

AN ANALYSIS OF DOG FAT IN BEEF MEATBALLS USING FOURIER-TRANSFORM INFRARED SPECTROPHOTOMETRY COMBINED WITH CHEMOMETRICS

(Analisis Lemak Anjing dalam Bakso Melalui Kaedah Spektrometri Inframerah Transformasi Fourier Gabungan Bersama Kemometri)

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Abstract

Bakso is a meatball made from beef and is very popular among Indonesians. However, the increasing number of cases of counterfeits and mixing of this meatball with dog meat in the city of Yogyakarta has caused significant unrest in several communities, especially Muslims. This study aimed to detect the fat content of dog meat in meatballs circulating in the city of Yogyakarta with an analysis using a combination of the FTIR method and the chemometric PCA. This research was designed with the making of a variety of meatballs consisting of 25 grams of beef and dog meat in calibrated samples of 0%, 20%, 40%, 60%, 80%, 90%, and 100%, as well as other ingredients, such as flour, garlic, and spices, as much as 5% of the meat weight. Three of the calibrated samples were validated and extracted with a Soxhlet extractor using the n-hexane solvent. The extracted fat was further analyzed by FTIR and processed with the Minitab19 software. The results showed that the wavenumbers ranged from 1,750 to 800 cm^{-1} , producing various peak intensities as well as, with the PLS calibration equation $y = 0.998206x + 0.999929$, an R^2 value of 0.9982, an RMSEC of 1.37%, an RMSEP of 1.19%, and an RMSECV of 2.32%. Furthermore, the dog and beef fats were successfully classified using the multivariate PCA. In conclusion, the analysis results showed that the FTIR spectrophotometric method combined with chemometrics was effective at classifying dog fat from other animal fats. Meanwhile, the analysis results showed that 2 out of 3 samples contained other meat contaminants.

Keywords: dog meat, meatball, Fourier transform infrared, principle component analysis, partial least squares

Abstrak

Makanan bakso yang diperbuat menggunakan daging lembu sangat popular di kalangan masyarakat Indonesia. Bagaimanapun, kes pemalsuan dan pencampuran daging lembu dengan daging anjing dalam pembuatan bakso yang berleluasa di bandar Yogyakarta telah menimbulkan keresahan yang ketara dalam sesetengah masyarakat khususnya di kalangan umat Islam. Kajian ini bertujuan untuk mengesan kandungan lemak anjing dalam daging bakso yang beredar di bandar Yogyakarta dengan menggunakan analisis gabungan kaedah FTIR dan kemometri PCA. Kajian ini direka bentuk dengan membuat variasi daging bakso yang terdiri daripada 25 gram daging lembu dan kandungan daging anjing yang telah ditetapkan kepada 0, 20, 40, 60, 80, 90 dan 100%, serta bahan-bahan lain seperti tepung, bawang putih dan perasa sebanyak 5% daripada berat daging. Tiga sampel yang telah ditetapkan diekstrak dengan Soxhlet menggunakan n-heksana sebagai pelarut. Lemak yang diekstrak kemudiannya dianalisis oleh FTIR dan diproses dengan perisian Minitab19. Keputusan menunjukkan julat gelombang antara 1750 hingga 800 cm^{-1} menghasilkan keamatan puncak yang berbeza-beza dan persamaan PLS $y = 0.998206x + 0.9999929$, nilai $R^2 = 0.9982$, RMSEC 1.37%, RMSEP 1.37%, RMSEP 1.52%. Tambahan pula, lemak anjing dan daging lembu berjaya dikelaskan menggunakan analisis PCA multivariate. Kesimpulannya, hasil analisis menunjukkan kaedah spektrofotometri FTIR yang digabungkan dengan kimometrik berkesan dalam mengklasifikasikan lemak anjing daripada haiwan lain. Sementara itu, analisis menunjukkan 2 daripada 3 sampel mengandungi bahan cemar daripada daging yang lain.

Kata kunci: bakso, daging anjing, inframerah transformasi Fourier, analisis komponen utama, kuasa dua terkecil separa

Introduction

According to Sahih Hadith Muslim no. 1933 *"The eating of all fanged beasts of prey is unlawful."* Additionally, Sahih Hadith Bukhari no. 3314 and Sahih Hadith Muslim no. 1198 state, *"There are five (harmful) things upon whose killer there is no sin whether he is in a state of ihram or otherwise: rats, scorpions, crows, kites, and voracious dogs (Kalb aqur)"*. In this regard, halal is a food requirement and is a mandatory provision for Muslims [1]. Therefore, food is said to be halal if there is no evidence forbidding it; however, it can also become haram if it is not good for consumption [2].

The Muslim community forbids the consumption of dog meat. However, dog meat adulteration in food products including buns, sausages, shredded meat, and meatballs has recently gained notice. This become quite profitable due to the trade-in of wild dog meat in several countries, which is carried out at low prices [3]. Furthermore, considering that the price of beef is more expensive compared to other varieties of meat, some traders have tried to minimize the cost of meatball production by mixing beef with other kinds of meat during the manufacturing process. This act is now considered to be an effective solution to reduce the production price of meatballs [4]. According to news reported by IDN Times Jogja published on January 13, 2020, dozens of dogs are slaughtered daily at various slaughterhouses in Bantul, Yogyakarta, Indonesia, to be served as dishes. In this regard, it is feared that there are meatball traders who

produce counterfeits by mixing beef with dog meat, and this has become very detrimental to the consumers, especially Muslim consumers who have been prohibited by the Islamic belief from the consumption of dog meat.

Several approaches are being used to detect and measure the fat content of dog meat and pork derivatives in food products. The first approach is to determine the ratio of several chemical constituents of the products and ensure that this ratio is constant. Secondly, it is to look for certain markers on food products, both in the form of chemical contents and morphological components that can prove the presence of pork derivatives in the food. Lastly, it is conducted in a physico-chemical analysis [5]. Subsequently, analytical methods have been developed for the analysis of non-halal products in raw materials and food products. These methods include Fourier-transform infrared (FTIR) spectrophotometry [6], chromatography [7], and differential scanning calorimetry (DSC) [8]. Furthermore, DNA-based methods such as polymerase chain reaction [9] and analysis methods based on odor identification (electronic nose) [10] are also used for the analysis.

FTIR was not able to distinguish dog fat from beef fat because they have peaks with the same wave number related to the functional groups of the compounds. The FTIR results differed in the peak intensity of each peak, but it is difficult to see the difference visually. The feasibility of FTIR spectroscopy in combination with

multivariate partial least squares (PLS) calibration was used for the quantitative analysis of dog meat in a binary mixture of beef in meatball formulations. The chemometric principal component analysis (PCA) was used for the classification of dog meat and beef meatballs [26-28].

Therefore, this research aimed to determine the presence of dog meat in meatball products with the partial least squares (PLS) model. The classification of dog fat and chicken fat was performed using a principal component analysis (PCA) with the FTIR method [11].

Materials and Methods

Materials

The main materials used in this research were reference

meatballs made from a mixture of beef obtained from Gedong Kuning Market, Rejowinangun, Kotagede District, Yogyakarta City, Special Region of Yogyakarta, Indonesia. Likewise, the dog meat ingredients were obtained from Jombor Lor, Mlati District, Sleman Regency, Special Region of Yogyakarta. The flour, as well as seasonings, was made in varying degrees of concentrations. In addition, the market sample beef meatballs were obtained by random selection of 3, out of the several meatball traders in various parts of Yogyakarta City, namely Timoho, Balirejo, and Glagahsari Streets, Special Region of Yogyakarta, Indonesia, and these samples were taken in October 2020. The solvents used in this research were technical n-hexane (Merck®) and Na₂SO₄ (Merck®). Research method (workflow analysis) is as shown in Figure 1.

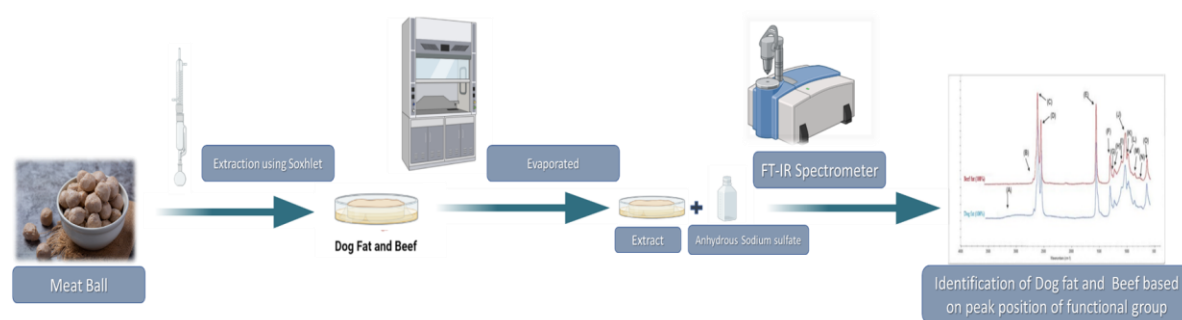


Figure 1. Workflow analysis of dog fat in beef meatballs using a Fourier-transform infrared spectrophotometer combined with chemometrics

Identification of dog species

Identification of dog species was carried out in the

Laboratory of Animal Systematics, Faculty of Biology, Gadjah Mada University, Yogyakarta.

Table 1. Variations in concentration of beef and dog meatball samples

Concentration (%)	Beef (grams)	Dog Meat (grams)
Cow 100	25	-
Dog 20	20	5
Dog 40	15	10
Dog 60	10	15
Dog 80	5	20
Dog 90	2.50	22.50
Dog 100	-	25

Meatballs production with variations in concentration

The meat ingredients were mashed, and additional

ingredients such as tapioca flour and spices, including shallots, garlic, ginger, and finely ground pepper, were added. The samples made in various concentrations can

be seen in Table 1. Meatballs were made by grinding beef and dog meat separately, consisting of 25 grams of meat. In addition, variations in concentration of dog meat in the beef meatballs made were: 0%, 20%, 40%, 60%, 80%, 90%, and 100% [12].

Fat extraction in meatballs

The meatballs were weighed according to the concentration made (Table 1), mashed, and extracted with a Soxhlet apparatus. Additionally, the solvent used was n-hexane, which was extracted for 4-7 hours at 70 °C. The extract was then added with anhydrous Na₂SO₄, which evaporated in a fume hood. The viscous extract was analyzed using an FTIR spectrophotometer [12].

Sample analysis with FTIR

The fat samples were analyzed using FTIR spectrophotometry. This analysis was carried out at a frequency of 4,000–650 cm⁻¹. Following this, the samples were dropped onto an ATR crystal at a controlled temperature (25 °C), and measurements were carried out on 32 scans at a resolution of 4 cm⁻¹ [13].

Statistical data analysis

The qualitative and quantitative statistical analysis of FTIR spectrophotometric test results on meatball samples combined with PLS and PCA multivariate chemometric calibration with the Minitab 19 software on a computer device was carried out. The partial least squares (PLS) method was used to determine the linearity. A Microsoft Excel 2010 software worksheet was also used to relate the actual sample to the predicted sample concentrations. The accuracy of the PLS model was evaluated by the coefficient of determination (R²), while that of the data analysis method was assessed using the root mean square error of cross-validation (RMSECV) and the root mean square error of prediction (RMSEP). The formula used to obtain the RMSECV (equation 1):

$$RMSECV = \sqrt{\frac{\sum_{i=1}^n (\hat{x}_i - x_i)^2}{n}},$$

where \hat{x}_i is the actual value of meatballs, x_i is the value calculated from cross-validation of meatballs, and n is the number of calibrations or validation samples [14]. Meanwhile, the formula used to obtain the RMSEP (equation 2):

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}},$$

where \hat{y}_i is the actual value of meatballs, x_i is the predictive value of meatballs, and n is the number of calibrations or validation samples [4].

Results and Discussion

Identification of dog species used as sample

The identification of dog species was conducted using pictures of several parts of the animal's body, such as the face, tail, legs, and ears, and pictures of the combined parts as a whole body [15]. The identification results indicated that the type of dog used was the mutt otherwise known as a local dog with the Latin name *Canis lupus familiaris*. The mutt is a dog species characterized by a skull with a relatively elongated snout and teeth adapted for eating meat. This dog species is generally not intentionally bred by humans but survives in areas where humans live, such as streets, cities, and villages [16].

Meatball fat extraction

The fat content in the meatballs was extracted using the Soxhlet extraction method. Similarly, a non-polar solvent such as n-hexane can also be used to extract fat. The extraction process was carried out at a temperature of about 70 °C, which corresponds to the boiling point of n-hexane [17]. For optimal extraction, this process was conducted for approximately 5 hours. Subsequently, whether the extraction process had been optimized was demonstrated by the turn of the color of n-hexane into dripping clear like its original color. Lastly, the addition of sufficient anhydrous Na₂SO₄ was intended for binding to the water molecules that may still be contained in the n-hexane as the presence of water in fat may interfere with the response of the FTIR spectrum [18].

Based on Figure 2(a), the FTIR spectra obtained were in the wavenumber range 4,000-600 cm⁻¹. It can be seen that there is no significant difference between the beef and dog meatball fat spectra because the main components of both fats, which are triglycerides, are the same, and both are regarded as animal fats. Therefore, it was deemed necessary to carry out a further analysis with the chemometric PCA to distinguish the intensity of infrared absorption peaks, which are more varied, making it easier to classify the beef and dog meatball fat

spectra. The descriptions of the absorption peaks and the identified functional groups are summarized in Table 2. From Table 2, the beef and dog fat shared a similarity in functional fat groups. Carbonyl, CH, and CO groups

appeared on the FTIR spectrogram. The difference in the variations of such functional groups can be seen with the chemometric PCA [29].

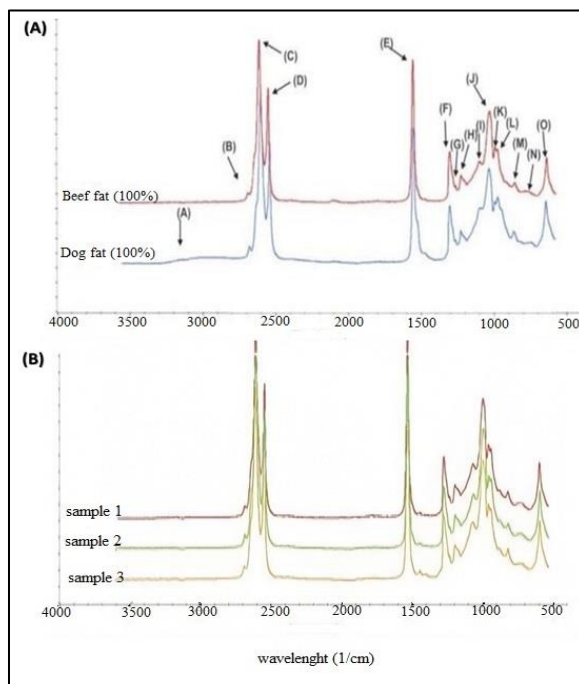


Figure 2. (a) Difference in the spectra of 100% beef meatball fat and 100% dog meatball fat and (b) the FTIR spectra of market beef meatball samples

Table 2. Identification of functional groups and vibrational types of the FTIR spectra of dog and beef fats

Peak Position (cm ⁻¹)		Functional Groups	Vibration Type	Intensity
Dog	Beef			
3,283	-	O-H	Stretching	Medium
3,007	3,003	C=C-H (cis)	Stretching	Medium
2,921	2,921	C-H(CH ₃)	Asymmetric stretching	Strong
2,852	2,852	C-H(CH ₂)	Asymmetric stretching	Strong
1,744	1,743	C=O (ester)	Stretching	Strong
1,461	1,462	C-H (CH ₂)	Bend scissoring	Strong
1,418	1,417	C=C-H (cis)	Bend (rocking)	Strong
1,376	1,376	C-H (CH ₂)	Bend Symmetrical	Strong
1,230	1,236	C-O (ester)	Stretching	Medium
1,160	1,159	C-O (ester)	Stretching	Medium
1,115	1,097	C-O (ester)	Stretching	Medium
968	965	C=C-H (trans)	Bend out	Medium
839	889	C=C-H (trans)	Bend out	Medium
721	721	C=C-H (cis)	Bend out	Strong

Wavenumber optimization as PLS calibration model

The results of the quantitative analysis which was carried out in the fingerprint area of the FTIR spectra to show a distinctive difference in the intensity of the absorption was significant and became the target for selecting the optimization wavenumbers [19]. The selection of these wavenumbers was intended for a calibration model that produces an R^2 value that is close to 1 and the smallest RMSEC [11]. The wavenumbers selected ranged from 1,750 to 800 cm^{-1} .

Additionally, the results of the optimization of the

calibration model showed an optimal range of wavenumbers from 800 to 1,750 cm^{-1} with the equation $y = 0.99820x + 0.99992$. Moreover, the resulting coefficient of determination (R^2) was 0.99820 (Figure 3), with an RMSEC value of 1.464435%. The optimization results obtained indicated the accuracy between the predicted value and the actual value, which was 99.82%. The random error value also indicated an error in the sample prediction from the calibration model equation with an RMSEP value of 1.52% and an RMSECV of 2.329%.

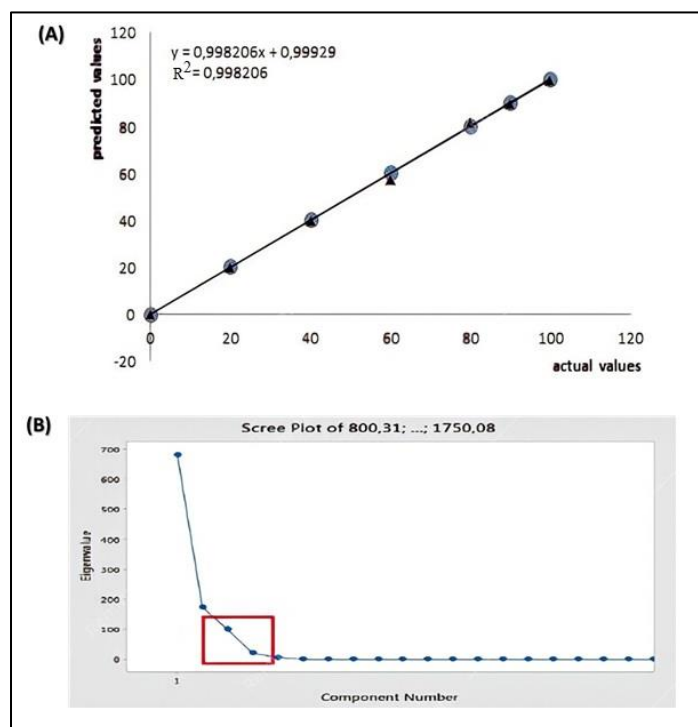


Figure 3. (a) The concentration variation of dog and beef fat resulted using the PLS data calibration model (0–100%) and (b) screen plot of the relationship between eigenvalues and PC results from the PCA

Pattern recognition analysis with the principal component analysis (PCA)

The PCA was performed using an absorbance data set of dog and beef fats, in addition to market samples, in the 1,750–800 cm^{-1} area, which was the fingerprint area. From this frequency range, information on which frequency contributes more to the PCA model will be obtained, and with the market samples, a clear separation between dog and beef fats will be provided. The PCA was performed with the help of Minitab19 and was integrated with Microsoft Excel 2010 [25]. The

chemometric PCA was selected for grouping the variables of each sample used. With the variable of fatty acids of each group of samples, the proximity of chemical properties will be known.

Scree plot

The selection of the number of main components (PC) was one of the aspects that contributed to the success of the PCA results. In addition, the choice of the number of PCs in the PCA could be determined from the eigenvalues generated by each of the main components.

Therefore, the number of PCs gained was relevant for explaining the initial information from PC data with eigenvalues > 1 [20].

Furthermore, the eigenvalue is used to describe a large number of variations and is said to be part of the total variation that can be explained by each PC. Based on Figure 3 (b), PC1 with an eigenvalue of 683.35 was able to explain 69.3% of the variance in the initial variable. Meanwhile, PC2 with an eigenvalue of 174.06 was able to explain 87.0% of the variance, PC3 with an eigenvalue of 99.76 was able to explain 97.1% of the variance, and PC4 with an eigenvalue of 21.17 was able to explain 99.2% of the variance, which was part of the

elbow, where there was a significant decrease in eigenvalues. Of the first 4 PCs, 99.2% of data variance was included and was relevant to explain the characteristics of the alert variable and the information contained [21].

Score plot

The PCA analysis was performed by the comparison of components after entering spectral data of 100% dog and beef fats. The analysis was then carried out by replication to ensure that the principal components were separated from other components using an optimized wavenumber [22]. The separation and grouping of the two score plots are presented in Figure 4 (a).

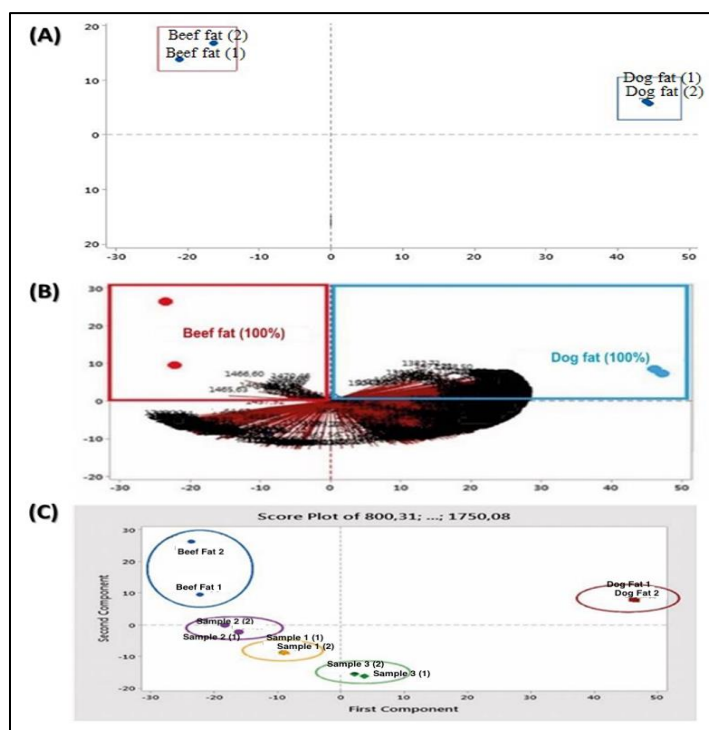


Figure 4. (a) Score plot PCA results in 100% dog fat and 100% beef fat. Note: red (beef fat) and blue (dog fat), (b) biplot of 100% dog fat and 100% beef fat, and (c) the score plot result of the PCA market sample, 100% dog, and 100% beef

Figure 4 (a) shows the results of the PCA analysis of two samples occupying different quadrants. Sample A (red) consisted of 2 beef tallows which were separated by 100% and had similar properties as a result of the close distance between the two fat plots which were also within the same quadrant, while sample B (blue) consisted of two 100% dog fat data which were found in different quadrants and at a great distance from sample

A. Additionally, the two samples in the biplot were shown to be sticking together. Hence, the closer the distance between the two plots, the more the fat similarities. Meanwhile, the farther apart the plots, the lesser the similarities between the fats. From this, it can be seen that sample A and sample B were well separated because they are in different quadrants [23].

Figure 4 (b) shows that 100% beef fat (red) and 100% dog fat (blue) both had special variables. The beef fat was in the same quadrant with several variables, including 1,348.96–1,360.53 cm^{-1} ; 1,453.10 cm^{-1} ; 1,456.96 cm^{-1} ; 1,457.92 cm^{-1} ; 1,465.63 cm^{-1} ; 1,466.60 cm^{-1} ; and 1,470.46 cm^{-1} . Based on this, it can be said that the wave numbers 1,348.96–1,360.53 cm^{-1} ; 1,453.10 cm^{-1} ; 1,456.96 cm^{-1} ; 1,457.92 cm^{-1} ; 1,465.63 cm^{-1} ; 1,466.60 cm^{-1} ; and 1,470.46 cm^{-1} are therefore characteristic of the beef fat.

Comparatively, the results of the dog fat were in the same quadrant with many variables, including 800.31–1,430.92 cm^{-1} ; 1,475.28–1,487.81 cm^{-1} ; and 1,520.60–1,727.91 cm^{-1} . Hence, it indicates that these wavenumber variables are characteristic of dog fat because they were all in the same quadrant.

Analysis of beef meatball samples circulating in Yogyakarta City

The grouping contained in the score plot of dog and beef fats has explained that the two samples were perfectly separated and were in different quadrants; hence, they can be applied to the market samples. The samples of market beef meatballs analyzed were 3 meatballs obtained from 3 different places in Yogyakarta city. The results of the spectra were then analyzed by the PCA together with a reference sample of 100% beef and dog fats at the wavenumber of the optimization results to determine the presence of dog meat adulteration in the market sample. The results of the FTIR spectra of the three samples are presented in Figure 2 (b).

At first glance, the spectra of the market samples looked the same, but the three fat spectra had different intensities for each wavenumber, especially in the fingerprint area. To further confirm the difference in the intensity of the three spectra, the score plot was used as shown in Figure 4 (c). The results of the PCA on the FTIR spectra of 100% dog and beef fats, with the three market samples, are shown in the score plot, with the five fats being in separate quadrants. None of the three market samples had proximity to both dog and beef fat standards.

Furthermore, all market sample plots were in different quadrants from the standard % dog fat plot, indicating that both plots did not share a similarity. The result of

this research therefore showed that the three meatball samples were not adulterated with dog meat. However, of the three market sample plots, only number 2 was in the same quadrant as the 100% beef fat plot. This shows that sample 2 had similar characteristics with beef fat. The further the plots of sample 1 and sample 3 from the 100% beef fat plot, the greater the difference in characteristics. In Figure 4 (C), the two samples (1 and 3) were most likely not pure beef and were likely to contain other types of meat contamination, which can be proven by further research.

Conclusion

The quantitative analysis of dog fat using PLS chemometrics resulted in optimization of wavenumbers in the range 1,750–800 cm^{-1} with the calibration model equation $y = 0.998206x + 0.99992$, which was quite accurate with a predicted value of 99.82% of the actual value. Furthermore, a coefficient of determination (R^2) of 0.998, an RMSEC of 1.46%, an RMSEP of 1.52%, and an RMSECV of 2.32% were obtained. In this regard, it can be concluded that the meatball samples in the market did not contain dog fat, but they were suspected to contain other types of fat.

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