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Validation of the Methods for Detection the Non-Milk Fat in a Mixture of Milk Fat and Palm Oil

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Abstract

Milk fat contains a variety of nutritive and health-promoting compounds that guard against some disease. In the current system of global competition, when the quality of milk and milk products is not an option but rather a requirement, therefore, determining the purity of milk fat is critical. This study aims to validate analytical methods for detecting palm oil in a mixture of milk fat and palm oil. Methods of this study was involved detection of non-milk fat in fat blinders by determining the saponification value, iodine number, refractive index, butyro refractometer reading, Gas chromatography, Reverse Phase High-performance liquid chromatography, and Fourier transforms Infrared. The results of this study revealed that the saponification value, Iodine number, refractive index, and Butyro Reading could be used to detect the addition of palm oil by a level of 10% - 20% or more to the milk. The level of some fatty acids in the milk as determined by GC, such as myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), is correlated well with the level of adding palm oil to milk fat. The determination of cholesterol and β-sitosterol content by RP-HPLC could be used for the detection of the addition of palm oil to milk fat. The spectrum behavior produced by FTIR spectroscopy in this adulterated sample is almost the same, so this technique could not be used to detect the palm oil in milk fat.

Keywords

Milk Fat, Palm Oil, Adulteration, Butyro Refractometer Reading, GC, RP-HPLC, FTIR

1. Introduction

Milk fat price is higher than other oils and fats; it occasionally gets mixed with

margarine to make unlawful gains. Although most adulteration of fats and oils does not endanger the public's health, it violates fundamental consumer rights [1]. Adulteration of dairy products is a common characteristic in different countries of the world [2] [3]. The replacement of milk fat by vegetable oil in dairy products processed by the dairy industry has been frequent for a long time, but this is usually noted on the label. Over the last few years, milk fat has increasingly been substituted without warning, especially when butter and cream with the word "natural" are involved, since vegetable oils are more widely available at lower prices [4]. Furthermore, the source and purity of vegetable fat are unknown and could have harmful health effects on consumers, as it was reported in milk fat replaced by palm oil [5].

Both fatty acids and sterols profiles are the usual analytical methods to detect milk fat adulteration in butter [6] [7]. Zachariah *et al.* [8] reported that the chemical constants were not effective for the detection of the palm and coconut oil. Although the problems which a high instrumental cost issues and cumbersome sample pre-treatment procedures, chromatographic techniques were used the most widely to check milk adulteration [9]. According to Nurseitova *et al.* [10] the gas chromatography with flame ionization detection was able to detect adulteration in butter. Palm oil can be modified using techniques like blending, fractionation, interesterification, and hydrogenation [11]. According to the Egyptian Standards (ES 154-7, 2005), butter oil should have a minimum milk fat content of 99.6%, maximum water content 0.3%, iodine value ranges from 30 - 45 and saponification value ranges from 218 - 228. In addition to, the product must be free from rancidity, foreign fat, residues, preservatives and thickeners (ES.154-7: 2005).

It is noteworthy that nutrition has a profound effect on the physical and chemical properties of milk fat. Therefore, the present study aims to validate recent trustworthy techniques to identify the non-milk fat in a mixture of milk fat and palm oil.

2. Materials and Methods

2.1. Materials

Butter oil (ghee) was made in the dairy pilot plant, Alexandria University starting by using the raw cow milk of the herd of the faculty of agriculture, Alexandria University. Palm oil (shortening, melting point 36˚C - 38˚C) was obtained from a local market and manufactured by Wilmar Company, Singapore. All chemicals were consumed analytical grade.Also, standards were utilized including Cholesterol solution, 10 mg/mL in chloroform (Supelco), β-Sitosterol, 100 µg/mL in chloroform (Supelco), and Fatty acid methyl esters standard mixture, It was produced by Sigma-Aldrich Co LLC, Merck KGaA, Darmstadt, Germany.

2.2. Methods

2.2.1. Preparation of Fat Mixtures

Formulated fat blends were prepared after the complete melting of milk fat

(99.6% MF) and palm oil (99.6% PO) at 65˚C for 10 min. The liquefied MF and PO were mixed in proportions to create 7 treatments (MF:PO 100%:0%, 95%:5%, 90%:10%, 80%:20%. 50%:50%, 20%:80%, and 0%:100%). Two of these samples represent the original components and five treatments were binary blends.

2.2.2. Samples Preparation

Lipids were extracted by solvents of methanol and chloroform (2:1) as described by [12].

2.2.3. Physicochemical Analysis

The saponification value was analyzed depending on Egyptian standard specification No:51-5:2005, the iodine value was analyzed depending on Egyptian standard specification No:51-4:2005, peroxide value was analyzed depending on Egyptian standard specification No:155-7:2006, and the refractive index was analyzed according to ISO 1739:2006, using Abbe refractometer model NAR-3T (ATAGO, Japan). Also, Butyro Readings and Refractometer index were measured at 40˚C, using ATAGO Digital Butyro-Refractometer CAT. No. 3454 (ATAGO, Japan).

2.2.4. Fatty Acid Profile Analysis

1) Preparation of fatty acid methyl esters (FAMEs):

The fatty acid methyl esters were prepared by dissolving the extracted fat in benzene (GC grade) and using -Methanol-H_2SO_4 for as described by [13].

2) Gas chromatography

Fatty acid profile analysis was achieved using ACME model 6100 GC (Young LIN Instrument Co., Korea) fitted with a split injector and FID detector. The carrier gas of nitrogen was used with a current flow rate of 0.5 ml/min. The vaporized materials were separated on a 30 m SP-2380 fused-silica capillary column with 0.25 mm i.d. and 0.2 μm film thickness (Supelco, Bellefonte, PA) and the detector temperature was adjusted to 260˚C. The injector temperature was adjusted to 220˚C and in split mode (split ratio 1:50). The column was initially maintained at 140˚C for 5 min, and the temperature was subsequently increased to 240˚C at a rate of 4˚C/min.

2.2.5. Determination of Cholesterol and *β*-Sitosterol by (RP-HPLC)

1) Extraction of Unsaponifiable Matter (USM) for RP-HPLC analysis:

The samples of fat were dissolved in 10 ml of isopropanol, then added one ml to methanolic solution of 10 mol/l potassium hydroxide (9:1) and refluxed for 30 minutes. After cooling, 5 ml of deionized water and 10 ml of n-hexane were added and intensively shaken at 150 rpm for 20 minutes at ambient temperature. The organic layer was separated, washed with deionized water and dried with sodium sulphate. The hexane solution was evaporated, and the remains were dissolved in 1 ml of methanol for HPLC analysis.

2) RP-HPLC determination:

Stock solutions of 2 different standards (Beta-Sitosterol and Cholesterol) in

methanol were prepared for standard solutions. Each of the standards was filtered by a 0.22 μm Nylon syringe filter then 10 μl was injected. Samples were prepared and filtered as it is using 0.22 μ Nylon syringe filter and 10 μL were injected. Samples were eluted by the reverse phase HPLC Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector at 205 nm. C18 Xterra: 4.6 × 250 mm, 5 μm Column was used. Isocratic elution with a mobile phase of water and methanol (15%:85%) mixture at a flow rate of 1ml/min was used. The column temperature was set up at ambient temperature [14].

2.2.6. FTIR Instrumental Analysis

All samples spectra (either pure or admixtures) were classified by FTIR spectrometer. The functional groups existent was described by a Bruker VERTEX 70v FT-IR Spectrometer connected with platinum ATR model V-100 in the range of wave numbers (400 - 4000 cm^{-1}).

2.3. Statistical Analysis

The observation data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The used tests were F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons. Significance of the obtained results was judged at the 5% level.

3. Results and Discussion

3.1. Validate Physicochemical Analytical Methods for Detecting Palm Oil and Milk Fat Mixing Ratios

The saponification value (SV) was 212.4 in butter oil (BO) and 165.6 in palm oil (PO) (Table 1). The saponification value in cow milk fat should be in the range of 218 - 228 mg KOH/g oil (ES154-P7-2005). The palm oil used in this study is shortening with a melting point of 36˚C - 38˚C. In the present study, there were significant differences between the blenders regarding saponification value ($P \leq 0.001$), iodine value ($P \leq 0.001$), and refractometer ($P \leq 0.001$). On the other hand, the peroxide value was zero in all treatments (Table 1). The saponification value of milk fat was much higher than that of palm oil as observed. The SV decreased as the percentage of palm oil in the mixtures of MF and PO increased. The saponification value is inversely proportional to molecular weight [15]. The saponification value is of little use in the detection of common adulterants in milk fat. However, Kumar *et al.* [16] noted that this method could be used for detecting the presence of mineral oils, such as liquid paraffin, in milk fat.

The Iodine value (IV) of milk fat was 34.48 g Iodine/100g fat as shown in Table 1. The IV of milk fat should be in the range of 30 - 45 g Iodine/100g fat according to ES:154-7:2005, while the IV of palm oil was 55.57 g Iodine/100g oil. The blenders had an IV ranging between 34.48 to 55.57 g Iodine/100g fat, dependent on the milk fat/palm oil ratio, but the IV is still in normal range (ES:154-7:2005) of MF in the mixture containing the addition of PO in the ratio 20%

Table 1. Saponification, the iodine value, refractometer index and PR-Butyro Reading and Refractometer index of mixing milk fat and palm oil at different ratios.

Treatment	MF:PO*	Saponification value mg KOH/g oil	Iodine value	Refractive index	Digital PR-Butyro-Refractometer	
					Refractometer index	Butyro Reading
1	100%:0%	212.4^{a}	34.48^{d}	1.4538^{d}	1.4555^{b}	39.10^{d}
2	95%:5%	197.8^{b}	35.17^{d}	1.4540^{d}	1.4558^{b}	43.30^{c}
3	90%:10%	197.0^{b}	38.37^{cd}	1.4542^{d}	1.4562^{b}	43.50^{bc}
4	80%:20%	187.9^{c}	42.07^{c}	1.4546^{d}	1.4562^{b}	45.23^{bc}
5	50%:50%	180.9^{d}	48.57^{b}	1.4570^{c}	1.4577^{b}	45.47^{b}
6	20%:80%	174.5^{e}	55.04^{a}	1.4585^{b}	1.4767^{a}	49.37^{a}
7	0%:100%	165.6^{f}	55.57^{a}	1.4622^{a}	1.4593^{b}	49.13^{a}

*MF: Milk fat, PO: Palm oil, Mean with Common letters are not significant (*i.e.* Means with Different letters are significant).

(42.07). According to these results of iodine value could be used to detect the adulteration of milk fat with palm oil if the PO is higher than 20% in the blend. These results agreed with Gandhi *et al.* [17] who studied the iodine value for detecting palm olein and sheep body fat in milk fat products, they found that the uncertainty of IV to detect the percentage of palm oil in milk fat was about 20%. Similar observations were observed by [18] [19] [20], as the IV was out of the normal range by adding PO to butter oil by about 40.0% or more.

The refractive index (RI) is simple, rapid, and widely used as a preliminary screening method for knowing the quality of milk fat. The RI of milk fat should be in the range of 1.4524 - 1.4552 at 40˚C [ES:154-7:2005]. Refractive indexes (RI) were 1.4538 for milk fat and 1.4622 for palm oil. The RI increased in blenders of milk fat with palm oil by increasing the level of palm oil, but the RI was still in the normal range of MF by adding up to 20% of PO, so the RI method could be used to detect the adulteration of palm oil with level more than 20% of adding PO in milk fat. Our results disagreed with Sharma and Singhal [21], who mentioned that a 5% - 20% presence of vegetable fat can be detected in milk using this method, depending upon the nature of the adulterant.

Butyro reading of different ratios of milk fat and palm oil mixtures using a digital Butyro-refractometer ranged from 39.10 in 100% MF to 49.13 in 100% PO (Table 1), while the highest value was in MF:PO 20%:80% ratio (49.37) and the lowest value was in MF:PO 100%:0% (39.10). No significant difference was found between the mixtures regarding to Butyro reader, except for MF: PO 20:80%.

The highest value of the refractometer index recorded by the Butyro-refractometer reader was in the 100% MF (1.4555) and the lowest value was in 100% PO (1.4593). Besides, there was no significant difference ($p \geq 0.05$) between all the studied mixtures regarding the refractometer index recorded by the Butyro-refractometer reader, so the refractometer index recorded by the Butyro-refractometer could not be used as indicator of replacing milk fat with palm oil.

3.2. Fatty Acid Profile Analysis by GC

The fatty acid profiles of MF analyzed by GC, (Table 2 and Figure 1), revealed that the capric acid (C10:0), lauric acid (C12:0), myristic (C14:0) acid, stearic acid (C18:0) and -linolenic acid (ALA; C18:3 n-3) were found at levels of 3.00%, 3.33%, 12.04%, 13.34%, and 1.23%, respectively. While all these corresponding values were not detected in 100% PO. Furthermore, while 100% MF had the highest levels of saturated fatty acids (SFA) (66.27%) compared to 47.36% in 100% PO, pure milk fat has a lower level of palmitic acid (C16) than pure palm oil. On the other hand, pure palm oil had high levels of oleic acid (C18:1 n-9) and linoleic acid (LA; C18:2 n-6) when compared with pure milk fat. Also, unsaturated fatty acids (USFA) and USFA/SFA were at high values in pure palm oil.

Fatty acids are usually analyzed by GC equipped with a flame ionization detector (FID). Fatty acid values in the literature have been reported as a percentage of total fatty acids; thus, individual fatty acid values may vary in different studies [15]. Increasing the levels of palm oil led to gradually decrease of myristic acid and stearic acid (Table 2), while palmatic acid, oleic acid and linoleic acid gradually increased. Furthermore, the levels of capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), and stearic acid (C18:0) could be used as an indicator for the detection of the adulteration of milk fat by palm oil. The same results were found by other researchers [16] [22]. In this study, the C14:0 level was used to detect the PO in milk fat as it correlated well with increasing the level of PO and could be used as an indicator of more than 20% of the PO. Due to the variation of breeding, season, and feeding, the low level (10% or lower) of adding PO in milk fat is difficult to detect by GC.

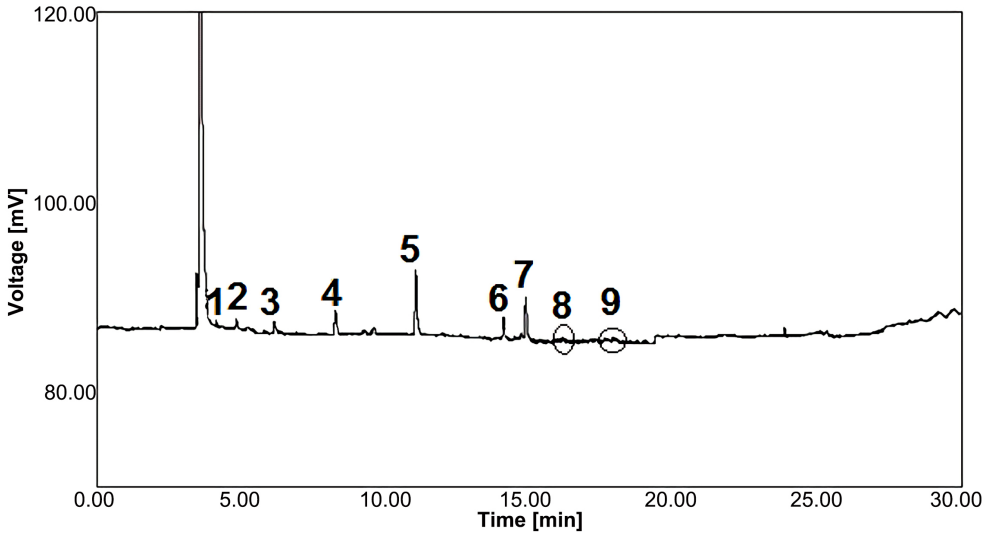
3.3. Determination of the Sterol Content of the Fat Blenders by RP-HPLC

The total content of cholesterol and β-sitosterol in pure milk fat was 252.76 μg/ml and 0.10 μg/ml, respectively, compared to 14.75 μg/ml and 1.73 μg/ml in pure

Table 2. Fatty acids profile analysis of mixtures of milk fat and palm oil as analyzed by GC.

Treatment	MF:PO*	C8:0%	C10:0%	C12:0%	C14:0%	C16:0%	C18:0%	C18: 1n9c%	C18: 2n6c%	C18: 3n3%	SFA%	USFA%	USFA/SFA
1	100%:0%	1.70	3	3.33	12.04	31.43	13.34	22.48	1.72	1.23	66.27	28.6	0.43
2	95%:5%	1.07	2.15	2.35	9.31	31.82	13.19	24.05	2.7	0.92	63.55	32.75	0.52
3	90%:10%	0.67	1.5	2.14	9.24	31.81	11.82	28.9	2.78	0.95	60.46	37.01	0.61
4	80%:20%	0.65	1.78	2.08	8.88	33.53	10.12	29.12	3.66	0.55	59.58	37.33	0.63
5	50%:50%	0.51	1.32	1.56	5.77	36.69	8.11	33.01	6.08	0.26	55.77	42.16	0.76
6	20%:80%	0.29	0.47	0.61	2.48	40.62	6.18	38.63	8.81	0.25	50.98	48.72	0.96
7	0%:100%	0.00	0	0	0	47.36	0	40.09	12.15	0	47.36	52.24	1.10

*MF: Milk fat, PO: Palm oil, C8:0 (Caprylic), C10:0 (Capric), C12:0 (Lauric), C14:0 (Myristic), C16:0 (Palmitic), C18:0 (Stearic), C18:1n9c (Oleic), C18:2n6c (Linoleic) C18:3n3 (Linolenic), SFA: saturated fatty acid, USFA: Unsaturated fatty acids, USFA/SFA: Unsaturated fatty acids/saturated fatty acid.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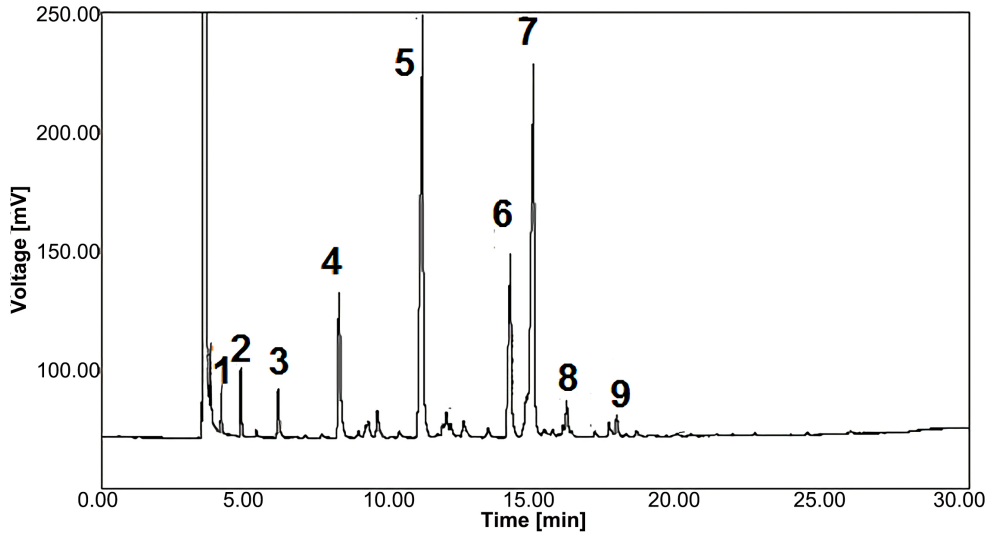
(a)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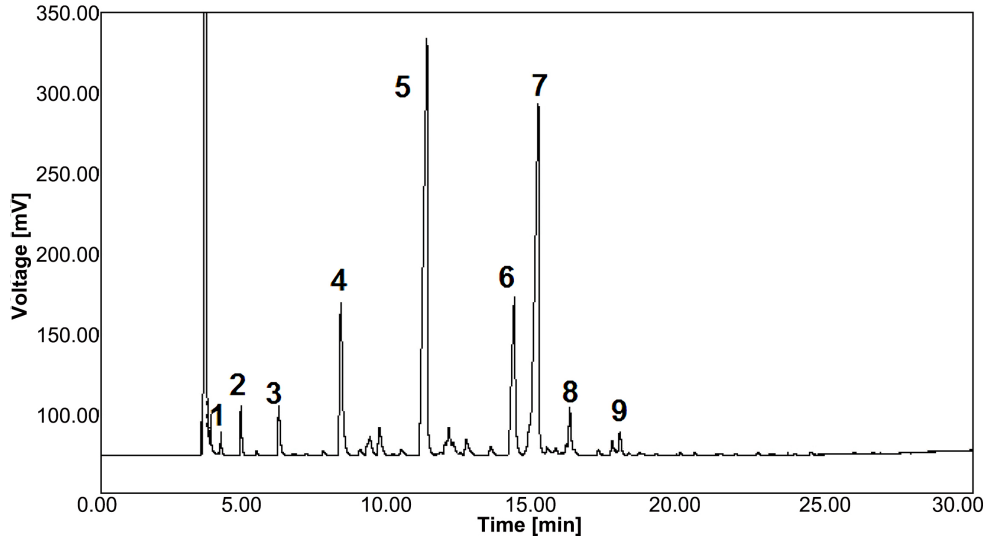
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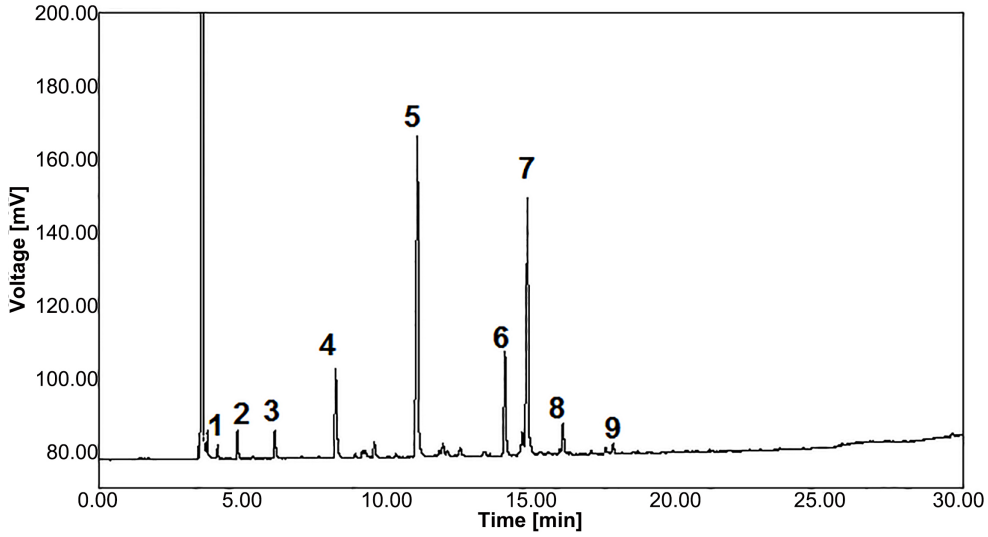
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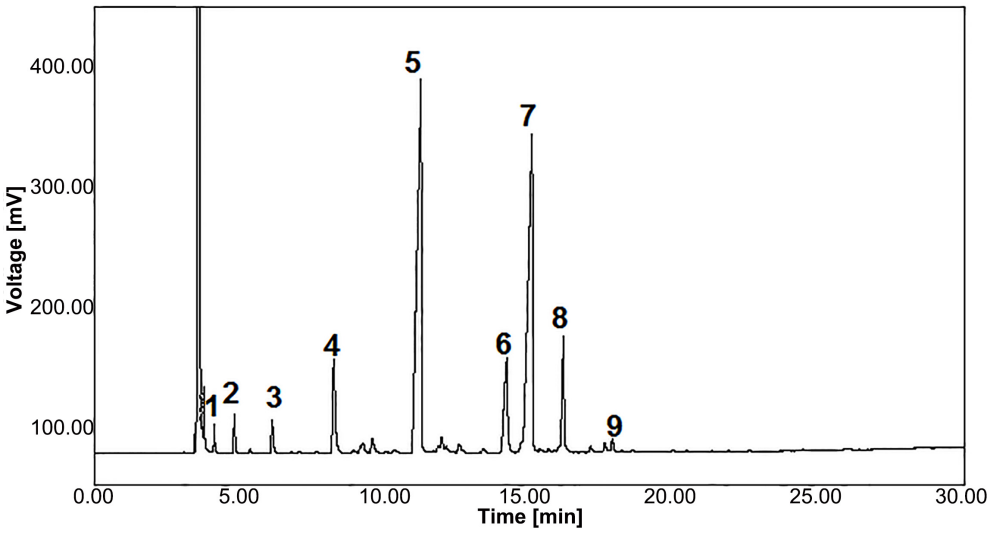
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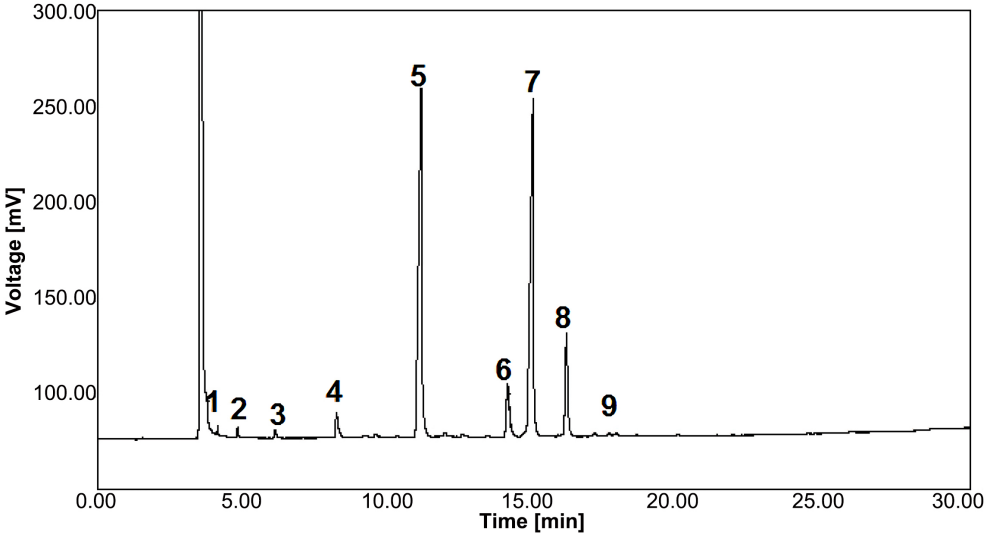
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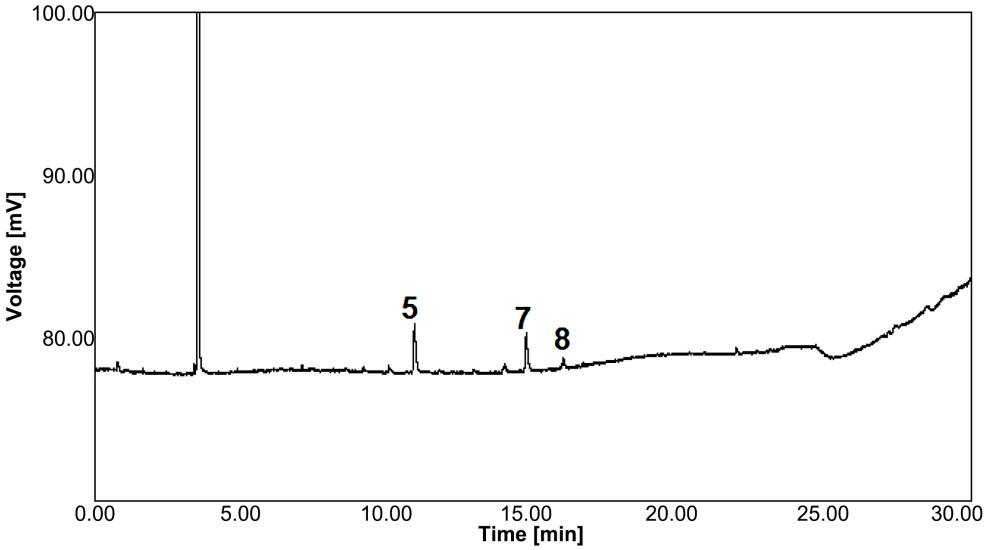


(g)

Figure 1. Gas chromatography (GC) of Fatty acids profiles analysis of mixtures of milk fat (MF) and palm oil (PO) in proportions respectively: (a) MF 100%:PO 0%, (b) MF 95%:PO 5%, (c) MF 90%:PO 10%, (d) MF 80%:PO 20%, (e) MF 50%:PO 50%, (f) MF 20%:PO 80%, (g) MF 0%:PO100%. 1—C8:0, 2—C10:0, 3—C12:0, 4—C14:0, 5—C16:0, 6—C18:0, 7—C18:1n9c, 8—C18:2n6c, 9—C18:3n3.

palm oil, respectively (Table 3). In this method, the lowest level of cholesterol was in pure palm oil, while the lowest β-Sitosterol was in pure milk fat.

The β-sitosterol was found as the major phytosterol in palm oil (3.45 μg/ml) and the same findings were concluded by Abd El-Aziz *et al.* [19]. They found that cholesterol and β-sitosterol contents were 231.0 g/ml and 8.5 g/ml in milk fat, respectively. As a result, when palm oil was added to the milk fat, it resulted in a progressive increase of β-sitosterol and a gradual dropping of cholesterol when compared to pure milk fat; the changes were proportionate to the addition level. Consequently, Contarini *et al.* [23] assumed that the determination of β-sitosterol content could be useful to uncover the adulteration of milk fat with palm oil when applied to an admixture containing 5.0% palm oil, based on the evidence for the presence of β-sitosterol content in pure milk fat and the highest limit of β-sitosterol in palm oil.

However, because of the large amounts and great variability of the cholesterol present in milk (204.3 to 382.4 mg/100g), which was reported by other researchers as well, [19] [24] [25], the determination of cholesterol content would be unsuccessful when administrated to a mixture containing a lower concentration of palm oil. Regardless of the cholesterol amount, the higher percentage of cholesterol fraction in milk (252.76 μg/ml) compared with the low level in palm oil (14.75 μg/ml), could be useful in detecting palm oil in milk. According to these results and the higher limit of cholesterol percentage reported by Contarini *et al.* [23] and Borkovcová *et al.* [14], the addition of 5.0% palm oil was sufficient to depress the percentage of cholesterol fraction to the lower limit even if the percentage was high initially.

Table 3. Sterols and cholesterol content of the mixed of milk fat and palm oil analyzed by HPLC.

Treatment	MF:PO	Cholesterol Conc. (μg/ml)	Beta-Sitosterol Conc. (μg/ml)
1	100%:0%	252.76	0.10
2	95%:5%	218.18	0.33
3	90%:10%	194.16	0.49
4	50%:50%	159.47	0.63
5	20%:80%	108.58	0.87
6	0%:100%	14.75	1.73

*MF: Milk fat, PO: Palm oil.

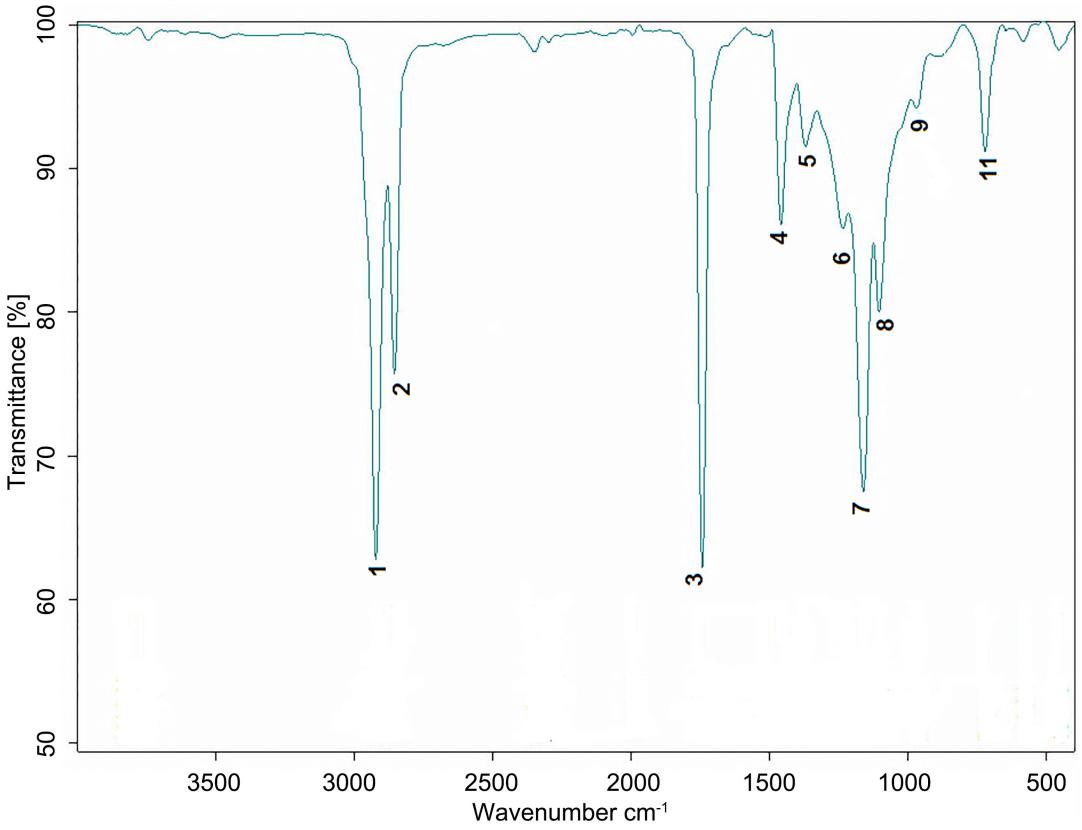
3.4. Identification of the Functional Groups in Milk Fat and Palm oil Mixtures Using FTIR

As observed in **Table 4** and **Figure 2**, different wavelengths and different levels of percentage of absorbance of each functional group were recorded. These functional groups exhibit the whole composition of milk fat, as well as the bonding behavior and absorption intensity of these functional groups. The primary ingredients in milk are recognized using fingerprint traits such as absorbance peak intensities, locations, wave numbers, and forms of the absorption peaks of the original FTIR spectra.

When comparing the mixed samples to a pure milk fat sample, the spectrum behavior produced in this adulterated sample is almost the same, but the wave numbers are somewhat different. The absorbing and relative intensities of wavenumbers differ slightly and the detectable variations could be recorded regarding CH_3 bending (86% in 100% MF and 92% in 100% PO groups), PO stretching (symmetric) of $>PO_2$ Polyphosphate bending (79% in 100 MF and 84% in 100% PO groups), phospholipid and methylene group CH2 (90% in 100% MF and 96% in 100% PO groups).

The wave number positions of absorbance peaks, peak intensities, and peak widths are specific to the functional groups of the sample; thus, each sample has a unique "fingerprint" absorbance spectrum [26]. Because of the complexity of milk, which can result in overlapping peaks of several elements, the primary ingredients in milk are recognized using fingerprint traits such as absorbance peak intensities, locations, wave numbers, and forms of the absorption peaks of the original FTIR spectra.

Our findings were in accordance with Rohman *et al.* [27] who carried out a general investigation through the spectra and showed that the spectra were very similar because the major component in fat and oil is fatty acids. Further investigation revealed some differences in several peaks, especially in the fingerprint region, Windarsih and Irnawati [28], suggested that deep investigation could differentiate between milk fat and palm oil spectra. However, in adulterated samples of milk fat with PO, it is very difficult to differentiate between authentic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Wavenumber cm-1
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3
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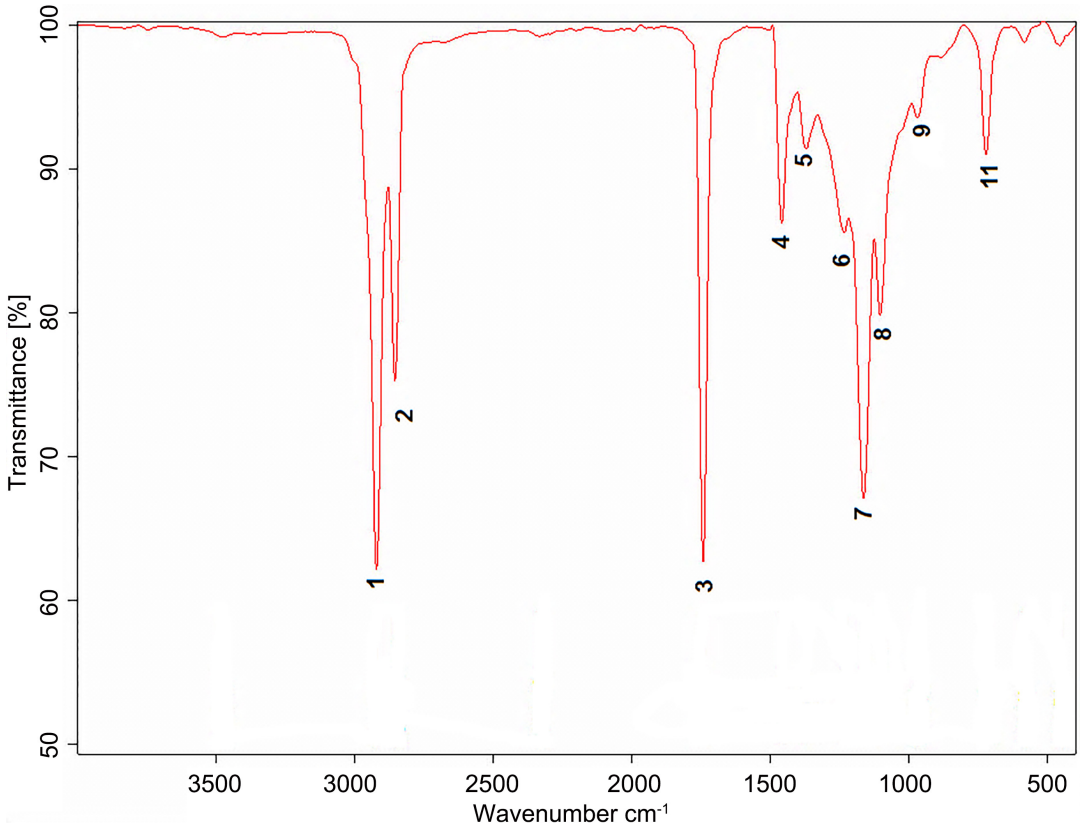
11

(a)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Wavenumber cm-1
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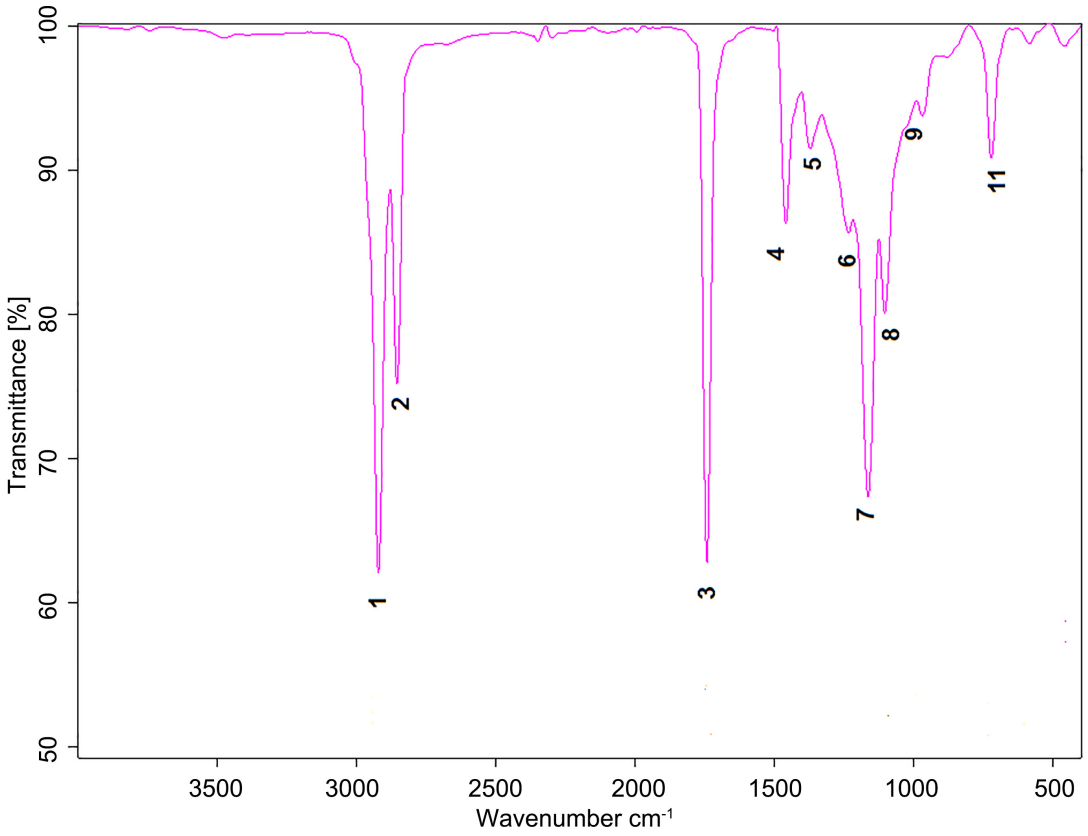
(b)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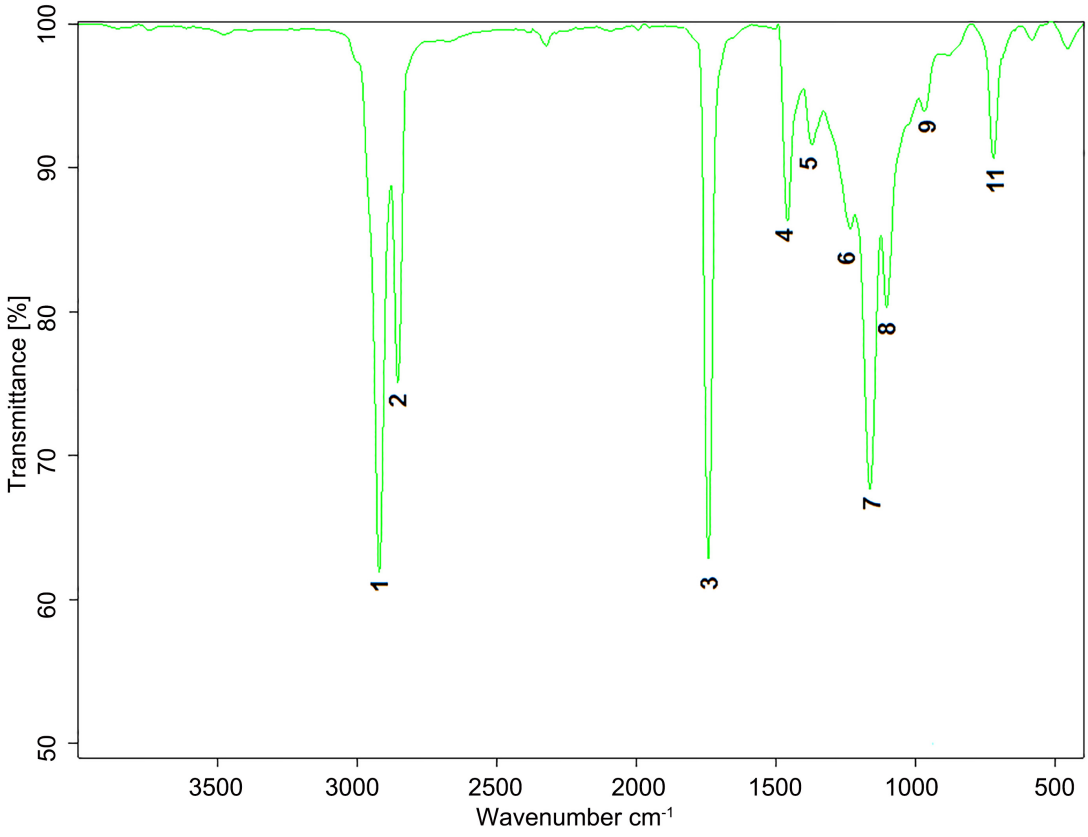
(c)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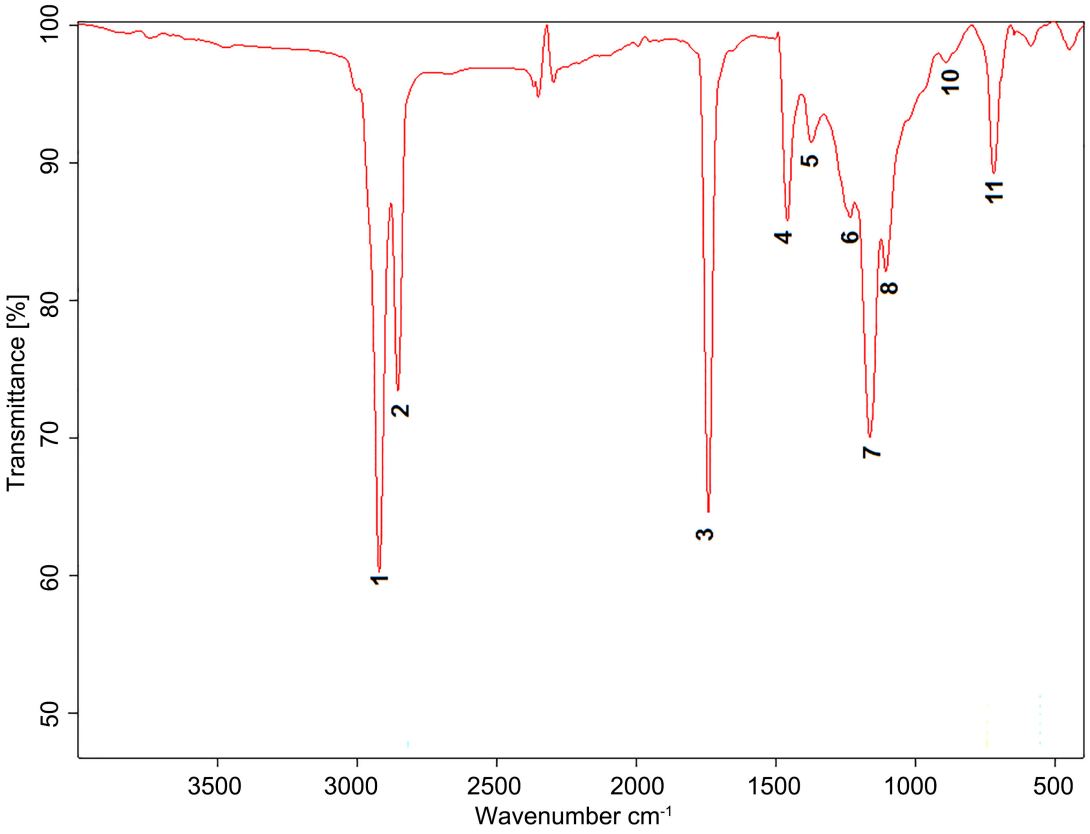
(d)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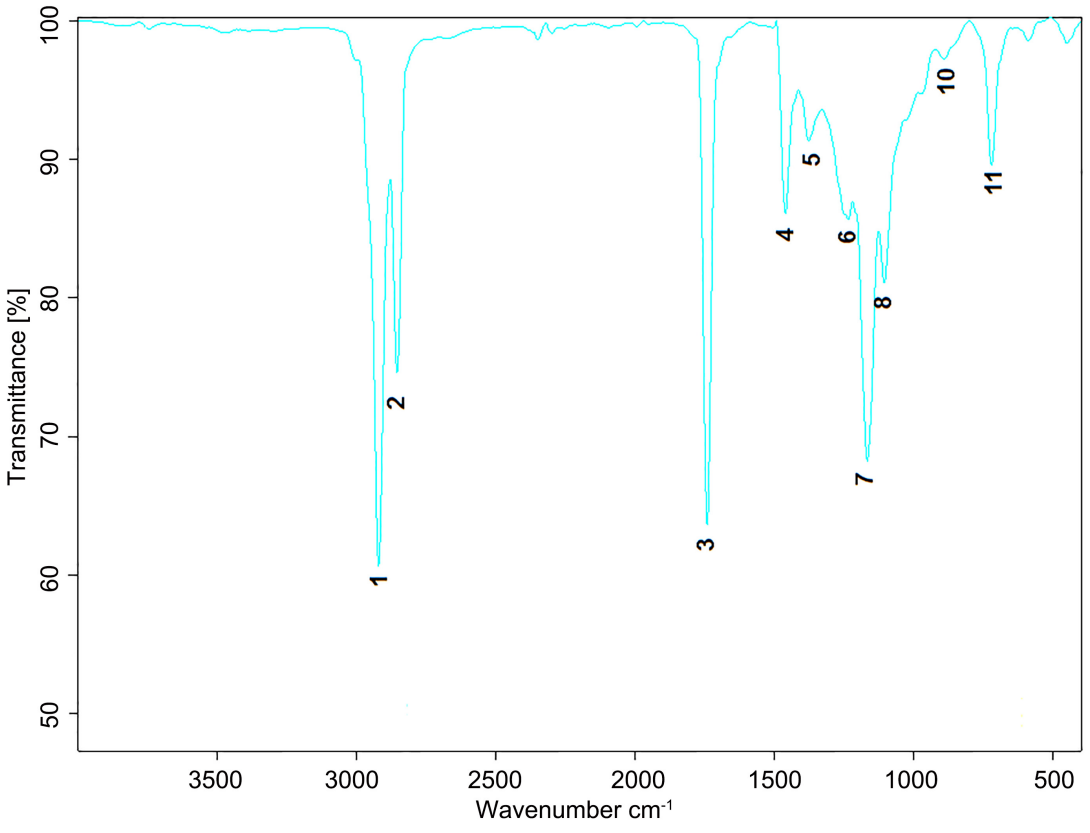
(e)


Transmittance [%]
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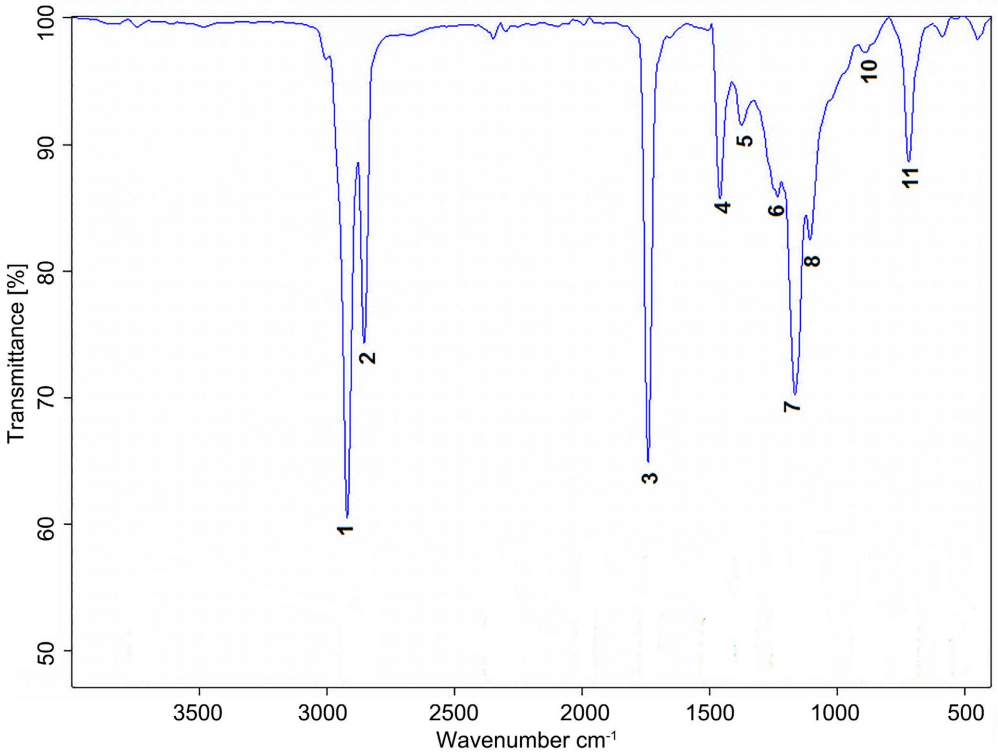
(f)



(g)

Figure 2. Functional groups identified by FTIR spectra of mixture groups of milk fat (MF) and palm oil (PO) in proportions respectively: (a) MF 100%:PO 0%, (b) MF 95%:PO 5%, (c) MF 90%:PO 10%, (d) MF 80%:PO 20%, (e) MF 50%:PO 50%, (f) MF 20%:PO 80%, (g) MF 0%:PO 100%. 1—Stretching of $>CH_2$ of acyl chains (assymetric), 2—Stretching of CH_2 of acyl chains, 3—C=O stretching, 4—CH_2 deformtion, 5—CH_3 bending, 6—P=O stretching (assymetric) of $>PO_2$ phosphodiesters Polyphosphate, phospholipid, 7—C-O-C stretching, 8—P=O stretching (symetric) of $>PO_2$ Polyphosphate, phospholipid, 9—Methylene group CH_2, 10—P-O-P stretching Polyphosphate, phospholipid, 11—CH_2 deformation.

Table 4. Functional groups identified by FTIR spectra of milk fat and palm oil mixture groups.

Functional Group	Wave Number	Mix fat (MF/PO)%*						
		100%:0%	95%:5%	90%:10%	80%:20%	50%:50%	20%:80%	0%:100%
C-H stretching-of the cis double bondc=o	3012	94%	98%	98%	98%	98%	98%	97%
C-H stretching-of the cis double bondc=o	3010	94%	98%	98%	98%	97%	97%	97%
C-H assymetric stretching of $-CH_3$	2955	84%	86%	86%	86%	86%	85%	87%
Stretching of $>CH_2$ of acyl chains (assymetric)	2920	63%	62%	62%	62%	61%	60%	61%
Stretching of CH_2 of acyl chains	2853	75%	75%	75%	75%	75%	74%	74%
C=O stretching	1741	66%	63%	63%	63%	64%	65%	65%
CH_2 deformtion	1458	86%	86%	86%	86%	86%	86%	86%
CH_3 bending	1375	86%	91%	92%	92%	91%	91%	92%
P=O stretching (assymetric) of $>PO_2$ phosphodiesters Polyphosphate, phospholipid	1236	82%	86%	86%	86%	86%	86%	86%
C-O-C stretching	1163	67%	67%	67%	68%	69%	70%	70%

Continued

P O stretching (symetric) of >PO_2 Polyphosphate, phospholipid	1095	79%	81%	82%	82%	83%	84%	84%
methylene group CH_2	970	90%	94%	94%	94%	95%	95%	96%
P-O-P stretching Polyphosphate, phospholipid	891	94%	98%	98%	98%	97%	97%	97%
CH_2 deformation	725	90%	91%	91%	91%	90%	90%	89%

*MF: Milk fat, PO: Palm oil.

milk fat spectra and adulterated milk fat spectra. So, FTIR spectra could not be used to detect adulterated milk by replacing milk fat with palm oil, but they could be used to detect some other additives. He *et al.* [26] suggested that some differences can be seen clearly between the milk and the adulterated samples. For example, there are two distinct auto-peaks at 2924 and 2840 cm^{-1} in the synchronous spectrum of the milk, while in the adulterant samples, including urea, glucose, and melamine, there are more than two auto-peaks between 3000 and 2700 cm^{-1}, and also, their intensities and positions vary from that of the raw milk.

4. Conclusion

This study concluded that the saponification value could be used to detect the replacing milk fat by palm oil in milk. The Iodine number, refractive index, and Butyro reading could be used to detect the replacing milk fat by palm oil at a level of 10% - 20% or more, but these techniques are not accurate to determine the adding palm oil levels. The level of some fatty acids in the milk, such as capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), could be used as an indicator for the detection of the replacing milk fat by palm oil using gas chromatography. Also, HPLC analysis could be used for the same purpose, as the addition of palm oil caused a gradual decrease in the cholesterol percentage and an increase in the β-sitosterol content of milk. FTIR spectroscopy, which revealed that comparing the mixed samples to a pure milk fat sample, showed the spectrum behavior produced in this adulterated sample is almost the same, but the wave numbers are somewhat different. The absorbing and relative intensities of wavenumbers differ slightly.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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