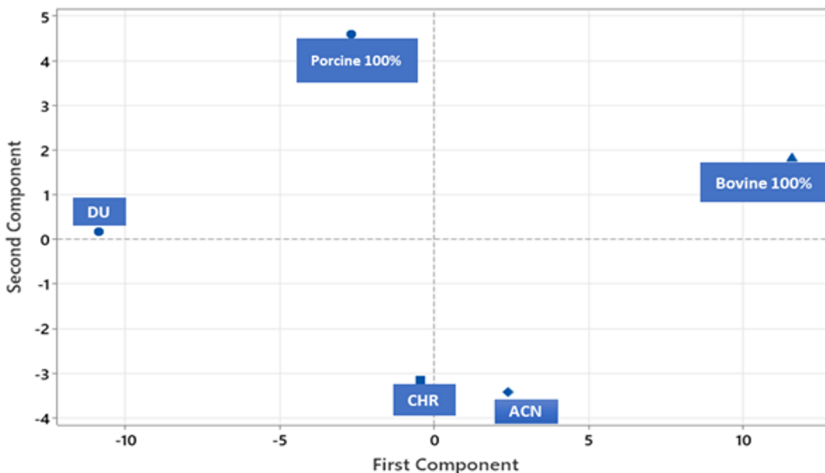


Fig. 4. PCA plot score 100 % bovine gelatin reference, 100 % porcine gelatin reference, ACN, CHR, and DU product gelatin samples

The results of the PCA plot based on Figure 4. show that the DU code sample is on the same quadrant as the 100% porcine gelatin reference. This means that the gelatin contained in the DU code sample has almost the same physical and chemical characteristics as porcine gelatin.

The reference gelatin face masks are made from a composition that resembles a face mask sold at Shopee. However, the manufacturing process is simple and incomplete like a factory-made face mask. This will affect the large difference in gelatin composition between samples. Besides, since gelatin has many brands on the market, there may be a brand difference between gelatin in reference face masks and gelatin inside facial masks sold at Shopee. The properties of collagen in gelatin can be affected by brand differences and will affect PCA plot score [20].

4 Conclusion

Gelatin analysis with FTIR results in chemical composition arrangements of groups O-H, C-N, C=O, C-H and C-O. The results of the optimization of the calibration analysis wave count using PLS at 1235-1077 cm⁻¹ showed a determination coefficient value (R²) of 0.9992 and an RMSEC value of 1.04% with the results of internal validation showing a R² value of 0.9991 and a RMSECV value of 0.78% and external validation resulted in a R²-value of 0.9995, and the RMSEP value of 0.094%. PCA multivariate chemometric analysis of the plot score quadrant indicates that one sample of facial mask gelatin is derived from porcine gelatin while the other two samples are not in the bovine gelatin or porcine gelatin area quadrants so the origin of the gelatin used is unknown.

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