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Spectra and carbonyl index changes on processed beef fats using fourier transform infrared spectrometer and principal component analysis

Aminullah Aminullah[a] (ID), Mardiah Mardiah[a] (ID), Lukmanul Hakim[a], Gustini Syahbirin[b] and Tetty Kemala[b]

[a]Department of Food Technology and Nutrition, Faculty of Halal Food Science, Djuanda University, Bogor, Indonesia; [b]Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia

ABSTRACT

Cooking processes such as frying, steaming, and roasting will modify or change the fatty acids composition in the fats. The objective of this research was to study and evaluate the changes in spectra profile included carbonyl index that occurs on cooked beef fats using Fourier Transform Infrared spectroscopy and principal component analysis, where the fat extraction using the Folch method. The results showed the spectra profiles of processed beef fats were modified at wavenumbers of $2028\,cm^{-1}$ which was C–H stretching vibrational, $1413\,cm^{-1}$ and $1218\,cm^{-1}$ of C–H (CH_2) scissoring bending, and $960\,cm^{-1}$ of CH=CH trans compared to raw beef fat. Steamed and fried beef fats have additional peaks at wavenumbers of $1239\,cm^{-1}$ and $757\,cm^{-1}$, respectively. These results also indicated that cooking processes have the potential to form trans fatty acids. In addition, the processes on beef fats also cause a decrease in the carbonyl index due to the formation of the volatile carbonyl. Finally, principal component analysis of infrared spectra showed that each processed beef fat can be clustered and classified as well as a fat extract from a beef meatball.

Introduction

High-temperature processing is widely applied to food materials for improving the hygiene quality by inactivating pathogenic microorganisms, improving flavors, as well as increasing the shelf life. However, generally, this processing will breakdown nutrient contents in foods such as protein and fat. The frying process is a process of food preparation by heating food in heat dispensing media in the form of oil. In this process, the oil is absorbed according to the amount of water that evaporates during frying. The amount of absorbed oil depends on the ratio between the middle and the inner layers. The thicker the layer leads to the more the oil will be absorbed.[1] Steaming is the process which utilizes water vapor as a conductor medium of heat. This process will change the texture of the meat to become soft gradually. In general, the frying temperatures are between 177–211 °C, while the steaming temperature is around 100 °C which is the boiling point of water.[2] Meanwhile, roasting is the cooking process using the heat of fire directly. The heat transfer is mostly through heat radiation where the heat can reach 260 °C.[3]

In general, the cooking processes which are applied on food will damage the fat properties. The degree of damage varies greatly depending on the processing temperature and time. The higher the temperature used, the more severe the fat damage.[4] One of the causes of damage to the fat is oxidation, where most of the unsaturated fatty acids will be damaged and produce volatile components such as ketone and aldehyde.[5] These components are related to carbonyl index, where it is the intensity ratio of the carbonyl absorption band (i.e. C=O) to that of another band (such as C–H stretching which occurs at $1462\,cm^{-1}$) in the spectrum.[6] Galliard[7] reported an increased carbonyl index once FFA reached a maximum, and that off-flavor scores observed by panelists dramatically

increased with increasing carbonyl formation. In addition, the cooking process can lower the fat content of food and its fatty acids, both essential and non-essential fatty acids. The fat content of uncooked beef was 17.2%, while if cooked at 60 °C, the fat content will drop to 11.2–13.2%. Ketaren[8] stated that the oil absorption into the material during the frying process will increase the saturated fatty acids concentration. FTIR spectrometer is used to analyze typical spectrum patterns that can be described as fingerprints of a fat product. Therefore, this method can be applied to test the authenticity and also to detect contamination in a food product.[9] Nurulhidayah et al.[10] analyzed the authenticity of beef butter using this method. Zahir et al.[11] reported the physicochemical properties and the quality evaluation of cooking oil using an FTIR spectrometer. In addition, This method was also used, with chemometric analysis, by Faridah et al.[12] to detect FFA and PV values in palm oil frying activities. Principal component analysis (PCA) is the most common method for processing multivariate data using data reduction, and it is often used in analyzing spectra data. Mueller et al.[13] used this analysis to identify the vegetable oil varieties in biodiesel. Selaimia et al.[14] used PCA in FTIR spectra to assess the oxidation effects on the characteristics of extra virgin olive oil. In addition, Dupuy et al.[15] classified edible fats and oils using PCA and FTIR spectra. However, data on spectra changes that are caused by cooking types in animal fats, included carbonyl index and classification and differentiation using chemometrics analysis, are less studied.

In this study, the different cooking processes such as frying, steaming, and roasting will be treated on raw beef fat, and the changes in the spectra profile will be studied and evaluated using Fourier Transform Infrared (FTIR) spectrometer and principal component analysis. For fat extraction, the Folch method from the previous study will be applied.[16,17]

Material and methods

Beef fats from the traditional market of Bogor Indonesia were fried, steamed, and roasted with 2 replication for each process. The meatball used was a commercial product taken from the market. First, the total lipid of all extracted beef fats using the Folch method was calculated. Then, infrared profiles of processed beef fats and BMB were analyzed using FTIR Nicolet i5 spectroscopy Thermo Fischer (Thermo Fischer Inc./PT. Alphasains Dinamika). The liquid of processed beef fats was placed on the plate of the FTIR spectrometer. Each time measuring the sample, the plate was cleaned using pure ethanol solvent pro-analysis, then the air spectrum was used as a background and recorded in graph and transmittance value. The FTIR spectrum was analyzed at wavenumbers of 4000–600 cm^{-1} with a 36 times scan.

Beef fat frying[18]

Fresh beef fat was weighed as much as 50 grams and then put into 160 °C of palm oil for 3–6 minutes. Then, the temperature in the middle of beef fat was measured using a thermometer. The beef fat was lifted and drained when the temperature was constant and then called fried beef fat (FBF).

Beef fat steaming[18]

Fifty grams of fresh beef fat was steamed at 100 °C for 30 minutes. Then, the temperature was measured using a thermometer in the middle of beef fat. After the temperature was constant, the beef fat was lifted and drained as steamed beef fat (SBF).

Beef fat roasting[19]

Fifty grams of fresh beef fat was washed and drained. Then, it was roasted at 73–82 °C for 3–5 minutes on a roaster with a distance of ±4 cm between the burned charcoal and the beef fat. After that, the beef fat was lifted and cooled as roasted beef fat (RBF).

Fat extraction method

The fat extraction method of beef fat was based on the previous studies where the selected method was the Folch method.[16,17]
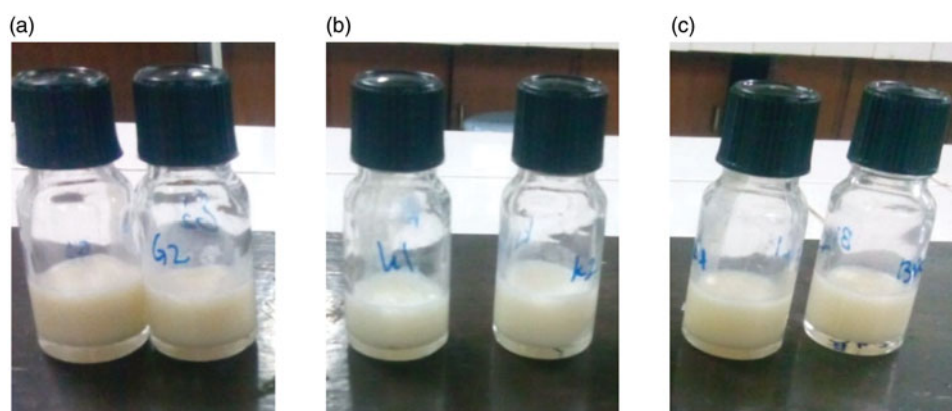
Figure 1. Extracts of (a) fried, (b) steamed, and (c) roasted beef fats using the Folch method.

Chloroform-methanol solvent (2:1 v/v) was added into a beaker glass of 5 grams sample until it reaches a final volume of 100 mL. The mixture was homogenized for 15–20 minutes using a magnetic stirrer at 300 rpm at room temperature. Then, it was filtered using a Buchner funnel with the help of a vacuum filter. Furthermore, it was washed by 20 mL of 0.88% NaCl solution. Then homogenized again for 1 minute and waited for 2 phase separation. The upper phase was removed and the chloroform phase containing lipid was evaporated.[20]

Estimation of carbonyl index[21]

The changes in carbonyl composition can be characterized by the carbonyl index, which was calculated by Eq. (1),

$$CI = A_{1700} / \sum A \tag{1}$$

where A_{1700} was absorption at around $1700 \, \text{cm}^{-1}$, and $\sum A$ was $A_{1700} + A_{1600} + A_{1460} + A_{1376} + A_{1030} + A_{864} + A_{814} + A_{743} + A_{724} + A_{(2953, 2923, 2862)}$.

Data analysis

Total lipids of raw and beef fats were analyzed using ANOVA of SPSS® ver 23 and Duncan Multiple Range Test (DMRT) for α of 5%. The infrared spectra were processed using OMNIC Software and Principal Component Analysis in The Unscrambler® X Software.

Table 1. Total lipid on extracts of raw and processed beef fats.

| Beef fat	Initial weight	Final weight	Total lipid (%)
Raw[15]	5.0305	4.1114	81.73 ± 0.4469[b]
Fried	5.1089	4.4154	86.43 ± 0.8466[c]
Steamed	5.0508	3.9947	79.09 ± 0.0109[a]
Roasted	5.2635	4.1412	78.68 ± 0.7159[a]

Note: Different superscripts show a significant difference at α of 5%. The value of total lipid is in mean ± standard deviation.

Result and discussion

Total lipid on processed beef fats

The extracted beef fats, which have been processed by frying, steaming, and roasting, can be seen in Fig. 1. Total lipids of these fats are then calculated and shown in Table 1. Analysis of variance shows that the type of processing has a significant effect ($p < 0.05$) on the total lipid of beef fat. The frying process increases the total lipid level from 81.73% to 86.43%. This process is very popular because it produces preferred flavor, color, and crunchy texture to the products.[22] It also increases total lipids in buffalo meat,[23] skipjack,[24] and duck meat[25] as a result of the absorption of cooking oil into food materials.[1,8]

In contrast to the frying process, total lipids of steamed and roasted beef fats are slightly lower than that of raw fat. This is due to several chemical reactions such as hydrolysis and oxidation during the cooking process. Hydrolysis, which is triggered by water vapor and heat, can break down the fat compounds into glycerol and fatty acids. Glycerol which has polar properties will dissolve in water or water vapor. In addition, Prabandari et al.[26] stated that the heating
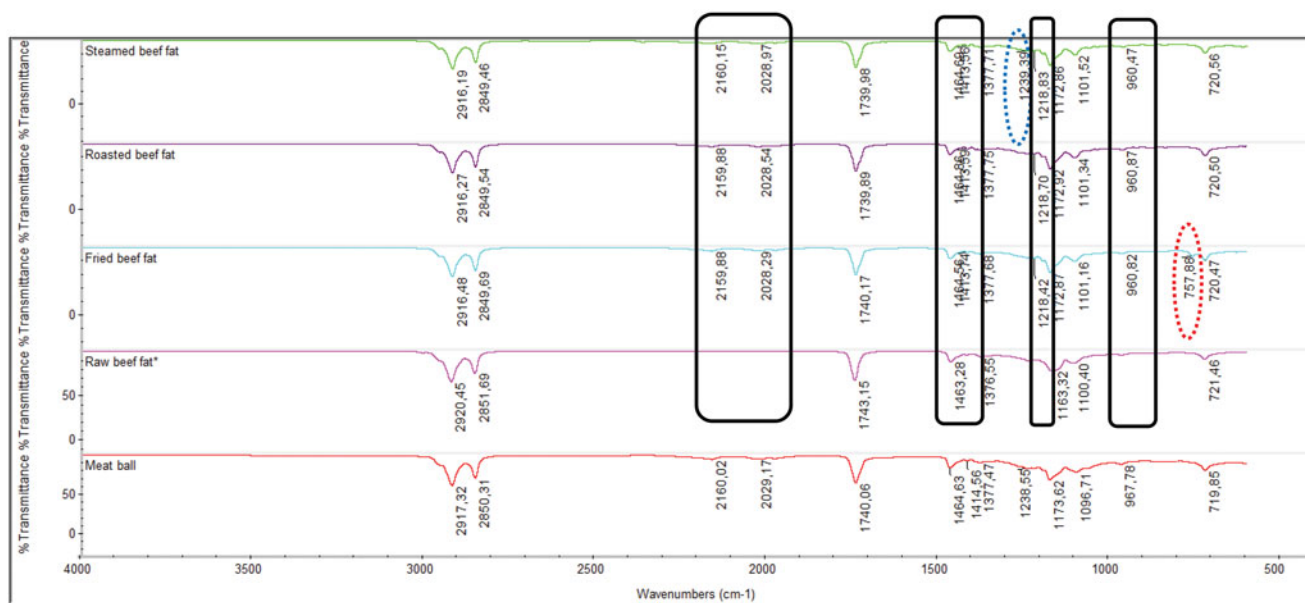
Figure 2. Infrared spectra of steamed, roasted, fried, raw beef fats and beef meatball. Raw beef fat was reported by Aminullah et al.[17] The thick line shows the difference between processed and raw beef fats. The dashed line shows the specific wavenumbers of steamed and fried beef fats. The infrared spectroscopy was overlaid in the 4000–600 cm^{-1} region with 36 times scan. FBF: fried beef fat; RBF: roasted beef fat; SBF: steamed beef fat.

process will break down fat components into volatile products such as aldehydes, ketones, alcohols, acids, and hydrocarbons. Fat levels of chicken sausage,[27] fish,[28,29] and golden snail[30] were decreased after the steaming process. In addition, Saldanha and Bragagnolo[31] reported that the roasting process decreased the fat contents in sardines and hake fishes.

## FTIR measurement on processed fats and beef meatball

The use of heat and water can trigger chemical reactions in fats, which cause chemical modifications in fatty acids. Based on the FTIR spectroscopy analysis of processed beef fats, several new transmittance peaks are formed on fried, roasted, and steamed beef fats compared to raw beef fat, which can be seen in Fig. 2. They are wavenumbers of 2028–2160 cm^{-1}, 1413 cm^{-1}, 1218 cm^{-1}, and 960–967 cm^{-1} for modes of C–H stretching, cis =C–H bending, C–H (CH$_2$) scissoring bending, and CH=CH trans, respectively,[32,33] which can be seen in Table 2. These data indicate that cooking processes produce trans compounds in processed beef fats. Kapitan[34] reported that the frying process caused the formation of absorption peaks at a

wavenumber of 966 cm^{-1} where it was related to 0.072–0.088% (w/w) of elaidic fatty acid (C18:1). Song et al.,[35] Żyżelewicz et al.,[36] and Tsuzuki et al.[37] also confirmed that elaidic trans fatty acids were formed in heated corn oil, roasted cocoa bean, and fried edible oils, respectively. Changes in cis to trans configuration in unsaturated fatty acids are inevitable steps during autoxidation. Choe and Min[38] stated that double bonds in the cis configuration are unstable and tend to form trans configuration where the higher temperature leads to the faster the reaction rates of oxidation and the more trans fat is generated. Table 2 also indicates the changes in transmittance peaks of fried and steamed beef fats at wavenumbers of 757 cm^{-1} and 1239 cm^{-1}, respectively, compared to raw beef fat. They refer to the =CH bending and –CH(CH$_2$) bending in weak intensity, according to Silverstein et al.[32] and Guillen and Cabo.[39] The heating process in oils and fats will accelerate the oxidation process, where the primary product is hydroperoxide. It may later break down to produce lower molecular weight compounds, such as alcohols, free fatty acids, ketones, and aldehydes, eventually leading to a rancid product.[8,40] Table 2 shows that raw and processed beef fats have transmittance peaks at wavenumbers of 1739–1743 cm^{-1}. Silverstein

Table 2. Functional and vibration mode of processed beef fats and beef meatball from their infrared spectra.

| Raw beef fat[15] | | Fried beef fat | | Roasted beef fat | | Steamed beef fat | | Beef meatball | | Functional group and mode |
Wave number (cm⁻¹)	Intensity (%T)	Wave number (cm⁻¹)	Intensity (%T)	Wave number (cm⁻¹)	Intensity (%T)	Wave number (cm⁻¹)	Intensity (%T)	Wave number (cm⁻¹)	Intensity (%T)	
721.33	88.8	720.47	78.6	720.50	79.0	720.56	78.7	719.85	79.3	=C–H, bending[30]
–	–	757.88	87.2	–	–	–	–	–	–	=C–H, bending[30]
–	–	960.82	88.8	960.87	88.6	960.47	88.7	967.78	87.5	CH=CH trans[31]
1100.40	84.5	1101.16	76.7	1101.34	76.4	1101.52	76.2	1096.71	77.7	C–O–C, stretching[30]
1163.32	74.8	1172.87	59.7	1172.92	59.8	1172.86	59.1	1173.62	67.4	–C–H, bending[30]
–	–	1218.42	82.1	1218.70	82.2	1218.83	82.2	1238.55	81.6	C–H (CH_2) scissoring bending[31]
–	–	–	–	–	–	1239.39	81.7	1238.55	82.3	–CH (CH_2) bending, weak[37]
1376.55	92.8	1377.68	88.1	1377.75	88.0	1377.71	88.1	1377.47	88.4	–C–H (CH_3), scissoring bending[31]
–	–	1413.74	89.8	1413.59	89.8	1413.56	89.7	1414.56	90.9	Cis=C–H bending[31]
1463.28	86.8	1464.56	81.5	1464.86	81.4	1464.69	81.4	1464.63	82.8	C–H (CH_2) scissoring bending[31]
1743.15	64.6	1740.17	56.4	1739.89	56.1	1739.98	55.6	1740.06	62.7	C=O stretching[30]
–	–	2028.29	93.9	2028.54	92.6	2028.97	93.3	2029.17	92.6	C–H stretching[30]
–	–	2159.88	92.7	2159.88	92.7	2160.15	93.1	2160.02	93.0	–C≡C–, stretching[30]
2851.69	74.2	2849.69	63.2	2849.54	63.1	2849.46	62.8	2850.31	68.9	–C–H (CH_2), stretching[31]
2920.45	64.6	2916.48	53.5	2916.27	53.5	2916.19	53.2	2917.32	59.3	–C–H (CH_2), stretching[31]

Note: %T is the transmittance intensity. These numbers are the mean from 2 replication samples. This table refers to Fig. 2 about the infrared spectra of steamed, roasted, fried, raw beef fats and beef meatball.
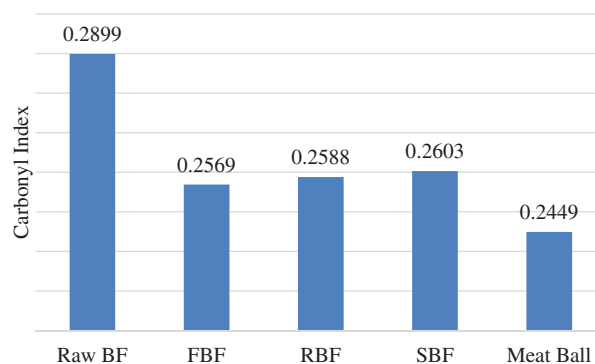

Figure 3. Carbonyl indices of raw, processed beef fats and beef fat of meatball. Carbonyl index (*CI*) is calculated from the absorbance at a wavenumber of 1700 cm⁻¹ (A_{1700}) and total absorbance ($\sum A$) at wavenumbers of 1700, 1600, 1460, 1376, 1030, 864, 814, 743, 724, 2953, 2923, and 2862 cm⁻¹ using $CI = A_{1700}/\sum A$. It refers to Table 2. FBF: fried beef fat; RBF: roasted beef fat; SBF: steamed beef fat; PC: principal component.

et al.[32] explained that carbonyl compounds such as aldehydes and ketones were formed at wavenumbers of 1870–1540 cm⁻¹ with groups and vibrational modes of C=O stretching. More specifically, Coates[41] explained that wavenumber of 1740 cm⁻¹ refers to aldehyde compounds. Brimberg[42] and Ahmed et al.[43] explained that raw fats, which do not undergo the cooking process, were undergone spontaneous oxidation (autoxidation), where oxygen promotes oxidation of poly and mono-unsaturated fatty acids of healthy oils and fats. Raw beef fats are taken from traditional markets where they are in direct contact with atmospheric oxygen. Changes in carbonyl compounds can be characterized by the carbonyl index which is calculated using Eq. (1), where the relationship between the absorbance and transmittance values can be explained by Eq. (2),

$$A = log(1/T) \qquad (2)$$

where A is the absorbance value and T is the transmittance value. By calculating the transmittance value from the FTIR spectrometer to the absorbance value using Eq. (2) and substituting the absorbance value into Eq. (1), the carbonyl indices of raw, fried, roasted, and steamed beef fats, as well as beef fat of meatballs, can be seen in Fig. 3.

It indicates that the cooking processes on beef fats cause a lower carbonyl index than that of raw fat. It is suspected that the formed carbonyl groups are mostly in the form of volatile carbonyl
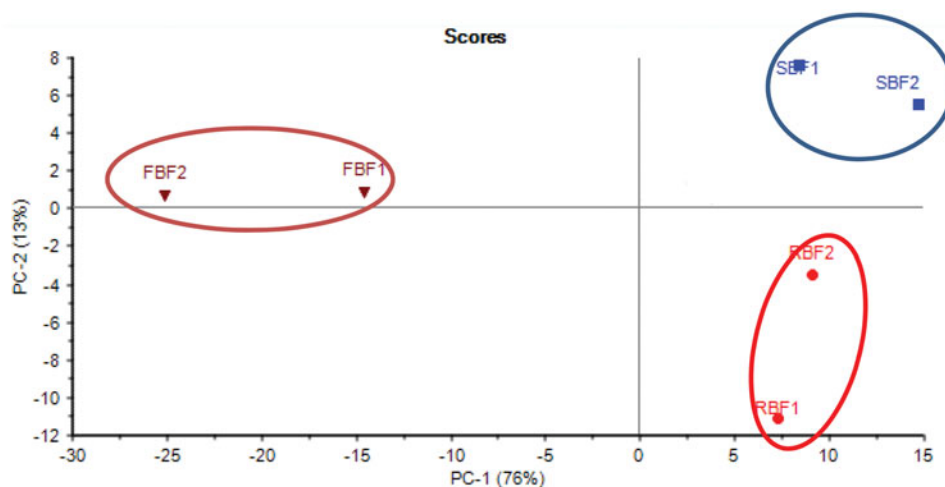
Figure 4. Principal component analysis of infrared spectra on processed beef fats. Two replication samples for each cooking process are generated using The Unscrambler® X Software. FBF: fried beef fat; RBF: roasted beef fat; SBF: steamed beef fat; BMB: beef meatball; PC: principal component.

components. Ohyabu et al.[44] explained the process and duration of heating increased volatile carbonyl formation. Also, Belitz et al.[45] reported that the rancid aroma of oils and fats was caused mainly by volatile carbonyl compounds, where heating was the accelerator of the rancidity process. There were large amounts of aldehydes such as hexanal, heptanal, pentanal, formaldehyde, acetaldehyde, etc in the fumes of cooking oil and fried foods.[46–48] Fullana et al.[49] also found six alkanals (C5–C10), seven 2-alkenals (C5–C11) and 3 alkadienals (C7, C9, and C10) in the fumes of canola, extra virgin olive, and refined olive oils. Kosowska et al.[50] specifically reported that the key volatiles of cooked beef include aldehydes, alcohols, and ketone such as octanal, nonanal, (E, E)-2,4-decadienal, methional, methanethiol, 2-furfurylthiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone, as well as the concentration of carbonyl compounds of hexanal, oxtanal and nonanal, are high in beef.

In addition to spectra profile changes of processed beef fats, Fig. 2 and Table 2 also show the peak pattern of extracted beef fats from beef meatball products. Beef meatballs are made from beef meat and fat which undergo a cooking process, especially heating using water (boiling process). Fig. 2 shows the similarity of spectra profiles between beef fat of meatballs and processed beef fats especially with steamed

beef fat with thirteen peaks, namely at wavenumbers of $719.85\,cm^{-1}$, $967.78\,cm^{-1}$, $1096.71\,cm^{-1}$, $1173.62\,cm^{-1}$, $1238.55\,cm^{-1}$, $1377.47\,cm^{-1}$, $1414.56\,cm^{-1}$, $1464.63\,cm^{-1}$, $1740.06\,cm^{-1}$, $2029.17\,cm^{-1}$, $2160.02\,cm^{-1}$, $2850.31\,cm^{-1}$, and $2917.32\,cm^{-1}$, where these are due to the cooking process which involves water as a cooking media.

Principal component analysis of beef fats spectra

The principal component analysis is then conducted on spectra profiles of fried, steamed, and roasted beef fats as well as spectra on beef meatball products. Principal component analysis (PCA) is a multivariate technique which reduces the dimensionality of data by transforming several related variables into a set of uncorrelated variables, while retaining as much variation as possible.[51] PCA aims to classify correlated variables and replace them with new groups called principal components.[52,53] In this study, PCA is accomplished using wavenumbers as the variable. Fig. 4 demonstrates the score plots of processed beef fats including roasted, fried, and steamed beef fats. These are describing the projection of samples defined by the first (PC1) and second (PC2) components, which accounted for 76% and 13% of the variation, respectively. These mean that all variables of wavelength data can be described by two PCs because these two PCs can describe almost 90% of the variation. Based on the score plots, it is known that each processed
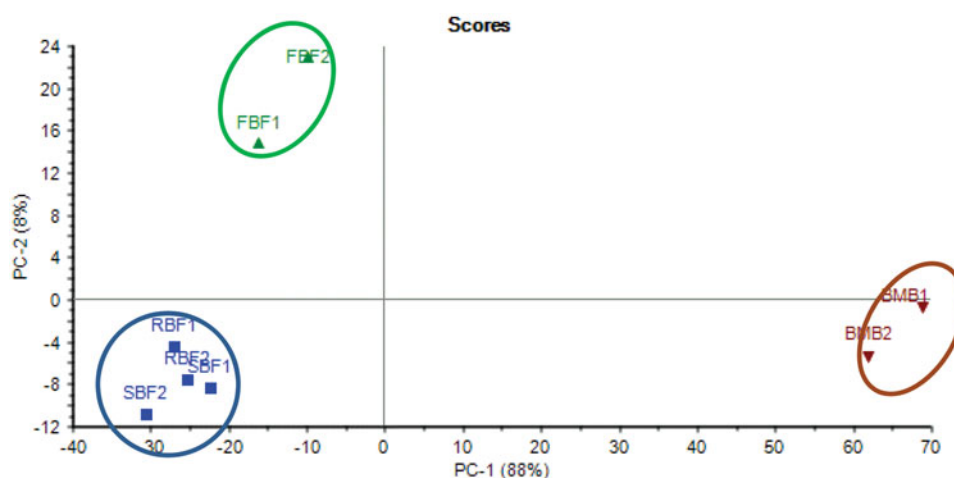
Figure 5. Principal component analysis of infrared spectra on processed beef fats and beef meatball. Two replication samples for each cooking process and beef meatball are generated using The Unscrambler® X Software. FBF: fried beef fat; RBF: roasted beef fat; SBF: steamed beef fat; BMB: beef meatball; PC: principal component.

beef fat can be separated from others, in which FBF has a negative side both in PC1 and PC2.

PC analysis is also conducted between the spectra of processed beef fats and beef meatballs, which can be seen in Fig. 5. PC1 and PC2 accounted for 88% and 8% of the variation, respectively, which means that all variables of wavelength data can be described by these two PCs for 96% of the variation. The score plot shows that the spectra of fat on beef meatballs are separated from those of all processed beef fats. The spectra of fried beef fats have PC1 on the negative side and PC2 on the positive side. Besides, roasted and steamed beef fats have similar spectra, which are indicated by a close distance.

Conclusion

The cooking processes on raw beef fats caused changes in the total lipid of fat samples, where frying increased the total lipid while steaming and roasting decreased it. The spectra analysis of processed beef fats using the FTIR spectrometer of Thermo Scientific Nicolet iS5 showed changes in the spectra of fat molecules, which were characterized by several new transmittance peaks such as a peak at a wavenumber of $960 \, cm^{-1}$. It meant that the cooking process has the potential to form trans compounds in beef fats. In addition, all processed beef fats have a lower carbonyl index than raw beef fat, which was suspected from the formation of volatile compounds. The principal component analysis showed that each processed beef fat, as well as a fat extract from a beef meatball, can be clustered and classified.

ORCID

Aminullah Aminullah ⓘ http://orcid.org/0000-0003-2354-6025
Mardiah Mardiah ⓘ http://orcid.org/0000-0002-5936-9633

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