

Methods For Detecting Butter Adulteration

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ABSTRACT

Twelve samples of butter were purchased from the local markets and compared with control butter sample made in the lab.. The samples were kept under cooling till analysis. The samples were analyzed by gas chromatography for the fatty acids content, and also chemically analyzed for cholesterol levels and fat content. The chromatographic analysis revealed that only three samples were identical to the control sample, while the other samples varied from the control regarding fatty acid composition. The results revealed that five samples showed a marked decrease in the total short chain acids when compared to the control sample. It could also be observed that four samples had higher content of lauric acid (C_{12}), while three samples possessed high content of palmitic acid ($C_{16:0}$). On the other hand,, two samples were characterized with higher levels of oleic acid ($C_{18:1}$), and stearic acid ($C_{18:0}$). These findings show that an adulteration with palm kernel oil, palm oil and tallow, was done, respectively. The results obtained from chromatographic analysis enabled to detect the adulteration by using the fatty acids ratios between certain fatty acids. The ratio between C_{12}/C_{10} , C_{14}/C_{12} , $C_{18:1}/C_{14}$, $C_{18:0}/C_{18:2}$, $C_{18:1}/C_{18}$ and the total saturated fatty acids/total unsaturated fatty acids, were used to detect the adulteration. The ratios between C_{12}/C_{10} , $C_{18:0}/C_{18:2}$, $C_{18:1}/C_{18:0}$, and total saturated fatty acids /total unsaturated fatty acids were useful in detecting the adulteration of butter fat with vegetable oils or tallow. Cholesterol content of the samples was carried out. The results obtained revealed that the addition of adulterants to the butter decreased the cholesterol level of the adulterated samples when compared to the control sample. The decrease of the cholesterol level seems to be proportional to the adulteration ratios. Also, calculating the cholesterol level of the suspected samples regarding the cholesterol level of the control samples helped to detect the ratios of adulterants in butter samples. Fat content of all samples did not differ.

INTRODUCTION

Because of the large price difference between butter fat substitute and pure butter fat, the adulteration of the later by substitutes is probably to occur. Vegetable oils and beef tallow are the common substitute of butter fat in Egypt. The mixing of animal fat with food products is a major concern to certain groups of consumers due to religious obligations and health complication. From religious perspectives, the source of fat that acts as adulterant is a serious issue of concern. In Islamic dietary laws, food containing porcine based substances are strictly forbidden, while in Hinduism, the consumption of beef fats in food is prohibited (Eliasi and Dweyer, 2002), Marikkrar *et al.* (2005).

However, several methods have been developed for the detection and qualification of adulterant in butter fat. Numerous authors (De peters 1993, Carisano and Riva 1976, Coleman 1961, Mattson and Luton 1958, Mattson 1963, Jensen *et al* 1969) have reported small amounts of beef tallow incorporated into butter by evaluating the fatty acids composition of the monoglycerids acquired by enzymatic hydrolysis. The addition of beef tallow in butter has been reported by Soliman and Younis (1986) by determining the cholesterol esters and diglycerides. However, differences in Fatty acids of vegetable oils and milk fat should be very distinct to be applicable to use as a detection tool (Fox *et al.* 1988, Ntakatrane *et al.* 2013; Ulberth 1994). Soybean and canola oils have high amount (7 – 10%) of linolenic acid ($C_{18:3}$), but milk fat has very low amount (0.9 – 1.2%). Therefore, detection of higher amount of linolenic acid in dairy products can be a sign of adulteration with soybean or canola oils (Clemente and Cahoon, 2009). Cottonseed, sunflower and corn oil have high amount (40 – 70%) of linolenic acid ($C_{18:2}$), but milk fat has low amount of this fatty acid (1 – 2%). Therefore, linoleic acid can also be used to detect some vegetable oils (Liu *et al.* 2002). Fatty acids composition of milk fat has long been used as a criterion to detect adulteration with vegetable oils mainly because milk fat is characterized by short chain fatty acids where are vegetable oils have

medium – to long chain fatty acids (Ntukatsane *et al.* (2013).

One of the most differences between milk fat and vegetable oils is cholesterol. The sterol of butter and margarine was isolated by saponification and chromatographic separation of the unsaponifiable matter in a florisil column, Eisner *et al.* (1962). Gas chromatography of the sterol fraction from six samples of butter indicated only one component, cholesterol. The samples of margarine apparently consisted of three major components of B- sitosterol, γ - sitosterol, and stigmasterol. Other trials have been carried out to detect the adulteration of butter fat with other foreign fats. The adulteration of butter fat was detected using the infra-red spectroscopy by measuring the transunsaturated acids in the authentic milk fat and hydrogenated vegetable oil, (Parodi and Dunstan, (1971). The unsaponifiable matter of butter contains 98.0 – 99.7% of cholesterol Torr *et al.* (1977). Analysis of the sterol fraction of butter and dried milk samples showed 98.5 – 99.5% cholesterol, 0.4 – 1.2% campesterol and ergosterol Guyot and sadin (1974). Since the plants are not a source of cholesterol, Enominger *et al.* (1983), the mixing of pure ghee with 5% of vegetable oils is resulting in bands on TLC chromatograms similar to those of the oils added, Sepasatian and Rao (1974), and it was found that the addition of vegetable fat to butterfat can be detected by the presence of sitosterol which is not present in pure butter fat, Hamberg and Bielefeld (1980). Ha- Jung Kim *et al.* (2016) suggested that oleic acid, linoleic acid and cholesterol are suitable indicators and can be used as biomarker to rapidly detect adulteration of milk fat.

Ratios between the fatty acids of fat can be used for check for addition of foreign fats to milk fat. Many investigator depend on the ratio between the fatty acids of fat to detect the adulteration of milk fat Echizen and Deki (1975) reported that butyric acid of butter fat decreased with the addition of other fats.

Gargano, (1979) found that the ratios of 1.01 – 1.38, 2.3 – 3.42 and 2.15 – 2.70 for $C_{12} : C_{10}$; $C_{14} : C_{12}$ and $C_{18:0} : C_{18:1}$, respectively, are usually considered characteristic of unadulterated butter. Chernev *et al.* (1979)

noted that the ratio of 0.81: 1 to 0.96: 1 between lauric and capric acids in milk fat are considered suitable for detection of adulteration of butter. Gosheva, (1979) reported that the ratio between $C_{12}: C_{10}$ should be 1.25 to obtain a good quality butter, but the $C_{14}: C_{12}$ ratio which should be 2.8 ranged from 2.42 to 3.7.

Farag *et al.* (1980) found that the ratios of $C_{18:0}: C_{18:2}$ and total saturated: total unsaturated fatty acids were effective for detecting the adulteration.

For detecting the adulteration of soybean oil and buffalo depot fat in milk fat, Anil Kumar *et al.* (2015) reported that the ratios of the individual fatty acids such as $C_{14:0}: C_{16:0}$, $C_{14:0}: C_{18:1}$, $C_{14:0}: C_{18:2}$, $C_{16:0}: C_{18:1}$, $C_{16:0}: C_{18:2}$ and $C_{18:0}: C_{18:2}$ were selected and compared with the respective overall range of all these fatty acid ratios of pure cow and buffalo milk fat, found to be ranged from 0.38 to 0.45, 0.44 to 0.54, 4.10 to 12.47, 1.04 to 1.3, 9.61 to 30.96 and 3.79 to 13.51, respectively. It also reported that the ratio of $C_{18:0}: C_{18:1}$ and $C_{14:0}: C_{18:2}$ were more helpful in detecting of adulteration. Therefore, by keeping all these aspects about fatty acid composition of milk fat an attempt was made to detect adulteration in butter with vegetable oils and buffalo depot fat using Gas liquid chromatography technique and chemical analysis, with the assesst of the distinct fatty acid of butter fat compared with the fatty acid profile of the suspected samples, also the ratios between the fatty acids was applied to detect the genuinity of the butter samples. Cholesterol content of the samples could be a good tool to detect the butter adulteration with other fats.

MATERIALS AND METHODS

1-Source of samples:

Twelve samples of buffalo butter were purchased from the local markets, located in the governments of Cairo, Giza and Qalubeya, whereas butter of the control sample was made from the cream of buffalo milk purchased from Mahalet Mousa, animal production research station / Kafr El-Shiekh governorate /Egypt. Buffalo milk sample was separated using cream separator (Alfa-Laval) and the cream was held overnight at 5°C and mechanically churned to obtain the butter. All samples were analyzed within the recommended time of consumption.

2-Extraction of milk fat:

Milk fat was extracted from each sample after centrifuging, by melting and the fat was filtered (Dorota Derewiaka, *et al.* (2011).

3-Methylation

Triglycerides fatty acids were converted to the corresponding methyl esters using methanol, zinc chloride and zinc dust as a catalyst. Shahin (1977).

4-Chromatographic analysis:

A stainless steel colum packed with 10% carbowax 20 M on cromosorb WHP 80-100 mesh 4 x 1/8 inch O.d. fitted with Kanik 3000c Gas chromatography under the following conditions:

Injection temperature: 220°C

Detector temperature: 240°C (FID)

Program: 130-220°C 4°C/min.

Air flow rate: 300 ml/min.

Nitrogen flow rate carrier 30 ml/min.

Hydrogen flow rate 30 ml/min.

5-Determination of cholesterol content:

Cholesterol content was determined in each sample according to Pantulu *et al.* (1975).

6-Iodine value:

The equation of Atrementova and Bogmolva, (1966) was applied to calculate the iodine value of each sample. $TUFA - 2.6424 + 0.911$ (I.V.).

7-Fat content:

Fat content was carried out according to Rose Gottlieb method. (AOAC 2012).

8- Statistical analysis:

The data obtain were statistical analyzed according to statistical analysis system user's guide (SAS, 1996).

RESULTS AND DISCUSSION

Table (1) illustrates the fatty acids profile of buffalo milk fat in the control and twelve suspected samples. From the Table it could be observed that the buffalo milk fat has short chain acids ($C_4 - C_8$) with value of 6.45 and medium chain acids ($C_{10}-C_{12}$), with value 3.7%, of the fatty acids, and the major fatty acids were C_{14} , C_{16} , C_{18} and $C_{18:1}$ Youniss and Soliman, (1986). Comparing the values of fatty acids reported in the table, with the level of buffalo milk fat fatty acids, it could be observed that the samples number 1, 7 and 8 had approximately the same content of the fatty acids of the control buffalo milk fat. Results in the same Table show that the other samples were greatly in contrast with the control sample.

The samples number 2,4,11 and 12 characterized with a high content of medium chain fatty acids ($C_{10}-C_{12}$) more than the control obtained, this increase is due to the high level of lauric acid (C_{12}). Furthermore, the fatty acid straric acid sharply decreased in the four samples to 9.7%, 8.9%, 10.2% and 9.1%, compared to the control $C_{18:0}$ acid which recorded 11.7%. The total saturated fatty acids of the samples 2,4,11 and 12 was in a high level than that of the control sample or the other samples1, 7 and 8 which seems to be unadulterated butter fat. The total saturated fatty acids of the samples number 2.4.11 and 12 were 67.35%, 70.74%, 67.9% and 70.4% respectively. While the total saturated fatty acids of the control sample and the samples number 1,7 and 8 were 65.6%, 66%, 65.37% and 65.47, respectively. From these previous findings it could be concluded that the samples number 2,4,11 and 12 were adulterated samples, because of the high content C_{12} lauric acid which considered as an evidence adulteration with palm kernel oil, which agree with Babyan (1981), Molkentin, and Precht (1988), Babyan and Rosenau (1991). Ntakasane, *et al.* (2013) and Ha-Jung Kim, *et al.* (2016), who obtained similar results.

The samples number 3,5 and 6 showed a fatty acids profile different from that of the control sample, the short chain fatty acids and the medium chain fatty acids of these samples were markedly lower than that of the control sample. They recorded 4.1%, 4.6%, 4.6% and 2.6%, 2.9%, 2.7% of total short chain acids and of total medium chain acids. On the other hand palmetic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$) and lenoleic acid ($C_{18:2}$) were found in high percentage for the samples. These findings led to suspect of the sample number 3, 5 and 6 that could be adulterated with palm oil in a different ratios. The high level palmetic, oleic and lenoleic and is evidence to the existing of palm oil

because it characterized with these acids, so the high level of the acids C₁₆, C_{18:1} and C_{18:2} refer to the presence of palm oil on the three samples number 3, 5 and 6.

Furthermore, the decrease observed in the short chain fatty acids and the medium chain fatty acid, the suspected samples emphasized the addition of foreign substances to the milk fat(Ramamuthy and Narayanan (1971), Juyoung *et al.* (2010) and Kumar *et al.* 2011). From the same Table, it is obvious that the two samples number 9 and 10, showed very low content of short chain fatty acids and of medium chain fatty acids 4.65%, 4.87% and 2.82%, 2.8% respectively, when compared to the control sample.

Also, it could be observed that the total saturated fatty acids were lower than that found in the control sample. Concerning the individual fatty acids stearic, oleic and linoleic acids were found of higher contents than that of the control sample. The samples 9 and 10, recorded 13.6% - 13.4%, 33.4%-33.5% and 2.08%-2.04%, for the stearic, oleic and linoleic, respectively.

From these results, it could be concluded that the samples number 9 and 10 were adulterated, and the adulteration could be with depot fat.

The high content of C₁₈, C_{18:1} and C_{18:2}, with a lower content of total short chain fatty acids and total medium chain fatty acids are considered an evident that the butter of the samples 9 and 10 was adulterated with depot fat (Mervat and Youness (1986), Youness and Mervat, (1986), Youness, (1991), Ulberth, (1994) Nurrulhidayah, *et al.* (2013), Ntakasan *et al.* (2013) and, Anil Kumar, *et al.* 2015).

Data given in Table (1) indicated that short chain fatty acids (C₄ – C₈), medium chain fatty acids (C¹⁰ – C₁₂), long chain fatty acids (C₁₄ – C₂₀), saturated fatty acids and unsaturated fatty acids differ according to the area from which the treatments were taken significantly (P > 0.0001).

Results in Table (2) reveal the iodine value and the ratios between fatty acids which can be used to check for butter fat adulteration. From these result, it is clear that the fatty acids of the control sample characterized with short chain fatty acids below (C₁₂) luric acid and the major fatty acids were C₁₄, C₁₆, C₁₈ and C_{18:1} acids (Stull and Brown (1964). Ratios between the fatty acids can be used for check for addition of foreign fat to milk fat.

Table 1. Fatty acids content the investigated samples compared with buffalo milk fat

Fatty acids	B.M.F.*	Number of Samples											
		1	2	3	4	5	6	7	8	9	10	11	12
C ₄	3.1	3.06	2.5	2	2.2	2.1	2.2	2.95	3.11	2.2	2.32	2.6	2.2
C ₆	1.95	2	1.6	1.2	1.5	1.5	1.4	1.99	2.01	1.42	1.5	1.7	1.6
C ₈	1.4	1.3	1.7	0.9	2.1	1	1	1.36	1.19	1.03	1.05	1.8	2.2
C ₁₀	1.8	1.9	2	1.3	2.6	1.5	1.4	1.9	1.96	1.32	1.3	2.2	2.6
C ₁₂	1.9	1.9	11.1	1.3	14.8	1.4	1.3	2	2.00	1.5	1.5	8.8	15.3
C ₁₄	10.6	10.95	11.3	6.0	11.6	6.2	6.1	10.7	10.6	9.1	9.05	11.2	11.6
C _{14:1}	1.7	1.5	1.3	1.1	1.2	1.3	1.07	1.53	1.45	1.4	1.4	1.4	1.2
C ₁₆	32.3	32.58	26.8	38.7	26.34	38.6	38.6	32.28	32.4	30.7	30.8	28.7	25.2
C _{16:1}	1.6	1.4	1.2	1.4	1.2	1.5	1.11	1.65	1.74	1.6	1.50	1.4	1.1
C ₁₈	11.7	11.5	9.7	8.51	8.9	9.1	9.3	11.33	11.4	13.6	13.4	10.2	9.1
C _{18:1}	29.8	29.7	28.75	33.54	25.3	32	32.6	29.9	29.8	33.4	33.5	28	25.8
C _{18:2}	1.3	1.4	1.4	3.5	1.56	3.2	3.3	1.55	1.54	2.08	2.04	1.3	1.5
C ₂₀	0.85	0.81	0.65	0.55	0.7	0.6	0.62	0.86	0.8	0.65	0.64	0.7	0.6
Total	100	100	100	100	100	100	100	100	100	100	100	100	100
S.C.F.A (C ₄ -C ₈)	6.45 ^a	6.36 ^{ab}	5.8 ^{bc}	4.1 ^c	5.8 ^{bc}	4.6 ^{de}	4.6 ^{de}	6.3 ^{ab}	6.31 ^{ab}	4.65 ^{de}	4.87 ^d	6.1 ^{abc}	6 ^c
M.C.F.A (C ₁₀ -C ₁₂)	3.7 ^{ed}	3.8 ^d	13.1 ^b	2.6 ^f	17.4 ^a	2.9 ^{ef}	2.7 ^f	3.9 ^d	3.96 ^d	2.82 ^{ef}	2.8 ^{ef}	11 ^c	17.9 ^a
L.C.F.A (C ₁₄ -C ₁₈)	89.85 ^d	89.84 ^d	81.1 ^f	93.3 ^a	76.8 ^g	92.5 ^c	92.7 ^b	89.8 ^d	89.73 ^d	2.53 ^e	92.33 ^a	82.9 ^e	76.1 ^h
S.F.A	65.6 ^c	66 ^{cc}	67.35 ^b	60.46 ^e	70.74 ^a	62 ^d	61.92 ^d	65.37 ^c	65.47 ^{ccf}	61.52 ^d	61.56 ^d	67.9 ^e	70.4 ^a
U.S.F.A	34.4 ^c	34 ^c	32.65 ^d	39.54 ^a	29.26 ^c	38 ^b	38.08 ^b	34.63 ^c	34.53 ^c	38.48 ^b	38.44 ^b	32.1 ^d	29.6 ^e

The letters possess treatments survey number. The means with the same letter at any position did not significantly differ (P > 0.05)

B.M.F.= Buffalo milk fat

S.C.F.A= Short chain fatty acids

M.C.F.A= Medium chain fatty acids

L.C.F.A= Long chain fatty acids

S.F.A= Saturated fatty acids

U.S.F.A= unsaturated fatty acids

Table (2) shows the ratios between fatty acids which can be used to check for detecting the adulterated butter fat with other fats. It's clear from these data that authentic milk fat has the ratios of 1.05, 5.57, 2.81, 9, 2.54 and 1.9 between C₁₂/C₁₀, C₁₄/C₁₂, C_{18:1}/C₁₄, C₁₈/C_{18:2}, C_{18:1}/C₁₈ and total saturated fatty acids/total unsaturated fatty acids, respectively. The ratios were considered characteristic of unadulterated butter. On the other hand the samples number 2,4,11 and 12 showed different ratios between those certain fatty acids. The ratios between C₁₂/C₁₀, C₁₄/C₁₂ and C₁₈/C_{18:2} were the most ratios changed according to the addition foreign fat to the butter. These samples 2,4,11 and 12 were suspected to the adulterated with palm kernel oil. Furthermore, the samples number 3,5 and 6 show the same dissimilarity to the normal butter. The ratios of this samples characterized with lower value on the

ratio C₁₂/C₁₀, C₁₄/C₁₂ and C₁₈/C_{18:2} while the ratios of C_{18:1}/C₁₄ and C_{18:1}/C₁₈ were higher than that observed in genuine butter. However these result emphasize the results obtained in Table (1) that the samples 3,5 and 6 were suspected to be adulterated with palm oil. The ratios of C₁₂/C₁₀, C₁₄/C₁₂ and C_{18:1}/C₁₈ were helped in detecting the adulteration. Data in the same Table showed the fatty acids ratios of the samples 9 and 10, it is clear that the fatty acids ratios were completely differ than the fatty acids ratios of the control milk fat. The ratios of C₁₂/C₁₀, C₁₄/C₁₂ and C_{18:1}/C₁₄, showed higher level, while the ratio of C₁₈/C_{18:2}, showed lower level when compared with the same ratios of the fatty acids of the control butter. The ratio of the total saturated fatty acids/ total unsaturated fatty acids did not widely affect. However, the ratios trend shows that the sample number 9 and 10 had the higher content of C₁₈,

C_{18:1} and C_{18:2} fatty acids, which indicated the presence of depot fat in the suspected samples similar results were reported by Gosheva (1979), Chernev *et. al.* (1979), Farrag *et. al.*, (1980), Youniss and Soliman (1986), Gunston *et. al.* (1995) and Anil Kumar *et.al.* (2015).

The iodine value of the control and the suspected samples are reported in Table (2). The iodine value did not assist in detecting the adulteration due to its wide range which mainly affected by the season, stage of lactation and

the plan of nutrition (Svensen and Yastgaard (1966), Hall, (1970), Gray, (1973), and Youniss, 1991).

Data given in Table (2) indicated that saturated fatty acids /unsaturated fatty acids and Iodine value differ according to the area from which treatments with taken significantly (P>0.0001)

Cholesterol is the principal sterol of butter. Usually the cholesterol content of butter ranged from 204 to 382.4 mg/100g fat (Pricht, 2001).

Table 2. Iodine value and the ratios between fatty acids which can be used to check for addition of other fat to butter fat

Samples number	C ₁₂ :C ₁₀	C ₁₄ :C ₁₂	C _{18:1} :C ₁₄	C ₁₈ :C _{18:2}	C _{18:1} :C ₁₈	Sat: UnSat	I.V.
B.M.F	1.05	5.57	2.81	9	2.54	1.9 ^c	34.86 ^c
1	1.1	5.47	2.71	8.2	2.58	1.94 ^c	34.42 ^c
2	5.55	1.01	2.54	6.92	2.96	3.06 ^b	32.94 ^d
3	1.00	4.6	5.59	2.43	3.94	1.52 ^d	40.50 ^a
4	5.69	0.78	2.18	5.7	2.84	2.41 ^a	29.21 ^e
5	0.93	4.4	5.16	2.84	3.51	1.63 ^d	38.81 ^b
6	0.92	4.69	5.34	2.81	3.5	1.62 ^d	38.90 ^b
7	1.05	3.35	2.79	7.3	2.64	1.88 ^c	35.11 ^c
8	1.02	5.14	2.81	7.4	2.61	1.89 ^c	35.00 ^c
9	1.13	6.06	3.67	6.5	2.49	1.59 ^d	39.33 ^b
10	1.15	6.46	3.7	6.56	2.5	1.6 ^d	39.29 ^b
11	4	1.27	2.5	7.84	2.74	2.11 ^b	32.34 ^d
12	5.88	0.75	2.22	6.06	2.83	2.37 ^a	29.59 ^e

The letters possess treatments survey number. The means with the same letter at any position did not significantly differ (P > 0.05)

B.M.F: buffalo milk fat Sat = Saturated fatty acids UnSat= unsaturated fatty acids I.V.= Iodine value

Table (3) showed the cholesterol content mg/100g butter of authentic butter sample, also twelve suspected butter samples. It observed that the cholesterol content of five analysed different genuine butter, it observed that the cholesterol content ranged from 230 to 244 with an average of 237mg/100g butter. From the Table, it is found that the samples number 1,7 and 8 were considered unadulterated samples, the cholesterol content of this samples were 232, 229 and 234mg/100g butter, respectively. The samples number 2,4,11 and 12 were found had lower cholesterol content 194, 173, 201 and 169mg/100g butter, respectively. These reported results were lower than the range of the cholesterol level of the authentic butter.

Furthermore, the samples number 3, 5 and 6 also recorded a lower content of cholesterol 149, 164.7 and 170mg/100g butter, than that observed to the control sample. From these previous results it is concluded that the cholesterol content of the suspected samples were decreased due to the addition of non milk fat substances such as vegetable oils to pure milk fat as an adulterant substances. However, cholesterol is very useful to detect

almost all types of vegetable oils in dairy products. Fox *et. al.* (1988), Oguntibeju *et. al.* (2009), Ntakasan *et. al.* (2013) and Ha. Jung kim *et. al.* (2016) found that the cholesterol concentration of the adulterated samples decreased proportionally to the mixing ratio. From the same Table the samples number 9 and 10 showed a cholesterol concentration lower than the cholesterol concentration of the cholesterol sample, but at the same time was higher than the cholesterol content of all the other samples.

The cholesterol concentration of the sample 9 and 10, were 202.2 and 203mg/100g butter. These results emphasize the hypothesis that the samples were adulterated with depot fat. Jin-Man Kim, *et. al.* (2015) and, Ha. Jung Kim, *et. al.* (2016) reported similar result when beef tallow mixed with pure milk fat in different proportions. The same table included the ratio of reduced cholesterol to the addition of vegetable oil or body fat (tallow) to the suspect butter.

Table (3) showed that Fat content of all samples did not differ considerably the butter control. It is obvious from the data in Table (3) that the treatments led significant differences (P>0.0001) but the fat % was not significant.

Table 3. Cholesterol, Fat content of buffalo butter and the reduction % occurred in cholesterol of the suspected samples

Constituents	Buffalo Butter	Number of Samples											
		1	2	3	4	5	6	7	8	9	10	11	12
Cholesterol Content mg/100g butter	237 ^a (230-244)	232 ^{ab}	194 ^d	149 ^h	173 ^{ef}	164.7 ^g	170 ^{fg}	229 ^b	234 ^{ab}	202.2 ^c	203 ^c	201 ^c	169 ^{ef}
Reduction in Cholesterol %	0 ^k	2.11 ⁱ	18.14 ^f	37.13 ^a	27 ^d	30.5 ^b	28.3 ^c	3.38 ^h	1.27 ^j	25.2 ^g	24.5 ^e	15.2 ^g	28.7 ^c
Fat %	80 ^a	80 ^a	81 ^a	83 ^a	80 ^a	81 ^a	80 ^a	79 ^a	80.5 ^a	80 ^a	80 ^a	82 ^a	81.5 ^a

The letters possess treatments survey number. The means with the same letter at any position did not significantly differ (P > 0.05)

* Average of five analysed samples.

CONCLUSION

Applying Gas –chromatography technique to detect butter adulteration using foreign fats or oils were helpful.

The fatty acids percentages and the ratios between fatty acids assessed to differentiate the adulterated butter from genuine butter. Furthermore, the chemical analysis to determine the cholesterol concentrations in the various samples facilitates the detection of the vegetable oils in butter.

The addition of beef tallow was also detected using the same chemical analysis. The calculated percentage of reduced cholesterol due to the addition of adulterants revealed the possibility to conclude the ratio of adulterants added.

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