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Development of A Protocol Based on Classic and Modern Techniques for Detection of Shortening and Hydrogenated Palm Kernel Oil Addition to Buffalo's Milkfat

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Abstract

In the Egyptian market, the most commonly used milkfat is buffalo's milkfat (BMF) as it is used by many consumers. Due to its high value and high cost, BMF is prone to adulteration with other cheap vegetable oils (VO). The common VO that are added to BMF are Shortening (SHO) and Hydrogenated Palm Kernel Oil (HPKO). Therefore, the current study was conducted to propose a two-steps protocol that can be used to detect adulteration of BMF with VO in general, and with SHO and HPKO in particular. SHO and HPKO were added, separately, to BMF by using different proportions (10, 20, 30, 40, and 50%). Reichert Meissl value (RM) and sterol fractions were used (first step) to detect the addition of VO to BMF. The addition of SHO and HPKO resulted in a significant decrease in the RM values when added with a proportion of 30% or more. The β-sitosterol values showed a significant increase when SHO or HPKO was added (10% or more). Fatty acids profile was used (second step) to determine the type of VO added to BMF. It was found that the addition of HPKO to BMF resulted in a significant increase in the levels of lauric acid (C12:0), palmitic acid (C16:0), and saturated fatty acids. While the addition of SHO to BMF resulted in a significant decrease in myristic acid (C14:0) and an elevation in palmitic acid (C16:0). Three different BMF samples from the local market were tested by using this two-steps protocol, and it succeeded to detect the addition of VO to BMF and to identify the type of VO that had been used. The obtained results suggested that the determination of β-sitosterol and fatty acids composition could be used for ascertaining the quality of milk fat.

Keywords: Buffalo's milkfat, Fatty acids composition, β-sitosterol, shortening, hydrogenated palm kernel oil

Introduction

Food fraud is a problem that can affect the food safety and marketing value of the final product [25, 31]. The problem of food fraud or food adulteration is considered a very serious problem that could affect the Food Industry either domestically or internationally due to its failure to meet consumer expectations. Milk is among the most foods that are commonly targeted by adulteration. The major milk component that is adulterated is milk fat (or milkfat) [21].

Milkfat is an important nutrient in milk that is separated from milk and sold as a milk product. In addition, it has been shown that the demand for butter is increased [16] as it can be used. In Egypt, the most commonly used milkfat is produced from buffalo's milk, and its price is higher than that of milkfat from cow's milk. Due to its high value and high price, milkfat is prone to be adulterated, and most of the milkfat is being adulterated by the addition of vegetable oils [21]. In Egypt, Buffalo's milkfat (BMF) is adulterated by mixing it with shortening and hydrogenated palm kernel oil that are very cheap compared to BBO to increase their profit margins [32]. From both legal and consumer protection points of view, it is crucial for milkfat to be correctly labeled [15].

Detection of adulteration of milk fat with vegetable oils has been studied by many investigators by using several classic and modern parameters [33]. Classic parameters such as the iodine value and refractive index, Reichert-Miessel value, Polenske value have been used [2, 7, 10, 23, 35]. Due to the wide range of these fat constants, they were not enough alone to detect adulteration of milkfat, as the milkfat may be adulterated and the fat constants are in the acceptable and normal range. Therefore, they should be used beside other methods. Especially if the milk fat was adulterated with vegetable oil with fat constant values close to those of milkfat.

In addition, fatty acid (FA) composition can be used to detect adulteration of milkfat. More precisely, these methods of detection are based on the measurement of individual, two, or more FA concentrations [5, 8, 10, 14]. Another method of

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detection is to measure triacylglycerols composition by GC analysis based on separation by carbon number, but this method showed some limitations [4, 6, 14, 27, 29].

Other methods that are used for the detection of adulteration of milkfat are based on the measurement of minor fat constituents such as diglycerides sterols, dehydrated forms of sterols (steradienes), and tocopherols [19, 26]. The main sterol fraction that is widely used for the detection of vegetable oil addition to milk fat is β-sitosterol [20]. Abd El-Malek [1] used cholesterol content as a tool for the detection of vegetable oils addition to butter from buffalo's milk.

Due to the widespread and low price of shortening (SHO) and hydrogenated palm kernel oil (HPKO) in the Egyptian market, it is more likely to be used for adulteration of BBO. Therefore, in the present study the authors attempted to develop a protocol for the detection of adulteration of BMF with SHO and HPKO. This protocol is based on the measurement of milk fat contestants (Reichert-Miessel) in addition to measurement of FA composition as well as sterol fractions (Stigmasterol and β -Sitosterol).

Experimental

Materials

Standard buffalo's milkfat:

Fresh buffalo's milk (6.3% fat, determined by using Gerber method [24]) was obtained from the herd of the faculty of agriculture, Cairo Univ., Giza, Egypt. The milk was separated using an Alfa-Laval separator and the obtained cream was churned to butter. The resultant butter was melted at 60°C; the butter oil layer was decanted and stored at 4°C until usage. This sample represents the standard Buffalo's milkfat (BMF).

Buffalo's milkfat samples from local market:

Three BMF samples were collected from three different local markets at Giza governorate, and stored at 4°C until usage.

Vegetable oils:

Four samples of oils namely Hydrogenated Palm kernel oil (HPKO) and Shortening (SHO) imported from Indonesia were purchased from the Consumer Association of Foodstuff, Feisal St., Giza, Egypt.

Preparation of vegetable oils and BMF mixtures:

The mixtures were prepared after complete melting of the standard BMF and vegetable oils (SHO and HPKO) at 70±0.5°C in an electrical water bath. The vegetable oils were mixed with BMF in different ratios (10, 20, 30, 40, 50%, w/w).

Method of analyses

Richert-Meissl number

Reichert-Meissl value (RM), an indication of soluble volatile fatty acids, was determined according to the method described by AOAC [3].

Determination of fatty acids composition:

Fatty acid compositions of samples were trans-esterified into their corresponding fatty acid methylesters (FAMEs) using methanolic NaOH and boron triflouride (BF3) with methanol as described by the AOAC [3].

The FAMEs were quantified by Shematizu Gas Chromatograph Series2010 equipped with a 2010 Sautosampler (Shematizu Co., Japan) and interfaced with a flame ionization detector (FID). The GC was equipped with a temperature programmable column. The column phase was SupplcoDB-Wax (carbowax) with the following dimensions: 30 m long, 0.25 mm i.e. with a 0.25µm phase thickness. Helium was used as a carrier gas with 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 at 2 psi was 0.68 mL/m. The column temperature was maintained from 50°C up to 200°C rate10°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).

Determination of sterol fractions (Stigmasterol, β – Sitosterol and Cholesterol):

Sample preparation and determination of sterol fractions were done according to the method fully described by Oh et al. [28]. In brief, 2 g of fat or oil was refluxed with 100 ml of alcoholic potassium hydroxide solution (2 mol/l) and a few anti-bumping granules for 2 hr. The unsaponifiable were first extracted 3 times with 10ml of petroleum ether; the extracts were combined and washed 3 times with water and then dried with anhydrous sodium sulphate, then ether was completely evaporated. Unsaponifiable residues were dissolved in methanol HPLC grade then filtrated with polytetrafluorethylene syringe filter (0.2µm pore size). HPLC conditions for injection: Agilent 1260 infinity HPLC Series (Agilent®, USA), equipped with Quaternary pump, a Kinetex XB-C18 column 100 mm × 4.6 mm (Phenomenex®, USA), operated at 35°C. The separation was achieved using isocratic elution with acetonitrile: methanol (3:1). The

injection volume was 20 µL. Detection: variable wavelength detector (VWD) set at 205 nm.

Statistical analysis

Results are presented as means ± SD. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons using a statistical package software (SPSS ver. 22.0). Differences were considered to be statistically significant when $P<0.05$.

RESULTS & DISCUSSION

1. Reichert-Meissl number

Data in Table (1) summarize the Reichert-Meissl number (RM) of BMF and its mixture with vegetable oils (SHO, HPKO). The RM for BMF was 29.22, while it was very low in SHO and HPKO (0.68 and 5.37, respectively) as the volatile fatty acids content is different in vegetable oils from that in milk fat. Therefore, the RM values of BMF decreased with the addition of both vegetable oils and it reached 21.08±0.36 and 16.56±0.31, when the percentage of both vegetable oils was 50% (for SHO and HPKO, respectively). The reduction in RM caused by vegetable oils was between 1.6 to 34.2% and 4.5 to 43.3% for SHO and HPKO, respectively. It is obvious that RM could detect adulteration of milk fat with small quantities (30% or more) of SHO and HPKO, only when both standard and adulterated samples are tested together. These results are in line with other investigators who found a very high detection limit of RM [12. 23].

2. Sterol fractions

Results of HPLC analysis of cholesterol and phytosterol (Stigmasterol and β-sitosterol) are presented in Table (2). It can be noticed that BMF samples is containing 289.9 mg/100g of cholesterol, and nearly no stigmasterol and β-sitosterol were found in both SHO and HPKO samples. Therefore, the addition of both vegetable oils resulted in a reduction in the contents of cholesterol and an increase in stigmasterol and β-sitosterol in the final BBO/vegetable oils mixtures.

Table (1): Values of Reichert-Meissl for BMF and vegetable oils (SHO and HPKO) mixtures.

Samples (BMF:SHO)	Reichert-Meissl value	Samples (BMF:HPKO)	Reichert-Meissl value
100:0	29.22^{a}±0.51	**100:0**	29.22^{a}±0.51
90:10	28.75^{a}±0.58	**90:10**	27.92^{b}±0.48
80:20	26.32^{b}±0.61	**80:20**	25.00^{c}±0.53
70:30	24.87^{c}±0.47	**70:30**	20.75^{d}±0.41
60:40	22.01^{d}±0.46	**60:40**	18.47^{e}±0.32
50:50	21.08^{e}±0.36	**50:50**	16.56^{f}±0.31
0:100	0.68^{f}±0.12	**0:100**	5.37^{g}±0.19

BMF (buffalo's milkfat), SHO (shortening), HPKO (hydrogenated palm kernel oil).
Values are Means ± standard deviation.
Values in the same raw bearing different superscript letters are different ($P<0.05$).

Accordingly, adulteration of BMF with both SHO and HPKO could be detected by determination of the presence of phytosterols, and in particularly β-sitosterol, which is the most abundant sterol in vegetable oils. These results are in agreement with what was previously reported by Hallabo and El-Nikeety [13] and Contarini et al. [36]. In addition, the International Dairy Federation (IDF) recommended the determination of sterols by using gas chromatography [17] or thin layer chromatography [18] as a routine method for detection of milk fat adulteration with vegetable oils.

Table (2): Sterol fractions in BO, SHO, HPKO and their mixtures.

Samples (BO:HPKO)	Cholesterol mg/100g	Stigmasterol mg/100g	β -Sitosterol mg/100g	Samples (BO:SHO)	Cholesterol mg/100g	Stigmasterol mg/100g	β -Sitosterol mg/100g
100:0	289.9^{a}±9.10	0.03^{f}±0.00	0.12^{g}±0.01	**100:0**	310.15^{a}±8.91	ND	ND
90:10	245.0^{b}±7.01	1.02^{e}±0.06	9.32^{f}±0.18	**90:10**	300^{a}±7.99	3.08^{e}±0.13	10.12^{f}±0.17
80:20	203.9^{c}±6.6	1.51^{d}±0.08	15.82^{e}±0.14	**80:20**	247.12^{b}±5.84	3.0^{e}±0.14	26.6^{e}±0.19
70:30	186.1^{d}±4.10	5.0^{c}±0.32	17.76^{d}±0.15	**70:30**	245.32^{b}±5.78	5.6^{d}±0.17	28.5^{d}±0.18
60:40	169.4^{e}±4.04	5.48^{c}±0.17	23.52^{c}±0.41	**60:40**	220.5^{c}±4.68	7.3^{c}±0.18	30.1^{c}±0.20
50:50	147.9^{f}±3.65	7.53^{b}±0.19	28.91^{b}±0.57	**50:50**	189.32^{d}±4.57	9.5^{b}±0.18	35.6^{b}±0.22
0:100	11.7^{g}±0.14	20.85^{a}±0.6	66.53^{a}±0.56	**0:100**	23.5^{e}±0.55	35.80^{a}±0.36	80.15^{a}±0.78

.BMF (buffalo's milkfat), SHO (shortening), HPKO (hydrogenated palm kernel oil)
.Values are Means ± standard deviation
Values in the same raw bearing different superscript letters are different ($P<0.05$).

3. Fatty acids composition

Data presented in Tables (3 & 4) show the fatty acids composition profile for both SHO and HPKO samples. It could be easily noticed that Lauric acid (C12:0) is found at a very high level in HPKO (41.9%, in Table 3), and it was very low in BMF samples (1.85%). On the other hand, Palmitic acid (C16:0) is the highest fatty acid in SHO (47.19%, in Table 4), while it was low in BMF samples (29.63%). As for stearic acid (C18:0), it was found to be 15.76% in BMF which was lower than that found in HPKO (21.94%), and higher than that found in SHO (4.64%).

On the other hand, Oleic acid (C18:1) was found to be 36.88% in SHO and 6.3% in HPKO compared to 28.05% in BMF. While, Linoleic acid (C18:2) was found in relatively low concentrations as it represents 2.81% in BMF, 8.66% in SHO, and 0.27% in HPKO. Levels of saturated (SFA) and unsaturated (UFA) fatty acids are shown in Table 5. It can be noticed that the BMF is high in SFA (63.63%) than in SHO (53.62%), and at the same time, it is lower than HPKO (92.45%). These results were found by other investigators [22].

Data in the same Tables show the fatty acids composition of the different mixtures ratios 10, 20, 30, 40, and 50% of HPKO (Table 3) and SHO (Table 4). It is noticeable that the fatty acid composition of each mixture completely changed when compared with the milk fat, also the total SFA gradually increased as the amount of added HPKO increased while the MUSFA and PUSFA took an opposite direction.

I It would be expected that when milk is mixed with vegetable oil a pronounced change in the fatty acids composition of the resultant mixture will be found. Accordingly, the fatty acid composition is a very important and dependable factor to detect adulteration of milk fat with vegetable oil, and it could be used to identify the type of vegetable oil used. Salem et al. [30] also found the FA composition of buffalo's milk fat is SFA (64%), USFA (33.0%), and MUSFA (28.08%) which was similar to that obtained for BMF in the present study. Similar results were also obtained for buffalo's milk fat by Abd El-Malek [1].

Other researchers found that the addition of lard or margarine to cow's or buffalo's milk fat resulted in significant changes in certain fatty acids (16:0, 18:0 and18:1) [9]. Moreover, the addition of vegetable oils to milk fat reduced levels of short-chain acids [11]. Sharma and Singhal [34] found significant changes in the fatty acid composition of ghee adulterated with hydrogenated vegetable oils when compared with control ghee. It is clear that vegetable oils are rich in linoleic acid (C18:2), palmitic acid (C16:0), and stearic acid (C18:0). Therefore, some official methods depending on fatty acid composition were found to be used for the detection of milkfat adulteration such as in Argentina [33].

4. Detection of milk fat adulteration in BMF samples from the local market

To apply the proposed two-steps protocol for detecting milk fat adulteration, three doubtful BMF samples were purchased from the local market (S1, S2 and S3). RM and sterol fractions were measured (first step).

As shown in Table (5), there is a great probability of adulteration particularly in samples S1 and S3 when compared to the standard BMF. RM number in these samples was lower than that of the standard BMF, in particular in sample S3 (RM reduced by 25.26% when compared with the BMF). It seems that RM is not sufficient to detect vegetable oils addition to BMF.

As shown in Table (5), cholesterol content of the three samples was lower than that of the BMF. It is obvious that cholesterol alone is not sufficient to detect adulteration with vegetable oils. However, evaluation of Stigmasterol and β-Sitosterol content of these samples clearly shows that the S2 sample was free of β-Sitosterol and showed the lowest

Stigmasterol content while the S1 and S3 showed higher content of both of β-Sitosterol (39.08 and 35.77 mg/100g fat, respectively), and Stigmasterol (7.45 and 5.05 mg/100g fat, respectively). These results indicate with no doubt that S1 and S3 samples are adulterated samples. To determine the type of vegetable oil that was added, the fatty acid composition was determined. Table (6) is showing the fatty acids composition of buffalo's milk fat samples (S1, S2 & S3) collected from local market compared with standard BMF. After comparing the fatty acid composition of these samples with the fatty acid composition of standard BMF (Table 6), HPKO (Table 3) and SHO (Table 4), it is most probable that Sample S1 and S3 might be adulterated by SHO. This is because SHO is characterized by the absence of (C6:0), (C8:0) and (C10:0), its low content of both Lauric acid (C12:0), Myristic acid (C14:0) and Stearic acid (C18:0), as well as its high levels of Palmitic acid (C16:0) and Linoleic acid (C18:2). Calculations of increasing and decreasing in fatty acid composition suggested that the highest ratio of adulteration is found in sample S1 followed by sample S3 while sample S2 seemed to be free of adulteration.

Table (3): Fatty acids composition of BMF as affected by the addition of HPKO at different levels

Fatty acids		BMF	Addition of HPKO (%) to BMF					HPKO
		100%	10%	20%	30%	40%	50%	100%
Caproic	C 6:0	1.09	1.32	1.12	1.04	0.84	0.68	0.25
Caprylic	C 8:0	0.63	1.17	1.38	1.75	1.87	2.52	3.77
Capric	C 10:0	1.30	1.68	1.72	1.94	1.90	2.32	3.16
Lauric	C 12:0	1.85	6.27	9.3	13.33	17.95	21.54	41.91
Myristic	C 14:0	9.41	9.38	9.37	9.77	10.35	11.13	12.47
Pentadecanoic	C 15:0	2.10	1.84	1.60	1.41	1.15	1.04	-
Palmitic	C 16:0	29.63	26.96	25.23	23.08	20.45	19.51	8.72
Heptadecanoic	C 17:0	1.6	1.36	1.25	1.09	0.90	0.81	-
Stearic	C 18:0	15.76	15.8	16.83	17.72	18.47	18.83	21.94
Arachidic	C 20:0	0.26	0.26	0.26	0.24	0.23	0.24	0.23
SFA (%)		**63.63**	**66.04**	**68.06**	**71.37**	**74.11**	**78.62**	**92.45**
Myristoleic	C 14:1	0.50	0.44	0.38	0.33	0.27	-	-
Palmitoleic	C 16:1	1.86	1.60	1.46	1.26	1.10	-	-
Oleic ω 9	C 18:1	28.05	24.76	23.38	21.44	19.27	6.30	6.30
Vaccinic ω 7	C 18:1	0.48	0.77	0.72	0.6	0.56	0.58	0.58
MUSFA		**30.89**	**27.57**	**25.94**	**23.63**	**21.2**	**18.29**	**6.88**
Linoleic ω 6	C 18:2	2.81	2.66	2.59	1.97	1.79	0.27	0.27
α-Linolenic	C 18:3	0.24	0.20	0.24	0.15	0.38	-	-
ω 3	C 18:4	0.56	0.44	0.47	0.37	0.30	-	-
PUSFA		**3.88**	**3.51**	**3.48**	**2.64**	**2.59**	**1.80**	**0.27**

BMF (buffalo's milkfat), HPKO (hydrogenated palm kernel oil)

Table (4): Fatty acids composition of BMF as affected by the addition of SHO at different levels

Fatty acids		BMF	Addition of SHO (%) to BMF					SHO
		100%	**10%**	**20%**	**30%**	**40%**	**50%**	**100%**
Caproic	C 6:0	1.09	2.16	1.2	1.13	0.92	0.99	0.01
Caprylic	C 8:0	0.63	1.06	0.63	0.6	0.51	0.52	0.03
Capric	C 10:0	1.30	1.79	1.19	1.11	0.95	0.92	0.02
Lauric	C 12:0	1.85	2.14	1.62	1.49	1.29	1.18	0.02
Myristic	C 14:0	9.41	8.93	7.61	6.83	5.96	5.74	1.16
Pentadecanoic	C 15:0	2.10	0.81	0.72	0.63	0.54	0.52	0.06
Palmitic	C 16:0	29.63	34.35	36.42	37.64	30.52	39.75	47.19
Heptadecanoic	C 17:0	1.6	0.46	0.45	0.38	0.34	0.33	0.11
Stearic	C 18:0	15.76	9.98	10.43	9.6	8.33	8.17	4.64
Arachidic	C 20:0	0.26	0.28	0.31	0.34	0.32	0.32	0.38
SFA (%)		**63.63**	**61.96**	**60.58**	**59.75**	**57.68**	**58.42**	**53.62**
Myristoleic	C 14:1	0.50	0.58	0.48	0.42	0.36	0.34	0
Palmitoleic	C 16:1	1.86	1.52	1.38	1.19	1.03	1.01	0.2
Oleic ω 9	C 18:1	28.05	0.17	0.18	0.13	0.12	0.11	0.02
Vaccinic ω 7	C 18:1	0.48	24.89	27.87	28.35	29.97	30.16	36.88
MUSFA		**30.89**	**27.16**	**29.91**	**30.09**	**31.48**	**31.62**	**37.1**
Linoleic ω 6	C 18:2	2.81	3.61	4.44	4.99	5.62	5.66	8.66
α-Linolenic	C 18:3	0.24	0.31	0.29	0.51	0.27	0.25	0.20
ω 3	C 18:4	0.56	0.37	0.32	0.30	0.22	0.22	0
PUSFA		**3.88**	**4.29**	**5.05**	**5.80**	**6.11**	**6.12**	**8.86**

BMF (buffalo's milkfat), HPKO (hydrogenated palm kernel oil)

Table (5): Sterols fraction in doubtful buffalo's milk fat collected from local markets compared with BO

Samples	Reichert Meissl	Cholesterol mg/100g	Stigmasterol mg/100g	β-Sitosterol mg/100g
BMF	29.22±0.51	289.91±9.1	0.03±0.0	0.12±0.01
S1	23.93±0.46	219.78±6.9	7.45±0.21	39.08±0.59
S2	25.50±0.48	254.49±6.85	0.95±0.01	ND
S3	21.75±0.39	232.20±7.02	5.05±0.19	35.77±0.68

.BMF (buffalo's milkfat), SHO (shortening), HPKO (hydrogenated palm kernel oil)
.Values are Means ± standard deviation
Values in the same raw bearing different superscript letters are different (P<0.05).

Table (6): Fatty acids composition in doubtful buffalo's milk fat collected from local markets compared with standard BMF.

Fatty acids		BMF	S1	S2	S3
			%		
Caproic	C 6:0	1.09	0.97	1.24	0.97
Caprylic	C 8:0	0.63	0.56	0.68	0.5
Capric	C 10:0	1.3	1.12	1.33	0.93
Lauric	C 12:0	1.85	1.52	1.83	1.31
Myristic	C 14:0	9.41	7.01	8.49	7.44
Pentadecanoic	C 15:0	2.1	1.7	1.85	1.92
Palmitic	C 16:0	29.63	35.4	29.52	37.76
Heptadecanoic	C 17:0	1.6	1.34	1.52	1.46
Stearic	C 18:0	15.76	8.89	13.79	10.39
Arachidic	C 20:0	0.26	0.23	0.21	0.24
SFA (%)		**63.63**	**58.74**	**60.46**	**62.92**
Myristoleic	C 14:1	0.5	0.46	0.66	0.37
Palmitoleic	C 16:1	1.86	1.41	2.21	1.5
Oleic ω 9	C 18:1	28.05	26.81	27.58	24.98
Vaccinic ω 7	C 18:1	0.48	2.91	3.13	3.4
MUSFA		**30.89**	**31.59**	**33.58**	**30.25**
	C 16:3	0.27	0.41	0.24	0.24
Linoleic ω 6	C 18:2	2.81	5.01	2.04	3.31
α-Linolenic	C 18:3	0.24	0.54	-	0.32
ω 3	C 18:4	0.56	0.58	0.63	0.57
PUSFA		**3.88**	**6.54**	**2.91**	**4.44**

BMF (buffalo's milkfat), HPKO (hydrogenated palm kernel oil), SHO (shortening) S1, S2 and S3 (BMF samples from local market).

CONCLUSION

In sum, the present study suggested a protocol for the detection of adulteration of Buffalo's milkfat (BMF) based on the measurement of Reichert Meissel value, sterol fractions and fatty acids composition. This protocol could be used for the detection of shortening and hydrogenated palm kernel oils addition to BMF. The obtained results showed that Reichert Meissel value alone could be used to detect adulteration of BMF only when a high quantity of vegetable oils was added, while sterol fractions could detect addition of vegetable oils with small quantities. Fatty acids composition is better in identifying the type of vegetable oil used for adulteration.

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