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Original Article

# IDENTIFICATION OF LARD ON PROCESSED PRODUCTS IN MEDAN CITY USING UV SPECTROPHOTOMETER WITH LINEAR DISCRIMINANT ANALYSIS AND PRINCIPAL COMPONENT ANALYSIS METHODS

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**ABSTRACT**

**Objective:** Processed meat products are highly popular among the community. However, deceptive traders sometimes adulterate these products with pork elements, necessitating thorough inspections. The qualitative detection of lard in processed products can be analyzed using UV spectrophotometry with chemometric techniques such as Linear Discriminant Analysis and Principal Component Analysis. These methods facilitate data analysis derived from spectra and wavelengths, enabling the categorization of objects and providing high accuracy.

**Methods:** This study aimed to determine whether processed products in Medan contain lard using UV spectrophotometry, Linear Discriminant Analysis, and Principal Component Analysis methods.

**Results:** The highest fat yield was obtained from lard at 14.24%, while the lowest was from chicken fat at 7.00%. The maximum wavelength results for control samples were 234 nm for chicken fat, 237 nm for beef fat, and 268 nm for lard. Data processing using Linear Discriminant Analysis and Principal Component Analysis showed that the processed products of three random samples, nugget, meatball, and sausage type A and C, fell within the same quadrant as chicken fat. Meatball and sausage type B were in the same quadrant as beef fat.

**Conclusion**: Based on the identification of lard in processed products in Medan City using UV spectrophotometer by LDA and PCA, all random samples of nuggets, meatballs, and sausages do not contain lard, and this method can classify chicken fat, beef fat, lard well.

**Keywords:** Identification, Lard, Processed products, UV Spectrophotometer, Chemometric


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## INTRODUCTION

One fundamental principle of food products is that their management and labelling should accurately reflect the ingredients present in the food. This is essential to ensure that consumers can easily identify the components within the food, especially given our society's diverse religious and cultural backgrounds. As highlighted in the guidance book on processed food labelling by the Indonesian Food and Drug Administration, labelling processed foods containing pork-derived ingredients requires a specific indication in the phrase "contains pork" [1].

The issue of dishonest traders adulterating processed products poses a significant problem within the community, leading to losses for consumers and food producers. Food contamination can occur due to mixing ingredients sourced from different parts of a pig into processed products. Furthermore, contamination can also arise during meat grinding using shared equipment, making it susceptible to mixing [2].

Many reasons cause the potential for pork contamination from the production process, one of which is that pork is cheaper due to the high birth rate of this animal. The current high price of beef has also triggered several producers to commit fraud by mixing pork into food preparations because there is much pork on the market, and it is sold at low prices to minimize production costs [3].

Various foods are often rumoured to contain lard, including meatballs, sausages, nuggets, and others. The cases of food contamination involve ingredients derived from pork, such as bacto-soytone, in seasoning products derived from pork. Contamination was also found in beef jerky, which contains traces of pork, and bulk meatballs containing pork DNA [2, 4].

Medan is the fourth largest city in Indonesia after Jakarta, Surabaya and Bandung. Located in North Sumatra and is one of the largest pork producers. This situation allows pork to be used in various food preparations. This fact underlies the selection of Medan as the sample area for this research [5].

Identifying fats in cattle, chicken, and pork involves Soxhlet extraction using the non-polar solvent n-hexane due to its non-reactive nature with other components, following the "like dissolved like." The continuous wetting of the sample with solvent in the Soxhlet process yields a more substantial extraction output. The instrument of choice is the UV spectrophotometer for its ability to analyze compounds with multiple bonds in fats (chromophores). It is known for its speed, affordability, and practicality in sample preparation. White (1965) reported maximum wavelength values for lard at 268 nm, chicken fat at 233 nm, and beef fat at 238 nm [6–8].

The dataset obtained from the UV spectrophotometer will be analyzed using chemometric methods, specifically Linear Discriminant Analysis (LDA) and Principal Component Analysis (PCA), to differentiate the content of chicken nuggets, meatballs, and sausages among chicken fat, beef fat, and lard. LDA offers the advantage of object classification and provides high accuracy in grouping results. On the other hand, PCA reduces the dataset dimensionality based on the principal components without sacrificing essential information, making analysis more manageable. A study by Geana *et al.* (2019) compared LDA results using UV-Vis spectrophotometry and FTIR spectroscopy showing superior classification results using UV-Vis spectrophotometry data, the accuracy of LDA using a UV-Vis spectrophotometer was 85.89% while using FTIR it was 51.50%, and research conducted by Suhandy and Yulia (2018), used coffee samples using a UV-VIS spectrophotometer and analyzed using LDA. The classification results can be successfully applied to classify luwak and non-luwak coffee samples with 100% accuracy for the LDA method [9–13].

Based on the description above, researchers are interested in identifying pork fat, chicken fat, and beef fat in processed products

using a UV spectrophotometer with the LDA and PCA methods using Unscrambler X 10.4 software.

## MATERIALS AND METHODS

### Tools and materials

The equipment used in this research included a UV-VIS spectrophotometer (Shimadzu UV Probe 1800) and Unscrambler X 10.4 software.

### Sample collection

Random sample collection was carried out purposively, with inclusion criteria being the absence of halal labelling and composition information on the nugget packaging. Chicken fat and beef fat were employed as negative controls, while lard was used as the positive control.

### Fat and sample preparation

Chicken, beef, and lard controls were cleaned of unused portions like meat and skin, washed, and finely chopped. The outer skin was peeled, and the inner portion was extracted for the nugget samples. The meatball and sausage samples were finely chopped, and each sample was homogenized using a chopper.

### Sample extraction using soxhlet

The cleaned and homogenized samples, wrapped in filter paper, totalling 50 grams, were placed into the thimble of the Soxhlet. Extraction was carried out with n-hexane solvent for 5 h at a temperature of 69 °C. Subsequently, the solvent was evaporated from the extracted material using a water bath to separate the fat from the solvent [8]. The obtained fat was cooled and weighed to determine the fat content in the sample using the equation:

$$\% \text{ Fat in Sample} = (b-a)/s \times 100\%$$

Description: a = weight of empty vial (g)

b = weight of empty vial (g)+fat (g)

s = weight of the sample (g)

### Qualitative test using UV spectrophotometer

The extracted fat, 0.1 ml in volume, was transferred into a 5 ml volumetric flask and dissolved in 5 ml of n-hexane. Subsequently, it was diluted by taking 0.1 ml from the 5 ml volumetric flask and dissolving it in 10 ml of n-hexane, resulting in a concentration of 0.2 µl/ml. The diluted solution was poured into a cuvette, and the wavelength was measured. This measurement process was repeated 15 times for each sample.

### Analysis of results using chemometrics

The UV spectrophotometer measurements yielded a dataset that will be further processed using chemometric analyses, specifically Linear Discriminant Analysis (LDA) and Principal Component Analysis (PCA), utilizing the Unscrambler X 10.4 software.

Linear Discriminant Analysis is one of the chemometric methods capable of distinguishing samples by grouping similar ones. The LDA output transforms the wave number data into two or three dimensions, providing a clearer visualization of grouping and the accuracy of differentiation among different samples. The steps for using LDA begin by inputting the dataset of wave number and absorbance results from the UV spectrophotometer. Then, transpose the data and add a column to the left of column 1 to input the sample categories. Fill in the category column according to the predetermined data. Once all categories are filled, click "define range" and specify the first column as the category column and absorbance based on the chosen wave number. Fill in the "rows" section according to the available data count, then analyze using Linear Discriminant Analysis.

Principal Component Analysis is a multivariate analysis method that transforms correlated original variables into new uncorrelated variables without losing essential information, thereby reducing dimensionality while retaining most of the variability of the original variables. The PCA method will also yield results in two and three dimensions. The initial steps for using PCA involve inputting the dataset of wave number and absorbance results from the UV spectrophotometer. Transpose the data and add a column to the left of column 1 for the sample categories. Populate the category column according to the established data. Once all categories are filled, click "define range" and specify the first column as the category column and absorbance based on the chosen wave number. Fill in the "rows" section according to the available data count, then analyze using Principal Component Analysis.

## RESULTS

### Sample extraction results using soxhlet

Extraction was performed using the Soxhlet method with n-hexane as the solvent. The highest yield was obtained from the positive control, lard, followed by the negative controls, beef fat and chicken fat. The percentage extraction yields can be observed in fig. 1 and table 1.

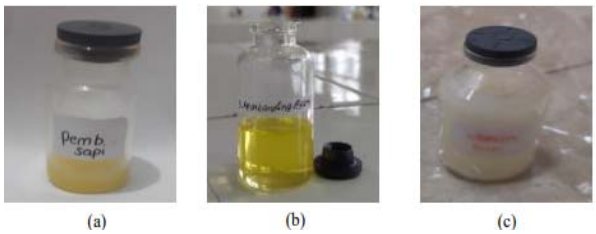


**Fig. 1: Result of extracted fat (a) beef fat (b) chicken fat (c) lard**

**Table 1: The wavelength of extracted fat**

| Group name | Description | Extraction yield (%) | Wavelength (nm) |
|---|---|---|---|
| Control | Chicken fat | 7,00 | 234 |
| | Beef fat | 12,25 | 237 |
| | Lard | 14,24 | 268 |
| Random Samples | Nugget A | 4,60 | 235 |
| | Nugget B | 4,35 | 234-235 |
| | Nugget C | 3,78 | 234 |
| | Meatball A | 3,33 | 233 |
| | Meatball B | 2,89 | 236 |
| | Meatball C | 1,25 | 233-234 |
| | Sausage A | 3,24 | 232 |
| | Sausage B | 3,03 | 240 |
| | Sausage C | 5,49 | 234 |

**Results of sample examination using uv spectrophotometer**

In this study, wavelength measurements were conducted on the extracted fats. The measurements were taken in the ultraviolet region within the 200-400 nm wavelength range, utilizing a sample concentration of 0.2 µl/ml. The specific wavelength results for each sample can be found in table 1, and a graphical representation is provided in fig. 2.

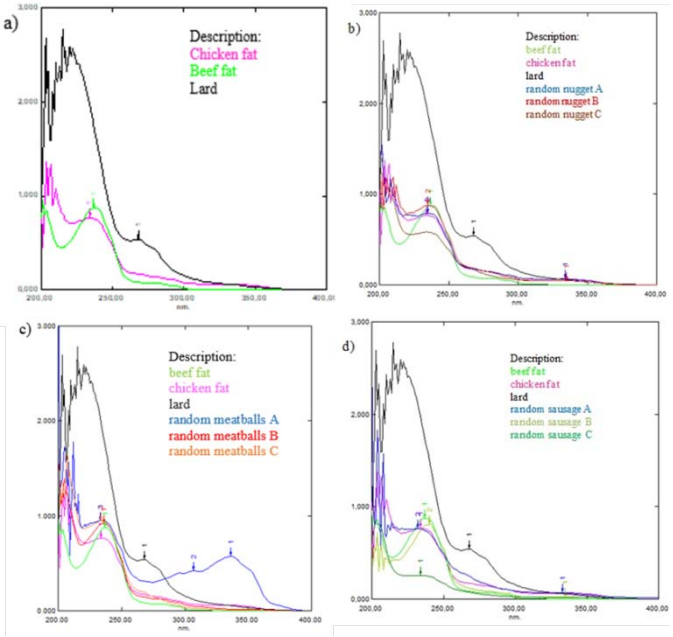


**Fig. 2: Overlay spectrum results: (a) fat control, (b) nugget sample, (c) meatball sample, (d) sausage sample**

**Linear discriminant analysis (LDA)**

The results of beef fat versus pork fat using random samples, the results obtained showed two groups: the blue circle group represents pork fat, while the orange circle group represents the beef fat group, which consists of beef fat itself and random samples A, B, and C from nuggets, meatballs, and sausages. The accuracy achieved from this initial analysis is 100%. 2D and 3D results can be seen in fig. 3.

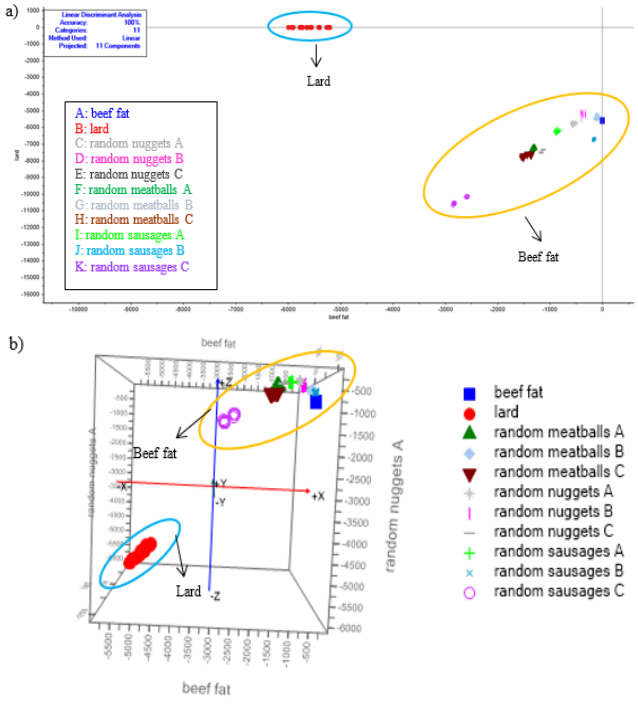


**Fig. 3: LDA results: (a) 2D beef fat, lard, and random samples; (b) 3D beef fat, lard, and random samples**

The results of Chicken fat versus pork fat using random samples obtained showed two groups: the light green circular cluster represents chicken fat, including chicken fat, random samples A, B, and C from nuggets, meatballs B and C, and sausage A and C. The light blue circular cluster represents the lard group. The accuracy achieved from this second analysis is 95.76%.

The 2D and 3D results can be observed in fig. 4.

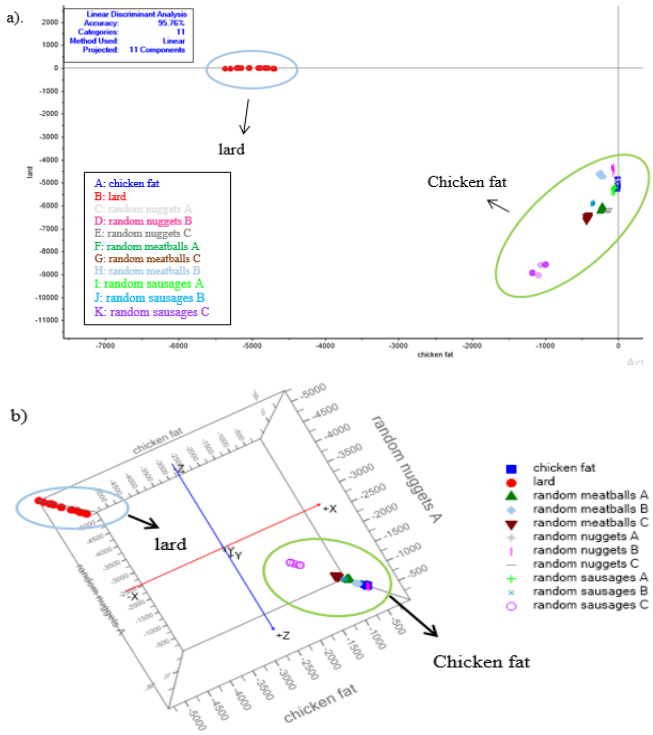


**Fig. 4: LDA results: (a) 2D chicken fat, lard, and random samples; (b) 3D chicken fat, lard, and random samples**

The results of Chicken fat versus beef fat using random samples showed two groups: the red circular cluster represents chicken fat, including chicken fat, random samples A, B, and C from nuggets, meatballs B and C, and sausage A and C. The green circular cluster represents the beef fat group, comprising beef fat, random samples of meatball B, and sausage B. The accuracy achieved from this third analysis is 100%. The 2D and 3D results can be observed in fig. 5.

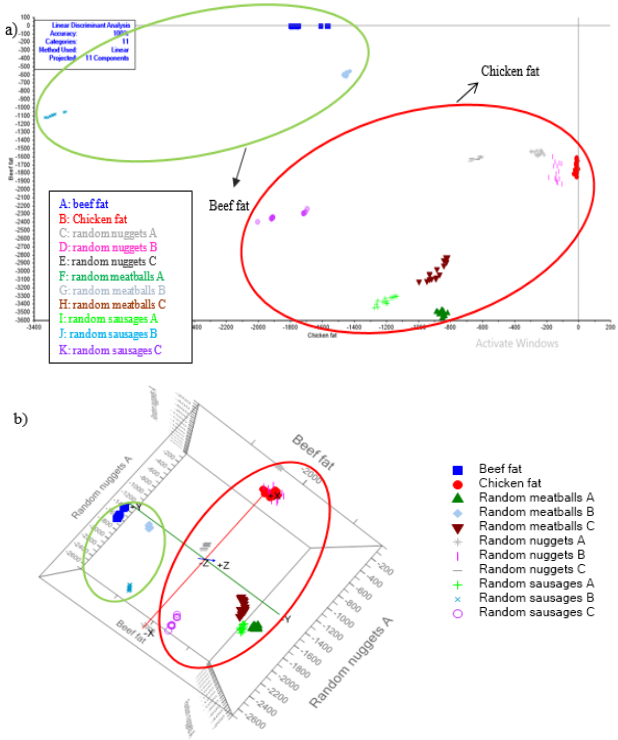


**Fig. 5: LDA results: (a) 2D chicken fat, beef fat, and random samples; (b) 3D chicken fat, beef fat, and random samples**

In the Principal Component Analysis (PCA) of beef fat, lard, and random samples, eigenvalues greater than one were obtained in PC 1, 10.8154. The PCA score plot results revealed two fat groups: the blue circular region (Quadrant I) represents the lard group, the orange circular region (Quadrant III) represents the beef fat group, including beef fat itself, and random samples A, B, and C. This implies that all these random samples are suspected to contain beef fat. The visual results can be observed in fig. 6.

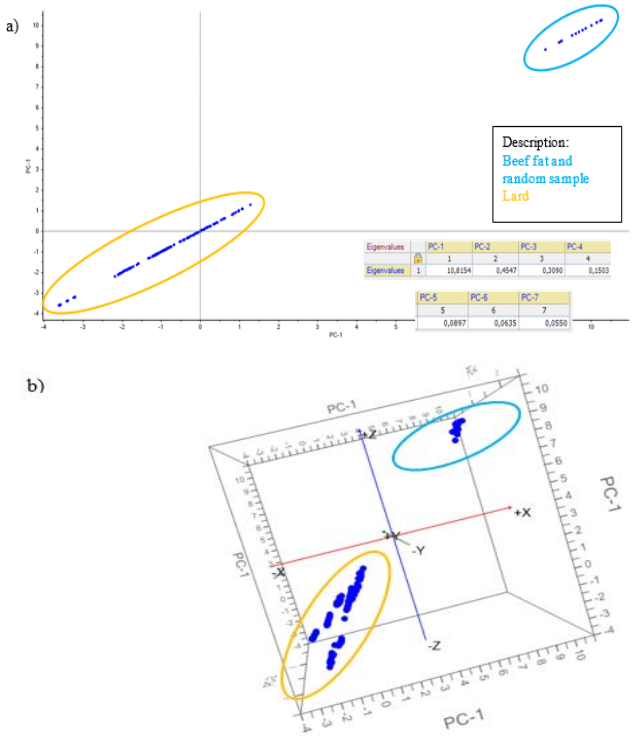


**Fig. 6: PCA results: (a) 2D beef fat, lard, and random samples; (b) 3D beef fat, lard, and random samples**

In the Principal Component Analysis (PCA) of chicken fat, lard, and random samples, eigenvalues greater than one were obtained in PC 1, 10.6797. The PCA score plot results revealed two fat groups: the green circular region (Quadrant III) represents the chicken fat group, including chicken fat itself and random samples A, B, and C. This suggests that all these random samples are classified as containing beef fat. The red circular region (Quadrant I) represents the lard group, comprising lard. The visual results can be observed in fig. 7.

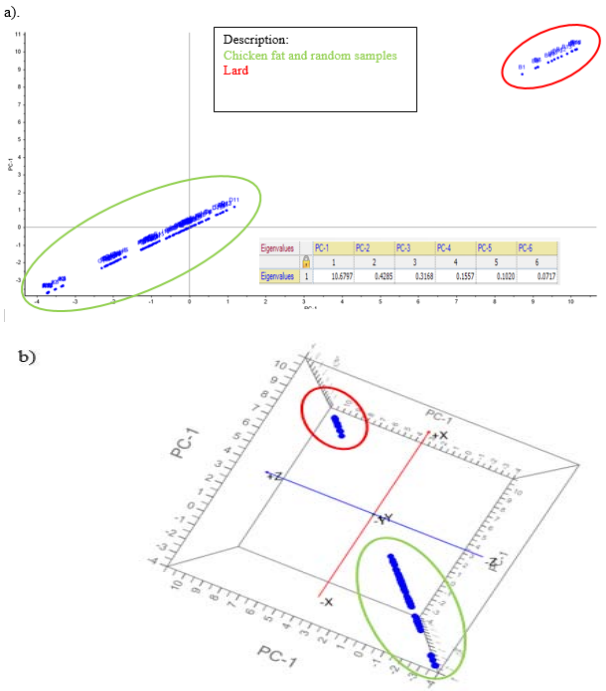


**Fig. 7: PCA results: (a) 2D chicken fat, lard, and random samples; (b) 3D chicken fat, lard, and random samples**

In the Principal Component Analysis (PCA) of chicken fat, beef fat, and random samples, eigenvalues greater than one were obtained in PC 1, 3.2891. The PCA score plot results revealed two fat groups: the purple circular region (Quadrant I) represents the chicken fat group, including chicken fat, random nugget samples A, B, and C, random meatball samples A and C, and random sausage samples A and C. The blue circular region (Quadrant III) represents the beef fat group, comprising beef fat, random meatball sample B, and random sausage sample B. The visual results can be observed in fig. 8.

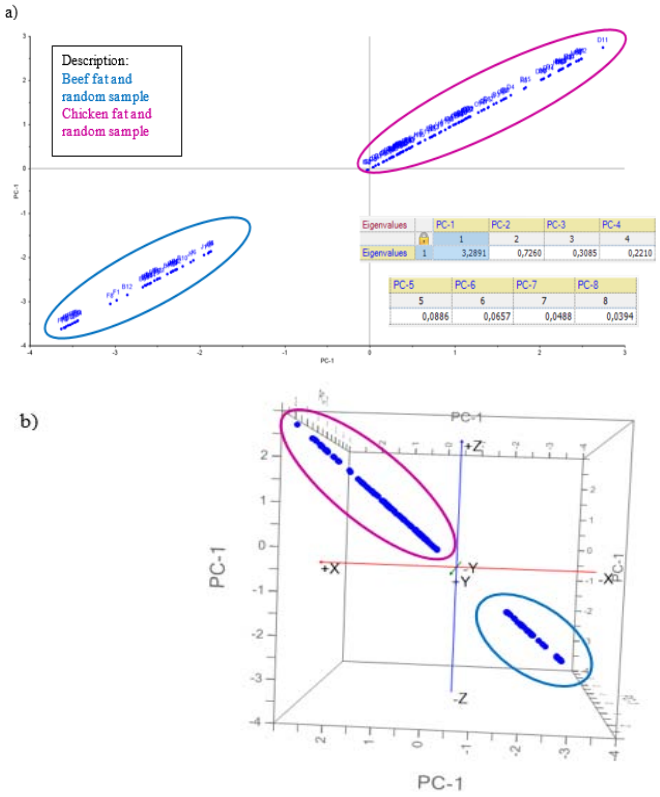


**Fig. 8: PCA results: (a) 2D chicken fat, beef fat, and random samples; (b) 3D chicken fat, beef fat, and random samples**

Dataset results from UV spectrophotometer measurements have been further processed using chemometric analysis, namely Linear Discriminant Analysis (LDA), and Principal Component Analysis (PCA) using Unscrambler X 10.4 software predict fat yields, which are classified into three, namely chicken fat, beef and pork fat. The prediction results of LDA and PCA can be seen in table 2.

**Table 2: Prediction of LDA and PCA outcome components**

| Place | Brand | Fat type | | |
|---|---|---|---|---|
| | | Chicken fat | Beef fat | Lard |
| Supermarket | Bulk nuggets | + | - | - |
| Supermarket | Bulk nuggets | + | - | - |
| Supermarket | Bulk nuggets | + | - | - |
| Traditional market | Homemade meatball | + | - | - |
| Traditional market | Homemade meatball | - | + | - |
| Traditional market | Homemade meatball | + | - | - |
| Supermarket | Bulk sausage | + | - | - |
| Supermarket | Bulk sausage | - | + | - |
| Supermarket | Bulk sausage | + | - | - |

## DISCUSSION

Extraction was carried out using the Soxhlet method with a non-polar solvent. This method was chosen for its ability to separate compounds effectively and yield a relatively high extraction of fatty acids. The extraction was performed using n-hexane solvent for 5 h, which indicated that the extraction yield increased with longer extraction times at the boiling point of n-hexane, 69 °C based on SNI 01-2891-1992 which explains that good extraction results require±6 h and strengthened by research conducted by Yuwana and Lesni (2019) stated that extraction results increased in number As the extraction time increases, this is proven by the research results which show that at the 2 hour the yield was 43.19%. At the 5 h, the yield was 44.25% [14]. N-hexane is a widely used solvent in previous research, being non-polar, does not react with other polar components due to the "like dissolved like" principle, and is insoluble in water, preventing water-soluble substances from being extracted and considered [9, 10].

Table 1 shows that the highest fat yield was obtained from the positive control of lard at 14.24%, followed by the negative control of beef fat at 12.25% and chicken fat at 7.00%. Chicken fat exhibited the lowest yield compared to pork and beef fat, attributed to its lower fat content compared to beef and pork. Chicken fat has a fat content of approximately 1.65%, beef fat is around 5%, and lard ranges from 6% to 11%. However, the results may be higher due to the lack of further testing on whether the solvent used in the Soxhlet process still contained fat, so it is recommended that to avoid an imperfect fat extraction process, an acrolein test ($KHSO_4$) can be carried out to detect triglyceride molecules contained in the solvent with the results negative if it has no odour and is brown [9, 10, 15].

UV spectrophotometry was chosen for identification due to its ability to analyze compounds with conjugated bonds in fats (chromophores), ease of sample preparation, practicality, and accurate precision. Based on fig. 1(a), The spectrum of each fat can be distinguished by looking at the shape and wavelength of the sample. Chickens have a sloping spectrum shape compared to cows,

with a maximum wavelength of 233 nm for chickens. Beef fat has a sharp spectrum shape with a maximum wavelength of 238 nm, and pork's spectrum shape is sloping compared to chicken and beef and has a maximum wavelength of 268 nm. The sample absorption value of each sample can vary because it is influenced by several variables, including the type of solvent, environmental pH, temperature and disturbing substances. The UV spectrophotometry maximum wavelength of chicken, beef, and lard controls aligns with previous studies, indicating chicken fat at 233 nm, lard at 268 nm, and beef fat at 238 nm. The tolerance limit for wavelength determination is not more than±2 nm from the specified wavelength, confirming that the identified wavelength of positive and negative control samples complies with the literature [6, 7, 11, 12].

Wavelength shifts can be attributed to bathochromic and hypsochromic effects. The bathochromic effect causes a shift in absorption towards longer wavelengths due to certain substituents/auxochromes on the chromophore. The hypsochromic effect involves a shift in absorption towards shorter wavelengths, which may result from changes in the solvent or the absence of certain substituents/auxochromes on a chromophore [6, 11, 16].

The results of the spectrum data obtained from the UV spectrophotometer test are then combined with chemometric analysis to make it easier to process the data obtained from the large and large value spectrophotometer into smaller ones. This model can also be used to estimate unknown samples. Chemometric testing using the Linear Discriminant Analysis method on beef, chicken, and pork fat samples and random samples. The analysis of beef fat, pork fat, and random samples found that the random samples were suspected to contain beef fat. This grouping is formed due to differences in characteristics between beef and pork fat, such as differences in wavelength and constituent groups, namely C20:4 (arachidonic acid) and C10:0 (capric acid). The analysis of chicken fat, pork fat, and random samples found that the random samples were suspected to contain chicken fat. This grouping was formed due to differences in characteristics between chicken and pork fat, such as differences in wavelength and constituent groups, namely C20:4 (arachidonic acid) and C10:0 (capric acid). The analysis of chicken fat, beef fat, and random samples found in the previous LDA experiment in fig. 2 showed that the random samples initially leaned towards chicken fat. However, after regrouping with chicken and lard, random samples of nuggets A, B, and C, meatballs B and C, and sausages A and C shifted closer to chicken fat. On the other hand, random samples of meatball A and sausage B are still aligned with chicken fat. This phenomenon is caused by differences in the constituents of chicken and beef fat in terms of wavelength and functional groups, namely C20:0 (arachidic acid) and C17:0 (margaric acid) [17–19].

Chemometric testing using the Principal Component Analysis method on beef, chicken, and pork fat samples and random samples. In testing beef fat, lard, and random samples, it can be concluded that random samples of nuggets, meatballs, and sausages are closer to beef fat, which indicates the presence of beef fat in the sample. The formation of different groups in different quadrants can be caused by differences in characteristics between beef fat and pork fat groups, especially in terms of wavelength and functional groups C20:4 (arachidonic acid) and C10:0 (capric acid). In testing chicken fat, pork fat, and random samples, it was concluded that the random samples of nuggets, meatballs, and sausages were closer to chicken fat, indicating beef fat in the samples. The formation of different groups in different quadrants can be caused by differences in characteristics between chicken fat and pork fat groups, especially in terms of wavelength and C10:0 (capric acid), C12:0 (lauric acid), C17: functional group 0 (acid margarat) and C20:0 (aracaric acid) [17–19].

In testing chicken fat, beef fat, and random samples, observations were made of two groups of random samples of nuggets, meatballs, and sausages in different quadrants. The formation of different groups in different quadrants can be caused by differences in characteristics between chicken fat and beef fat groups, especially in terms of wavelength and functional groups C20:0 (arachidic acid) and C17:0 (margaric acid) [17–19].

In the overall previous PCA results, comparing lard and beef fat revealed that the random samples were positioned in the same quadrant as beef fat. Similarly, in comparing lard and chicken fat, the random samples were positioned in the same quadrant as chicken fat. Moreover, the random samples could be separated by comparing chicken and beef fat.

Consequently, it can be concluded that the LDA result from random samples from nuggets A, B, C, meatballs, and sausages A and C fall within the chicken fat group, and random samples of meatballs B and sausage B are included in the beef fat group with high accuracy values. This is in accordance with research conducted by Suhandy and Yulia (2017) which states that the results of the LDA classification were successfully applied to classify luwak and non-luwak coffee samples with 100% accuracy, while the PCA results above show the quadrant differences between chicken, beef and fat. pork can be caused by variations in wavelength and functional groups found in various types of fat [12, 13].

## CONCLUSION

Based on tests carried out on processed products in Medan City using a UV spectrophotometer, the wavelength of samples suspected to contain chicken fat was 232-235 nm, beef fat 236-240 nm, pork fat 268 nm and grouping results using the Linear discriminant analysis method and Principal Component Analysis, this method can classify processed fat products well so that the results of the analysis that have been carried out on processed products in the city of Medan do not contain pork fat.

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## AUTHOR CONTRIBUTIONS

Concept–H. S., S. Y.; Design–H. S., F. R. H.; Supervision–H. S., S. Y.; Resources–H. S., F. R. H.; Materials–H. S., S. Y.; Data Collection and/or Processing–F. A. S., D. E. S., A. N. A.,; Analysis and/or Interpretation–H. S., F. A. S., D. E. S., A. N. A.,; Literature Search–H. S., F. A. S., D. E. S.; Writing–H. S., F. A. S., D. E. S., A. N. A.; Critical Reviews–S. Y., F. R. H.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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