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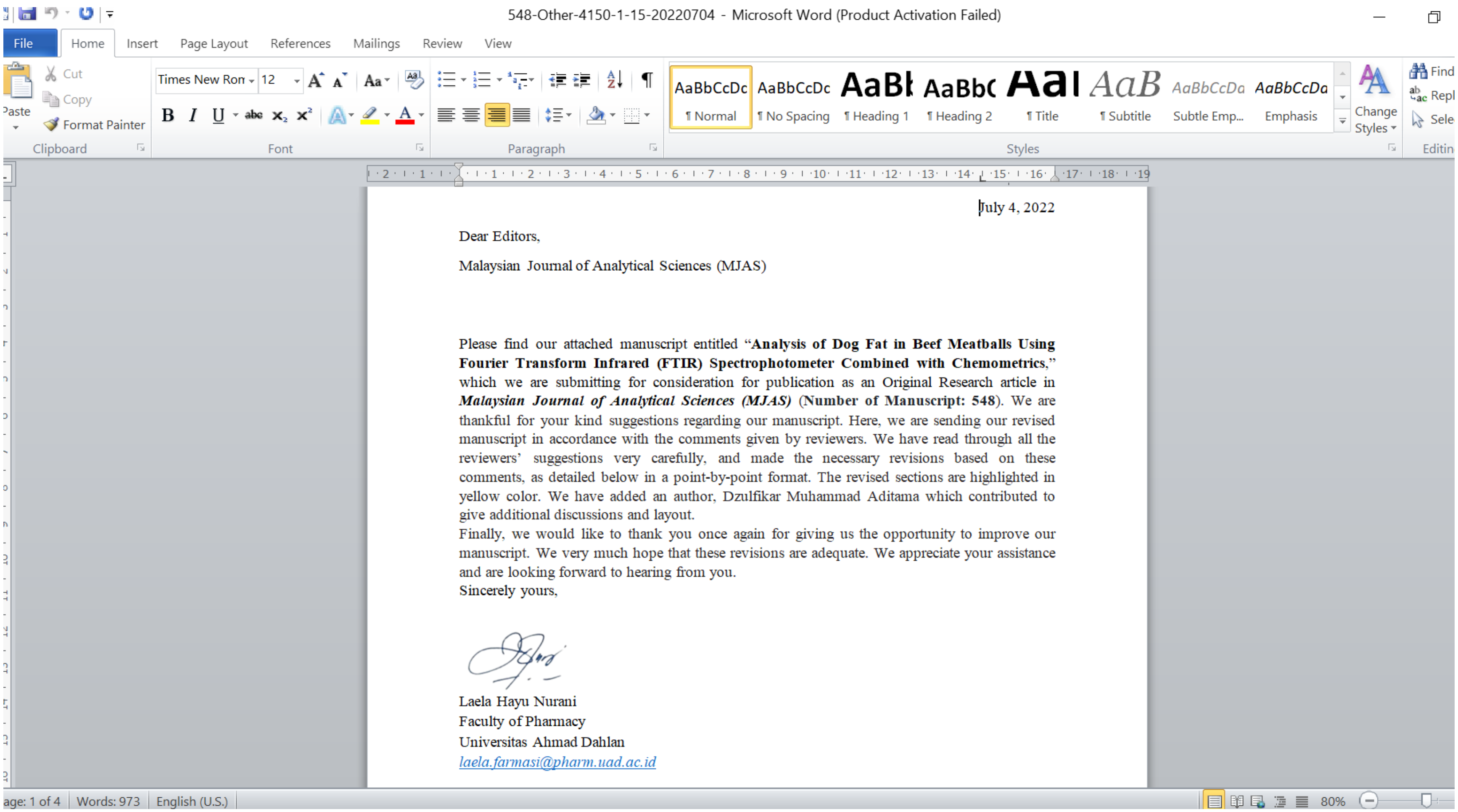

July 4, 2022

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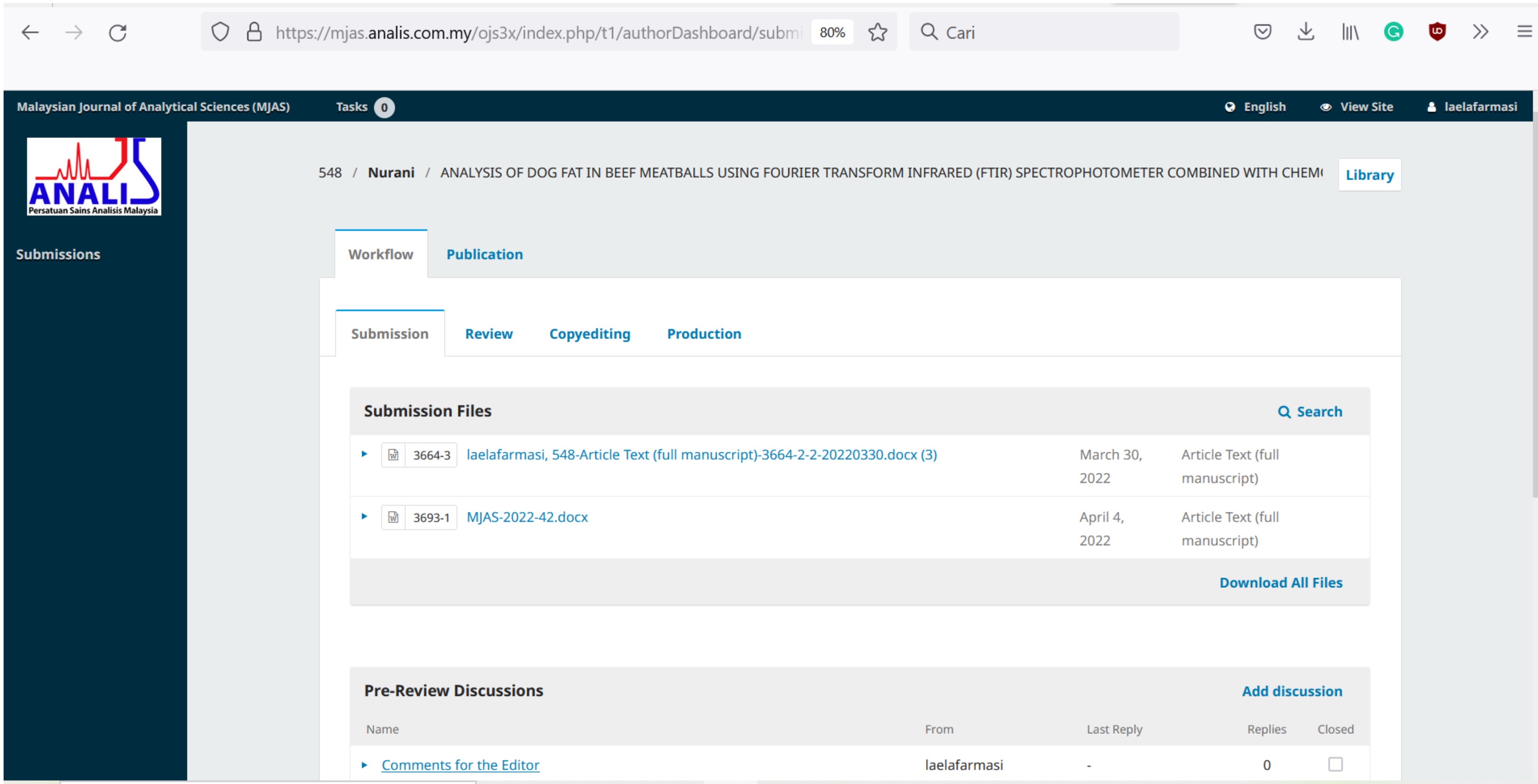
Finally, we would like to thank you once again for giving us the opportunity to improve our manuscript. We very much hope that these revisions are adequate. We appreciate your assistance and are looking forward to hearing from you.

Sincerely yours,

Laela Hayu Nurani
Faculty of Pharmacy
Universitas Ahmad Dahlan
laela.farmasi@pharm.uad.ac.id

Submission



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Workflow | Publication

Submission | Review | Copyediting | Production

Round 1

Round 1 Status
Submission accepted.

Notifications

[MJAS] Editor Decision	2022-05-16 11:49 PM
[MJAS] Editor Decision	2022-07-04 03:39 PM
[MJAS] Editor Decision	2022-08-06 12:04 AM

Reviewer's Attachments

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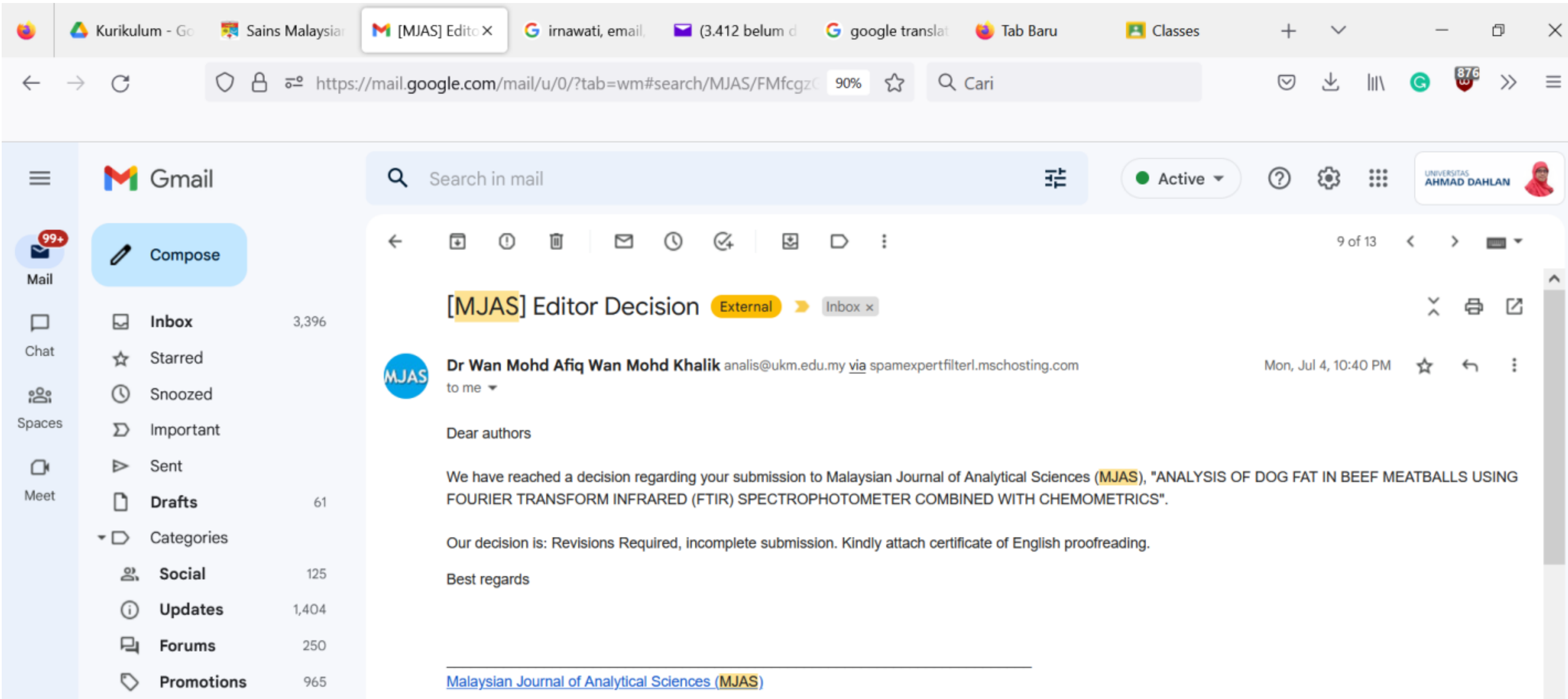
Name	From	Last Reply	Replies	Closed
Revision	laelafarmasi 2022-07-04 11:53 AM	-	0	
Manuscript revision_Professional proofread	laelafarmasi 2022-07-19 11:26 AM	-	0	



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Dr Wan Mohd Afiq Wan Mohd Khalik analis@ukm.edu.my via spamexpertfilterl.mschosting.com Mon, Jul 4, 10:40 PM

to me

Dear authors

We have reached a decision regarding your submission to Malaysian Journal of Analytical Sciences (MJAS), "ANALYSIS OF DOG FAT IN BEEF MEATBALLS USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROPHOTOMETER COMBINED WITH CHEMOMETRICS".

Our decision is: Revisions Required, incomplete submission. Kindly attach certificate of English proofreading.

Best regards

Malaysian Journal of Analytical Sciences (MJAS)

Round 1 dari reviewer B dan C:

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Malaysian Journal of Analytical Sciences (MJAS)

Submissions

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[MJAS] Editor Decision

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Dear authors

We have reached a decision regarding your submission to Malaysian Journal of Analytical Sciences (MJAS), "ANALYSIS OF DOG FAT IN BEEF MEATBALLS USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROPHOTOMETER COMBINED WITH CHEMOMETRICS".

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Best regards

Editor Malaysian Journal of Analytical Sciences

Reviewer B:

Recommendation: Revisions Required

----

1. Is subject matter falls in the scope of the journal?

1. Is subject matter falls in the scope of the journal?

Yes

2. Is the title of the article appropriate? (*If not, please suggest the alternative*)

Yes

Comments for the Author

3. Is abstract summarises clearly and concisely the major findings of the article?

No

4. Are objectives/aims of the study clearly defined?

No

5. Is study design appropriate with regard to type of study?

Yes

6. Is study design appropriate with regard to sampling?

Yes

7. Is study design appropriate with regard to variables measured?

Yes

8. Is study design appropriate with regard to data collection techniques?

Yes

9. Is study design appropriate with regard to moral and ethical issues?

Yes

10. Are results presented in a clear manner?

Yes

11. Are quantitative results statistically supported by appropriate statistical tests?

Yes

12. Are figures appropriate and correctly labelled?

Yes

13. Are tables appropriate and correctly labelled?

13. Are tables appropriate and correctly labelled?

Yes

14. Is discussion relevant with appropriate comparisons with similar studies?

Yes

15. Are conclusions made valid based on findings?

Yes

16. Are references correctly referred to, adequate, up to date and according to *M. Jour. Analy. Scien.*?

No

17. Recommendation

A. As a Research Article

Accept for publication, after necessary revisions,

B. As a Non-Research Article*

*Please specify as below:

Research Perspective,

Reviewer C:

1) This paper is interesting to read because the title is attractive and related to muslim issues (haram and halal food).

2) The abstract is not written well because no data about FTIR results.

3) Bahasa Melayu abstract not written properly because author wrote in Bahasa Indonesia.

4) Introduction too simple, need to write more about background of study and problem statement in details. Author need to relate the current method as well.

5) No information about Principles Component Analysis, PCA was selected and compare with others method.

6) Figure 1 has been labelled with a-om but no information related with this label in the text, no explanation.

7) Values for significant figures in Figure 2 is quite big which is 6 significant figure. need to shorten it just about 3 significant figures is enough.

8) Comparison study with the aid of Table is needed to proof that this study is equivalent or give a good results comparable to the previous study.

9) Graphical picture that summarize overall project is strongly recommended to ensure the reader understand with this method.

Recommendation: Revisions Required

Revisi 4 Juli 2022:

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Revision

Participants Edit

Dr Wan Mohd Afiq Wan Mohd Khalik (wanafiq)

ChM. Dr. Noorfatimah Yahaya (noorfatimah)

Laela Hayu Nurani (laelafarmasi)

Messages

Note	From
Dear Editors, Malaysian Journal of Analytical Sciences (MJAS) Please find our attached manuscript entitled "**Analysis of Dog Fat in Beef Meatballs Using Fourier Transform Infrared (FTIR) Spectrophotometer Combined with Chemometrics**," which we are submitting for consideration for publication as an Original Research article in ***Malaysian Journal of Analytical Sciences (MJAS)*** (**Number of Manuscript: 548**). We are thankful for your kind suggestions regarding our manuscript. Here, we are sending our revised manuscript in accordance with the comments given by reviewers. We have read through all the reviewers' suggestions very carefully, and made the necessary revisions based on these comments, as detailed below in a point-by-point format. The revised sections are highlighted in yellow color. We have added an author, Dzulfikar Muhammad Aditama which contributed to give additional discussions and layout. Finally, we would like to thank you once again for giving us the opportunity to improve our manuscript. We very much hope that these revisions are adequate. We appreciate your assistance and are looking forward to hearing from you. Sincerely yours, Laela Hayu Nurani Faculty of Pharmacy	laelafarmasi 2022-07-04 11:53 AM

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Best regards

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Participants Edit

Dr Wan Mohd Afiq Wan Mohd Khalik (wanafiq)

ChM. Dr. Noorfatimah Yahaya (noorfatimah)

Laela Hayu Nurani (laelafarmasi)

Messages

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Sincerely yours,

Associate Professor Dr Laela Hayu Nurani M.Si
Faculty of Pharmacy,
Universitas Ahmad Dahlan, Yogyakarta, Indonesia
Jl. Prof. DR. Soepomo Sh, Warungboto,
Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta. Indonesia

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Associate Professor Dr Laela Hayu Nurani M.Si

Faculty of Pharmacy,

Universitas Ahmad Dahlan, Yogyakarta, Indonesia

Jl. Prof. DR. Soepomo Sh, Warungboto,

Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta. Indonesia

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Our decision is to: Accept submission for publication. The final acceptance is subjected to the final proof. It will deliver to authors when it ready, expected in September/October 2022.

Best regards

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Dr Wan Mohd Afiq Wan Mohd Khalik analis@ukm.edu.my via spamexpertfilterl.mschosting.com · Sat, Aug 6, 7:04 AM

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Dear authors

We have reached a decision regarding your submission to Malaysian Journal of Analytical Sciences (MJAS), "ANALYSIS OF DOG FAT IN BEEF MEATBALLS USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROPHOTOMETER COMBINED WITH CHEMOMETRICS".

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MJAS <analis@ukm.edu.my>

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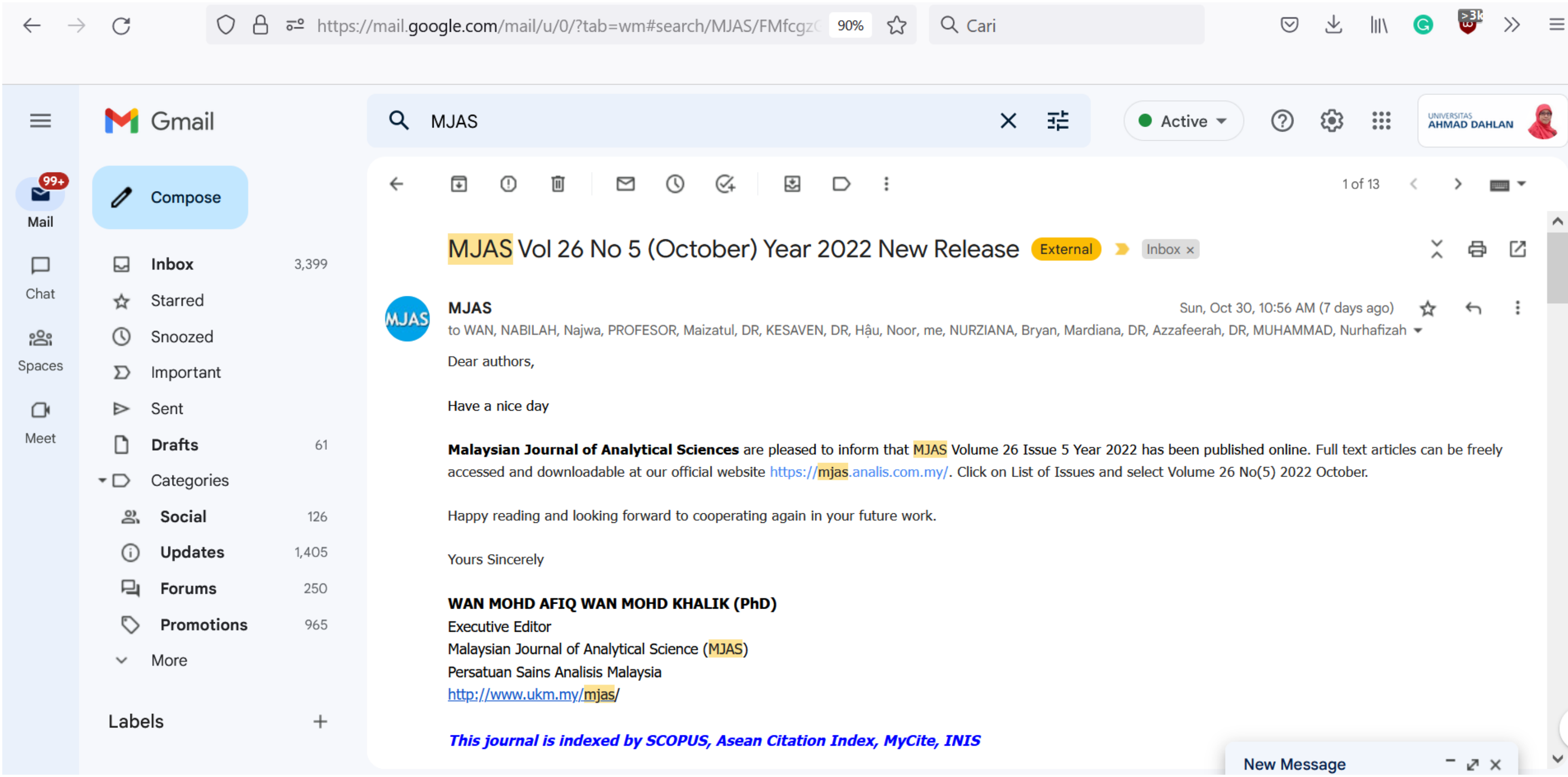

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MJAS
Sun, Oct 30, 10:56 AM (7 days ago)
to WAN, NABILAH, Najwa, PROFESOR, Maizatul, DR, KESAVEN, DR, Hậu, Noor, me, NURZIANA, Bryan, Mardiana, DR, Azzafeerah, DR, MUHAMMAD, Nurhafizah

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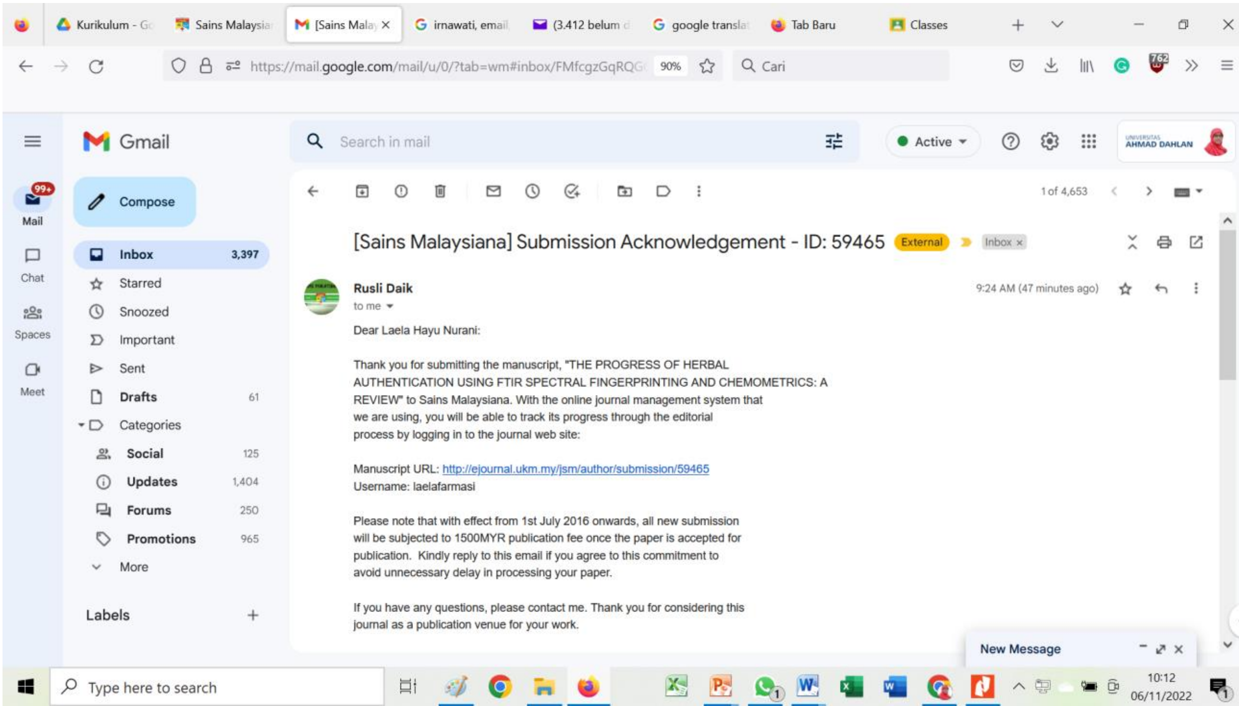

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Rusli Daik
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9:24 AM (47 minutes ago)

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Manuscript URL: http://ejournal.ukm.my/jsm/author/submission/59465
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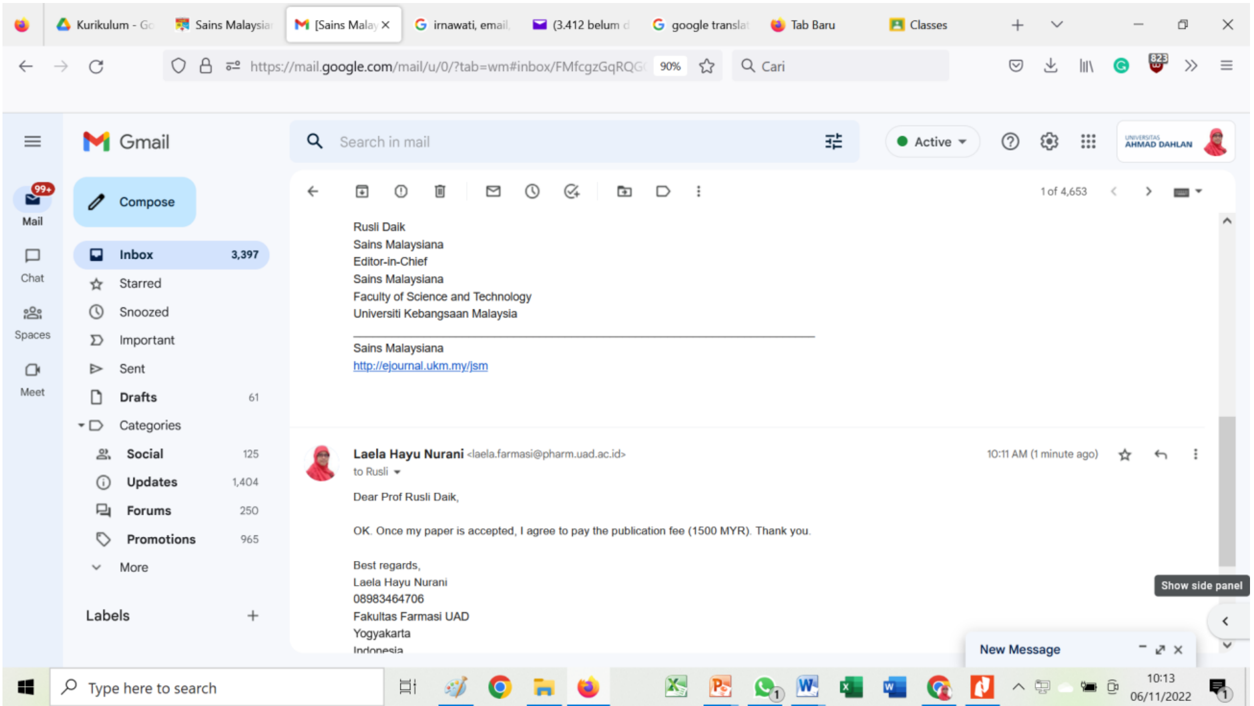
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Laela Hayu Nurani <laela.farmasi@pharm.uad.ac.id> 10:11 AM (1 minute ago)
to Rusli

Dear Prof Rusli Daik,

OK. Once my paper is accepted, I agree to pay the publication fee (1500 MYR). Thank you.

Best regards,
Laela Hayu Nurani
08983464706
Fakultas Farmasi UAD
Yogyakarta
Indonesia

Malaysian Journal of Analytical Sciences
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Published by Malaysian Analytical Sciences Society

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)

Laela Hayu Nurani[1,2*], Any Guntarti[1,2], Achmad Rizaldy[1], Citra Ariani Edityaningrum[1], Nina Salamah[1,2], Lalu Muhammad Irham[1], Dzulfikar Muhammad Aditama[1], Abdul Rohman[3,4]

[1]Faculty of Pharmacy,
Universitas Ahmad Dahlan Yogyakarta 55164, Indonesia
[2]Ahmad Dahlan Halal Center,
Universitas Ahmad Dahlan Yogyakarta 55164, Indonesia
[3]Center of Excellence, Institute for Halal Industry and Systems,
Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[4]Faculty of Pharmacy,
Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

**Corresponding author: laela.farmasi@pharm.uad.ac.id*

Received: 30 March 2022; Accepted: 6 August 2022; Published: xx October 2022

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 Yogjakarta 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 Yogjakarta 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 Na_2SO_4 (Merck®). The research method (workflow analysis) is as shown in Figure 1.

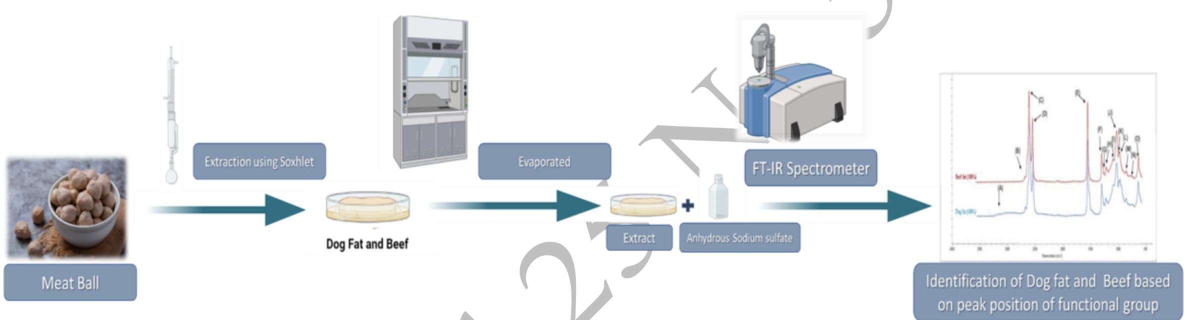

Figure 1. Workflow analysis of dog fat in beef meatballs using a Fourier-transform infrared spectrophotometer combined with chemometrics

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 Na_2SO_4, 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 (R^2), 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):

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

where $\hat{x}i$ is the actual value of meatballs, xi 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):

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

where $\hat{y}i$ is the actual value of meatballs, xi 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 Na_2SO_4 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].

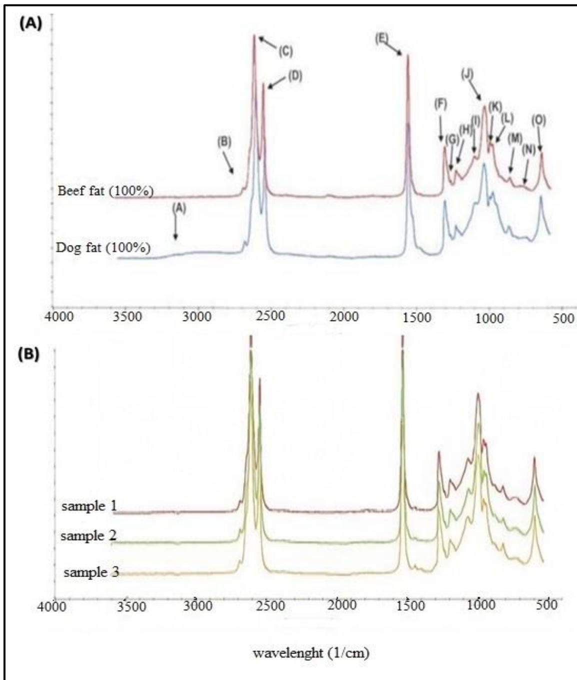

Figure 2. (a) Difference in the spectra of 100% beef meatball fat and 100% dog meatball fat and (**b**) the FTIR spectra of market beef meatball samples

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%.

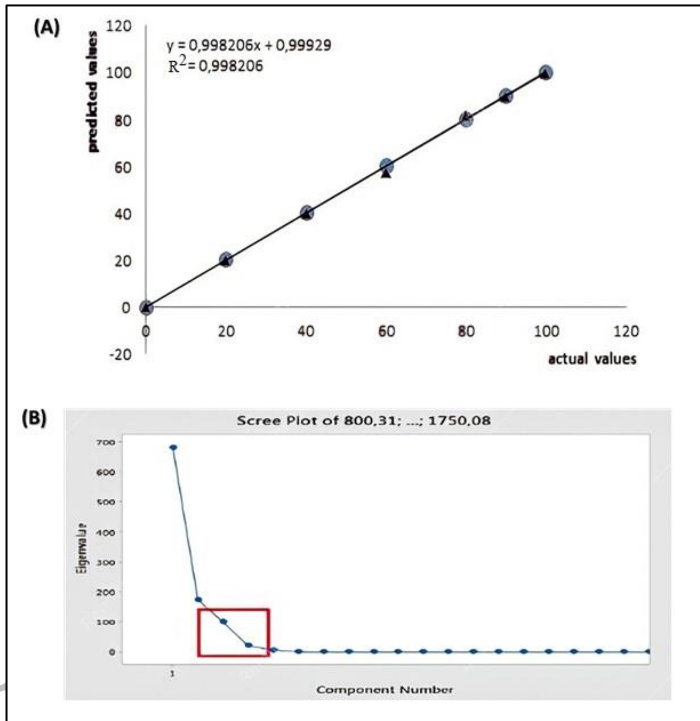

Figure 3. (a) The concentration variation of dog and beef fat resulted using the PLS data calibration model (0–100%) and (b) screen plot of the relationship between eigenvalues and PC results from the PCA

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).

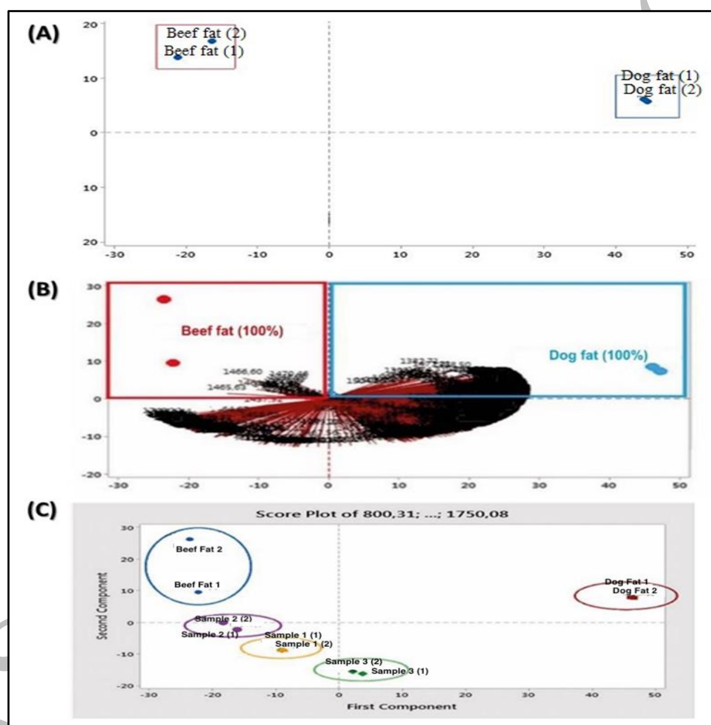

Figure 4. (a) Score plot PCA results in 100% dog fat and 100% beef fat. Note: red (beef fat) and blue (dog fat), (b) biplot of 100% dog fat and 100% beef fat, and (c) the score plot result of the PCA market sample, 100% dog, and 100% beef

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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AN ANALYSIS OF DOG FAT IN BEEF MEATBALLS USING FOURIER~~-~~-TRANSFORM INFRARED (FTIR) SPECTROPHOTOMET~~ER~~RY COMBINED WITH CHEMOMETRICS

Analisis Lemak Anjing dalam Bakso Sapi dengan Metode *Fourier Transform InfraRed* (FTIR) Dikombinasikan dengan Kemometrika

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Abstract

Bakso is a meatball made from beef ~~which~~ and is very popular among Indonesians. However, the increasing number of cases of counterfeit~~sing~~ ~~cases~~ and mixing of this meatball with dog meat in the city of Yogyakarta has caused significant unrest in several communities, especially ~~among~~ Muslims. This study aimed~~s~~ to detect the fat content of dog meat~~s~~ in meatballs circulating in the city of Yogyakarta ~~by~~ with an analysis using a combination of the FTIR method~~s~~ and the chemometric PCA ~~chemometrics~~. This research was designed ~~by~~ 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% ~~from~~ 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 ~~and~~as well as, with ~~obtained~~ the PLS calibration equation ~~of~~ 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 ~~analysis~~. In conclusion, the analysis results showed that the FTIR spectrophotometric method combined with chemometrics ~~is~~ was effective ~~in~~ at classifying dog fat from other animal fats. Meanwhile, the analysis results showed that 2 out of 3 samples contained other meat contaminants.

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Keywords: Dog meat, meatball, FTIR, PCA, PLS

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 Yogjakarta 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 Yogjakarta dengan menggunakan analisis gabungan kaedah FTIR dan kimometrik 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, FTIR, PCA, PLS

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~~d~~,~~;~~ *"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 ~~that should be consumed~~ and is a mandatory provision for Muslims [1]. Therefore, food is said to be halal if there is no evidence forbidding it; ~~but~~ 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 ~~between~~ 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 ~~and~~ 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 ~~chemometrics~~ (PCA) was used for the classification ~~between~~ of dog meat and beef meatballs [26–28].

Therefore, this research aim~~s~~eds to determine the presence of dog meat in meatball products with the partial least squares (PLS) model. The classification of ~~the~~ dog fat ~~with~~ and chicken fat ~~is~~ was performed using a principal ~~Principles~~ 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 ~~the~~ 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, ~~were~~ 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 ~~the~~ technical n-hexane (Merck®)~~,~~ and Na_2SO_4~~.~~ (Merck®). The research method (workflow analysis) is as shown in Figure 1.

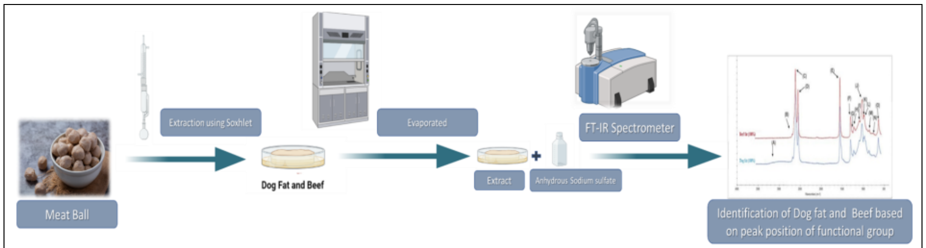

Figure 1. Workflow Analysis of Dog Fat in Beef Meatballs Using a Fourier-Transform Infrar~~-R~~ed (FT-IR) Spectrophotometer Combined with Chemometrics

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 the~~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 ~~with~~ in various concentrations can be seen in Table 1. ~~The~~ Meatballs were made by grinding beef and dog meat separately, ~~and it consisted~~consisting of 25 grams of meat. In addition, variations in ~~the~~ 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 Na_2SO_4, which evaporated in a fume hood. The viscous extract was analyzed ~~by~~ 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 ~~was~~ were dropped onto ~~the~~ 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. ~~The~~ A Microsoft Excel 2010 software worksheet was also used to relate the actual sample ~~(actual value)~~ to the predicted sample ~~(predicted value)~~ concentrations. The accuracy of the PLS model was evaluated by the coefficient of determination (R^2), 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 is

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

where~~:~~ $\hat{x}i$ is the~~=~~ actual value of meatballs,~~;~~ xi is the~~=~~ value calculated from cross-validation of meatballs,~~;~~ and n is the number of calibrations or validation samples [14]. ~~While~~ Meanwhile, the formula used to obtain the RMSEP is

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

where~~:~~ $\hat{y}i$ is the~~=~~ actual value of meatballs,~~;~~ xi 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 ~~through~~ 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]. ~~Furthermore,~~ The identification results indicated that the type of dog used was thea mutt otherwise known as a local dog with the Latin name *Canis lupus fa-miliaris*. 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].

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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, ~~to note~~whether the extraction process had been optimized~~,~~ was demonstrated by the turn of the color of ~~the~~ n-hexane ~~became~~ into dripping clear like its original color. Lastly, the addition of sufficient anhydrous Na_2SO_4 was intended ~~to bind~~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].

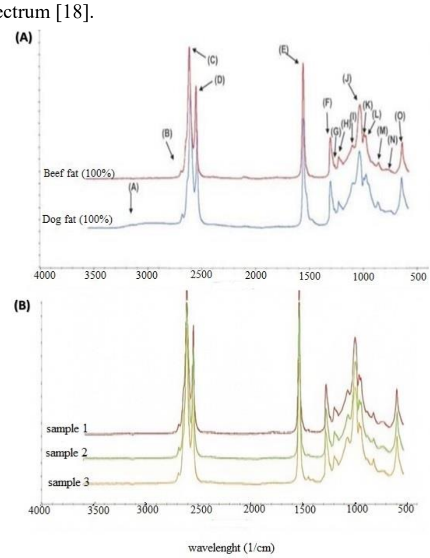

Figure 2 (**A**). Difference in the spectra of 100% beef meatball fat and 100% dog meatball fat (**B**). The FTIR

spectra ~~results from~~of market beef meatball~~s~~ samples.

Based on Figure 2 (A), the FTIR spectra ~~result~~ 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 ~~is~~ was deemed necessary to carry out a further analysis with the chemometric PCA ~~chemometrics~~ to distinguish the intensity of infrared absorption peaks, which are more varied, making it easier to classify ~~between~~ 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 ~~had~~ shared a similarity in functional fat groups. Carbonyl, CH, and CO groups appeared on the FTIR spectrogram. The difference ~~of~~ in the variations of such functional groups can be seen ~~the difference~~ with the chemometric PCA ~~chemometrics~~ [29].

Table 2. Identification of functional groups and vibrational types of the FTIR spectr~~um~~a 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 i~~o~~n 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 wave–numbers [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 ~~selection in the~~ wavenumbers selected ranged from 1,750 to ~~-~~800 cm^{-1}.

Additionally, the results of the optimization of the calibration model showed ~~the~~ an optimal range of wavenumbers ~~at~~ from 800 to ~~-~~1,750 cm^{-1} with the equation y = 0.99820x + 0.99992. Moreover, ~~; and~~ the resulting coefficient of determination (R^2) was 0.99820 (Figure 3), ~~;~~ with an RMSEC value of 1.464435 %. ~~Moreso,~~ 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%.

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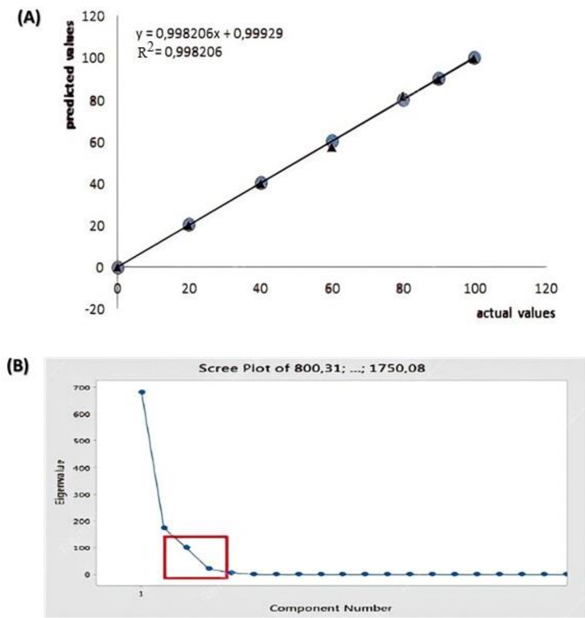

Figure 3 (**A**). The ~~results~~ concentration variation of dog and beef fat ~~of processing~~ resulted using the PLS data calibration model ~~concentration variation of dog and beef fat~~ (0–100%) (**B**). Screen plot of the relationship between eigenvalues and PC results from the PCA.

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 ~~the~~ market samples, in the 1,750–800 cm^{-1} area, which was the fingerprint area. ~~Subsequently,~~ From this frequency range, information ~~will be obtained~~ on which frequency contributes more to the PCA model will be obtained, and with the market samples, ~~would provide~~ a clear separation between dog and beef fats will be provided~~, with the market samples~~. The PCA was performed with the help of Minitab19 and was integrated with Microsoft Excel 2010 [25]. The chemometric PCA ~~chemometrics were~~ was selected ~~to~~ for grouping the variables of each sample used. With the variable of fatty acids ~~owned by~~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 ~~can~~ could be determined from the eigenvalues generated by each of the main components. Therefore, the number of PCs ~~gotten~~ gained was relevant ~~in~~ 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. ~~While~~ 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 ~~and is~~ was part of the elbow, where there ~~is~~ was a significant decrease~~.~~ ~~of the~~in eigenvalues.~~,~~ ~~with~~ Of the first 4 PCs, 99.2% of ~~the~~ data variance was included and ~~is~~ 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).

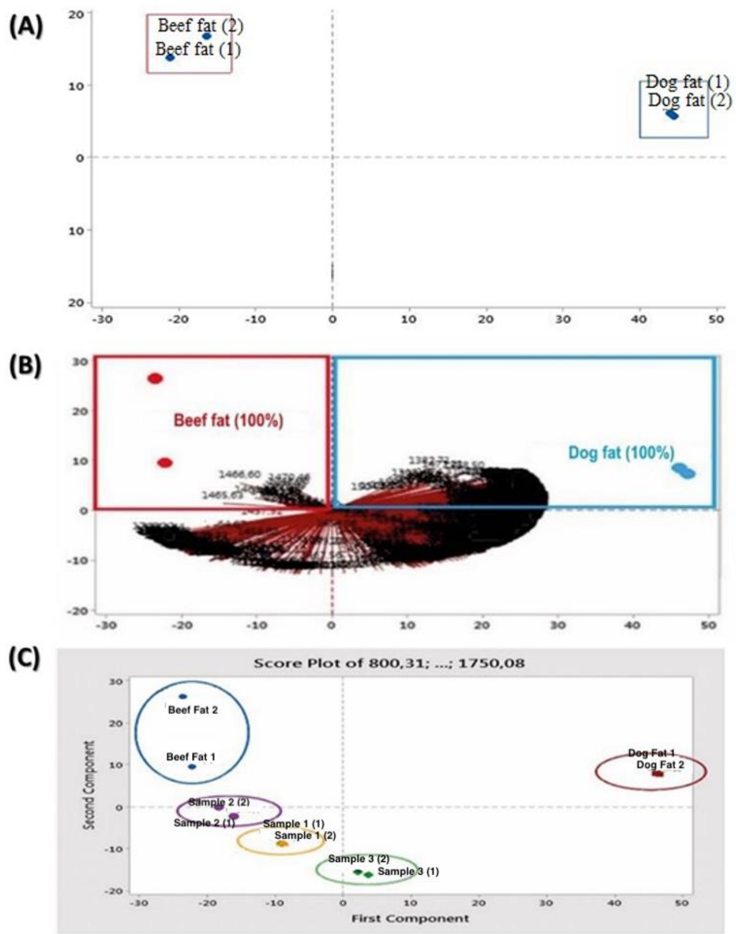

Figure 4 (**A**)~~.~~ Score plot PCA results in 100% dog fat and 100% ~~cow~~ beef fat. Note: red (~~cow~~ beef fat) and blue (dog fat), (**B**)~~.~~ Biplot of 100% dog fat and 100% beef fat, (**C**) The score plot result of the PCA market sample, 100% dog, and 100% beef

Figure 4 (A) shows the results of the PCA analysis of two samples occupying different quadrants. Sample A (red) consisteds of 2 beef tallows which are were separated by 100% and have had similar properties as a result of the close distance between the two fat plots which are were also within the same quadrant,. while sample B (blue) consisteds of two 100% dog fat data which is were found in different quadrants and at a great distance from sample A. Additionally, the two samples in the biB plots 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 are 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 have had special variables. The beef fat showsare was in the same quadrant with several variables, including 1,348.96 cm^{-1} – -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 which are 1,348.96 – cm^{-1}-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}, and are therefore all characteristics of the beef fat.

Comparatively, the results of the dog fat are were in the same quadrant with many variables, including 800.31 cm^{-1} –1,430.92 cm^{-1}; 1,475.28 – cm^{-1}-1,487.81 cm^{-1}; and 1,520.60 – cm^{-1}-1,727.91 cm^{-1}. Hence, it indicates that these wavenumber variables area characteristic of dog fat because they are 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 are were perfectly separated and are 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 several areas 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 have 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 analysis 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 have the sameshare 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. Therefore, This shows that sample 2 had similar characteristics with the beef fat. The further the plots of sample 1 and sample 3 from the 100% beef fat plot, the greater the difference in characteristics. Moreso. In the Figure 4 (C), the two samples (1 and 3) were most -likely not pure beef and were likely to containeed 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 thea calibration model equation y = 0.998206x + 0.99992, which was quite accurate with a predicted value of 99.82% of the actual value. This further obtained a value of theFurthermore, a coefficient of determination (R^2) of 0.998206,; an RMSEC by of 1.46%,; an RMSEP of 1.52%,; and an RMSECV by of 2.32% were obtained. In this regard, it can be concluded that the meatball samples in the market do did not contain dog fat, but are they were suspected to contain other types of fat.

Acknowledgements

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publication.

ANALYSIS OF DOG FAT IN BEEF MEATBALLS USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROPHOTOMETER COMBINED WITH CHEMOMETRICS

Analisis Lemak Anjing dalam Bakso Sapi dengan Metode *Fourier Transform InfraRed* (FTIR) Dikombinasikan dengan Kemometrika

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Abstract

Bakso is a meatball made from beef surimi which is very popular among Indonesians. However, the increasing number of counterfeiting cases and mixing of meatball with dog meat in the city of Yogyakarta have caused significant dismay among several communities, especially among Muslim community. This research aims to detect the content of dog fat in bakso circulating in the city of Yogyakarta. This research was designed by making 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% from the meat weight. Three of the calibrated samples were validated and extracted with Soxhlet using n-hexane solvent. The extracted fat was further analyzed by FTIR and processed with Minitab19 software. The results showed that the wavenumber ranged from 1750 to 800 cm^{-1} and obtained the PLS calibration equation of $y = 0.998206x + 0.999929$, R^2 value = 0.9982, RMSEC 1.37%, RMSEP 1.19%, and RMSECV 2.32%. Furthermore, the dog and beef fats were successfully classified using multivariate PCA analysis. In conclusion, the analysis results show that the FTIR spectrophotometric method combined with chemometrics is effective in classifying dog fat from other animals. Meanwhile, the analysis showed that 2 out of 3 samples contained other meat contaminants.

Keywords: Dog meat, meatball, FTIR, PCA, PLS

Abstrak

Bakso terbuat dari surimi daging sapi yang sangat populer di kalangan masyarakat Indonesia. Namun maraknya kasus pemalsuan dan pencampuran bakso dengan daging anjing di Kota Yogyakarta telah menimbulkan keresahan yang cukup signifikan di beberapa masyarakat khususnya di kalangan umat Islam. Penelitian ini bertujuan untuk mendeteksi kandungan lemak anjing pada bakso yang beredar di kota Yogyakarta. Penelitian ini dirancang dengan membuat variasi bakso yang terdiri dari 25 gram daging sapi dan daging anjing dalam sampel terkalibrasi 0, 20, 40, 60, 80, 90 dan 100%, serta bahan lain seperti tepung, bawang putih dan bumbu sebanyak 5% dari berat daging. Tiga sampel terkalibrasi divalidasi dan diekstraksi dengan Soxhlet menggunakan pelarut n-heksan. Lemak hasil ekstraksi selanjutnya dianalisis dengan FTIR dan diolah dengan *software* Minitab19. Hasil penelitian menunjukkan bilangan gelombang berkisar antara 1750 sampai 800 cm^{-1} dan diperoleh persamaan kalibrasi PLS y = 0,998206x + 0,9999929, nilai R^2 = 0,9982, RMSEC 1,37%, RMSEP 1,19%, dan RMSECV 2,32%. Selanjutnya, lemak anjing dan daging sapi berhasil diklasifikasikan menggunakan analisis PCA multivariat. Kesimpulannya, hasil analisis menunjukkan bahwa metode spektrofotometri FTIR yang dikombinasikan dengan kemometrik efektif dalam mengklasifikasikan lemak anjing dari hewan lain. Sementara itu, analisis menunjukkan bahwa 2 dari 3 sampel mengandung kontaminan daging lainnya.

Kata kunci: Daging anjing, bakso, FTIR, PCA, PLS

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 Muslim no. 1198 stated; *"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 requirement for food consumption and is a mandatory provision for Muslims [1]. Therefore, food is said to be halal if there is no evidence forbidding it but 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 trading 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 than other kinds 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 are prohibited by Islam belief to consume dog meat.

Several approaches are being used to detect and measure the fat content of dog and pork derivatives in food products. The first approach is done by determining the ratio among several chemical constituents of the products and ensuring that this ratio is constant. Secondly, it is done by finding certain markers on food products, both in the form of chemical content 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.

Therefore, this research aims to determine the presence of dog meat in meatball products with the Partial Least Square (PLS) model and to classify dog fat with chicken fat using Principles Component Analysis (PCA) with the FTIR method [11].

Materials and Methods

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The main materials used in this research were reference meatballs made from a mixture of beef obtained from the 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 of 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 street Special Region of Yogyakarta, Indonesia, and these samples were taken in October 2020. The solvents used in this research were the technical n-hexane (Merck®), and Na_2SO_4. (Merck®).

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The fat samples were analyzed using FTIR spectrophotometry. This analysis was carried out at a frequency of 4000-650 cm^{-1}. Following this, the sample was dropped onto the ATR crystal at a controlled temperature (25°C) and measurements were carried out on 32 scans at a resolution of 4cm^{-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 Minitab 19 software on a computer device was carried out. Partial Least Square (PLS) was used to determine the linearity. The Microsoft Excel 2010 software worksheet was also used to relate the actual sample (actual value) to the predicted sample (predicted value) 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 RMSECV is

$$\text{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{x}i - xi)^2}{n}}$$

Where: $\hat{x}i$= actual value of meatballs; xi=value calculated from cross-validation of meatballs; and n is the number of calibration or validation samples [14]. While the formula used to obtain the RMSEP is

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}i - yi)^2}{n}}$$

Where: $\hat{y}i$= actual value of meatballs; xi= predictive value of meatballs; and n is the number of calibration or validation samples [4].

Result and Discussion

Identification of Dog Species used as Sample

The identification of dog species was conducted through pictures of several parts of the animal's body such as the face, tail, legs, ears, and pictures of the combined parts as a whole body [15]. Furthermore, the identification results indicated that the type of dog used was a mutt otherwise known as a local dog with the Latin name Canis lupus fa-miliaris. 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, to note the extraction process had been optimized, the color of the n-hexane became dripping clear like its original color. Lastly, the addition of sufficient anhydrous Na_2SO_4 was intended to bind 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].

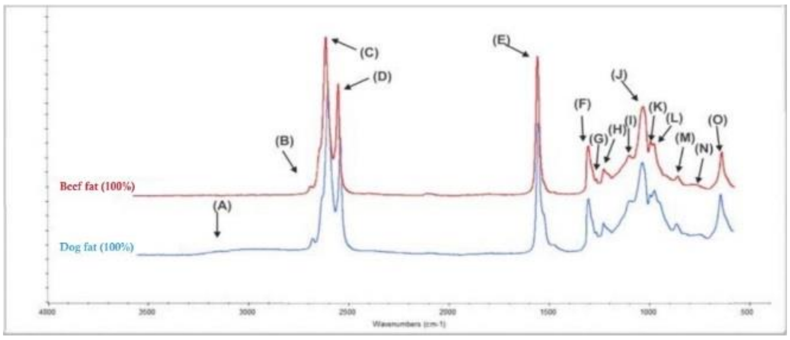

Figure 1. Difference spectra of 100% beef meatball fat and 100% dog meatball fat

Based on Figure 1, it can be seen that there is no significant difference between 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. The descriptions of the absorption peaks and the identified functional groups are summarized in Table 2.

Table 2. Identification of functional groups and vibrational types of the FTIR spectrum of Dog and Beef Fats

Peak position (cm^{-1})		Functional groups	Vibration type	Intensity
Dog	Cow			
3283	-	O-H	Stretching	Medium
3007	3003	C=C-H (cis)	Stretching	Medium
2921	2921	C-H(CH3)	Asymmetric stretching	Strong
2852	2852	C-H(CH2)	Asymmetric stretching	Strong
1744	1743	C=O (ester)	Stretching	Strong
1461	1462	C-H (CH2)	Bend scissoring	Strong
1418	1417	C=C-H (cis)	Bend (rocking)	Strong
1376	1376	C-H (CH2)	Bend Symmetrical	Strong
1230	1236	C-O (ester)	Stretching	Medium
1160	1159	C-O (ester)	Stretching	Medium
1115	1097	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 quantitative analysis which was carried out on 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 wave number [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 selection in the wavenumber ranged from 1750-800 cm^{-1}.

Additionally, the results of the optimization of the calibration model showed the optimal range of wavenumbers at 800-1750 cm^{-1} with the equation y= 0.99820x+0.99992; and the resulting coefficient of determination (R^2) was 0.99820 (Figure 2); with an RMSEC value of 1.464435 %. More so, the optimization results obtained 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%.

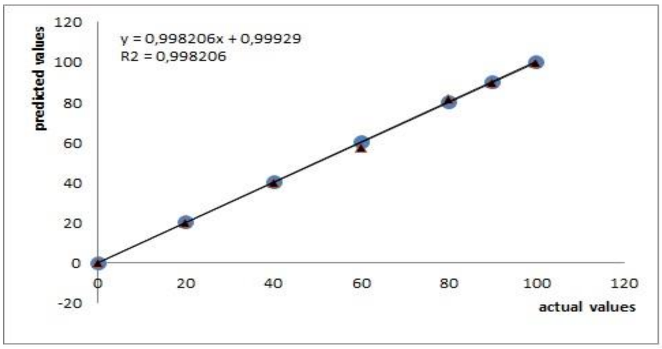

Figure 2. The results of processing the PLS data calibration model concentration variation of dog and beef fat (0-100%)

Pattern recognition analysis with Principal Component Analysis (PCA)

The PCA was performed using an absorbance dataset of dog and beef fats, in addition to the market samples in the 1750-800 cm^{-1} area which was the fingerprint area. Subsequently, from this frequency range, information will be obtained on which frequency contributes more to the PCA model and would provide a clear separation between dog and beef fats, with the market samples. The PCA was performed with the help of minitab19 and was integrated with Microsoft Excel 2010 [25].

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 PCA can be determined from the eigenvalues generated by each of the main components. Therefore, the number of PCs gotten was relevant in explaining the initial information from PC data with eigenvalue>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, PC1 with an eigenvalue of 683.35 was able to explain 69.3% of the variance in the initial variable. While 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% and PC4 with an eigenvalue of 21.17 was able to explain 99.2% and is part of the elbow, where there is a significant decrease. of the eigenvalues. With the first 4 PCs, 99.2% of the data variance was included and is relevant to explain the characteristics of the alert variable and the information contained [21].

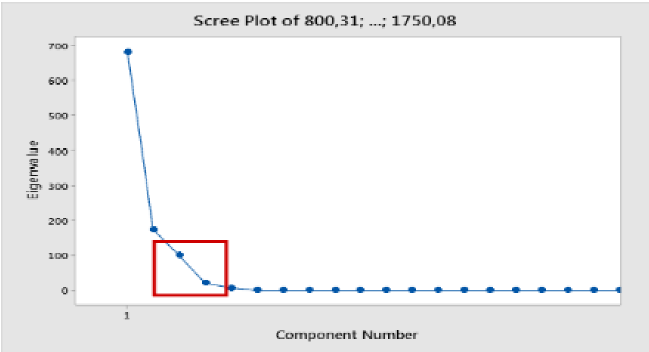

Figure 3. Scree plot of the relationship between eigenvalues and PC results from PCA

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.

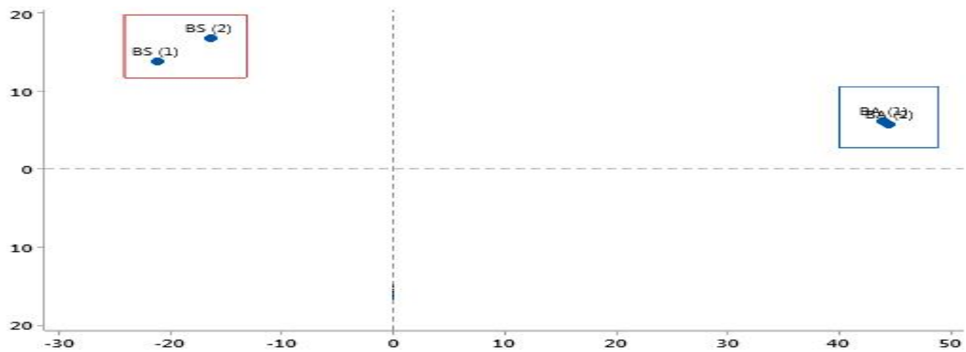

Figure 4. Score plot PCA results in 100% dog fat and 100% cow fat. Note: red (cow fat) and blue (dog fat)

Figure 4 shows the results of the PCA analysis of two samples occupying different quadrants. Sample A (red) consists of 2 beef tallows which are separated by 100% and have similar properties as a result of the close distance between the two fat plots which are also within the same quadrant. While sample B (blue) consists of two 100% dog fat which is found in different quadrants and at a great distance from sample A. Additionally, the two samples in the B plots were shown to be sticking together. Hence, the closer the distance between the two plots, the more the fat similarities while the farther apart the plots, the lesser the similarities between the fat. From this, it can be seen that sample A and sample B are well separated because they are in different quadrants [23].

Figure 5 shows that 100% beef fat (red) and 100% dog fat (blue) both have special variables. The beef fat shows the same quadrant with several variables, including 1348.96 cm^{-1} - 1360.53 cm^{-1}; 1453.10 cm^{-1}; 1456.96 cm^{-1}; 1457.92 cm^{-1}; 1465.63 cm^{-1}; 1466.60 cm^{-1} and 1470.46 cm^{-1}. Based on this, it can be said that the wave numbers which are 1348.96 cm^{-1}-1360.53 cm^{-1}; 1453.10 cm^{-1}; 1456.96 cm^{-1}; 1457.92 cm^{-1}; 1465.63 cm^{-1}; 1466.60 cm^{-1} and 1470.46 cm^{-1}, and are therefore all characteristics of the beef fat.

Comparatively, the results of the dog fat are in the same quadrant with many variables, including 800.31 cm^{-1}-1430.92 cm^{-1}; 1475.28 cm^{-1}-1487.81 cm^{-1}; 1520.60 cm^{-1}-1727.91 cm^{-1}. Hence, it indicates that these wavenumber variables area characteristic of dog fat because they are all in the same quadrant.

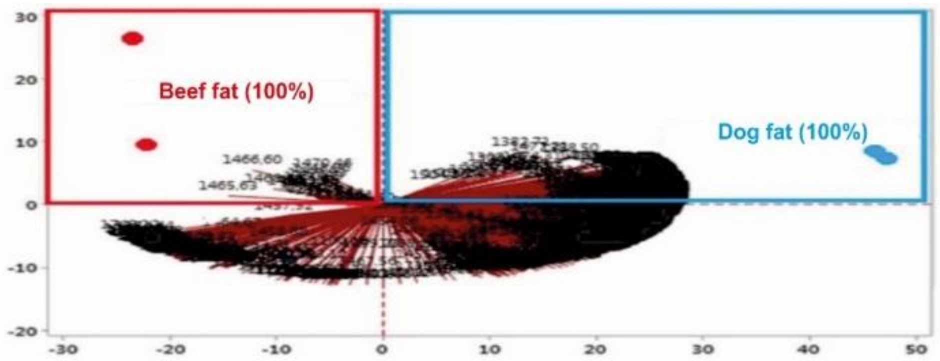

Figure 5. Biplot of 100% dog fat and 100% beef fat

Analysis of Beef Meatball Samples Circulating in Yogyakarta City

The grouping contained in the score plot of dog and beef fat has explained that the two samples are perfectly separated and are 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 several areas in Yogyakarta city. The results of the spectra were then analyzed by 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 6.

At first glance, the spectra of the market samples look the same, but the three fat spectra have 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 7.

The results of PCA analysis 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 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 have the same 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. Therefore, this shows sample 2 had similar characteristics with the beef fat. While the plots of sample 1 and sample 3 were very far from the beef fat plot, the further the sample plot is from the 100% beef fat plot, the greater the difference in characteristics. More so, the two samples were most not pure beef and contained other types of meat contamination, which can be proven by further research.

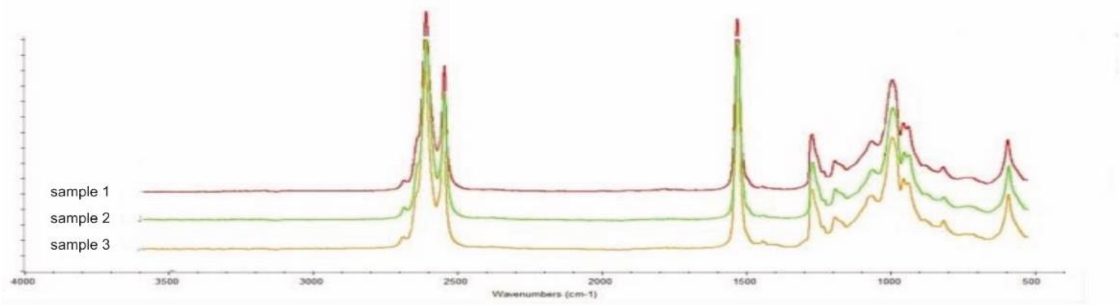

Figure 6. FTIR spectra results from market beef meatballs samples

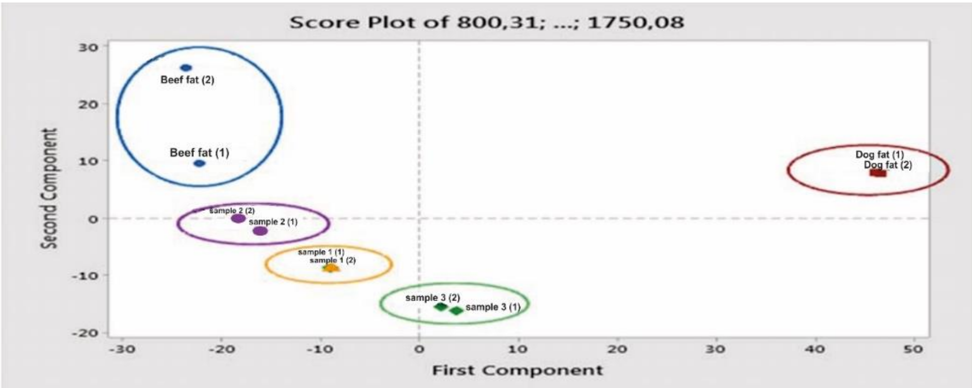

Figure 7. The score plot result of PCA market sample, 100% dog and 100% beef

Conclusion

The quantitative analysis of dog fat using PLS chemometrics resulted in optimization of wavenumbers in the range 1750-800 cm^{-1} with a calibration model equation y=0.998206x+0.99992 which was quite accurate with a predicted value of 99.82% of the actual value. This further obtained a value of the coefficient of determination (R2) of 0.998206; RMSEC by 1.46%; RMSEP 1.52%; and RMSECV by 2.32%. In this regard, it can be concluded that the meatball samples in the market do not contain dog fat but are suspected to contain other types of fat.

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ANALYSIS OF DOG FAT IN BEEF MEATBALLS USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROPHOTOMETER COMBINED WITH CHEMOMETRICS

Analisis Lemak Anjing dalam Bakso Sapi dengan Metode *Fourier Transform InfraRed* (FTIR) Dikombinasikan dengan Kemometrika

Abstract

Bakso is a meatball made from beef surimi which is very popular among Indonesians. However, the increasing number of counterfeiting cases and mixing of meatball with dog meat in the city of Yogyakarta have caused significant dismay among several communities, especially among Muslim community. This research aims to detect the content of dog fat in bakso circulating in the city of Yogyakarta. This research was designed by making 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% from the meat weight. Three of the calibrated samples were validated and extracted with Soxhlet using n-hexane solvent. The extracted fat was further analyzed by FTIR and processed with Minitab19 software. The results showed that the wavenumber ranged from 1750 to 800 cm^{-1} and obtained the PLS calibration equation of y = 0.998206x + 0.999929, R^2 value = 0.9982, RMSEC 1.37%, RMSEP 1.19%, and RMSECV 2.32%. Furthermore, the dog and beef fats were successfully classified using multivariate PCA analysis. In conclusion, the analysis results show that the FTIR spectrophotometric method combined with chemometrics is effective in classifying dog fat from other animals. Meanwhile, the analysis showed that 2 out of 3 samples contained other meat contaminants.

Keywords: Dog meat, meatball, FTIR, PCA, PLS

Abstrak

Bakso terbuat dari surimi daging sapi yang sangat populer di kalangan masyarakat Indonesia. Namun maraknya kasus pemalsuan dan pencampuran bakso dengan daging anjing di Kota Yogyakarta telah menimbulkan keresahan yang cukup signifikan di beberapa masyarakat khususnya di kalangan umat Islam. Penelitian ini bertujuan untuk mendeteksi kandungan lemak anjing pada bakso yang beredar di kota Yogyakarta. Penelitian ini dirancang dengan membuat variasi bakso yang terdiri dari 25 gram daging sapi dan daging anjing dalam sampel terkalibrasi 0, 20, 40, 60, 80, 90 dan 100%, serta bahan lain seperti tepung, bawang putih dan bumbu sebanyak 5% dari berat daging. Tiga sampel terkalibrasi divalidasi dan diekstraksi dengan Soxhlet menggunakan pelarut n-heksan. Lemak hasil ekstraksi selanjutnya dianalisis dengan FTIR dan diolah dengan *software* Minitab19. Hasil penelitian menunjukkan bilangan gelombang berkisar antara 1750 sampai 800 cm^{-1} dan diperoleh persamaan kalibrasi PLS y = 0,998206x + 0,9999929, nilai R^2 = 0,9982, RMSEC 1,37%, RMSEP 1,19%, dan RMSECV 2,32%. Selanjutnya, lemak anjing dan daging sapi berhasil diklasifikasikan menggunakan analisis PCA multivariat. Kesimpulannya, hasil analisis menunjukkan bahwa metode spektrofotometri FTIR yang dikombinasikan dengan kemometrik efektif dalam mengklasifikasikan lemak anjing dari hewan lain. Sementara itu, analisis menunjukkan bahwa 2 dari 3 sampel mengandung kontaminan daging lainnya.

Kata kunci: Daging anjing, bakso, FTIR, PCA, PLS

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 Muslim no. 1198 stated; *"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 requirement for food consumption and is a mandatory provision for Muslims [1]. Therefore, food is said to be halal if there is no evidence forbidding it but 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 trading 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 than other kinds 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 are prohibited by Islam belief to consume dog meat.

Several approaches are being used to detect and measure the fat content of dog and pork derivatives in food products. The first approach is done by determining the ratio among several chemical constituents of the products and ensuring that this ratio is constant. Secondly, it is done by finding certain markers on food products, both in the form of chemical content 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.

Therefore, this research aims to determine the presence of dog meat in meatball products with the Partial Least Square (PLS) model and to classify dog fat with chicken fat using Principles 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 the 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 of 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 street Special Region of Yogyakarta, Indonesia, and these samples were taken in October 2020. The solvents used in this research were the technical n-hexane (Merck®), and Na_2SO_4. (Merck®).

Identification of dog species

Identification of dog species was carried out in the Laboratory of Animal Systematics, Faculty of Biology, Gadjah Mada University, Yogyakarta.

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 with various concentrations can be seen in Table 1. The meatballs were made by grinding beef and dog meat separately and it consisted of 25 grams of meat. In addition, variations in the concentration of dog meat in 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 Na_2SO_4, which evaporated in a fume hood. The viscous extract was analyzed by FTIR Spectrophotometer [12].

Table 1. Variations in the Concentration of Beef and Dog Meatball Samples

Concentration	Beef (grams)	Dog Meat (grams)
Cow 100 %	25	-
Dog 100%	-	25
Dog 20 %	20	5
Dog 40%	15	10
Dog 60 %	10	15
Dog 80 %	5	20
Dog 90 %	2,50	22,50

Sample analysis with FTIR

The fat samples were analyzed using FTIR spectrophotometry. This analysis was carried out at a frequency of 4000-650 cm^{-1}. Following this, the sample was dropped onto the ATR crystal at a controlled temperature (25°C) and measurements were carried out on 32 scans at a resolution of $4cm^{-1}$ [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 Minitab 19 software on a computer device was carried out. Partial Least Square (PLS) was used to determine the linearity. The Microsoft Excel 2010 software worksheet was also used to relate the actual sample (actual value) to the predicted sample (predicted value) 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 RMSECV is

$$\text{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{x}i - xi)^2}{n}}$$

Where: $\hat{x}i$= actual value of meatballs; xi=value calculated from cross-validation of meatballs; and n is the number of calibration or validation samples [14]. While the formula used to obtain the RMSEP is

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}i - yi)^2}{n}}$$

Where: $\hat{y}i$= actual value of meatballs; xi= predictive value of meatballs; and n is the number of calibration or validation samples [4].

Result and Discussion

Identification of Dog Species used as Sample

The identification of dog species was conducted through pictures of several parts of the animal's body such as the face, tail, legs, ears, and pictures of the combined parts as a whole body [15]. Furthermore, the identification results indicated that the type of dog used was a mutt otherwise known as a local dog with the Latin name Canis lupus fa-miliaris. 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, to note the extraction process had been optimized, the color of the n-hexane became dripping clear like its original color. Lastly, the addition of sufficient anhydrous Na_2SO_4 was intended to bind 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].

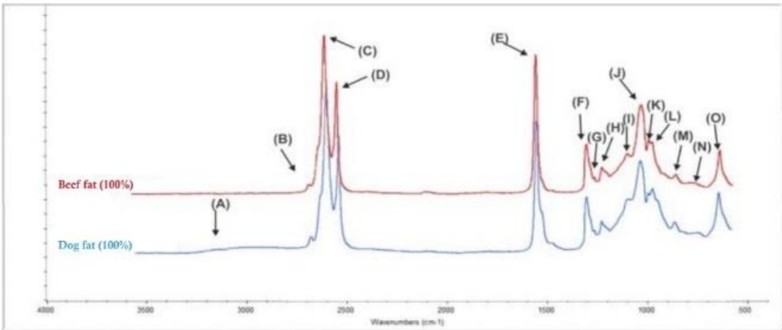

Figure 1. Difference spectra of 100% beef meatball fat and 100% dog meatball fat

Based on Figure 1, it can be seen that there is no significant difference between 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. The descriptions of the absorption peaks and the identified functional groups are summarized in Table 2.

Table 2. Identification of functional groups and vibrational types of the FTIR spectrum of Dog and Beef Fats

Peak position (cm^{-1})		Functional groups	Vibration type	Intensity
Dog	Cow			
3283	-	O-H	Stretching	Medium
3007	3003	C=C-H (cis)	Stretching	Medium
2921	2921	C-H(CH3)	Asymmetric stretching	Strong
2852	2852	C-H(CH2)	Asymmetric stretching	Strong
1744	1743	C=O (ester)	Stretching	Strong
1461	1462	C-H (CH2)	Bend scissoring	Strong
1418	1417	C=C-H (cis)	Bend (rocking)	Strong
1376	1376	C-H (CH2)	Bend Symmetrical	Strong
1230	1236	C-O (ester)	Stretching	Medium
1160	1159	C-O (ester)	Stretching	Medium
1115	1097	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 quantitative analysis which was carried out on 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 wave number [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 selection in the wavenumber ranged from 1750-800 cm^{-1}.

Additionally, the results of the optimization of the calibration model showed the optimal range of wavenumbers at 800-1750 cm^{-1} with the equation y= 0.99820x+0.99992; and the resulting coefficient of determination (R^2) was 0.99820 (Figure 2); with an RMSEC value of 1.464435 %. More so, the optimization results obtained 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%.

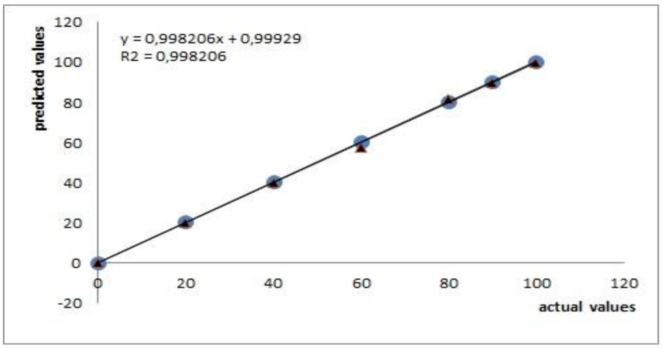

Figure 2. The results of processing the PLS data calibration model concentration variation of dog and beef fat (0-100%)

Pattern recognition analysis with Principal Component Analysis (PCA)

The PCA was performed using an absorbance dataset of dog and beef fats, in addition to the market samples in the 1750-800 cm^{-1} area which was the fingerprint area. Subsequently, from this frequency range, information will be obtained on which frequency contributes more to the PCA model and would provide a clear separation between dog and beef fats, with the market samples. The PCA was performed with the help of minitab19 and was integrated with Microsoft Excel 2010 [25].

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 PCA can be determined from the eigenvalues generated by each of the main components. Therefore, the number of PCs gotten was relevant in explaining the initial information from PC data with eigenvalue>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, PC1 with an eigenvalue of 683.35 was able to explain 69.3% of the variance in the initial variable. While 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% and PC4 with an eigenvalue of 21.17 was able to explain 99.2% and is part of the elbow, where there is a significant decrease. of the eigenvalues. With the first 4 PCs, 99.2% of the data variance was included and is relevant to explain the characteristics of the alert variable and the information contained [21].

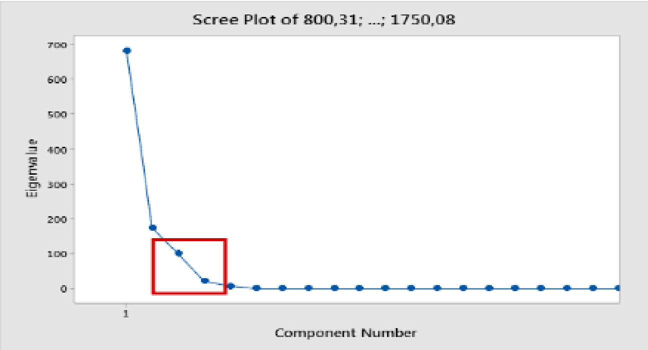

Figure 3. Scree plot of the relationship between eigenvalues and PC results from PCA

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.

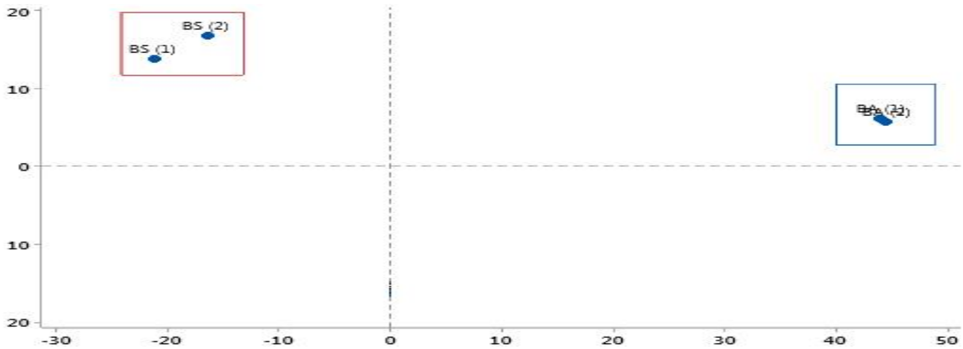

Figure 4. Score plot PCA results in 100% dog fat and 100% cow fat. Note: red (cow fat) and blue (dog fat)

Figure 4 shows the results of the PCA analysis of two samples occupying different quadrants. Sample A (red) consists of 2 beef tallows which are separated by 100% and have similar properties as a result of the close distance between the two fat plots which are also within the same quadrant. While sample B (blue) consists of two 100% dog fat which is found in different quadrants and at a great distance from sample A. Additionally, the two samples in the B plots were shown to be sticking together. Hence, the closer the distance between the two plots, the more the fat similarities while the farther apart the plots, the lesser the similarities between the fat. From this, it can be seen that sample A and sample B are well separated because they are in different quadrants [23].

Figure 5 shows that 100% beef fat (red) and 100% dog fat (blue) both have special variables. The beef fat shows the same quadrant with several variables, including 1348.96 cm^{-1} - 1360.53 cm^{-1}; 1453.10 cm^{-1}; 1456.96 cm^{-1}; 1457.92 cm^{-1}; 1465.63 cm^{-1}; 1466.60 cm^{-1} and 1470.46 cm^{-1}. Based on this, it can be said that the wave numbers which are 1348.96 cm^{-1}-1360.53 cm^{-1}; 1453.10 cm^{-1}; 1456.96 cm^{-1}; 1457.92 cm^{-1}; 1465.63 cm^{-1}; 1466.60 cm^{-1} and 1470.46 cm^{-1}, and are therefore all characteristics of the beef fat.

Comparatively, the results of the dog fat are in the same quadrant with many variables, including 800.31 cm^{-1}-1430.92 cm^{-1}; 1475.28 cm^{-1}-1487.81 cm^{-1}; 1520.60 cm^{-1}-1727.91 cm^{-1}. Hence, it indicates that these wavenumber variables area characteristic of dog fat because they are all in the same quadrant.

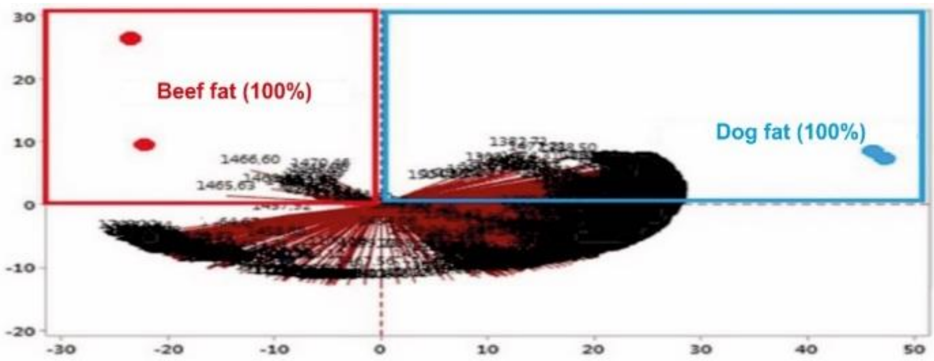

Figure 5. Biplot of 100% dog fat and 100% beef fat

Analysis of Beef Meatball Samples Circulating in Yogyakarta City

The grouping contained in the score plot of dog and beef fat has explained that the two samples are perfectly separated and are 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 several areas in Yogyakarta city. The results of the spectra were then analyzed by 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 6.

At first glance, the spectra of the market samples look the same, but the three fat spectra have 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 7.

The results of PCA analysis 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 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 have the same 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. Therefore, this shows sample 2 had similar characteristics with the beef fat. While the plots of sample 1 and sample 3 were very far from the beef fat plot, the further the sample plot is from the 100% beef fat plot, the greater the difference in characteristics. More so, the two samples were most not pure beef and contained other types of meat contamination, which can be proven by further research.

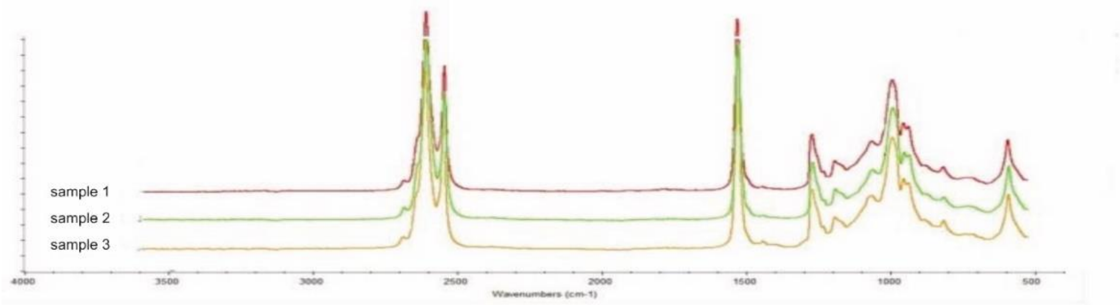

Figure 6. FTIR spectra results from market beef meatballs samples

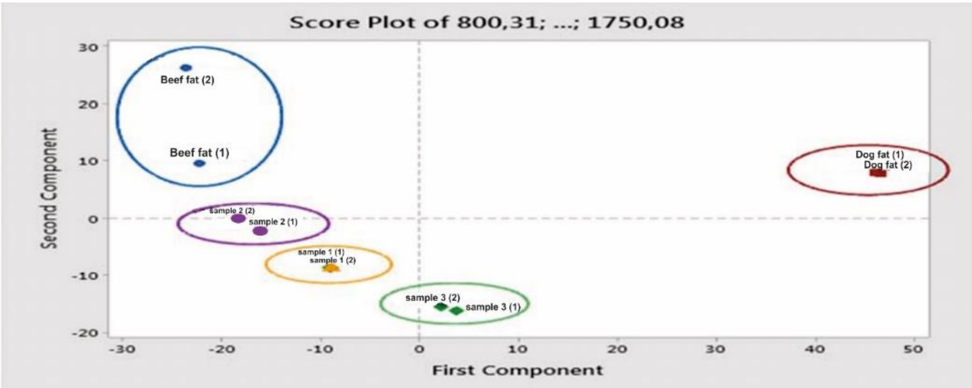

Figure 7. The score plot result of PCA market sample, 100% dog and 100% beef

Conclusion

The quantitative analysis of dog fat using PLS chemometrics resulted in optimization of wavenumbers in the range 1750-800 cm^{-1} with a calibration model equation y=0.998206x+0.99992 which was quite accurate with a predicted value of 99.82% of the actual value. This further obtained a value of the coefficient of determination (R2) of 0.998206; RMSEC by 1.46%; RMSEP 1.52%; and RMSECV by 2.32%. In this regard, it can be concluded that the meatball samples in the market do not contain dog fat but are suspected to contain other types of fat.

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