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Comparative study for the detection of Egyptian buffalo butter adulteration with vegetable oils using conventional and advanced methods

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Abstract

Adulteration of butter can occur when milk fat is replaced with cheaper oils due to the economic advantage of such substitution. With potential health risks on consumers, the issue raises alarming public health concerns. The adulteration of the Egyptian buffalo butter (EBB) with vegetable oils (VOs) was detected by conventional and advanced techniques. EBB was mixed with VOs at different ratios 25, 50, and 75% (w/w). The conventional methods (saponification number, iodine value, polenskee value, and Reichert-Meissl value) were compared with advanced methods (GC, HPLC, and FTIR). The results indicated that the assurance of conventional methods is not adequate at expansion levels under 50.0% VOs in EBB. In light of the progressions prompted by advanced methods using GC and HPLC, the debasement of EBB with 25.0% VOs can be detected and are more accurate in detecting the sterols and their fractions in butter.

1 | INTRODUCTION

Butter is a dairy product produced by the churning of milk or cream, and it is characterized by varying properties based on the qualities of the raw material from which it is produced (Dvorak, Lu zov, & Sustova, 2016). According to Council Regulation (EC) No. 2991/94, it is a product made entirely from milk. Butter is a valuable product which has a high percentage of fat (ranging between 80–90 g/100 g milk fat in net weight) used directly as a breakfast favorite, as well as an ingredient in the preparation of many dishes or as cooking oil for some hot dishes (Fındık & Andiç, 2017). Butter oil is manufactured in various countries in Africa, the Middle East, and Asia by melting the butter at 110–140°C. This end product is named differently in various countries: "ghee" of India, "maslee" of the Middle East and "roghan" of Iran (Atasoy & Türkoglu, 2010).

As milk butter is an expensive product, it may be subjected to adulteration by addition of cheaper fats in order to reduce the price of butter during manufacturing (increasing the margin of profit), while neglecting to inform the consumer of that fact through placing the respective information on the label, (Tomaszewska-Gras, 2016). The need to follow uniform food standards, including the detection of adulteration, have become important concepts in quality control and food safety especially following the emergence and proliferation of a multitude of foodborne diseases around the world (De la Guardia & Illueca, 2013).

Various methods are used for the detection of butter adulteration which are classified as traditional and advanced techniques. As well, the physicochemical methods are considered the most commonly used ones for authenticating dairy products (Karoui, Dufour, & De Baerdemaeker, 2007). Saponification number (SN), iodine number (IN), Polenske Value, Reichert-Meissel value, and insoluble impurities are relevant quality parameters and are considerably important in measuring the chemical nature and determining the final quality of the oil (Lawson, 1985).

Currently, some advanced techniques such as liquid chromatography (HPLC) and gas chromatography (GC), isotope ratio analysis, and DNA based method have also been found extremely helpful in dealing with the problems related to adulteration, and authentication of dairy products (Pajor, Galló, Steiber, Tasi, & Póti, 2009). These methods involved the detection of milk fat components such as TAG, fatty

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acids, and sterols. Determination of the milk fat purity according to the official European Community method is based on TAG composition analyzed by GC on a packed column (Derewiaka, Sosin´ska, Obiedzin´ski, Krogulec, & Czaplicki, 2011). In a different approach, Molkentin (2007) has used GC to detect the adulteration of butter with foreign fat and satisfactory results were obtained. However, some limitations need to be considered with technologically processed milk fat, fat from low-fat milk products such as skim milk or buttermilk, as well as with milk fat from other species than cows. Derewiaka et al. (2011) pointed out that the analysis of fatty acid composition was not an effective tool to detect the adulteration of milk fat in butter due to the variability of the fatty acid composition which is influenced by various factors. Conversely, it was reported that, FAs have been applied for the analysis of milk fat content, discrimination of animal fat, and determination of free FAs. Related studies have been developed for the discrimination of foreign fats in milk fat (Fontecha, Mayo, Toledano, & Juárez, 2006; Gutiérrez et al., 2009).

HPLC is one of the analytical techniques used to separate, identify, and quantify the components in a mixture (Hamed, Abdel-Hamid, Gámiz-Gracia, García-Campaña, & Arroyo-Manzanares, 2019; Hamed, Moreno-González, García-Campaña, & Gámiz-Gracia, 2017; Hamed, Moreno-González, García-Campaña, & Gámiz-Gracia, 2017a; Kamal & Karoui, 2015). For milk and dairy products, HPLC methods could be considered as useful tools to perform several measurements related to the quantification and detection of parameters such as (i) dairy product components; (ii) levels of additive used; and (iii) environmental pollutants and xenobiotics. The determinations of tocochromanols or phytosterols and the foreign vegetable fats in butter have also been performed by HPLC.

In addition, Fourier transform infrared (FTIR) spectroscopy, a fingerprint technique, is a rapid, simple, sensitive, advanced, and accurate analytical tool that finds its application in the wide areas of detection of adulteration in various food commodities (Rodriguez-Saona & Allendorf, 2011). The technique has been widely applied, because once the instrument has been calibrated; it can be used for routine analyses. FTIR coupled with chemometric techniques has been extensively used to discriminate different edible oils and fats (Nurrulhidayah et al., 2013).

In view of the above mentioned, the aim of this study was to identify the adulteration of the buffalo butter available in the Egyptian market by different analytical methods including milk fat standards, FTIR, chromatographic analysis of fatty acids, and sterols. Palm oil (PO) and hydrogenated palm kernel oil (HPKO) were used due to their common use as additives or adulterants in the Egyptian food industry sector.

2 | MATERIALS AND METHODS

2.1 | Materials

2.1.1 | Egyptian buffalo butter (EBB)

Standard EBB was obtained from the Faculty of Agriculture, Cairo University, Giza, Egypt.

2.1.2 | Vegetable oils

PO and HPKO, imported from Indonesia, were purchased from the Consumer Association of Foodstuff, Faisal St., Giza, Egypt.

2.1.3 | Preparation of the mixtures of EBB with VOs (PO and HPKO)

The mixtures were prepared after complete melting of the standard Egyptian buffalo's butter (EBB) and VOs at 70 ± 0.5°C in a water bath (Jenway, Cole-Parmer, Beacon Road, Stone, Staffordshire, ST15 OSA, UK). The VOs (PO and HPKO) were mixed with EBB in different ratios (25, 50, and 75%, w/w) as follow: for EBB with PO were: EBB/$PO_{25,}$ EBB/PO_{50}, and EBB/PO_{75} and for EBB with HPKO were EBB/$HPKO_{25,}$ EBB/$HPKO_{50}$, and EBB/$HPKO_{75.}$ In addition, two samples represent the standard EBB and VOs as Control samples. All samples were stored at 4°C until analysis. All experiments were carried out in triplicates and each analysis in triplicates.

2.2 | Methods of analysis

2.2.1 | Chemical properties

Reichert-Meissl value (soluble volatile fatty acids), Polenske value (insoluble volatile fatty acids), Iodine value, and Saponification value were determined in triplicates according to the method described by AOAC (2005).

Reichert Meissl value and Polenske value

Reichert Meissl and Polenkse values were determined as follows: A portion of sample (5 g) was saponified using glycerol and 50% NaOH, diluted with water and acidified, and thereafter steam-distilled in a glass apparatus at a controlled rate. The condensed and cooled distillate was filtered and the water-soluble acids that pass through were estimated by titration with 0.1 N NaOH to give the Reichert-Meissl value. While the water-insoluble acids collected on the filter paper were dissolved out in alcohol and titrated to give the Polenske value. Blank tests were made without fat sample.

$$\text{Reichert}-\text{Meissl value} = 1.10(\text{T1}-\text{T2})$$

$$\text{Polenske value} = (\text{T3}-\text{T4}).$$

Where,

T1 = volume in mL of 0.1 N NaOH used for sample (water soluble).

T2 = volume in mL of 0.1 N NaOH used for blank (water soluble).

T3 = volume in mL of 0.1 N NaOH used for sample (water insoluble).

T4 = volume in mL of 0.1 N NaOH used for blank (water insoluble).

Saponification value

Saponification value was determined as follows: 2 g of fat sample were saponified with an excess of alkali (0.5 N KOH) dissolved in alcohol. Some of the alkali was used up in saponifying the fat to soap of its fatty acids and glycerol; the remainder was estimated by titrating against standard acid (0.5 N HCL) using I% phenolphthalein as indicator. Blank determination was made upon the same quantity of the KOH solution at the same time under the same conditions.

Saponification value was determined by the following formula

$$\text{Saponification value} = 28.05(\text{T2}-\text{T1})/\text{W}.$$

Where,

T2 = volume in mL of 0.5 N acid required for the blank.

T1 = volume in mL of 0.5 N acid required for the sample, and W = weight of the sample taken.

Iodine value

Iodine value was determined as follows: 0.40–0.45 g of clear fat were taken in a dried conical flask and added with 15 mL of carbon tetrachloride and 25 mL of Wijs' reagent. The content were kept in the flask in the dark for 1 hr. 20 mL of KI solution were added to approximately 150 mL of distilled water and mixed. Titration with 0.1 N sodium thiosulphate solution using 2 mL of starch as indicator was then performed. A blank test was also performed, using the same quantities of the reagents. Iodine value was determined by the following formula

$$\text{Iodine value} = 12.69(\text{B}-\text{S})\text{N}/\text{W}.$$

Where,

B = volume in mL of standard sodium thiosulphate solution required for blank.

S = volume in mL of standard sodium thiosulphate solution required for sample.

N = normality of the standard sodium thiosulphate solution, and.

W = weight in g of material taken for the test.

2.2.2 | Sample preparation for determination of sterols fractions

A portion of 1 g of the sample was taken. Then, a solution of methanol/aqueous potassium hydroxide (10 M) (9/1, v/v) was added and refluxed for 30 minutes. After cooling, 5 mL of deionized water and 10 mL of n-hexane were added and intensively shaken for 20 min. The organic layer was separated, washed with deionized water to the neutral reaction and dried with sodium sulfate. The hexane solution was evaporated and the residue dissolved in 1 mL of methanol for HPLC analysis (Borkovcová, Janoušková, Draþková, Janštová, & Vorlová, 2009).

2.2.3 | HPLC conditions for identification and determination of sterols fraction

Analyses were determined by reverse phase HPLC on a Gemini-Nx C18 column (5u, 250 × 4.6 mm). Analyses were performed on a Knauer liquid chromatograph (Germany) with UV detector at 250 nm. Isocratic elution with mobile phase of methanol and water (95:5) mixture at a flow rateof 0.7 mL/min was used. The column temperature was set up at 35°C; injection volume was 20 μL. Data were collected and evaluated by software claritychrome (Knauer, Germany).

The calculation of these method was done as follows:

$$\text{Weight } \% \text{ of each sterol} = (\text{A}/\text{As}) \times (\text{V}/\text{Vs}) \times (\text{Ms}/\text{M}) \times 100$$

Where:

A = peak area of sterols in the sample.

As = peak area of sterols in standard.

V = total volume of sample solution.

Vs = total volume of standard solution.

Ms = content of sterols of standard.

M = content of sterols of the sample.

2.2.4 | Determination of fatty acids composition

Fatty acid compositions of samples were determined as described by the AOAC (2012). The Fatty Acid Methyl Esters (FAMEs) were quantified by Thermo Gas Chromatograph with a flame ionization detector (FID). The column phase was Supelco DB-Wax (Carbowax) with the following dimensions: 30 m long, 0.25 mm, that is, with a 0.25 μm phase thickness. Helium was used as a carrier gas with a flow rate of 40 mL/min. One μL was injected using the inlet in a split mode.

The head pressure was set at 2 psi, and the split vent flow was 7 mL/m. The injector temperature was 250°C. The column flow rate was 40 mL/min at 2 psi was 0.68 mL/m. The column temperature was maintained from 50 to 200°C rate 10°C/s and was held at 260°C for 80 min. The detector was operated in the selected ion monitoring mode. Fatty acids were identified by retention times obtained from the FAME standards (Sigma Company, St. Louis, MO).

2.2.5 | IR measurements

Fourier transform infrared spectra were collected between 4,000 and 400 nm at a resolution of 4 nm on a Fourier transforms infrared (FT-IR) FTIR spectrometer (A Nicolet 6,700 from Thermo Nicolet Corp., Madison, WI) A crystal was preheated to 65 ± 2°C. A 100 μL fat sample at 65°C was placed on the preheated crystal. The temperature controller allowed the stabilization of analysis temperature at 65 ± 2°C for each spectrum. The absorbance spectrum was obtained by rationing the single beam spectrum against that of the air background. For the classification analysis, three independent spectra were recorded from each sample.

3 | RESULTS AND DISCUSSIONS

3.1 | Chemical properties

It is well-known that there are various factors influencing the chemical properties of milk fat such as the singularity of dairy animals, stage of lactation, breed contrasts, diurnal, and accidental (sporadic) everyday impacts and feed type and quantity.

3.1.1 | Saponification number

It is considered as a standout among the most vital compound properties for quality affirmation of lipids and as a decent effective measure for corruption of fats and oils particularly margarine fat with VOs (Zaidul, Norulaini, Omar, & Smith Jr, 2007). The saponification number of EBB and its blends with PO and HPKO are given in Table 1.

Saponification number of milk fat was 226 and it was in the normal range reported by (Dhurvey, Kawtikwar, & Sakarkar, 2012; Ozkanli & Kaya, 2007; Samet-Bali, Ayadi, & Attia, 2009). The data revealed that the saponification number is gradually reduced by increasing the added quantity of VOs. The decreasing in saponification number of the blends could be correlated with the high molecular weight of VOs fatty acids, where VOs contain a high level of medium and long-chain fatty acids (Table 1). However, the data confirmed that the addition of VOs to the EBB in the ratios 25.0 and 50.0% did not affect the normal range (210 to 233) of saponification number; where it reduced to 223 and 214 for the blends containing HPKO and to 220 and 212 for the blends containing PO, respectively. Also, the findings demonstrated that the blends with more than 50% of VOs have a saponification number out of the normal range of pure EBB. These findings are in line with those previously reported by many researchers (Abd El-Aziz, Kholif, & Morsy, 2012; Dhurvey et al., 2012).

3.1.2 | Reichert-Meissl values

The data illustrated in Table 1 outline the Reichert-Meissl number of EBB, VOs, and the different blends. These data reported the changes occurred the quality of the raw materials and the blends due to the mixing process.

It is clear from the current data that the average value of the Reichert-Meissl value of standard buffalo's milk fat (EBB) was 29.6 while VOs values were very low when compared with the EBB value. When VOs were mixed with EBB, the Reichert-Meissl value of the mix was gradually reduced with the increasing in the oil added. Addition of 25% of HPKO and PO oils led to a reduction of Reichert-Meissl number to 6.1 and 7.4 respectively.

3.1.3 | The Polenske value

The Polenske value is a measure of the unsoluble unpredictable unsaturated fats and is valuable to distinguish oils with the medium chain unsaturated fatty acids (USFA) (Sheppard, Shen, & Rudolf, 1985). The data appeared in Table 1 showed the Polenske value of EBB, VOs, and the various blends. The obtained data revealed that Polenske values have a similar trend that found of the values of saponification number. The Polenske value was considerably higher (1.63) for EBB as compared with that of HPKO (0.37) and PO (0.35). A gradual decrease of the Polenske values was obtained by the addition of VOs to EBB. The decreasing rate was proportional to the added level of VOs. However, the addition of VOs to EBB in the ratio of 25.0 to 50% did not affect the normal range of Polenske values (1.2–2.4) of the resultant mixture (Abd El-Aziz, Mahran, Asker, El-Hadad, & Sayed, 2013; Singhal, 1980). The addition of VOs to EBB in a ratio of more than 50.0% increased Polenske value. Previously reported data obtained by (Dhurvey et al., 2012; Fatouth, Singh, Koehler, Mahran, & Metwally, 2005; Park, Juárez, Ramos, & Haenlein, 2007; Samet-Bali et al., 2009) revealed a similar effect of VOs on Polenske values. In view of the aforementioned data, it can be concluded that Polenske value and Recherit-Miesssel number cannot be considered suitable methods for detection of fat adulteration when VOs are added at a level less than 50%.

3.1.4 | Iodine value

Iodine value, another fat constant, is helpful in deciding the level of hardness as high iodine number means the presence of a high amount of unsaturated fatty acids in the fat. (Zaidul et al., 2007). Iodine value of EBB and its blends with VOs are presented in Table 1.

TABLE 1 Chemical constants of EBB, HPKO, PO, and its mixtures

Treatment	Saponification number	Iodine number	Reichert-Meissl number	Polenske number
EBB	226 ± 3.4	34.2 ± 0.3	29.6 ± 0.4	1.63 ± 0.1
EBB/HPKO$_{25}$	223 ± 2.3	42.2 ± 0.3	24.5 ± 0.3	0.72 ± 0.1
EBB/HPKO $_{50}$	214 ± 4.3	44.1 ± 0.5	18.0 ± 0.4	0.55 ± 0.2
EBB/HPKO $_{75}$	192 ± 1.2	47.1 ± 1.2	6.1 ± 2.0	0.41 ± 0.01
HPKO	168 ± 1.1	49.4 ± 1.2	0.5 ± 1.5	0.37 ± 0.2
EBB/PO$_{25}$	220 ± 2.3	41.1 ± 0.9	23.9 ± 0.8	0.69 ± 0.2
EBB/PO$_{50}$	212 ± 3.2	43.5 ± 0.2	19.1 ± 0.3	0.53 ± 0.2
EBB/PO$_{75}$	195 ± 2.1	46.3 ± 0.8	7.4 ± 0.5	0.39 ± 0.09
PO	194 ± 3.4	48.5 ± 0.6	0.5 ± 0.9	0.35 ± 0.8

Inversely to saponification, Reichert-Meissl and Polenske values; iodine value of EBB was 35.2 which is lower than those of HPKO (49.4) and PO (48.5). These findings were in agreement with (Abd El-Aziz et al., 2013; Singhal, 1980). The increase in iodine number value of VOs could be attributed to the high content of unsaturated fatty acid in these oils, especially oleic, and linoleic acids, as compared with EBB (Table 1). The addition of VOs to EBB caused a gradual increase in iodine value of EBB. The increase was proportional to the added level. According to the data obtained by (Abd El-Aziz et al., 2012; Kumar, Sharma, Lal, Kumar, & Seth, 2010), the addition of VOs to EBB in the ratio 50.0% or more got the iodine value out of the normal range of EBB (27–35). Therefore, our findings demonstrated that this method cannot be successfully used in the detection of adulteration of buffalo butter if the added level of VOs was 25.0%.

3.2 | Chromatographic methods

3.2.1 | Sterol determination by HPLC

Sterols are vital lipids that should be routinely monitored in nourishment. Cholesterol, sitosterol, and stigmasterol are polycyclic steroid mixes with comparative chemical structure. Cholesterol is an ordinary creature sterol, for example, its substance in drain fat is 95–98% of the aggregate sterol, stigmasterol, and sitosterol are alluded to as phytosterols. Both cholesterol and phytosterols are presented in free and esterified forms. In this sense, milk fat is considered the main source of dietary cholesterol where its content in bovine milk fat obtained from butter is ranging between 204.3–382.4 mg/100 g with an average equal to 265.5 mg/100 g (Molkentin, 2007). Currently, there is a growing interest in the detection of sterols in dairy products by various techniques aiming for one or more of the following objectives: to measure the total cholesterol content to obtain nutritional information, to detect the presence of vegetable fats in dairy products, and measuring specific phytosterols (β-sitosterol and stigmasterol). HPLC is among the advanced techniques used in sterol and cholesterol determination, being a fast and reliable alternative to GC (Borkovcová et al., 2009).

Sterols content in EBB, VOs, and their blends are presented in Table 2. The data obtained from the determination of Reichert-Meissl number clearly pointed out to milk fat adulteration and the data indicated that adulteration probably occurred through the addition of VOs. Therefore, to make sure of this probability, the detection and determination of sterols in these samples should be done. Accordingly, all samples used in Reichert-Meissl determination were subjected to HPLC analysis for the determination of cholesterol and phytosterol (Stigmasterol and β-Sitosterol). Data present in Table 2 revealed that buffalo butter contains cholesterol at a rate of 278.34 mg/100 g fat; while stigmasterol and β-Sitosterol have been found at low concentrations (0.05 and 0.10 mg/100 g fat, respectively). Conversely, HPKO contains 12.33, 22.32, and 63.01 mg/100 g oil of cholesterol, stigmasterol, and β-Sitosterol, respectively, while PO contains 11.81, 21.34, and 61.54 mg/100 g oil of cholesterol, stigmasterol, and β-Sitosterol, respectively.

TABLE 2 HPLC analysis for cholesterol and phytosterol (Stigmasterol and β-Sitosterol) determination in EBB and the mixture of PO and HPKO

Sample	Cholesterol mg/100 g	Stigmasterol mg/100 g	β–Sitosterol mg/100 g
EBB	278.34 ± 2.8	0.05 ± 0.01	0.1 ± 0.01
HPKO	12.33 ± 0.1	22.32 ± 0.22	63.01 ± 0.63
EBB/HPKO$_{25}$	210.33 ± 2.1	1.88 ± 0.02	18.46 ± 0.18
EBB/HPKO$_{50}$	145.89 ± 1.5	9.12 ± 0.09	28.54 ± 0.29
EBB/HPKO$_{75}$	119.5 ± 1.2	11.43 ± 0.11	40.82 ± 0.41
PO	11.81 ± 0.1	21.34 ± 0.21	61.54 ± 0.62
EBB/PO$_{25}$	207.45 ± 2.1	1.78 ± 0.02	17.67 ± 0.18
EBB/PO$_{50}$	144.56 ± 1.4	8.56 ± 0.09	27.56 ± 0.28
EBB/PO$_{75}$	117.5 ± 1.2	10.29 ± 0.10	39.65 ± 0.40

As presented in Table 2 when buffalo's butter oil was mixed with HPKO at levels of 25, 50, and 75%, cholesterol content was decreased from 278.34 to 119.5 mg/100 g fat at 75% mixing ratio while the content decreased from 278.34 to reach 117.5 mg/100 g fat when 75% of PO was added to buffalo butter. Conversely, stigmasterol gradually increased in butter-VOs mixture from 0.05 to be 1.88 mg/100 g fat at 25% mixture to reach 11.43 mg/100 g fat at 75% mixture with HPKO while gradually increased from 0.05 to be 1.78 mg/100 g fat at 25% mixture to reach 10.29 mg/100 g fat at 75% mixture of PO. Also, β-Sitosterol in buffalo's butter increased from 0.10 mg/100 g fat in the buffalo butter to reach 40.82 mg/100 g fat at 75% mixture of HPKO and increased from 0.10 mg/100 g fat in the buffalo butter to reach 39.65 mg/100 g fat at 75% mixture of PO.

In view of these result, it can be concluded that the addition of VOs to buffalo butter led to reducing the cholesterol content and increasing the phytosterol content and this change in their contents is related to the added level of VOs. Thus, VOs could be detected in buffalo butter by determining the presence of phytosterols, particularly β-Sitosterol, which is the most abundant sterol in VOs. The current findings are in agreement with those previously obtained by (Hallabo & El-Nikeety, 1987).

3.2.2 | Fatty acids composition by GC

Milk fat is one of the most complex edible fats, consisting of more than 400 fatty acids (Jensen, 2002) Table 3 show the fatty acids profile of EBB and the blends with HPKO and PO. These data indicated that EBB was characterized by its content of short chain (SCFAs) medium-chain (MCFAs) and long-chain (LCFAs) fatty acids. While in VOs, LCFAs were the most abundant fatty acids, especially palmitic, oleic and linoleic acids but SCFAs and MCFAs, except myristic fatty acid, were not found.

The data present in Table 3, Figures 1 and 2 showed that EBB contained a higher portion of saturated fatty acids (SFA; 67.94%) and a lower portion of unsaturated fatty acids (28.9%) than HPKO (51.8 and 37.55%, respectively) and PO (52.53 and 47.47%, respectively).

TABLE 3 Fatty acid profile of EBB, HPKO, PO, and its blends

Fatty acids		Treatment								
		EBB % of total	EBB/HPKO$_{25}$	EBB/PO$_{25}$	EBB/HPKO$_{50}$	EBB/PO$_{50}$	EBB/HPKO$_{75}$	EBB/PO$_{75}$	HPKO	PO
C4	Butyric	1.95	1.63	1.68	0.71	0.74	0.42	0.49	-	-
C6	Caproic	1.03	0.88	0.93	0.35	0.41	0.22	0.25	-	-
C8	Caprylic	0.71	0.51	0.54	0.23	0.27	-	-	-	-
C10	Capric	1.46	1.18	1.21	0.56	0.64	0.47	0.52	-	-
C12	Lauric	1.8	1.66	1.76	1.01	1.05	0.55	0.69	-	-
C14:0	Myristic	11.85	9.1	9.75	4.91	5.15	3.2	3.11	0.99	1.09
C14:1	Myristoleic	0.68	0.53	0.51	0.33	0.29	0.14	0.12	-	-
C16:0	Palmitic	35.4	37.71	37.78	39.18	39.9	41.4	40.92	42.55	43.13
C16:1	Palmitioleic	1.42	1.31	1.36	1.02	1.16	0.69	0.72	0.11	0.25
C18:0	Stearic	13.19	9.08	10.03	9.43	9.33	7.88	7.92	7.87	7.93
C18:1	Oleic	26.8	30.2	29.04	33.51	32.81	36.12	36.63	37.44	37.56
C18:2	Linoleic	1.48	4.21	3.27	7.02	6.46	8.26	8.03	10.41	9.55
C18:3	Linolenic	1.68	1.54	1.66	1.29	1.32	0.25	0.21	0.24	0.21
C20:0	Arachidic	0.55	0.46	0.48	0.45	0.47	0.4	0.39	0.39	0.38

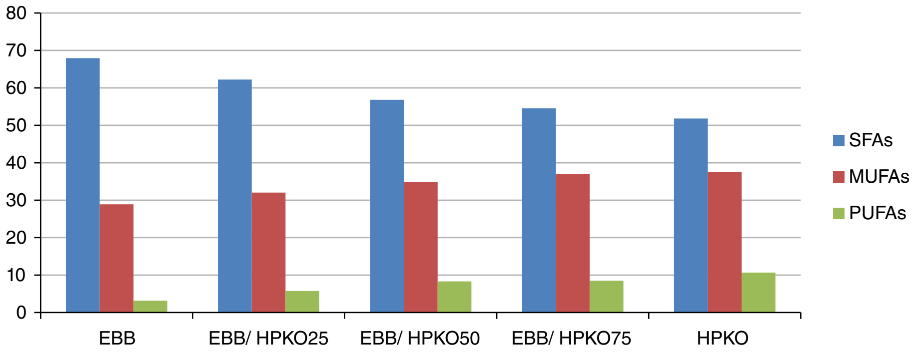

FIGURE 1 Fatty acid distribution of EBB, HPKO, and its blends

These results are similar to those obtained by other authors (Abdul Azis et al., 2011; Graill, 1992 & Lopez, bourgaux, Lesieur, Riaublanc, & Ollivon, 2006).

In addition, palmitic acid (35.4%) was the most abundant saturated fatty acid in EBB. Palmitic acid as an SFA and oleic acid as a USFA was the most abundant in both EBB and HPKO. However, palmitic and oleic acids of VOs were considerably higher as compared to the original EBB. Linoleic acid was also considerably lower in EBB (1.48%) as compared to HPKO (10.41%) and PO (9.45%).

Therefore, the adulteration of EBB with different levels of HPKO caused considerable changes in certain fatty acids; that is, butyric, myristic, palmitic, stearic, oleic, and linoleic acids. The higher the level of addition, the higher the change occurred in fatty acid profile (Table 3). In particular, with increasing levels of VOs, butyric and myristic acids gradually decreased, while palmitic, stearic, oleic, and linoleic acids gradually increased. A similar observation was found by Fox, Duthie, and Wulff (1989) in a blend of milk fat with soybean oil. Calvo et al. (2007) found that replacement of milk fat with VOs in many processed cheeses within the Egyptian market changed markedly the fatty acids profile of the cheese fat. Cheeses containing HPKO showed a high content of C16, C18:1, and C18:2 acids.

3.2.3 | Characterization of Fourier transform infrared (FT-IR) spectra

In fact, every oil/fat differs on its composition, length, and degree of unsaturation of the fatty acids as well as their positions in the chain. IR spectra represent a combined fingerprint pattern unique to each oil/fat and were used for discriminate analysis (Yang, Irudayaraj, & Paradkar, 2005). The spectra of both studied VOs were very similar. Strong absorption of bands for both EBB and VOs were observed in the regions of 3,000–2,800, 1800–1,600, 1,500–1,300 and 1,250–1,100 nm, which correspond to C—H stretching vibrations, C═O ester, C═C cis stretching and C—O stretching according to Christy and Egeberg (2006), respectively. A weak signal at 3006 nm, which associated with —C═C—H stretching groups of cis-unsaturation was observed. However, FT-IR spectral peak heights of EBB was lowest compared with those of VOs, which increased slightly with addition

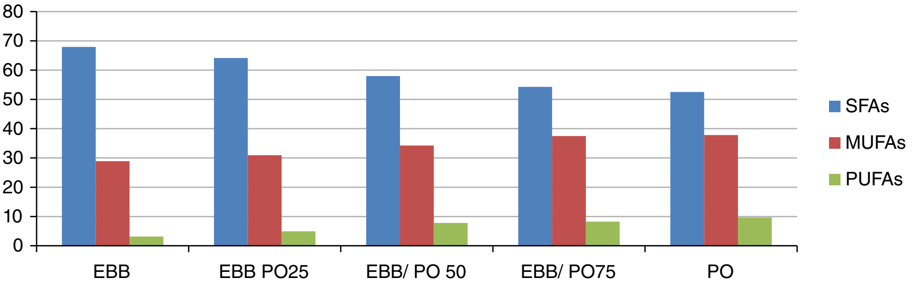

FIGURE 2 Fatty acids distribution of EBB, PO, and its blends

of VOs. A similar observation was found by Koca, Kocaoglu-Vurma, Harper, and Saona (2010) in butter and margarine. The exact position and intensity of bands are affected by proportion of unsaturated fatty acids (Vlachos et al., 2006). Vlachos et al. (2006) found that sunflower oil, soybean oil, corn oil, and sesame seed oil showed a maximum absorbance at 3009 nm whereas olive oil had a maximum band absorbance at 3006 nm and this spectral band shift was likely due to differences in the proportion of oleic acid acyl groups and linoleic and linolenic acyl groups. In the same trend, some differences were observed in FT-IR spectral bands at 1659 nm, which also associated with —C═C—H (cis) stretching vibration for EBB and VOs. While it was very difficult to differentiate between EBB, VOs and its mixtures based on the spectra from observation, FT-IR spectral bands of EBB were lower in absorption in the regions of 3,000–2,800 nm than those of VOs. The maximum absorption of bands for EBB was at 2,925 and 2,855, which shifted to 2,928 and 2,857 nm for VOs, respectively. Also, FT-IR spectral bands of EBB gradually increased with increasing the addition level of PO. Yang et al. (2005) reported that the major peaks that represent triglyceride functional groups could be observed around 2,937 nm (C—H stretching, asymmetry), and 2,856 nm (C—H stretching, symmetry). At 1745 nm, another strong band was found in both EBB and PO, which is reported to be associated with —C═O stretching vibrations of acids and esters (Koca et al., 2010). FT-IR spectra of BO at this band were lower than those of VOs. However, when EBB adulterated with VOs EBB had almost similar FT-IR spectra at 1745 nm. Strong absorption of bands for both EBB and VOs were observed in the region of 1,500 and 1,350 nm. VOs showed spectral bands with higher maximum absorbance at 1,465 and 1,378 nm than EBB. Another major difference occurs in spectra region at 1,250 to 1,050 nm; the FT-IR spectra peaks of VOs were higher than these of EBB. Guillen and Cabo (1997) reported that the stretching vibrations of the C—O bond of esters composed of two asymmetric vibrations consisting of C—C(═O)—O and O—C—C, occur in the region between 1,300 and 1,000 nm. However, there were no major differences in these spectra peaks between EBB and EBB adulterated with VOs. AOAC (2005) reported the band at 966 nm, associated with —HC═CH out-of-plane deformation vibrations, could be used as a marker band for the determination of trans fats. However, EBB, VOs, and their mixtures showed low absorption at this spectrum 966 nm, which shifted to 972 nm. However, it was very difficult to differentiate between EBB, VOs and its mixtures based on this spectrum from observation. Generally, all above FT-IR spectra regions can be used to differentiate between EBB and VOs. However, FT-IR spectra regions at 3006, 3000–2,800, 1800–1,600, and 1,500–1,300 nm can be used to detect adulteration of EBB with VOs especially at higher concentrations.

4 | CONCLUSION

A comparative study between some advanced and traditional methods for detection of milk fat adulteration. The precision and sensitivity of some methods for detecting VOs in EBB were investigated. The liquefied EBB and VOs were mixed in proportions of 0:100, 25:75, 50:50, 75:25, and 100:0 (w/w) to create five treatments. Two treatments represent the original components and three treatments were binary blends. The samples were analyzed for chemical properties (Iodine value, saponification number, Richert-Missel and Polenske values) and some main components such as fatty acids, cholesterol, and sterols) were determined using GC and HPLC. The obtained findings revealed that as the proportion of VOs being added to EBB increased, saponification, Reichert-Meissl, and Polenske values decreased, while iodine value increased. The addition of VOs to EBB in ratios of more than 50.0% lead to the increase of saponification, Reichert-Meissl, Polenske, and iodine values to beyond the normal range of pure EBB. Therefore, these properties cannot be used to detect the adulteration with VOs when applied to a mixture containing less than 50.0% VOs. By increasing the proportion of VOs in EBB, cholesterol content, and cholesterol percentage decreased while phytosterol contents increased. In view of the obtained findings, it can be concluded that the advanced techniques such as HPLC and GC can be successfully used in the detection of butter adulteration through their capability to detect the lower content of phytosterols which served as an indicator of using VOs as adulterants, even if added in very low concentrations. FT-IR spectra regions at 3006, 3000–2,800 and 1800–1,600 nm can be used to detected adulteration EBB with VOs especially at higher concentration and it is not sensitive to detect the difference between PO and HPKO.

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AUTHOR CONTRIBUTION

Ahmed M.Hamed: conceived of the presented idea, conceived and planned the experiments and was involved in supervised the work. Mahmoud Aborass, Israa El-Kafrawy: carried out the experiment Gehan Safwat: wrote the manuscript with support from Ahmed M. Hamed. All authors discussed the results and contributed to the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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