International Journal of

Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Dairy Science

ISSN 1811-9743
DOI: 10.3923/ijds.2019.36.44

Research Article
Detection of Milk Fat Adulteration with Coconut Oil Depending on Some Physical and Chemical Properties

Eid Ragab Salem, Rizk Azab Awad and Osama Ibrahim El Batawy

Department of Food Science, Faculty of Agriculture, Ain Shams University, Shoubra Khaima, 11241, Cairo, Egypt

Abstract

Background and Objective: Milk fat adulteration has always been a serious problem in the food industry because of the economic advantages of partly replacing high-priced fats with low-priced oils. So, the aim of this research was to attempt the detection of milk fat adulteration with coconut oil depending on some physical and chemical properties of different adulterated products. **Methodology:** Different adulterated milk fat treatments with coconut oil were prepared using cow, (CB) and buffalo milk fat. Six treatments of adulterated milk fat were prepared according to incorporate the ratio added of coconut oil. Milk fat samples of either cow as buffalo milk were adulteration by adding coconut oil in ratios of 25, 50 and 75% of total fat. Puree cow, buffalo and coconut oils (100%) were used as reference fats (controls). All samples were analyzed for chemical (acid value, iodine value, saponification number, peroxide value, fatty acids profile) and physical (refractive index, melting point) properties. **Results:** The results indicated that, most of the milk fat properties were significantly affected with adulteration with different ratios of coconut oil. Increasing the percentage of adulterated coconut oil into milk fat either cow or buffalo gradually decreased the refractive index, melting point, acid value, iodine value, peroxide value and unsaturated fatty acids (%). On the other hand, addition of coconut oil to pure milk fat either cow or buffalo led to significant increase of saponification number, total short chain (TSC) and saturated fatty acids (%). However, these values were gradually increased by increasing the ratio of added coconut oil to milk fat. **Conclusion:** It could be concluded that, some physical and chemical methods such as; melting point, iodine value, saponification number, percentage of TSC, saturated and unsaturated fatty acids may be useful to determine the adulterated of milk fat with different ratios of coconut oil. it could be calculated the ratios of adulteration in the cow and buffalo's milk fat with coconut oil by using different equations depended on the different of some physical and chemical properties between milk fat and coconut oil.

Key words: Adulteration, cow milk fat, buffalo milk fat, coconut oil, physo-chemical properties

Citation: Eid Ragab Salem, Rizk Azab Awad and Osama Ibrahim El Batawy, 2019. Detection of milk fat adulteration with coconut oil depending on some physical and chemical properties. Int. J. Dairy Sci., 14: 36-44.

Corresponding Author: Osama Ibrahim El-Batawy, Department of Food Science, Faculty of Agriculture, Ain Shams University, Shoubra Khaima, 11241, Cairo, Egypt Tel: 00201118623388

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Milk fat is one of the most complex fats found in nature. This complexity stems from the extreme diversity of its fatty acids (FA) (e.g., chain length, degree of saturated and unsaturation and branching) and more than 400 of these have been recently identified[1]. Milk fat also contains thousands of triacylglycerol (TAG) species. Price and functionality are important factors that affect the use of milk fat. Milk fats have the disadvantage in both high price and limited functionality compared to tailored vegetable fats and oils. Its flavor and reputation as a natural product are the biggest advantages of milk fat. Moreover, milk fat is play an important role for flavor in certain applications and high-quality foods, such as dairy and bakery products. Milk fat considered as one of the most expensive commodity fats on the market; therefore, the detection of foreign fat in milk fat is an important research problem. Milk fat can be adulterated by different ways such as direct incorporation of foreign fat in butterfat or by homogenization of skimmed milk with less expensive foreign fat. Among foreign fats, vegetable oil such as palm and coconut can be used to adulterate the milk fat.

Coconut oil is edible oil derived from the kernel of *Cocos nucifera* L., a tropical plant and is largely consumed for edible and non-edible purposes which include cooking, confectionary, bakery, cosmetics and pharmaceutical. It is a clear liquid at room temperature and has a pleasant aroma. It is mainly consists of saturated fatty acids (>91%) and the major part of the saturated fatty acids are medium chain fatty acids (MCFA) (>51%) which are easily digestible and easily absorbed into the body through the portal vein and produce energy[2]. Coconut oil contains a high content of low molecular weight saturated fatty acids, the distinctive characteristic of lauric oil. Coconut oil is characterized by unique properties such as; pleasant odor, bland flavor, high resistance to rancidity, a narrow temperature range of melting, easy digestibility and absorbability, high gross for spray oil use and superior foam retention capacity for whip-topping use[3].

Milk fat adulteration has always been a serious problem because of the economic advantages of partly replacing high-priced fats with low-priced oils (e.g., palm oil, coconut oil etc) without labeling the product[4]. Accordingly, blends of milk fat and vegetable fats are increasingly used in the food industry. There are several complex food systems in which the fats of different origin are in a mixed form. Many scientific findings are available about the characteristics of natural fats but there is limited information on the physical properties of fat mixtures. The common methods of detecting such adulteration has consisted in determining the fatty acid composition of the fat obtained from the suspected product. However, the microscopic appearance and the melting point of fat could greatly help in detecting the vegetable fat in butter fat. Methods of detecting food adulteration are depending on physical, chemical, biochemical and other techniques. Many of the methods described involve physical properties such as viscosity, refractive index or melting point of the fat. However, there were also many chemical tests such as iodine value, saponification number, Reichert, Polenske and acetyl values[5,6]. In the experiments performed so far, attempts have been made to detect vegetable or animal foreign fats in milk fat, e.g., by analyzing the fatty acids, the triacylglycerols, the sterols and sterol acetate or by thermoanalytical methods such as differential scanning calorimetry. Other findings have been derived from analysis of unsaponifiables or a-tocopherol[7].

Therefore, the aim of this study was to describe different methods to investigate the adulteration of cow and buffalo butter fat with coconut oil (as vegetable oil) through testing some physical and chemical characteristics of the fat.

MATERIALS AND METHODS

Materials: Fresh cow and buffalo butter were obtained from processing milk unit, Faculty of Agriculture, Cairo University, Giza, Egypt. Cow and buffalo butter oil (Anhydrous milk fat) ware prepared by the method of Amer *et al.*[8]. Fresh butter was melted at 60°C, removing the top oil layer, filtering through glass wool and drying the resulting oil over anhydrous sodium sulphate. The oil then refiltered (under vacuum, Whatman no 41 paper) to obtain clear oil (~99.5% milk fat) and stored at -20°C until used. Coconut oil made in Indonesia, was obtained from the Abou El Houl for Import and Export Co., 815 Port Said St., Ghamra, Hadayk El Kobba, Cairo, Egypt. The sample flushed with nitrogen and stored at -20°C until used. All chemicals used in analytical methods were analytical grade. Solvents (Hexane, chloroform, petroleum ether and ethyl alcohol) were distilled before use. The chemicals were purchased from the following sources: Sigma Chemicals Company and El-Gamhouria Trading Chemicals and Drugs Company, Egypt.

This study was carried out in Food Science Department, Faculty of Agricultural, Ain Shams University, Cairo, Egypt during September, 2017-September, 2018. The different samples of pure and adulterated milk fat were prepared in Dairy production Unit, Faculty of Agricultural, Ain Shams University, Cairo, Egypt. Fatty acids content of the tested samples were determined in National Research Center, Dokki, Giza, Egypt.

Methods

Experimental procedures: Different fat treatments were prepared after complete melting of butter oil (cow butter, (CB), buffalo butter, (BB)) and coconut oil (CO) at 70±0.5°C. There were three control treatments, the first control treatment was pure cow butter, second control treatment was pure buffalo butter and the third control treatment was pure coconut oil. Six treatments of adulterated butter milk fat were prepared according to incorporate the coconut oil into either buffalo o r cow butter at three different levels i.e., C C25 (25% coconut oil and 75% cow butter), CC50 (50% coconut oil and 50% cow butter), CC75 (75% coconut oil and 25% cow butter), Also, BC 25 (25% coconut oil and 75% buffalo butter), BC50 (50% coconut oil and 50% buffalo butter), BC75 (75% coconut oil and 25% buffalo butter). All samples were stored at -20°C until analyses.

Analytic methods: The refractive index of tested samples was estimated using a Carl Zeiss Refractometer (JENA, Type 337397, Germany) at 40°C, melting point (MP), acid value, iodine value, saponification number and fatty acids content of the tested samples were determined according to the method described in AOAC[9].

Statistical analysis: Statistical analysis was performed according to SAS[10] using General Linear Model (GLM) with main effect of treatments. Duncan's multiple range was used to separate among of three replicates at $p \leq 0.05$.

Equations of milk fat adulteration were performed according to Microsoft Office Excel[11] program for creating values and calculating the slope and intercept.

RESULTS AND DISCUSSION

Physical properties

Refractive index: There are many physical characteristics of the edible fats and oils such as refractive index and melting point, which are played an important role in assessing their quality and palatability, as well as the consumer acceptability of these products. The physical characteristics of fat or oil are dependent on the degree of unsaturation, the carbon chain length, the isomeric fatty acid forms and molecular configuration[12].

As shown in Table 1, no significant difference was observed between the RI value in cow and buffalos milk fat. It is clear from the results that coconut oil showed the lowest (RI) value while cow milk fat had the lowest. The obtained data agreed with Ariponnammal[13] who observed that, the reflective index of pure milk fat was higher than that of coconut oil. Addition of coconut oil to milk fat either cow or buffalo led to decrease the RI value. Also, RI value was decreased with increasing ratio added of coconut oil into milk fat either cow or buffalo. This decrease in RI values with increasing the ratios of coconut oil added could be due to that the coconut oil contained short chain and saturated fatty acids compared with milk fat which may let to increase RI values of treatments. From the obtained data, it could be observed that there were non-significant differences in RI between pure milk fat and adulterated milk fat with coconut oil up to 50%. The refractive index of fats and oils may be correlated with fatty acids content, free fatty acids content, saturation degree and oxidative state[14,15]. However, the slope of the change in the refractive index, with the addition of coconut oil, decreases slightly at higher values of the addition. The refractive index of adulterated cow and buffalo milk fat with different levels of coconut oil was within the natural variation of pure milk fat samples. Therefore, this analytical characteristic did not appear to show any significance in detecting adulteration of milk fat with coconut oil.

Table 1: Refractive index and melting point (°C) of milk fat either pure or adulterated with different ratios from coconut oil

	Refractive index		Melting point	
Adulteration (%)	Cow (C)	Buffalo (B)	Cow (C)	Buffalo (B)
Pure milk fat	1.44680[a]	1.44678[a]	35.0[a]	34.5[a]
25% coconut	1.44577[a]	1.44535[a]	32.8[b]	32.3[b]
50% coconut	1.44442[a]	1.44455[a]	29.6[c]	29.1[c]
75% coconut	1.44352[b]	1.44340[b]	27.0[d]	26.2[d]
Pure coconut oil	1.44212[b]	1.44212[b]	24.0[e]	24.0[e]

[a,b,c]Means with same letter among treatments are not significantly different at $p \leq 0.05$

From the statistical analysis of the obtained data, it could be calculated the ratios of adulteration in the cow and buffalo's milk fat with coconut oil using the following equation:

For cow milk fat:

$$RI = 1.446848\text{-}\left(\frac{4.60 \times Y}{100000}\right)$$

For buffalo milk fat:

$$RI = 4.446694\text{-}\left(\frac{4.5 \times Y}{100000}\right)$$

Where:

Y = Adulteration (%) of milk fat with coconut oil
RI = Refractive index value of milk fat

Melting point: Data in Table 1 indicated that, the pure coconut oil showed the lowest melting point among all treatment, this could be due to the lower percentages of long chain fatty acids in coconut oil which led to lower it melting point. On the other side, pure cow's milk fat had the highest melting point compared with other treatments. The results agreed with Celik and Bakirci[16]. From the data, it could be noticed that adulteration of milk fat with coconut oil decreased the melting point in both cow and buffalo milk fat. Gradually increasing percentage of added coconut oil into milk fat either cow or buffalo gradually decreased the melting point. Decreasing melting point of pure milk fat blended with coconut oil could be attributed to the decrease in solid fat content of coconut oil as compared to pure milk fat either cow and buffalo. De Martini Soares *et al*.[17] reported the melting point depends on the solid to liquid phase transition of triacylglycerols which are already present in greater amounts when the blend of coconut oil. According to the data obtained from Table 1, it could be found that, there were significant differences between pure milk fat either cow or buffalo and all adulterated milk fat with 25-75% coconut oil. Therefore, this method may be useful to determine the adulterated the butter fat with different ratios of coconut oil.

From the statistical analysis of the obtained data, it could be calculated the ratios of adulteration the cow and buffalo's milk fat with coconut oil using the following equation:

For cow milk fat:

$$MP = 35.24 - 0.11 \times Y$$

For buffalos milk fat:

$$MP = 34.64 - 0.11 \times Y$$

Where:

Y = Adulteration (%)of milk fat with coconut oil
MP = Melting point (°C) of milk fat

Chemical properties

Acid value: As illustrated in Table 2, it could be noticed that, acid value was significant lower in pure coconut oil than that of milk fat either pure control or adulterated with different ratios of coconut oil. These results agreed with Park *et al*.[18] who found that the acid value of pure milk fat was 0.48%. Also, Odoom *et al*.[19] observed that, acid value of collected coconut oil samples were 0.5 mg KOH/g oil (0.05%). Addition of coconut oil to milk fat either cow or buffalo resulted in significant lower acid value being decreased with increasing the ratio added. The acid values were slightly higher in cow milk fat than buffalo milk fat at any ratio added of coconut oil. According to Nielsen[20], acid values are dependent on different factors such as FFA, acid phosphate and conditions of the storage. Therefore, the acid value cannot be successfully used to detect the blending of cow or buffalo milk fat with coconut oil.

Table 2: Acid value and iodine value (g I_2 absorbed\100 g fat) of milk fat either pure or adulterated with different ratios from coconut oil

	Acid value		Iodine value	
Adulteration (%)	Cow (C)	Buffalo (B)	Cow (C)	Buffalo (B)
Pure milk fat	0.45[a]	0.24[a]	31.7[a]	33.1[a]
25% coconut	0.37[b]	0.19[b]	27.6[b]	25.0[b]
50% coconut	0.25[c]	0.18[b]	22.0[c]	21.4[c]
75% coconut	0.20[c]	0.13[c]	16.3[d]	15.2[d]
Pure coconut oil	0.09[d]	0.09[d]	8.5[e]	8.5[e]

[a,b,c]Means with same letter among treatments are not significantly different at $p \leq 0.05$

From the statistical analysis of the obtained data, it could be calculated the ratios of adulteration in cow and buffalo's milk fat with coconut oil using the following equation:

For cow milk fat:

$$AV = 0.45 - 0.004 \times Y$$

For buffalos milk fat:

$$AV = 0.24 - 0.0014 \times Y$$

Where:

Y = Adulteration (%) of milk fat with coconut oil
AV = Acid value (%)

Iodine value: Iodine value (IV) is a measure of the extent of fat unsaturation. The value varies with the type and proportion of unsaturated fatty acids present in fat. From the obtained data in Table 2, it could be observed that, the pure coconut oil showed the lowest iodine value among all treatment. On the other side, pure cow's milk fat had the highest iodine value compared with other treatments. The decrease in iodine value of coconut oil could be attributed to the lower unsaturated fatty acid content in coconut oil than pure milk fat either cow or buffalos. These results found to be in agreement with Bolton[21] who found that, pure milk fat contained about 35% of total saturated acids, most of which consists of oleic acid, the iodine value of milk fat lies around 30. Marina *et al*.[22] stated that, iodine value in coconut oil ranged from 4.47-8.55. The addition of coconut oil to pure milk fat caused a gradual and significant decrease in iodine value of adulterated milk fat. The decrease was proportional to the add level. Iodine value (IV) is a measure of the extent of unsaturation of fat and is a structure index. This value for milk fat ranges from 32-37, which is low in comparison to most other fats[23]. Kehar *et al.*[24] also reported that the IV of cow ghee ranged from 27.4-40.5. The average IV of ghee of Deoni cow[25] is 27.7 . Many common vegetable oils have high IVs, ground nut 90, sesame 108, linseed 180. Coconut oil is unique in having an IV of only about 9. IV furnishes very clear information on the actual nature of the fat. High values characterize the liquid vegetable fats except the coconut oil and myristica groups and few other fats[21]. Therefore, lower iodine value can be taken as an index of adulteration of ghee with coconut oil.

From the statistical analysis of the obtained data, the ratios of adulteration could be calculated of cow and buffalo's milk fat with coconut oil using the following equation:

For cow milk fat:

$$IV = 32.8-0.23\times Y$$

For buffalos milk fat:

$$IV = 32.45-0.24\times Y$$

Where:

Y = Adulteration (%) of milk fat with coconut oil

IV = Iodine value (g I_2 absorbed/100 g fat)

Saponification number: Data in Table 3 indicated that, saponification number was significantly affected by adulterating milk fat (cow and buffalo) with different coconut oil ratios. The data showed that coconut oil possessed higher saponification number than that of milk fat either cow or buffalo. Saponification value of buffalo milk fat was slightly lower than that of cow milk fat. These results agreed with Javeed *et al.*[25] and Singhal[23] who reported that, the range of saponification values in cow ghee falls within the lower (222.80) and upper (237.00), respectively. On the other hand, Marina *et al.*[22] observed that saponification value in coconut oil ranged from 250.07-260.67 mg KOH g oil. Addition of coconut oil to pure milk fat either cow or buffalo led to significant increase of saponification number in mixture of fat. However, saponification value was gradually increased by increasing the ratio of added coconut oil to milk fat either cow or buffalo. The increase in saponification number of blends pure milk fat coconut oil could be correlated to the low molecular weight of coconut oil fatty acids. Saponification value (SV) gives an indication of average molecular weight of fatty acids present in the fat. Since coconut oil may be containing a high proportion of fatty acids of low molecular weight compared with pure milk fat either cow or buffalo. Also, coconut oil was exceptional in having an SV of 243-262, due to its high content of lauric (12:0) and myristic (14:0) acids[23,26]. From the data in Table 3, it could be observed that, adulterating the pure milk fat with different coconut oil caused a significant effect on saponification. Therefore, saponification number could be used for detecting adulteration of milk fat with different ratios coconut oil.

Table 3: Saponification number (mg KOH \g fat) and peroxide value of milk fat either pure or adulterated with different ratios from coconut oil

	Saponification number		Peroxide value	
Adulteration (%)	Cow (C)	Buffalo (B)	Cow (C)	Buffalo (B)
Pure milk fat	229.45[c]	226.91[c]	1.20[a]	1.80[a]
25% coconut	234.16[bc]	232.45b[c]	0.92[a]	1.25[b]
50% coconut	240.12[b]	238.73[b]	0.64[b]	1.00[c]
75% coconut	246.82[ab]	245.16[ab]	0.40[c]	0.70[c]
Pure coconut oil	252.62[a]	252.62[a]	0.15[d]	0.15[d]

[a,b,c]Means with same letter among treatments are not significantly different at $p\leq 0.05$

From the statistical analysis of the obtained data, the ratios of adulteration the cow and buffalo's milk fat with coconut oil could be calculated using the following equation:

For cow milk fat:

$$SN = 228.8+0.24\times Y$$

For buffalos milk fat:

$$SN = 226.3+0.26\times Y$$

Where:

Y = Adulteration (%) of milk fat with coconut oil

SN = Saponification number (mg KOH/g fat) of milk fat

Peroxide value: It is clear from the data in Table 3 that, peroxide value was lower in coconut oil than that of pure milk fat either cow or buffalo milk fat. These results confirmed with Celik and Bakirci[16] who found that, peroxide value of the milk fat recorded 1.3 mEq Kg and Marina *et al.*[22], who observed that, coconut oil samples characterized with low peroxide value (0.21-0.57 mequiv oxygen kg). Adulteration of pure fat samples either cow or buffalo milk fat with coconut oil caused a significant decrease in peroxide value of the mix. This is due to high saturated fatty acids content of coconut oil compared to buffalo or cow milk fat. Many research reported that, coconut oil addition to other vegetable oils improves their oxidative stability indicating that coconut oil can be used as a natural antioxidant through the blending process.

From the statistical analysis of the obtained data, the ratios of adulteration the cow and buffalo's milk fat with coconut oil could be calculated using the following equation:

For cow milk fat:

$$PV = 1.19-0.010\times Y$$

For buffalos milk fat:

$$PV = 1.75 - 0.015\times Y$$

Where:

Y : Adulteration (%) of milk fat with coconut oil
PV : Peroxide value (mEq kg)

Fatty acids profile: Fatty acid profile of cow and buffalo milk fat either pure or adulterated with different ratios from coconut oil are presented in Table 4 and 5. The obtained data showed that, in coconut oil butyric, heptadecenoic Cis 10, linoleic (trans), linolenic (cis), linolenic and docosanoic fatty acids were not found, while caprilic, lauric, myristic and oleic fatty acids were the most abundant. Percentage of caprilic, capric, lauric, myristic and oleic fatty acids in coconut oil were higher than that in pure fat milk either cow or buffalo. While other fatty acids (butyric, caproic, tridecylic, tetradecenoic, pantadecylic, pentadecanoic ME, palmitic, palmitoleic, heptadecanoic, heptadecanoic Cis 10, stearic, oleic, linolenic (cis), linolenic, arachidic, eicosenoic and docosanoic) in pure coconut oil were lower than that in pure cow and buffalo milk fat. The results are agreement with the results obtained by Lopez *et al.*[27] who reported that, milk fat is characterized by short-chain (C4-C8, 8.3%), medium-chain (C10-C12, 6.6%) and long-chain (C14-C18, 81.9%) length fatty acids. Moreover, milk fat is a relatively high saturated fat about 65% saturated fatty acids (mainly C16:0, C18:0 and C14:0) and about 35% unsaturated fatty acids (mainly C18:1). Shin *et al.*[28] also found that the fatty acids composition of butter fat contained 3.9% butyric acid, 2.9% caproic acid, 2.1% caprylic acid, 4.4% capric acid, 6.2% lauric acid, 14.0% myristic acid, 6.2% lauric acid, 14.0% myristic acid, 29.5% palmitic acid, 2.0% palmitoleic acid, 11.0% stearic acid, 17.9% oleic acid, 1.0% linoleic acid and 0.5 % linolenic acid.

Table 4: Fatty acids content of cow milk fat either pure or adulterated with different ratios from coconut oil

		Test results of fatty acids				
Fatty acids	Test items	Cow milk fat	25% coconut	50% coconut	75% coconut	Pure coconut oil
Butyric	C4:0	3.10	2.32	1.52	0.78	ND
Caproic	C6:0	1.73	1.21	1.01	0.70	0.67
Caprilic	C8:0	0.89	2.64	4.36	5.77	7.81
Capric	C10:0	1.71	2.80	3.80	4.51	6.01
Lauric	C12:0	2.17	15.05	25.46	34.39	46.48
Tridecanoic	C13:0	0.08	0.06	0.06	0.05	0.04
Myristic	C14:0	10.01	12.30	14.17	16.04	17.64
Tetradecenoic	C14:1	0.68	0.60	0.51	0.31	0.01
Pentadecanoic	C15:0	1.78	1.27	0.86	0.49	0.01
Pentadecenoic	C15:1	0.39	0.29	0.18	0.10	0.01
Palmitic	C16:0	30.92	24.93	19.84	15.29	9.01
Palmitoleic	C16:1	1.83	1.34	0.91	0.53	0.03
Heptadecanoic	C17:0	1.21	0.91	0.57	0.34	0.01
Heptadecenoic Cis 10	C17:1	0.38	0.31	0.19	0.11	ND
Stearic	C18:0	11.17	8.97	7.02	5.37	2.86
Oleic	C18:1	24.12	20.13	16.20	12.53	7.49
Linoleic(trans)	C18:2T	0.50	0.33	0.23	0.12	ND
Linoleic	C18:2	1.64	1.67	1.69	1.75	1.76
Linolenic (cis)	C18:3n6	0.18	0.15	0.10	0.05	ND
Linolenic	C18:3n3	0.62	0.46	0.30	0.18	ND
Arachidic	C20:0	0.45	0.33	0.27	0.19	0.09
Eicosanoic	C20:1	0.22	0.21	0.16	0.13	0.05
Docosanoic	C22:0	0.24	0. 18	0.13	0.08	ND
Unknown	-	3.90	1.36	0.45	ND	ND
TSC	$C_{4:0}$-$C_{8:0}$	5.72^{c}	6.17^{bc}	6.89^{b}	7.25^{b}	8.48^{a}
TLC	$C_{10:0}$-$C_{22:0}$	90.30^{a}	92.29^{a}	92.65^{a}	92.56^{a}	91.5^{a}
TSC\TLC (%)		6.30^{c}	6.60^{c}	7.40^{b}	7.80^{b}	9.20^{a}
USFA	$C_{16:1}$-$C_{20:1}$	30.56^{a}	25.94^{b}	20.47^{c}	15.81^{d}	9.35^{e}
SFA	$C_{4:0}$-$C_{22:0}$	65.46^{e}	72.97^{d}	79.07^{c}	84.0^{b}	90.63^{a}
USFA\SFA (%)		46.60^{a}	35.50^{b}	25.80^{c}	18.80^{d}	10.30^{e}

TSC: Total short chain, TLC: Total long chain, USFA: Unsaturated fatty acids, SFA: Unsaturated fatty acids, [a,b,c]Means with same letter among treatments are not significantly different at $p\leq 0.05$. ND: Not detected

Table 5: Fatty acids content of buffalo milk fat either pure or adulterated with different ratios from coconut oil

		Test results of fatty acids				
Fatty acids	Test items	Buffalo milk fat	25% coconut	50% coconut	75% coconut	Pure coconut oil
Butyric	C4:0	2.57	2.21	1.31	0.97	ND
Caproic	C6:0	1.52	1.50	0.96	0.91	0.67
Caprilic	C8:0	0.85	1.81	4.48	5.99	7.81
Capric	C10:0	1.60	2.21	4.01	4.97	6.01
Lauric	C12:0	2.44	8.39	27.59	35.76	46.48
Tridecanoic	C13:0	0.08	0.06	0.05	0.04	0.04
Myristic	C14:0	10.10	11.00	14.58	15.56	17.64
Tetradecenoic	C14:1	0.59	0.51	0.25	0.14	0.01
Pentadecanoic	C15:0	1.57	1.34	0.69	0.38	0.01
Pentadecenoic	C15:1	0.36	0.30	0.15	0.08	0.01
Palmitic	C16:0	28.88	26.80	17.94	13.64	9.01
Palmitoleic	C16:1	1.59	1.35	0.70	0.40	0.03
Heptadecanoic	C17:0	1.20	0.85	0.50	0.26	0.01
Heptadecenoic Cis 10	C17:1	0.37	0.24	0.13	0.07	ND
Stearic	C18:0	12.65	11.12	7.35	5.21	2.86
Oleic	18:1	25.17	22.95	15.67	11.78	7.49
Linoleic(trans)	C18:2T	1.06	0.76	0.48	0.28	ND
Linoleic	C18:2	1.90	1.88	1.81	1.78	1.76
Linolenic (cis)	C18:3n6	1.16	0.53	0.27	0.04	ND
Linolenic	C18:3n3	0.53	0.46	0.23	0.14	ND
Arachidic	C20:0	0.45	0.39	0.22	0.18	0.09
Eicosanoic	C20:1	0.27	0.23	0.11	0.09	0.05
Docosanoic	C22:0	0.21	0.18	0.10	0.07	ND
Unknown	-	2.83	2.67	0.40	0.86	ND
TSC	$C_{4:0}$-$C_{8:0}$	4.94^{c}	5.52^{bc}	6.75^{b}	7.87^{ab}	8.48^{a}
TLC	$C_{10:0}$-$C_{22:0}$	92.18^{a}	91.55^{a}	92.83^{a}	90.87^{a}	91.5^{a}
TSC\TLC (%)		5.30^{d}	6.00^{c}	7.20^{b}	8.60^{ab}	9.20^{a}
USFA	$C_{16:1}$-$C_{20:1}$	33.00^{a}	29.21^{a}	19.80^{b}	14.80^{c}	9.35^{d}
SFA	$C_{4:0}$-$C_{22:0}$	64.12^{e}	67.86^{d}	79.78^{c}	83.94^{b}	90.63^{a}
USFA\SFA (%)		51.40^{a}	43.00^{b}	24.80^{c}	17.60^{d}	10.30^{e}

TSC: Total short chain, TLC: Total long chain, USFA: Unsaturated fatty acids, SFA: Unsaturated fatty acids [a,b,c]Means with same letter among treatments are not significantly different at $p \leq 0.05$. ND: Not detected

From the data it could be noticed that, pure milk fat either cow or buffalo was characterized by lower content of short-chain (TSC) fatty acids than that of coconut oil. There were non-significant differences in content of TLC fatty acids between coconut oil and pure milk fat either cow or buffalo. Coconut oil also contains lower USFA (%) than pure milk fat (cow and buffalo). On the other hand, the content of SFA in coconut oil was higher compared to pure cow or buffalo milk fat. Such results have been reported by other researchers as well Krishna *et al.*[29], who stated that, coconut oil contains 92% of saturated fatty acids, most of them (about 70%) are lower chain saturated fatty acids known as medium chain fatty acids (MCFAs). Therefore, adulteration of pure milk fat either cow or buffalo with different ratios of coconut oil caused considerable changes in certain fatty acids; i.e., butyric, caprilic, lauric, palmatic, stearic and oleic acids, the change was increased with increasing percentages of coconut oil (Table 4, 5). In particular, with increasing levels of coconut oil, butyric, palmetic and oleic acids were gradually decreased, while caprilic, lauric and myristic acids gradually increased. Moreover, TSC and SFA gradually increased while USFA gradually decreased with the increasing of coconut oil levels in milk fat. The obtained results agree with Farag *et al.*[30], who found that adulteration of cow and buffalo milk fat with various levels of lard or margarine caused significant changes in certain fatty acids, i.e., 22:0, 18:1, 18:0 and 16:0. Fox *et al.*[31] found that the addition of vegetable fat will, in effect, dilute the concentrations of the short-chain acids by an amount equal to the concentration of the added vegetable fat. Sharma and Singhal[32] found that the fatty acid composition of ghee containing hydrogenated vegetable oil was significantly different from that of control ghee containing no additional fats.

From the data in Table 4 and 5 it could be noticed that, pure milk fat either cow or buffalo was characterized by lower percentage of TSC/TLC and higher percentage of USFA/SFA compared with pure coconut oil. Adulteration of pure milk fat, either cow or buffalo with various levels of coconut oil caused increase in TSC/TLC% and decrease the percentage of USFA/SFA. This may be due to the addition of coconut oil in milk fat increase the concentrations of the TSC and SFA fatty acids while dilute the concentrations of the TLC and USFA fatty acids.

From the statistical analysis of the obtained data, the ratios of adulteration the cow and buffalo's milk fat with coconut oil could be calculated using the following equation:

For cow milk fat:

$$TSC = 5.59+0.03\times Y$$

For buffalos milk fat:

$$TSC = 4.83+0.04\times Y$$

Where:

Y = Adulteration (%)of milk fat with coconut oil
TSC = Total short chain fatty acids (%)

For cow milk fat:

$$USFA = 31-0.21\times Y$$

For buffalos milk fat:

$$USFA = 33.6-0.25\times Y$$

Where:

Y = Adulteration (%) of milk fat with coconut oil
USFA = Unsaturated fatty acids (%)

For cow milk fat:

$$SFA = 66.2+0.25\times Y$$

For buffalos milk fat:

$$SFA = 63.5+0.28\times Y$$

Where:

Y = Adulteration (%) of milk fat with coconut oil
SFA = Saturated fatty acids (%)

For cow milk fat:

$$TSC/TLC\ (\%) = 6+0.03\times Y$$

For buffalos milk fat:

$$TSC/TLC\ (\%) = 5.2+0.04\times Y$$

Where:

Y = Adulteration (%)of milk fat with coconut oil
TSC/TLC (%) = (Total long chain/Total long chain) fatty acids (%)

CONCLUSION

Finally, it could be concluded that, some physical and chemical methods such as determination of melting point, iodine value, saponification number and percentage of TSC, saturated and unsaturated fatty acids may be useful to determine the adulteration of butter fat with different ratios of coconut oil.

SIGNIFICANCE STATEMENTS

This study discovers the importance of using some physical and chemical methods such as determination of melting point, iodine value, saponification number and percentage of TSC, saturated and unsaturated fatty acids to determine the adulteration of milk fat with different ratios of coconut oil. From the obtained data, it could be calculated the ratios of adulteration in the cow and buffalo's milk fat with coconut oil by using different equations (to the best of researchers' knowledge, no researchers used such additives in previous studies) depended on the different of some physical and chemical properties between milk fat and coconut oil. This study will help the researchers and food analysts to detect the adulteration of milk fat with coconut oil by using some equations; therefore, this may be help to solve the serious problem in the food industry.

REFERENCES

1. Jensen, R.G., 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci., 85: 295-350.
2. Mu, H. and C.E. Hoy, 2004. The digestion of dietary triacylglycerols. Progr. Lipid Res., 43: 105-133.
3. Che Man, Y.B. and A.M. Marina, 2006. Medium Chain Triacylglycerol. In: Neutraceutical and Specialty Lipids and their Co-Products, Shaidi, F. (Ed.). Taylor and Francis Group, Boca Raton, FL., USA., pp: 27-56.
4. Lipp, M., 1996. Comparison of PLS, PCR and MLR for the quantitative determination of foreign oils and fats in butter fats of several European countries by their triglyceride composition. Zeitschrift Lebensmittel-Untersuchung Forschung, 202: 193-198.
5. Valentino, F.P., L. Boniforti and R. Monicelli, 1965. Reichert-Meissl and Polenske values: Composition and gas chromatographic determination of soluble and insoluble acids in butter. Dairy Sci. Abstr., 28: 1008-1008.
6. Youness , N. A., 1991. Adulteration of butterfat: Validity of Reichert-Meissl, Polenske and iodine values. Grasas Aceites, 42: 267-270.

7. Precht, D., 1992. Detection of foreign fat in milk fat I. Qualitative detection by triacylglycerol formulae. Zeitschrift Lebensmittel-Untersuchung Forschung, 194: 1-8.
8. Amer, M.A., D.B. Kupranycz and B.E. Baker, 1985. Physical and chemical characteristics of butterfat fractions obtained by crystallization from molten fat. J. Am. Oil Chem. Soc., 62: 1551-1557.
9. AOAC., 2005. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
10. SAS., 1999. SAS User's Guide for Personal Computers. Version 8.2, SAS Instintute Inc., Cary, NC., USA.
11. Microsoft® Office Excel, 2013. Microsoft® office excel custom guide. Microsoft® Office Excel, Saint Louis Park, USA.
12. Zaidul, I.S.M., N.A.N. Norulaini, A.K.M. Omar and R.L. Smith Jr., 2007. Blending of supercritical carbon dioxide (SC-CO_2) extracted palm kernel oil fractions and palm oil to obtain cocoa butter replacers. J. Food Eng., 78: 1397-1409.
13. Ariponnammal, S., 2012. A novel method of using refractive index as a tool for finding the adultration of oils. Res. J. Recent Sci., 1: 77-79.
14. Kiritsakis, A.K., C.M. Stine and L.R. Dugan, 1983. Effect of selected antioxidants on the stability of virgin olive oil. J. Am. Oil Chem. Soc., 60: 1286-1290.
15. Fatouh, A.E., R.K. Singh, P. E. Koehler, G. A. Mahran and A.E. Metwally, 2005. Physical, chemical and stability properties of buffalo butter oil fractions obtained by multi-step dry fractionation. Food Chem., 89: 243-252.
16. Celik, S. and I. Bakirci, 2000. A study on the physical and chemical properties of cookery-type butter. Pak. J. Biol. Sci., 3: 596-598.
17. De Martini Soares, F. A. S., R. C. da Silva, K. C. G. da Silva, M.B. Lourenco, D.F. Soares and L.A. Gioielli, 2009. Effects of chemical interesterification on physicochemical properties of blends of palm stearin and palm olein. Food Res. Int., 42: 1287-1294.
18. Park, Y.W., M. Juarez, M. Ramos and G.F.W. Haenlein, 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res., 68: 88-113.
19. Odoom, W., A. Bart-Plange, J.O. Darko and A. Addo, 2014. Quality assessment of moisture content, free fatty acids and acid value of coconut oil produced in the Jomoro district of the Western region of Ghana. J. Res. Agric., 3: 205-210.
20. Nielsen, S.S., 1994. Introduction to the Chemical Analysis of Foods. Chapman and Hall, New York, USA., ISBN-13: 9780412137716, pp: 93-207.
21. Bolton, E.R., 1999. Oils Fats and Fatty Foods: Their Practical Examination. 1st Edn., Biotech Books, Delhi, India, ISBN-13: 9788176220286, pp: 22-301.
22. Marina, A.M., Y.B. Che Man, S.A.H. Nazimah and I. Amin, 2009. Chemical properties of virgin coconut oil. J. Am. Oil Chem. Soc., 86: 301-307.
23. Singhal, O.P., 1980. Adulterants and methods for detection. Indian Dairyman, 32: 771-774.
24. Kehar, N.D., S.N. Ray, T.S. Krishnan, B.C. Joshi and B. C. Rauaskar, 1956. Studies on Fats, Oils and Vanaspatis. Manager of Publications, Delhi, India, pp: 5-12.
25. Javeed, S.I., S. D. Rathi and V.M. Ingle, 1986. Physico-chemical constants of ghee prepared from Deoni cow, cross-bred cow and Surti buffalo milk. Indian J. Dairy Sci., 4: 491-492.
26. Rangappa, K. S. and K.T. Achaya, 1974. Indian Dairy Products. Asia Publishing House, India, pp: 295-326.
27. Lopez, C., C. Bourgaux, P. Lesieur, A. Riaublanc and M. Ollivon, 2006. Milk fat and primary fractions obtained by dry fractionation: 1. Chemical composition and crystallisation properties. Chem. Phys. Lipids, 144: 17-33.
28. Shin, J.A., C.C. Akoh and K.T. Lee, 2010. Enzymatic interesterification of anhydrous butterfat with flaxseed oil and palm stearin to produce low-*trans* spreadable fat. Food Chem., 120: 1-9.
29. Krishna, A. G. G., G. Raj, A.S. Bhatnagar, P.P.K. Kumar and P. Chandrashekar, 2010. Coconut oil: Chemistry, production and its applications-a review. Indian Coconut J., 53: 15-27.
30. Farag, R.S., S.H. Abo-Raya, F.A. Ahmed, F.M. Hewedi and H.H. Khalifa, 1983. Fractional crystallization and gas chromatographie analysis of fatty acids as a means of detecting butterfat adulteration. J. Am. Oil Chem. Soc., 60: 1665-1669.
31. Fox, J.R., A.H. Duthie and S. Wulff, 1988. Precision and sensitivity of a test for vegetable fat adulteration of milk fat. J. Dairy Sci., 71: 574-581.
32. Sharma, R. and O.P. Singhal, 1996. Fatty acid composition, Bomer value and opacity profile of ghee prepared from milk adulterated with foreign fats. Indian J. Dairy Sci., 49: 62-67.