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# Detection of vegetable oil and animal depot fat adulteration in anhydrous milk fat (Ghee) using fatty acid composition

## Abstract

Two type of adulterants i.e. soybean oil (SO) and buffalo depot fat (BDF) along with pure cow and buffalo milk fats, collected and prepared after every two months of interval for a complete one year, were analyzed for their fatty acid composition using gas liquid chromatography. Both the adulterants were added individually at 5, 10 and 15 percent levels (v/v) as well as in their combinations at 5+5 (10), 10+10 (20) and 15+15 (30) percent levels (v/v) in both types of milk fat separately. It was observed that soybean oil consisted of high amount (51.86 percent) of linoleic ($C_{18:2}$) acid, while buffalo depot fat possessed high content (49.17 percent) of oleic ($C_{18:1}$) acid. Milk fats from both the species of cow and buffalo were found containing more of myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acids. The results revealed that the SO was detected even at 5 percent level using linoleic ($C_{18:2}$) acid as marker, while BDF was detectable at 5 percent level using oleic ($C_{18:1}$) acid as the base. When the ratios of some fatty acids ($C1_{4:0}/C_{16:0}$, $C_{14:0}/C_{18:1}$, $C_{14:0}/C_{18:2}$, $C_{16:0}/C_{18:1}$, $C_{16:0}/C_{18:2}$ and $C_{18:0}/C_{18:2}$) were calculated for detecting adulteration, it was noticed that two fatty acid ratios ($C_{14:0}/C_{18:1}$ and $C_{14:0}/C_{18:2}$) were found more useful in detecting adulteration in maximum number (78 percent) of samples. Whereas, on the basis of the ratios of sum of $C_{4:0}$ to $C_{14:1}$/sum of $C_{15:0}$ to $C_{20:0}$ fatty acids and vice-versa, addition of both the adulterants at all the levels (added individually as well as in their combinations) in both the milk fats was easily detected.

**Keywords:** adulteration, milk fat (cow and buffalo), soybean oil, buffalo depot fat, fatty acids, gas liquid chromatography

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## Introduction

Milkfat is a highly valuable and costlier product consumed throughout the world from antiquity as it imparts good sensory and nutritional properties and also adds economyto the milk and other food products. Therefore it is highly prone to adulteration with cheaper oils/fats such as vegetable oils, animal depot fats, hydrogenated fats, interesterified fats and inedible mineral oils etc. has economic advantages when the product is not labeled accordingly. In recent years, the problem of adulteration has assumed a very serious dimension. Such a situation could tarnish the image of dairy Industry both in domestic and international market. In India, milk fat is consumed as Ghee (clarified butter fat) and no important religious or festive Hindu rite omits the use of ghee. By adding animal depot fats in ghee, the sacred holy food, the unscrupulous traders are not only robbing the people of their money, but also playing with the religious sentiments, especially of the vegetarian section of the society, besides adversely affecting their health.

In order to ensure a genuine product to the consumer, legal and quality limits for the characteristics of ghee have been set in the various states/regions of India by Government of India, under the Food Safety and Standards Rules[1] and Ghee Grading and Marketing rules[2] and under this the offenders are liable to penalty. The problem of detection of milk fat adulteration has assumed a very serious dimension in today's preview especially in the regime of global competitiveness, where quality of milk and milk products is not an option, but an obligation. However, establishing the purity of milk fat is a very complex phenomenon.[3–5] It is as complicated as detecting sea water in ocean water.

Fatty acid composition of milk fat is also greatly affected by season, breed, species, parity, stage of lactation etc.,[6–8] which further complicate the situation of adulteration because it broaden the overall range of each fatty acid. Milk fat is uniquely distinct from other fats being the only fat containing short chain fatty acids.[9–12] Whereas, vegetable oils/fats are rich in linoleic acid[13,14] and animal depot fat are rich in oleic acid.[15] Therefore, by keeping all these aspects about fatty acid composition of different oils/fats, an attempt was made to detect adulteration in milk fat with soybean oil and buffalo depot fat using Gas liquid chromatography technique.

## Materials and methods

### Samples preparation

Cow and buffalo milk fat samples were prepared from their respective milks using creamery butter method.[16] Refined soybean oil of a well-known brand was procured from the local market of Karnal, Haryana. Buffalo adipose tissue was collected from abattoir located at Keshavpuram, New Delhi and its fat was extracted by heat clarification at 140˚C followed by filtration using ordinary filter paper and finally with Whatman No. 4 using vacuum filter assembly. Cow milk was a mixture of the milk obtained from the herd of Karan Swiss, Karan Fries, Sahiwal and Tharparkar breeds. Buffalo milk was also herd milk from the Murrah breed only. In order to eliminate the effects due to season, samples were collected at two months interval over a period

of one year in January, March, May, July, September and November. The mean of the above period is presented in the study. Cows and buffaloes were maintained under identical conditions of feeding and management. The fodder/roughage given to animals throughout the year consisted of oat, maize and berseem in March; maize and silage in May; maize, silage and jowar in June; maize and jowar from July to September; jowar, bajra and silage from October to December; berseem and oat in January and February. The concentrate mixture comprised of mainly maize, barley, paddy or wheat or oat, groundnut cake, mustard cake, cottonseed cake, wheat bran, rice bran, a minerals mixture and common salt. Adulterated milk fat samples were prepared by adding soybean oil and buffalo depot fat individually @5, 10 and 15%(v/v) and in combination @5+5(10), 10+10(20) and 15+15(30)% (v/v) to pure cow and buffalo milk fat in a completely molten stage and mixed properly.

## Preparation of fatty acid methyl esters

Methyl esters of pure milk fat (cow and buffalo), adulterants fat/oil (soybean oil and buffalo depot fat), milk fat samples added with adulterants individually and also with their combinations were prepared by the method as described below:

Methylation of above samples for making esters was done by the sealed tube method of deMan[17] as modified by Luddy et al.[18] Incubation temperature of 75°C was used instead of 60°C. Approximately 0.2g of the melted milk fat sample was introduced into a freeze drying tube by means of pasture pipette directly to the bottom without sticking milk fat inside the inner wall of tubes followed by addition of approximately 0.3ml of 0.2N sodium methoxide and 0.1ml of benzene by making about double the quantity of solvents (sodium methoxide and benzene) to that of fat. The tube was sealed and placed in an oven maintained at 75°C and shaking was done at regular intervals of 10 to 15minutes. The methylation was completed in an hour, as evident from the change of two phase system into one phase system. At the time of analysis, ester tubes were broken and sample was injected into the GLC column without any further treatment. Fatty acids of above said samples in methylated form were analyzed in glass column packed with diethyl glycol succinate (10 percent) using gas chromatograph (NETEL Chromatograph, Michro-9100), equipped with flame ionization detector and temperature control modules.

A programme of total 37min was standardized. The sample was injected at an initial temperature of 70°C maintained for 3min and then raised after the emergence of butyric acid peak to 150°C by holding for 3min till the emergence of myristic acid ($C_{14:0}$) peak with a ramp rate of 5°C/min, then again raised up to 200°C with same ramp rate till the emergence of oleic acid ($C_{18:1}$) and then maintained at 200°C for 5min until emergence of the peak of linolenic acid on chromatograph.

The reference standards of different saturated and unsaturated fatty acids (Sigma Aldrich, Germany) were also run under the similar conditions of the gas liquid chromatography for identifying the particular fatty acid by comparing their retention time with that obtained for fatty acids in case of milk fat samples. By using above specifications, chromatographs of different fatty acids profile were obtained and the percentage of each fatty acid was calculated and compared.

## Results and discussion

Pure milk fats, vegetable oils and fats, and depot fats exhibit wide difference in their fatty acid composition.[12,19–21] Therefore, in the present investigation, it was planned to use these differences to assess the possibilities of detecting the adulterant oil and fat in milk fat.

Table 1 show that there were slight differences in fatty acid composition of milk fat from the two species (buffalo and cow). The average percentage of unsaturated fatty acids of buffalo and cow milk fat were 28.73 and 32.21, respectively. This indicated that cow milk fat was slightly more unsaturated than buffalo milk fat. It was also noticed that buffalo milk fat contained slightly higher proportion of $C_{4:0}$, $C_{6:0}$, $C_{16:0}$ and $C_{18:0}$ fatty acids than those of cow milk fat. A lower level of $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:0}$ fatty acid in buffalo milk fat than in cow milk fat was also observed. Similar differences in fatty acid profile of milk fat from the two species have also been reported by earlier workers.[10,12,21] However, some workers[9,22] did not notice any difference in the percentage of $C_{18:1}$ fatty acid of buffalo and cow ghee. On the other hand, some workers[10,12,23] did not observe $C_{20:0}$ fatty acid, but other workers[24,25] have reported the presence of $C_{20:0}$ in milk fat, which corroborate the observation made in the present study. The differences noticed in the present study in the fatty acid composition of buffalo and cow pure milk fats may be attributed to the species characteristics, since the study was carried out under identical conditions of feeding and management.

Summer season (May-June) showed a slight increase in the unsaturated fatty acids, whereas the monsoon (July-August) and winter (January-February) seasons marginally decreased the unsaturated fatty acids.[9,12,26] A similar trend of changes in the fatty acid composition of buffalo and cow pure milk fat with respect to the seasons was observed in the present investigation.

The buffalo depot fat and soybean oil were devoid of short chain ($C_{4:0}$-$C_{12:0}$) fatty acids which were otherwise uniquely present in milk fat (Table 1). Even myristic acid ($C_{14:0}$) was also found to be absent in the soybean oil studied. The four main fatty acids of the depot fat and soybean oil were observed to be palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. Buffalo depot fat showed the higher percentage of palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acid. On the other hand, the soybean oil showed an exceptionally higher level of $C_{18:2}$ than other fats with second highest level of $C_{18:1}$, in addition to the higher level of $C_{18:2}$ as reported above.

On comparing the fatty acid composition of the pure milk fat (buffalo and cow) with that of the adulterant oil and fat, it was found that in addition to the differences observed in terms of presence of short chain fatty acids ($C_{4:0}$ to $C_{12:0}$) in pure milk fat, and the absence of the same in the adulterant fat/oil (depot fat and vegetable oil), the contents of $C_{14:0}$ and $C_{16:0}$ fatty acids were also higher in buffalo and cow pure milk fat. Whereas, the $C_{18:0}$ content was found to be lower in pure milk fat than depot fat but higher than that of the vegetable oil studied in the present investigation.

In pure milk fat samples adulterated with individual depot fat or vegetable oil (Tables 2-4), the short and medium chain fatty acids ($C_{4:0}$ to $C_{15:0}$) decreased quantitatively with a concomitant increase in long chain fatty acids, depending upon the amount of the adulterant oil/fat added to milk fat. Linoleic acid ($C_{18:2}$) is a common long chain unsaturated fatty acid present in all the natural oils and fats including milk fat, though in varying amounts. However, its amount is predominantly higher in vegetable oils, while it is comparable in milk fats and animal depot fat. Therefore, its differential content has been used as an index for the detection of vegetable oils added to milk fat. The $C_{18:2}$ content in buffalo milk fat ranged from 0.93 to 1.62percent

with an average of 1.33 percent, while in cow milk fat, it ranged from 2.39 to 2.75 percent with an average of 2.53percent. In soybean oil and buffalo depot fat, the content of $C_{18:2}$ ranged from 49.93 to 53.27, and 2.43 to 3.78 percent with an average of 51.86 and 3.11 percent, respectively (Table 1). The present study revealed that on the basis of an increase in $C_{18:2}$ content in milk fat samples adulterated with soybean oil as compared to the overall range of $C_{18:2}$ content (0.93 to 2.75 percent) in buffalo and cow pure milk fat, it might be possible to detect addition of soybean oil even up to 5 percent level in both types of milk fats. But using this as the basis, addition of buffalo depot fat even up to 10 percent level in cow milk fat and 15 percent level in buffalo milk fat may not be detected because the $C_{18:2}$ content of buffalo depot fat was not much different from that of milk fat. Panda et al.[21] & Kumar et al.[12] have also reported that using $C_{18:2}$ as a marker fatty acid, adulteration of milk fat with vegetable oil even up to 5 percent level can be easily detected which corroborates our findings. On the other hand, it was observed in the present study that adulterants added in combination could be detected at all the levels (5, 10 and 15 percent of each adulterant) studied.

Oleic acid ($C_{18:1}$) is another long chain mono-unsaturated fatty acid commonly present in high amount in different natural oils and fats including milk fat, though in varying amounts. It was observed from the present investigation on fatty acid composition of fats/oil that oleic acid ($C_{18:1}$) which is present in very high concentration in buffalo depot fat (about 50 percent of total fatty acids) could be used as the base for its detection in milk fat, in which its concentration is less than 25 percent of total fatty acids. The content of oleic acid in buffalo milk fat ranged from 22.34 to 23.50 percent with an average of 22.85 percent, while in cow milk fat, it ranged from 23.41 to 25.54 percent with an average of 24.52 percent. In soybean oil and buffalo depot fat, the content of $C_{18:1}$ ranged from 21.47 to 26.34, and 47.59 to 50.47 percent with an average of 23.88 and 49.17 percent, respectively (Table 1). The present study revealed that on the basis of an increase in $C_{18:1}$ content in milk fat samples adulterated with buffalo depot fat as compared to the overall range of $C_{18:1}$ content (22.34 to 25.54 percent) in buffalo and cow pure milk fats, as low as 5 percent addition of buffalo depot fat in cow milk fat and 10 and 15 percent levels in buffalo milk fat were detectable. But using this as the basis, addition of soybean oil, even up to 15 percent level in both cow and buffalo milk fats may not be detected because the $C_{18:1}$ content of soybean oil was not much different from that of milk fat. On the other hand, mixture of adulterants (5, 10 and 15 percent of each adulterant) could be detected in both types of milk fats. Comparison of the results on use of $C_{18:2}$ and $C_{18:1}$ for the detection of adulteration with soybean oil and buffalo depot fat showed that $C_{18:2}$ is suitable as a marker fatty acid for detection of soybean oil in milk fat, while $C_{18:1}$ is useful for detecting buffalo depot fat in milk fat.

In addition to the study of profile of individual fatty acids, some of the workers[19-21,27–29] have noted that any change in the ratio of major fatty acids before and after addition of adulterant fats/oils could be used as a criteria for detecting adulteration in milk fat. Keeping these aspects in view, some of the fatty acids ratios were calculated which are given in Table 5.

**Table 1** Fatty acid profile (percent weight) of pure milk fat (cow and buffalo), soybean oil and buffalo depot fat

| Fatty acids* | Type of fat/oil** | | | |
|---|---|---|---|---|
| | PCG | PBG | SO | BDF |
| $C_{4:0}$ | 3.79±0.03 | 4.83±0.08 | - | - |
| $C_{6:0}$ | 2.29±0.03 | 2.64±0.12 | - | - |
| $C_{8:0}$ | 1.39±0.03 | 1.27±0.04 | - | - |
| $C_{10:0}$ | 3.11±0.07 | 2.43±0.05 | - | - |
| $C_{12:0}$ | 3.54±0.06 | 3.15±0.04 | - | - |
| $C_{14:0}$ | 11.73±0.22 | 11.69±0.17 | - | 2.24±0.17 |
| $C_{14:1}$ | 1.38±0.03 | 1.01±0.04 | - | - |
| $C_{15:0}$ | 0.89±0.02 | 1.04±0.06 | - | - |
| $C_{16:0}$ | 26.90±0.24 | 28.37±0.34 | 12.72±0.40 | 24.59±0.44 |
| $C_{16:1}$ | 1.98±0.05 | 2.13±0.09 | - | - |
| $C_{17:0}$ | 0.38±0.02 | 0.35±0.04 | - | - |
| $C_{18:0}$ | 10.73±0.15 | 12.58±0.12 | 3.28±0.29 | 18.84±0.23 |
| $C_{18:1}$ | 24.52±0.35 | 22.85±0.19 | 23.88±0.71 | 49.17±0.40 |
| $C_{18:2}$ | 2.53±0.07 | 1.33±0.10 | 51.86±0.55 | 3.11±0.20 |
| $C_{18:3}$ | 1.81±0.05 | 1.41±0.06 | 6.23±0.34 | 2.06±0.18 |
| $C_{20:0}$ | 3.05±0.06 | 2.94±0.12 | 2.03±0.24 | - |
| Total saturated fatty acids | 67.79±0.51 | 71.27±0.29 | 18.03±0.37 | 45.66±0.43 |
| Total unsaturated fatty acids | 32.21±0.50 | 28.73±0.27 | 81.97±0.39 | 54.34±0.45 |
| Sum of $C_{4:0}$ to $C_{14:1}$ fatty acids | 27.24±0.23 | 27.01±0.36 | 0 | 2.24±0.17 |
| Sum of $C_{15:0}$ to $C_{20:0}$ fatty acids | 72.77±0.24 | 72.99±0.38 | 100 | 97.76±0.19 |

PCG, pure cow ghee; PBG, pure buffalo ghee; SO, soybean oil; BDF, buffalo depot fat; (-): Fatty acid was absent

*The first figure refers to the number of carbon, and the second figure to the number of double bonds.**Data represent the mean±SE of six determinations.

**Table 2** Fatty acid profile (percent weight) of cow milk fat added individually with soybean oil and buffalo depot fat

| Fatty acids* | Level of adulteration** | | | | | |
|---|---|---|---|---|---|---|
| | 5%SO | 10%SO | 15%SO | 5%BDF | 10%BDF | 15%BDF |
| $C_{4:0}$ | 3.54±0.04 | 3.36±0.07 | 3.19±0.08 | 3.58±0.04 | 3.32±0.07 | 3.25±0.05 |
| $C_{6:0}$ | 2.14±0.05 | 2.02±0.08 | 1.92±0.06 | 2.16±0.07 | 2.08±0.06 | 1.98±0.08 |
| $C_{8:0}$ | 1.28±0.03 | 1.13±0.06 | 1.06±0.05 | 1.22±0.08 | 1.16±0.05 | 1.01±0.06 |
| $C_{10:0}$ | 2.95±0.06 | 2.76±0.09 | 2.64±0.08 | 2.88±0.08 | 2.78±0.06 | 2.67±0.08 |
| $C_{12:0}$ | 3.35±0.08 | 3.16±0.12 | 3.07±0.06 | 3.38±0.09 | 3.14±0.08 | 3.08±0.09 |
| $C_{14:0}$ | 11.12±0.32 | 10.31±0.30 | 9.72±0.26 | 10.96±0.45 | 10.46±0.62 | 9.97±0.56 |
| $C_{14:1}$ | 1.25±0.06 | 1.17±0.05 | 1.11±0.06 | 1.21±0.05 | 1.13±0.04 | 1.02±0.05 |
| $C_{15:0}$ | 0.86±0.05 | 0.76±0.06 | 0.67±0.08 | 0.88±0.03 | 0.73±0.05 | 0.62±0.04 |
| $C_{16:0}$ | 26.34±0.64 | 25.43±0.65 | 24.58±0.47 | 26.72±0.58 | 26.62±0.69 | 25.46±0.73 |
| $C_{16:1}$ | 1.92±0.08 | 1.86±0.06 | 1.78±0.08 | 1.86±0.05 | 1.81±0.06 | 1.72±0.05 |
| $C_{17:0}$ | 0.31±0.04 | 0.26±0.06 | 0.20±0.05 | 0.33±0.04 | 0.24±0.05 | 0.22±0.06 |
| $C_{18:0}$ | 10.38±0.91 | 9.88±1.13 | 9.56±0.87 | 11.58±0.63 | 11.66±0.73 | 11.98±0.82 |
| $C_{18:1}$ | 24.57±0.76 | 24.51±0.69 | 24.48±0.54 | 26.08±0.48 | 27.78±0.85 | 29.91±0.93 |
| $C_{18:2}$ | 5.16±0.12 | 8.54±0.43 | 11.13±0.63 | 2.51±0.07 | 2.63±0.07 | 2.78±0.12 |
| $C_{18:3}$ | 1.95±0.06 | 2.12±0.07 | 2.23±0.04 | 1.84±0.06 | 1.78±0.07 | 1.82±0.08 |
| $C_{20:0}$ | 2.88±0.05 | 2.73±0.07 | 2.66±0.08 | 2.81±0.08 | 2.68±0.09 | 2.51±0.05 |
| Total saturated fatty acids | 65.1±50.65 | 61.80±0.58 | 59.27±0.52 | 66.50±0.64 | 64.87±0.53 | 62.72±0.68 |
| Total unsaturated fatty acids | 34.85±0.38 | 38.20±0.33 | 40.73±0.42 | 33.50±0.28 | 35.13±0.32 | 37.25±0.23 |
| Sum of $C_{4:0}$ to $C_{14:1}$ fatty acids | 25.63±0.38 | 23.91±0.52 | 22.71±0.57 | 25.39v0.65 | 24.07±0.48 | 22.98±0.43 |
| Sum of $C_{15:0}$ to $C_{20:0}$ fatty acids | 74.37±.78 | 76.09±0.51 | 77.29±0.45 | 74.61±0.68 | 75.93±0.42 | 77.02±0.58 |

PCG, pure cow ghee; PBG, pure buffalo ghee; SO, soybean oil; BDF, buffalo depot fat

*The first figure refers to the number of carbon, and the second figure to the number of double bonds.

**Data represent the mean±SE of six determinations.

**Table 3** Fatty acid profile (percent weight) of buffalo milk fat added individually with soybean oil and buffalo depot fat

| Fatty acids* | Level of adulteration** | | | | | |
|---|---|---|---|---|---|---|
| | 5%SO | 10%SO | 15%SO | 5%BDF | 10%BDF | 15%BDF |
| $C_{4:0}$ | 4.33±0.05 | 4.11±0.06 | 3.98±0.05 | 4.36±0.04 | 4.16±0.06 | 3.92±0.05 |
| $C_{6:0}$ | 2.48±0.05 | 2.28±0.05 | 2.04±0.04 | 2.48±0.05 | 2.21±0.05 | 1.98±0.04 |
| $C_{8:0}$ | 1.08±0.04 | 1.02±0.05 | 0.88±0.04 | 1.10±.03 | 1.03±0.03 | 0.95±0.03 |
| $C_{10:0}$ | 2.07±0.06 | 1.88±0.07 | 1.58±0.03 | 2.12±0.04 | 1.93±0.04 | 1.87±0.05 |
| $C_{12:0}$ | 2.68±0.07 | 2.54±0.06 | 2.23±0.06 | 2.71±0.07 | 2.52±0.06 | 2.36±0.06 |
| $C_{14:0}$ | 10.68±0.25 | 10.19±0.27 | 9.94±0.19 | 11.15±0.11 | 10.72±0.16 | 9.25±0.13 |
| $C_{14:1}$ | 0.88±0.04 | 0.76±0.05 | 0.65±0.05 | 0.87±0.06 | 0.80±0.06 | 0.71±0.04 |

Table Continued

| Fatty acids* | Level of adulteration** | | | | | |
|---|---|---|---|---|---|---|
| | 5%SO | 10%SO | 15%SO | 5%BDF | 10%BDF | 15%BDF |
| $C_{15:0}$ | 0.94±0.03 | 0.84±0.04 | 0.72±0.04 | 0.94±0.03 | 0.82±0.03 | 0.75±0.03 |
| $C_{16:0}$ | 27.53±0.36 | 26.61±0.37 | 26.33±0.42 | 28.13±0.38 | 27.76±0.48 | 27.58±0.53 |
| $C_{16:1}$ | 1.93±0.05 | 1.82±0.06 | 1.74±0.04 | 1.95±0.06 | 1.78±0.05 | 1.69±0.05 |
| $C_{17:0}$ | 0.34±0.03 | 0.26±0.04 | 0.21±0.02 | 0.31±0.04 | 0.25±0.02 | 0.18±0.04 |
| $C_{18:0}$ | 12.31±0.14 | 11.58±0.18 | 11.24±0.16 | 13.25±0.12 | 13.41±0.17 | 13.85±0.19 |
| $C_{18:1}$ | 23.35±0.43 | 23.41±0.52 | 23.57±0.63 | 25.15±0.68 | 27.23±0.63 | 29.51±0.72 |
| $C_{18:2}$ | 4.88±0.08 | 7.91±0.09 | 9.95±0.11 | 1.42±0.04 | 1.48±0.05 | 1.67±0.04 |
| $C_{18:3}$ | 1.66±0.07 | 1.98±0.05 | 2.16±0.06 | 1.34±0.05 | 1.28±0.05 | 1.21±0.05 |
| $C_{20:0}$ | 2.86±0.06 | 2.81±0.06 | 2.78±0.06 | 2.72±0.07 | 2.62±0.06 | 2.52±0.06 |
| Total saturated fatty acids | 63.93±0.72 | 59.72±0.68 | 55.67±0.62 | 64.84±0.63 | 60.95±0.53 | 56.95±0.73 |
| Total unsaturated fatty acids | 36.07±0.31 | 40.28±0.42 | 44.33±0.53 | 35.16±0.28 | 39.05±0.21 | 43.05±0.32 |
| Sum of $C_{4:0}$ to $C_{14:1}$ fatty acids | 23.43±0.25 | 21.22±0.31 | 18.39±0.23 | 23.81±0.31 | 21.36±0.28 | 18.58±0.18 |
| Sum of $C_{15:0}$ to $C_{20:0}$ fatty acids | 76.57±0.68 | 78.78±0.74 | 81.61±0.75 | 76.19±0.72 | 78.64±0.63 | 81.42±0.66 |

PCG, pure cow ghee; PBG, pure buffalo ghee; SO, soybean oil; BDF, buffalo depot fat

*The first figure refers to the number of carbon, and the second figure to the number of double bonds.

**Data represent the mean±SE of six determinations.

**Table 4** Fatty acid profile (percent weight) of cow and buffalo milk fat added with mixture of soybean oil and buffalo depot fat

| Fatty Acids* | Level of adulteration | | | | | |
|---|---|---|---|---|---|---|
| | Cow Ghee** | | | Buffalo ghee** | | |
| | 5+5% (SO+BDF) | 10+10% (SO+BDF) | 15+15% (SO+BDF) | 5+5% (SO+BDF) | 10+10% (SO+BDF) | 15+15% (SO+BDF) |
| $C_{4:0}$ | 3.27±0.06 | 3.16±0.06 | 2.69±0.05 | 4.21±0.06 | 3.75±0.04 | 3.34±0.07 |
| $C_{6:0}$ | 2.04±0.05 | 1.71±0.05 | 1.63±0.03 | 2.21±0.04 | 1.93±0.04 | 1.78±0.06 |
| $C_{8:0}$ | 1.15±0.04 | 1.06±0.04 | 0.83±0.03 | 1.12±0.03 | 0.98±0.03 | 0.77±0.04 |
| $C_{10:0}$ | 2.73±0.06 | 2.42±0.06 | 2.14±0.06 | 2.18±0.05 | 1.85±0.04 | 1.62±0.05 |
| $C_{12:0}$ | 3.05±0.07 | 2.73±0.07 | 2.33±0.05 | 2.98±0.07 | 2.66±0.06 | 2.26±0.06 |
| $C_{14:0}$ | 9.98±0.11 | 9.13±0.14 | 7.91±0.09 | 9.95±0.16 | 9.21±0.09 | 7.98±0.12 |
| $C_{14:1}$ | 1.21±0.05 | 1.01±0.04 | 0.86±0.05 | 1.16±0.07 | 0.98±0.04 | 0.83±0.03 |
| $C_{15:0}$ | 0.81±0.05 | 0.75±0.06 | 0.69±0.04 | 0.86±0.05 | 0.81±0.03 | 0.70±0.04 |
| $C_{16:0}$ | 26.19±0.45 | 24.64±0.39 | 23.74±0.43 | 26.42±0.52 | 24.79±0.42 | 23.92±0.37 |
| $C_{16:1}$ | 1.55±0.06 | 1.26±0.06 | 1.12±0.05 | 1.45±0.04 | 1.19±0.04 | 1.06±0.05 |

Table Continued

| Fatty Acids* | Level of adulteration | | | | | |
|---|---|---|---|---|---|---|
| | Cow Ghee** | | | Buffalo ghee** | | |
| | 5+5% (SO+BDF) | 10+10% (SO+BDF) | 15+15% (SO+BDF) | 5+5% (SO+BDF) | 10+10% (SO+BDF) | 15+15% (SO+BDF) |
| $C_{18:0}$ | 11.64±0.14 | 11.39±0.21 | 11.14±0.19 | 11.92±0.25 | 12.28±0.28 | 12.12±0.16 |
| $C_{18:1}$ | 25.58±0.52 | 27.26±0.63 | 28.47±0.67 | 26.81±0.47 | 28.88±0.39 | 30.28±0.33 |
| $C_{18:2}$ | 5.65±0.08 | 8.57±0.09 | 11.37±0.08 | 4.28±0.08 | 6.32±0.07 | 8.87±0.09 |
| $C_{18:3}$ | 2.08±0.06 | 2.18±0.07 | 2.51±0.05 | 1.46±0.04 | 1.68±0.04 | 2.01±0.04 |
| $C_{20:0}$ | 2.76±0.06 | 2.48±0.08 | 2.36±0.06 | 2.67±0.07 | 2.41±0.05 | 2.28±0.04 |
| Total saturated fatty acids | 67.30±0.53 | 64.12±0.66 | 61.93±0.51 | 69.27±0.56 | 67.43±0.48 | 65.21±0.39 |
| Total unsaturated fatty acids | 32.70±0.48 | 35.88±0.39 | 38.07±0.43 | 30.73±0.35 | 32.57±0.41 | 34.79±0.37 |
| Sum of $C_{4:0}$ to $C_{14:1}$ fatty acids | 24.20±0.31 | 22.78±0.27 | 21.30±0.22 | 24.79±0.35 | 23.37±0.38 | 21.04±0.25 |
| Sum of $C_{15:0}$ to $C_{20:0}$ fatty acids | 75.80±0.64 | 77.22±0.59 | 78.70±0.49 | 75.21±0.46 | 76.63±0.53 | 78.96±0.62 |

SO, soybean oil; BDF, buffalo depot fat

*The first figure refers to the number of carbon, and the second figure to the number of double bonds.

**Data represent the mean±SE of six determinations

For detecting the adulteration of soybean oil and buffalo depot fat in milk fat, 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. It can be seen from the Table 5 that the overall range of $C_{14:0}/C_{16:0}$, $C_{14:0}/C_{18:1}$, $C_{14:0}/C_{18:2}$, $C_{16:0}/C_{18:1}$, $C_{16:0}/C_{18:2}$ and $C_{18:0}/C_{18:2}$ of both pure cow and buffalo milk fat ranged from 0.38 to 0.45, 0.44 to 0.54, 4.10 to 12.47, 1.04 to 1.30, 9.61 to 30.96 and 3.79 to 13.51, respectively.

Using the range of these fatty acid ratios of cow and buffalo milk fat and their comparison with the average values of cow and buffalo milk fats added with adulterants individually as well as in their combination, the results revealed that the ratio of $C_{14:0}/C_{18:1}$ and $C_{14:0}/C_{18:2}$ were found more helpful in detecting the maximum number (78 percent) of adulterated samples (Table 6), as compared to the other ratios studied. It was also observed that both these ratios ($C_{14:0}/C_{18:1}$ and $C_{14:0}/C_{18:2}$) were able to detect the equal number (78 percent) of adulterated samples.

It can be inferred from the above part of the study that $C_{18:1}$ and $C_{18:2}$ are the important long chain fatty acids, as discussed before, for the detection of adulterants (animal depot fat and vegetable oil) in milk fat. In addition to the above ratios studied, the ratios of the sum of the total of $C_{4:0}$ to $C_{14:1}$/sum of the total of $C_{15:0}$ to $C_{20:0}$ fatty acids and vice-versa were also compared between the pure and adulterated milk fat samples. The sum of the total of $C_{4:0}$ to $C_{14:1}$/sum of the total of $C_{15:0}$ to $C_{20:0}$ fatty acids for pure cow and buffalo milk fat was found to vary between 0.35 to 0.40, while the ratio of sum of the total of $C_{15:0}$ to $C_{20:0}/C_{4:0}$ to $C_{14:1}$ fatty acids varied between 2.49 to 2.86.

It was interesting to note that when the average ratio of sum of the total of $C_{4:0}$ to $C_{14:1}$/sum of the total of $C_{15:0}$ to $C_{20:0}$ fatty acids of adulterated milk fat samples was compared, with the overall range of the ratio of sum of the total of $C_{4:0}$ to $C_{14:1}$/sum of the total of $C_{15:0}$ to $C_{20:0}$ fatty acids of pure cow and buffalo milk fats, all the types of adulteration levels studied, irrespective whether individual or in combination, were detectable both in cow and buffalo milk fats. The same was found to be true when the vice-versa ratios (sum of the total of $C_{15:0}$ to $C_{20:0}/C_{4:0}$ to $C_{14:1}$ fatty acids) were used as the basis for comparison, as here also all the types of adulteration cases were detected (Table 7) & (Table 8).

**Table 6** Certain fatty acid ratios of milk fat (cow and buffalo) added with adulterants individually and in combination thereof

| Type of ghee | Type of adulterants fat/oil | Level of adulterants (%) | Fatty acid ratios* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $C_{14:0}/C_{16:0}$ | $C_{14:0}/C_{18:1}$ | $C_{14:0}/C_{18:2}$ | $C_{16:0}/C_{18:1}$ | $C_{16:0}/C_{18:2}$ | $C_{18:0}/C_{18:2}$ |
| PCG | SO | 5 | 0.42 | 0.45 | 2.16 | 1.07 | 5.1 | 2.01 |
| | SO | 10 | 0.41 | 0.42 | 1.21 | 1.04 | 2.98 | 1.16 |
| | SO | 15 | 0.4 | 0.4 | 0.87 | 1 | 2.21 | 0.86 |
| | BDF | 5 | 0.41 | 0.42 | 4.37 | 1.02 | 10.65 | 4.61 |
| | BDF | 10 | 0.39 | 0.38 | 3.98 | 0.96 | 10.12 | 4.43 |
| | BDF | 15 | 0.39 | 0.33 | 3.59 | 0.85 | 9.16 | 4.31 |
| | SO+BDF | 5+5 | 0.38 | 0.39 | 1.77 | 1.02 | 4.64 | 2.06 |
| | SO+BDF | 10+10 | 0.37 | 0.33 | 1.07 | 0.9 | 2.88 | 1.33 |
| | SO+BDF | 15+15 | 0.33 | 0.28 | 0.7 | 0.83 | 2.09 | 0.98 |
| PBG | SO | 5 | 0.39 | 0.46 | 2.19 | 1.18 | 5.64 | 2.52 |
| | SO | 10 | 0.38 | 0.44 | 1.29 | 1.14 | 3.36 | 1.46 |
| | SO | 15 | 0.38 | 0.42 | 1 | 1.12 | 2.65 | 1.13 |
| | BDF | 5 | 0.4 | 0.44 | 7.85 | 1.12 | 19.81 | 9.33 |
| | BDF | 10 | 0.39 | 0.39 | 7.24 | 1.02 | 18.76 | 9.06 |
| | BDF | 15 | 0.34 | 0.31 | 5.54 | 0.93 | 16.51 | 8.29 |
| | SO+BDF | 5+5 | 0.38 | 0.37 | 2.32 | 0.99 | 6.17 | 2.79 |
| | SO+BDF | 10+10 | 0.37 | 0.32 | 1.46 | 0.86 | 3.92 | 1.94 |
| | SO+BDF | 15+15 | 0.33 | 0.26 | 0.9 | 0.79 | 2.7 | 1.37 |

PCG, pure cow ghee; PBG, pure buffalo ghee; SO, soybean oil; BDF, buffalo depot fat

**Table 7** Sum of C4:0 to C14:1/ sum of C15:0 to C20:0 fatty acids and vice-versa ratios of pure milk fats (cow and buffalo) and adulterant fat/oil

| Type of adulterant | Sum of $C_{4:0}$ to $C_{14:1}$/ Sum of $C_{15:0}$ to $C_{20:0}$ fatty acids | | Sum of $C_{15:0}$ to $C_{20:0}$/ Sum of $C_{4:0}$ to $C_{14:1}$ fatty acids | |
|---|---|---|---|---|
| | Range* | Average±SE | Range* | Average±SE |
| PCG | 0.36-0.39 | 0.37±0.01 | 2.58-2.77 | 2.67±0.03 |
| PBG | 0.35-0.40 | 0.37±0.01 | 2.49-2.86 | 2.71±0.05 |
| SO | 0.00-0.00 | 0 | 0.00-0.00 | 0 |
| BDF | 0.02-0.03 | 0.02±0.001 | 34.97-53.95 | 44.92±2.73 |

PCG, pure cow ghee; PBG, pure buffalo ghee; SO, soybean oil; BDF, buffalo depot fat

*Data represent the mean±SE of six determinations

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**Table 8** Sum of C4:0 to C14:1/sum of C15:0 to C20:0 fatty acids and vice-versa ratios of milk fats (cow and buffalo) added with adulterants individually and in combination thereof

| Type of adulterant | Level | Sum of $C_{4:0}$ to $C_{14:1}$/ Sum of $C_{15:0}$ to $C_{20:0}$ fatty acids | | Sum of $C_{15:0}$ to $C_{20:0}$/ Sum of $C_{4:0}$ to $C_{14:1}$ fatty acids | |
|---|---|---|---|---|---|
| | | Cow ghee | Buffalo ghee | Cow ghee | Buffalo ghee |
| SO | 5 | 0.34 | 0.32 | 2.9 | 3.13 |
| SO | 10 | 0.31 | 0.3 | 3.18 | 3.39 |
| SO | 15 | 0.29 | 0.27 | 3.4 | 3.69 |
| BDF | 5 | 0.33 | 0.33 | 2.99 | 3.03 |
| BDF | 10 | 0.32 | 0.3 | 3.15 | 3.28 |
| BBF | 15 | 0.3 | 0.27 | 3.35 | 3.75 |
| SO+BDF | 5+5 | 0.27 | 0.31 | 3.27 | 3.2 |
| SO+BDF | 10+10 | 0.23 | 0.27 | 3.71 | 3.68 |
| SO+BDF | 15+15 | 0.31 | 0.23 | 4.44 | 4.38 |

SO, soybean oil; BDF, buffalo depot fat

## Conclusion

The present study revealed that the soybean oil could easily be detected at 5 percent level in milk fat when linoleic acid was used as a marker, while on the basis of oleic acid;buffalo depot fat was detected easily at the same level. On the other side, when the ratios of the certain fatty acids were tried for detecting adulteration, it was noticed that the two fatty acid ratios ($C_{14:0}/C_{18:1}$ and $C_{14:0}/C_{18:2}$) were found useful for detecting most of the adulteration levels. The best results of the study were come out when the ratios of the sum of $C_{4:0}$ to $C_{14:1}$ and $C_{15:0}$ to $C_{20:0}$ fatty acids and vice-versa were calculated and it was found that the adulteration of both the adulterants, whether added individually or in combination were detected at all the levels studied.

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## Conflict of interest

The author declares no conflict of interest.

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