

Research Article

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Study of different extraction methods on finger print and fatty acid of raw beef fat using fourier transform infrared and gas chromatography-mass spectrometry

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Abstract: Beef fat was an extracted fat that a byproduct of beef processing industry. In this research, the objective was to study the effect of extraction methods on finger prints and fatty acid profiles of beef fat and to obtain the chosen extraction method. This study consisted of two steps namely extracting beef fat using Soxhlet, Folch, Bligh and Dyer, and Hara and Radin methods as well as characterizing the finger print using FTIR and fatty acid profiling using GCMS. The total lipid by Soxhlet, Folch, Bligh & Dyer, and Hara & Radin methods were 89.67%, 81.73%, 55.16%, and 84.40%, respectively. Statistical analysis showed that the saturated fatty acid in Folch was not significantly different with that in Soxhlet and Hara and Radin at a 5% level. Polyunsaturated fatty acids such as linoleic and linolenic acids are present in all methods, where linoleic acid was the highest in Folch and linolenic acid was the lowest in Bligh and Dyer. The PCA analysis showed that only Folch that can be differentiated and has minimal residual error values. The results of the finger print analysis showed the extraction methods which were used are not different. These data showed that Folch was the chosen method for extracting beef fat.

Keywords: beef fat; extraction; fatty acid; finger print analysis; force constant.

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1 Introduction

The analysis method of fatty acid composition by utilizing Gas Chromatography - Mass Spectrometry or GC-MS is developed in the verification of the purity of food products containing animal fat [1]. Nina Naqiyah et al. [2] also used this method to compare fatty acid composition of beef fat, lard, chicken fat, and goat fat. Even so, Hermanto et al. [3] characterized beef fat by GC-MS analysis which is combined with Fourier Transform InfraRed (FTIR) analysis. The FTIR method is utilized to analyze the spectrum patterns of a fat product. Typical spectral patterns can describe the animal finger prints [4]. Nurulhidayah et al. [5] analyzed the authenticity of beef fat butter by using this method. Rohman et al. [6] and Guntarti et al. [7] reported pig contamination in beef meatballs accurately using FTIR. Meanwhile, Rohman et al. [6] developed FTIR method to detect beef fat contamination in cod liver oil. In addition, the interaction of infrared radiation with the material can be understood in terms of changes in molecular dipoles associated with vibration and rotation. A molecule can be viewed as a mass system connected by a bond with a springlike property, in which the stiffness of this bond can be characterized by a constant, the force constant, k (derived from Hooke's law).

The fatty tissue needs to be prepared by sampling with an extraction process. The selection of methods will affect the results of extraction processes and extracted components. Manirakiza et al. [8] showed that the Soxhlet method was better than Bligh & Dyer for chicken meat, while Ewald et al. [9] found opposite results for fish meat. Pérez-Palacios et al. [10] evaluated six methods of extraction and showed that the Folch method obtained the best results in various meats and processed meat products. Based on these, the study focusses on different extraction methods that will give the specific profiles and characteristics of beef fat. In this study, four extractions by Soxhlet, Folch, Hara and Radin, and Bligh and Dyer methods are used. The Soxhlet method is the most

common semi-continuous method used for fat extraction from food materials [11]. Pérez-Palacios et al. [10] reported that the Folch method is effective, efficient, and fast for fat extraction on a variety of meats and their preparations with low, moderate, high to very high fat contents. In addition, Aminullah et al. [12] also reported that Folch was the selected method for extracting raw chicken fat. The Bligh and Dyer method can produce an enduement of about 95% of the total fat even with the reduction of the solvent [13]. While in the Hara and Radin Method, two phases were separated rapidly during the washing step, the solvent density was low enough to allow centrifugation of homogenates as an alternative to filtration and the solvent being cheaper. The objective of the research is to study and evaluate the effect of extraction method on finger print and fatty acid profiles of beef fat using FTIR and GC-MS, respectively, and to obtain the chosen extraction method for extracting beef fat.

2 Methods

In this research, there were two parts of research namely extracting beef fat tissue (abdominal fat) using four extraction methods and measuring fatty acid and finger print profiles using GC-MS and FTIR, respectively. The samples were collected at same day and same market and then stored in a freezer. Before the samples were extracted in two replications, the samples were thawed first.

2.1 Extraction method

There were four extraction methods which were used in this research namely Soxhlet (SO), Folch (FO), Bligh and Dyer (BD), and Hara and Radin (HR) methods with two replications. The first, second, third, and fourth samples of beef fat were extracted by using Soxhlet method based on AOAC [14], Folch et al. [15], Bligh and Dyer [16], and Hara and Radin [17] methods, respectively. Beef fat was prepared by rendering the adipose tissue of beef, obtained from the meat market in Pasar Bogor, West Java, Indonesia. Then, the percentage of the total lipid was calculated.

2.2 Analysis of fatty acid composition using GC-MS

After the extraction, the extracted beef fats were transesterified according to Metcalfe and Schmitz [18], separated by gas chromatography – mass spectrometry (Agilent Technologies 7890A Gas Chromatography with

Auto Sampler and 5975C Mass Selective Detector and Chemstation data system) as follows: a 1 μ L fame was then injected into the GC column by the autosampler method. Separation was done in the HP Innnowax capillary column with the injector temperature was set at 260°C. The oven temperature was programmed where the initial temperature was 50°C for 0.5 min, then raised to 195°C (8°C/min), then raised again to 225°C (1°C/min) and held for 22 minutes. Helium gas flow rate of 1.86 mL/min. The MS detector used was Electron Multifier Detector (EMD) 70eV. The mass spectral results were processed by GCMS postrun analysis software. The fatty acid data were expressed as the peak area percentage.

2.3 Analysis of finger print using FTIR

The liquid extracted beef fat from previous extractions were placed on the plate of Thermo Scientific Nicolet iS5 FT-IR spectrometer (Thermo Fischer Inc./PT. Alphasains Dinamika). Each time measuring the sample, the plate was cleaned by using pure ethanol solvent pro-analysis then air spectrum was used as a background and recorded in graph and absorbance or transmittance value. The FTIR spectrum was analyzed at wave numbers of 4000-600 cm^{-1} with 36 times scan using OMNIC Software. The results were in the form of an infrared spectra graph where the graph was the relationship between wave numbers with the transmittance intensity.

2.4 Statistical Analysis

Statistical analysis using analysis of variance (ANOVA) and Duncan's posthoc test of SPSS version 21[®] if $p < 0.05$ or significantly difference. In order to differentiate fatty acids of beef fat using SO, FO, BD, and HR extraction methods, the chemometrics of principal component analysis was performed with the aid of OpenChrom Community Edition (Diels)

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Total lipid on beef fat

The beef fat tissue is extracted by the four previously mentioned methods to determine the total lipid content that can be seen in Table 1.

Based on the results, it can be seen that the total lipid percentage of BD is the lowest than the other extraction methods. This is consistent with the results of Iverson et al. [13] which showed that samples containing more than 2% lipid, the BD method is significantly lower in lipid content than the FO method and the higher the lipid level leads to the greater the difference. In addition, initially BD method was developed for fish samples with fat content less than 1%, and samples with higher content of fat may require modification [16]. Aminullah et al. [20] also stated that total lipid in BD was the lowest than other methods in chicken fat as well as in pork fat. Total lipid of HR method is slightly higher than that of FO method. According to Schlectriem et al. [19], this may be due to the extract of HR method containing less non-lipid than FO extract, so the total lipid in FO will lower than that in HR method. Meanwhile, SO method yields the highest value of 89.67%.

3.2 Fatty acid composition of beef fat

Beef fat has diverse fatty acids which is shown in Table 2. Capric acid (C10:0) to 11-eicosanoic (C20:1) acid can be detected using GCMS. According to Sharma et al. [21], beef fat typically contains 9 or 10 dominant fatty acids and estimated contains about 180-200 other fatty acids in very small amounts.

There is little difference in the fatty acid composition of beef fat extracted by various methods. There are six dominant fatty acids which are present in all extraction method namely myristic, palmitic, palmitoleic, margaric, stearic, oleic, and linoleic acids. These results in accordance to Rohman et al. [22] which stated that these fatty acids are dominant in beef fat. The SO method produces a composition similar to that of FO except for cis-10-Nonadecanoic acid (C19:1) which is not detected in the sample. Meanwhile, HR and BD methods give similar results which can detect capric acid (C10:0) to linolenic acid (C18:3). Statistical analysis shows that saturated fatty acid (SFA) in FO is no significantly different with that in SO and HR at 5% level. While, SFA in FO is significantly different with that in BD which SFA in BD is higher than that in FO. Polyunsaturated fatty acid (PUFA) such as linoleic and linolenic acids are present in all extraction method, where linoleic acid in FO is the highest and linolenic acid in BD is the lowest than others. In addition, cis-10-nonadecanoic acid only presents in FO in small amount.

FO method is an effective and efficient extraction method to extract fat from various tissues. Pérez-Palacios et al. [10] also suggested that although the SO method is

Table 1: Total lipid of beef fat using four extraction methods.

Extraction Method	Initial Weight	Final Weight	Total Lipid (%)
SO	5.0033	4.4865	89.67 ^d
FO	5.0305	4.1114	81.73 ^b
BD	5.0451	2.7827	55.16 ^a
HR	5.0051	4.2242	84.40 ^c

Different superscript means significant difference at α of 5%

also very good for quantifying the total lipid other than the FO method, but the long use of heat in SO can induce lipid oxidation, phospholipid hydrolysis and other lipid chemical modifications. According to Alm [23], fatty acid composition is strongly influenced by several factors such as species, feeding method (intensive or extensive), and the origin of fat (e.g. meat, bone, skin). These can lead to differences in fatty acid composition from different experiments, for example, pentadecanoic acid is measured with a percentage of 1.07-1.08%. Ahmad Nizar et al. [24] and Guntarti et al. [7] reported that pentadecanoic acid (C15:0) was detected in beef fat, while this fatty acid was not found in Hermanto et al. [3]. In addition, arachic acid (C20:0) of 0.14-0.16% is detected in this research which is also reported by Hermanto et al. [3] with percentage of 0.33%. In contrast to Guntarti et al. [7] which stated this fatty acid is undetected. While Ahmad Nizar et al. [24] reported that the composition only until linoleic acids (C18:2).

In order to make the differentiation and classification, the fatty acid profiles of beef fat in SO, FO, BD, and HR are subjected to the chemometrics technique of principal component analysis (PCA). PCA is an unsupervised pattern recognition technique widely used in chemometrics study. PCA projects the original data in reduced dimensions defined by the principal components (PCs). This technique is useful when there are correlations present among studied data [25]. Figure 1 demonstrates the score plot of PCA of beef fat in SO, FO, BD, and HR describing the projection of samples defined by the first (PC1) and second (PC2) components.

Figure 1 demonstrates the score plot of PCA of four extraction methods describing the projection of samples defined by the first (PC1) and second (PC2) components. Based on the score plots, it is known that FO can be separated from others in which FO has positive side either in PC1 and PC2. In order to know the number of PCs suggested by the software to be used in PCA model, the residual analysis was constructed. Based on the predicted residual error sum of square values (Figure 2), it can be stated that 3 PCs is necessary for PCA model,

Table 2: Fatty acid composition of beef fat in four extraction methods using GC-MS.

Fatty acid		Peak area (%)			
		SO	FO	BD	HR
Capric	C10:0	0.10 ± 0.01 ^{a,b}	0.10 ± 0.00 ^b	0.09 ± 0.01 ^a	0.10 ± 0.00 ^b
Lauric	C12:0	0.26 ± 0.01 ^a	0.26 ± 0.01 ^a	0.24 ± 0.01 ^a	0.26 ± 0.01 ^a
Myristic	C14:0	6.71 ± 0.09 ^b	6.48 ± 0.04 ^a	6.76 ± 0.06 ^b	6.70 ± 0.08 ^b
Myristoleic	C14:1	0.81 ± 0.03 ^a	0.79 ± 0.00 ^a	0.73 ± 0.01 ^a	0.81 ± 0.06 ^a
Pentadecanoic	C15:0	1.08 ± 0.04 ^a	1.08 ± 0.03 ^a	1.07 ± 0.01 ^a	1.08 ± 0.01 ^a
Palmitic	C16:0	27.17 ± 0.08 ^a	26.34 ± 0.37 ^a	27.65 ± 0.25 ^a	27.38 ± 0.93 ^a
Palmitoleic	C16:1	2.09 ± 0.03 ^a	2.37 ± 0.06 ^b	2.12 ± 0.04 ^a	2.42 ± 0.11 ^b
Margaric	C17:0	3.01 ± 0.06 ^a	3.06 ± 0.05 ^a	2.98 ± 0.01 ^a	2.99 ± 0.01 ^a
Cis-10-heptadecanoic	C17:1	0.64 ± 0.00 ^a	0.65 ± 0.01 ^a	0.61 ± 0.04 ^a	0.66 ± 0.01 ^a
Stearic	C18:0	23.01 ± 0.00 ^a	23.33 ± 0.52 ^a	23.19 ± 0.33 ^a	23.05 ± 0.53 ^a
Oleic	C18:1	31.56 ± 0.31 ^a	31.70 ± 0.45 ^a	30.75 ± 0.66 ^a	31.74 ± 0.31 ^a
Linoleic	C18:2	2.56 ± 0.03 ^b	2.71 ± 0.02 ^c	2.48 ± 0.04 ^a	2.55 ± 0.02 ^{a,b}
Linolenic	C18:3	0.32 ± 0.01 ^b	0.33 ± 0.01 ^b	0.27 ± 0.02 ^a	0.30 ± 0.00 ^b
Nonadecanoic	C19:0	0.15 ± 0.00 ^a	0.16 ± 0.01 ^a	nd	nd
Cis-10- Nonadecanoic	C19:1	nd	0.11 ± 0.01	nd	nd
Arachic	C20:0	0.15 ± 0.01 ^a	0.16 ± 0.01 ^a	nd	nd
Cis-11-eicosenic	C20:1	0.18 ± 0.00 ^a	0.19 ± 0.01 ^a	nd	nd
Saturated Fatty Acid		61.62 ± 0.14 ^{a,b}	60.94 ± 0.21 ^a	61.96 ± 0.02 ^b	61.55 ± 0.49 ^{a,b}
Unsaturated Fatty Acid		38.16 ± 0.23 ^{a,b}	38.82 ± 0.35 ^b	36.94 ± 0.78 ^a	38.47 ± 0.51 ^b

The value of each fatty acid is a mean ± standard deviation of two replications. Each row with different letters is significantly different (P < 0.05). nd is not detected

because at this PC number, PRESS value reach minimal [26]. The extraction of fat from food is crucial in the food industry for product formulation and because food labels must report both saturated and unsaturated fat content. From these data (it can be seen that), the Folch method and GCMS measurements can analyze fatty acids both saturated and unsaturated better than other solvent-based extraction methods. In addition, the Folch method can extract rapidly low, moderate, high and very high fat contents in food [10].

3.3 Finger print analysis of beef fat

The use of FTIR spectroscopy as a fat and oil analysis technique is being developed. The simple method, which does not require much preparation, short time and relatively cheap are the advantages of this technique [27]. The extracted beef fat samples are scanned with Thermofisher Scientific Nicolet iS5 FTIR, in IR area with wavenumbers of 4000-600 cm⁻¹ and the resulted FTIR spectra which can be seen in Figure 3. Based on these,

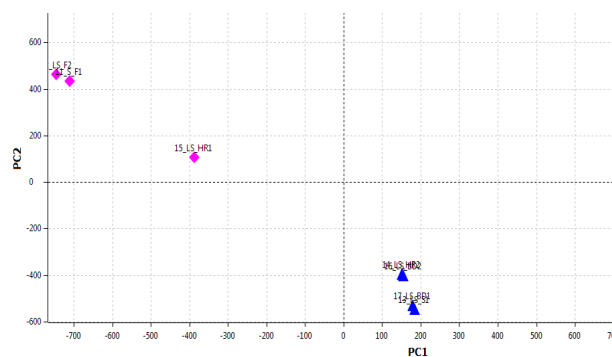


Figure 1: The score plot of PCA model using fatty acid composition of beef fat in SO, FO, BD, and HR.

several functional groups can be seen which are contained in beef fat (Table 3).

Triglycerides which are fat-forming components are reflected in the obtained FTIR spectra. High intensity is seen in the region of the wave numbers 2851-2921 cm⁻¹, this area indicates the presence of -C-H(CH₂) with vibration mode of asymmetric or symmetric stretching. This is consistent with the fatty acid composition which is

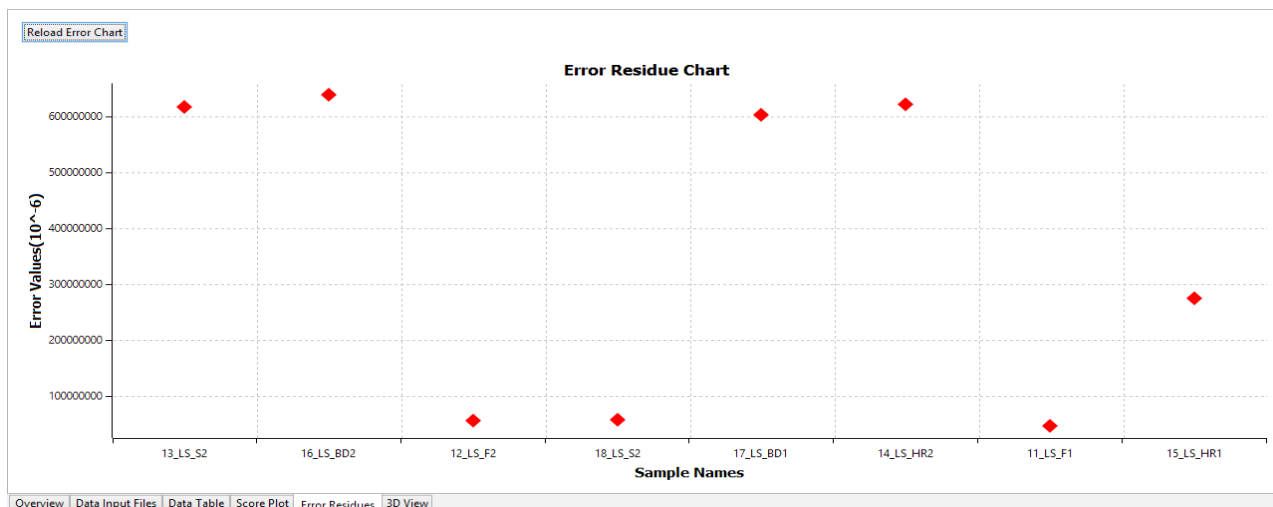


Figure 2: The residual error of PCA model for determination of optimum principle components used in PCA model.

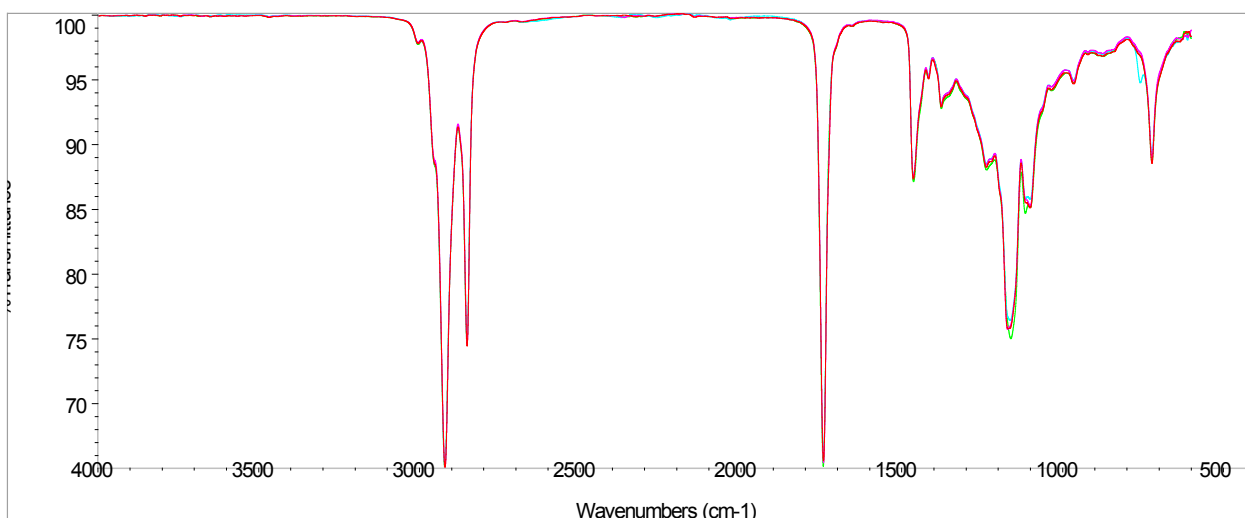


Figure 3: FTIR spectra of beef fat with four extraction method.

dominated by long chain single fatty acids. High intensity at the wavenumber of 1741-1743 cm⁻¹ indicates a high content of stretching -C = O. This group is a functional group for the ester compound which in this case is a fatty acid [28], whereas in the wavenumbers of 1099-1171 cm⁻¹ indicates the presence of stretching C-O from C-O-C. This cluster connects fatty acid esters with glycerol. The content of many unsaturated fatty acids is also reflected in the wavenumbers of 721 cm⁻¹ which shows the presence of bending =C-H group. In addition, from these data show that the obtained spectra are no significantly different from all extraction method.

Vibration mode of stretching C-O-C is found in beef fat samples using FTIR. It can be determined the magnitude of the force constant by assuming inharmonic oscillation is shown in Table 4. The force constant is

Table 3: Functional group and vibration mode on beef fat based on FTIR spectra.

Wavenumber (cm ⁻¹)				Functional group and vibrational mode
SO	FO	BD	HR	
721.3	721.33	721.32	721.25	=C-H, bending
1114.92	1099.88	1100.01	1099.94	C-O-C, stretching
1159.97	1162.01	1170.24	1169.08	C-O-C, stretching
1376.3	1376.49	1376.59	1376.55	=C-H-(cis), bending
1462.7	1463.14	1463.26	1463.22	-C-H- (CH ₂ -CH ₃) bending
1743.39	1743.01	1742.59	1742.78	C=O, stretching
2851.89	2851.66	2851.42	2851.5	-C-H (CH ₂), stretching
2920.8	2920.4	2919.97	2920.14	-C-H (CH ₂), stretching

Table 4: Anharmonic constant value (Nm^{-1}) with asymmetry stretching vibration of C-O from C-O-C.

Extraction method	$\Delta\varepsilon_1$ (cm^{-1})	$\Delta\varepsilon_2$ (cm^{-1})	x_e	$\bar{\omega}_e$	f (10^{13} Hz)	k (Nm^{-1})
SO	1114.92	1159.97	0.2448	2184.79	6.5544	1929.351
FO	1099.88	1162.01	0.2427	2137.63	6.4129	1846.958
BD	1100.01	1170.24	0.2418	2129.79	6.3893	1833.435
HR	1099.94	1169.08	0.2419	2130.74	6.3922	1835.071

Example calculation of force constant, k, (SO extraction):

- Using equations (1) and (2) and comparing them, where $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ are 1114.92 cm^{-1} and 1159.97 cm^{-1} , respectively: are obtained
 - $\frac{1114.92}{1159.97} = \frac{\bar{\omega}_e(1-2x_e)}{2\bar{\omega}_e(1-3x_e)}$
 - $(1114.92) \times 2 \times (1-3x_e) = (1159.97) \times (1-2x_e)$
 - $x_e = 0.2448$
- So:
 - $\bar{\omega}_e(1-2x_e) = 1114.92$
 - $\bar{\omega}_e(1-2(0.2448)) = 1114.92$
 - $\bar{\omega}_e = \frac{1114.92}{(1-2(0.2448))} = 2184.79\text{ cm}^{-1}$
- By using equation (3):
 - $f = \frac{c}{\lambda} = c\bar{\omega}_e = 3 \times 10^{10}\text{ cm s}^{-1} \times 2184.79\text{ cm}^{-1} = 6.5544 \times 10^{13}\text{ Hz}$
- and:
 - $\mu = \frac{Mm}{M+m} = \frac{1.944 \times 10^{-26}\text{ kg} \times 2.655 \times 10^{-26}\text{ kg}}{(1.944 \times 10^{-26}\text{ kg} + 2.655 \times 10^{-26}\text{ kg})} = 1.5444 \times 10^{-26}\text{ kg}$
- So by substituting them into equation (4), then
 - $k = 4\pi^2 f^2 \mu = 4 \times (3.14)^2 \times (6.5544 \times 10^{13})^2 \times 1.5444 \times 10^{-26}\text{ N/m}$
 - $k = 1929.531\text{ N.m}^{-1}$

identified as the bonding strength of the molecule. The inharmonic constant (X_e) of C-O from C-O-C can be calculated using the comparison of equations (1) and (2)

$$\Delta\varepsilon = \bar{\omega}_e(1-2x_e)\text{ cm}^{-1} \quad (1)$$

$$\Delta\varepsilon = 2\bar{\omega}_e(1-3x_e)\text{ cm}^{-1} \quad (2)$$

where $\Delta\varepsilon$ and $\bar{\omega}_e$ are wavenumbers (cm^{-1}). So, the value of inharmonic constant (X_e) of C-O are 0.2418 - 0.2448 and $\bar{\omega}_e$ values are $2129.79 - 2184.79\text{ cm}^{-1}$. Brooks [29] stated that $\bar{\omega}_e$ value of C-O is 2170 cm^{-1} . The given infrared rays will be absorbed by the molecular vibrations if the frequency of infrared is equal to the frequency of the molecular vibration or is called resonance. The frequency that occurs can be calculated using equation (3)

$$f = \frac{c}{\lambda} = c\bar{\omega}_e \quad (3)$$

where c is the speed of light ($3 \times 10^{10}\text{ cm.s}^{-1}$), so that the frequency values are $6.3893 - 6.5544 \times 10^{13}\text{ Hz}$ is obtained. Then, this value is substituted into equation (4) to obtain the force constant of C-O which are in the range of $1833.435 - 1929.351\text{ N.m}^{-1}$. These are consistent with Brooks [29] which reported that force constant of C-O is 1860 N.m^{-1} .

$$f = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \text{ or } k = 4\pi^2 f^2 \mu \quad (4)$$

where $\mu = \frac{Mm}{M+2m}$, with M and m are C mass ($1.944 \times 10^{-26}\text{ kg}$) and O mass ($2.655 \times 10^{-26}\text{ kg}$), respectively.

4 Conclusion

The percentages of total lipid in raw beef by Soxhlet, Folch, Bligh & Dyer, and Hara & Radin methods were 89.67%, 81.73%, 55.16%, and 84.40%, respectively. Statistical analysis showed that saturated fatty acids in almost all methods were not significantly different than a 5% level, while polyunsaturated fatty acid in Folch showed a higher amount than the others. In addition, PCA analysis showed that only the Folch method can be differentiated and has minimal residual error values. The results of finger print analysis showed that the extraction methods which were used were not significantly different. The peaks were formed at wavenumbers of about 721 cm^{-1} , 1100 cm^{-1} , 1160 cm^{-1} , 1376 cm^{-1} , 1463 cm^{-1} , 1743 cm^{-1} , 2851 cm^{-1} , and 2920 cm^{-1} which showed stretching vibration modes of C-O ester groups from C-O-C at 1100 and 1160 cm^{-1} where force constant of C-O was about 1860 N.m^{-1} . These data showed that Folch was the best method for extracting beef fat.

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