

## A study on change of fatty acids profile in ghee adulterated with palm oil

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**Abstract:** Ghee, is clarified butter or anhydrous milk fat, has an integral relation with Indian culinary culture. Due to the importance and seasonal decline in the production; unscrupulous traders tend to add cheap vegetable oils like palm oil in ghee. The composition of milk fat and oil vary in terms of fatty acids. Milk contains mainly short and medium chain fatty acids whereas vegetable oil contains long chain fatty acids. Due to different fatty acids composition between milk fat and palm oil, we tried to exploit this fatty acids difference to identify the adulteration of palm oil in ghee, using Gas chromatography. Study suggested that due to palm oil adulteration in ghee, the concentration of short chain fatty acids get decreased and long chain saturated and unsaturated fatty acids showed an increase. In addition to the above, change was also observed in the ratio of the total of saturated fatty acids to unsaturated fatty acids. Fatty acids analysis could be very effective for the detection of palm oil adulteration in ghee to the tune of 5 %. Our current study suggested that fatty acids study was able to detect foreign fat like palm oil in milk fat but this study is not suitable to confirm the type of foreign fat in milk fat or ghee.

**Keywords:** Adulteration, Butyric acid, Ghee, Gas liquid Chromatography, Linoleic acid, Palm oil

India is the highest milk producing country in the world. In India after liquid milk (~ 46 %), ghee (~ 28 %) is the second largest dairy product that is consumed throughout the country (GAIN, 2014). A very complex situation arises during the lean season of the year i.e., in the summer months (May to August) when the supply of milk and ghee drastically reduces. In this situation, unscrupulous traders use to add cheaper fats such as vegetable oils (palm oil, cottonseed oil etc.) in ghee, for gaining more money. Traditionally such type of adulteration is checked by analysing different fat constants of milk fat like- Reichert–Meissl value (RM value), Polenske value and Butyro Refractometer (BR) reading. Recently, dry fractionation technique coupled with fat constants analysis, was proved more suitable than simple fat constants analysis, to detect adulteration in ghee (Kumar et al. 2017); however, these technique are time consuming.

Recent trend being followed by the unscrupulous traders adulterate ghee in such a way that cannot be detected by simple fat constant analysis based approaches. Very recently, Pathania et al. (2020) applied a new approach of zooming in and superimposing of selected peaks in the chromatograms of triglycerides (using Gas chromatography), to detect refined palm oil along with other manipulated fats. Detection of palm oil adulteration in ghee is always a challenge for the scientific community; as there are very few studies available for the detection of oil in ghee (Ramani et al. 2019). Bector and Sharma (2002) were able to detect palm oil adulteration in ghee using a colorimetric test ; however these test was unable to detect BHA added ghee and ghee adulterated with palm oil. Researchers developed methods using fourier transform - near infrared (FT-NIR) and fourier transform - middle infrared (FT-MIR) spectroscopy to identify palm oil adulteration in ghee (Mehta et al. 2018; Aparnathi et al. 2019). However, FT-IR based analytical approaches were not suitable to detect palm oil in ghee lower than 10% level.(Aparnathi et al. 2019)

Fatty acid composition of milk fat is greatly affected by season, breed, species, parity, stage of lactation etc. (Kumar et al. 2015). Bharwade et al. (2017) reported that short and medium chain fatty acids were predominated in ghee. Butyric acid is a unique fatty acid in milk fat; however, change in butyric acid indicated the presence of adulteration in ghee (Mehta, 2013). Upadhyay et

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al. (2018) reported that for both pure cow and pure buffalo ghee, the major fatty acids were myristic acid (C<sub>14:0</sub>), palmitic acid (C<sub>16:0</sub>), stearic acid (C<sub>18:0</sub>) and oleic acid (C<sub>18:1</sub>). Orsavova et al. (2015) reported that major fatty acids in palm oil were palmitic acid, oleic acid, linoleic acid and stearic acid; however, those researchers reported the absence of short chain fatty acids. Rebechi et al. (2015) reported that fatty acids ratio (C10:0/C8:0, C12:0/C10:0, C14:0/C12:0 and C14:0/C18:0) could not able to detect body fat adulteration in milk fat. In a different study Kumar et al. (2015) successfully used selected fatty acid ratios (C14:0/C18:1 and C14:0/C18:2) as a marker for the detection of soybean oil adulteration in ghee.

Milk fat is unique and distinct from other fats. It is the only fat that contains short chain fatty acids, while vegetable oils are rich in linoleic acid (Kumar et al. 2015). Therefore, by keeping all these aspects of the fatty acid composition of different oils/fats, an attempt was made to detect adulteration in ghee with palm oil by fatty acid analysis using Gas chromatography technique.

Mix milk were collected from a local dairy farmers of Amreli district, Gujarat. The ghee was prepared by creamery butter method (De, 2011). The final temperature of clarification was 110°C. The clarified fat was filtered using coarse filter paper to obtain ghee and stored in airtight glass bottles at room temperature.

Palm oil was procured from a local market of Amreli city. For the preparation of adulterated ghee samples, pure ghee samples were heated to 60-70°C for 10 min before adding and mixing of palm oil. The adulterated ghee samples were prepared by the addition of

palm oil in four different proportions (5, 10, 15 and 20 % w/w) into pure ghee.

Methylation of the above samples for making esters was done by the sealed tube method of DeMan (1964) as modified by Luddy et al. (1968). An incubation temperature of 75°C was used instead of 60°C.

Ester tubes were broken at the time of analysis and the sample was injected into the Gas chromatography column without any further treatment. Fatty acid profile of the above said samples in methylated form were analyzed in the capillary column (length 30 m, internal diameter 0.25 mm) using Gas Chromatography (Thermo Fisher Scientific – Trace 1110) equipped with a flame ionization detector (240°C). The other conditions like Total Flow: 79 ml/min (N<sub>2</sub>), Column Flow: 1.49 ml/min, Pressure: 13.4 Kpa and Purge flow: 3 ml/min were set for further analysis.

The reference standards (Loba Chemical) of different fatty acids methyl esters were used to standardize a 37 minutes Gas chromatography programme. The sample was injected at an initial temperature of 70°C maintained for 3 min and then raised after the emergence of butyric acid peak to 150°C by holding for 3 min till the emergence of myristic acid (C<sub>14:0</sub>) 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<sub>18:1</sub>) and then maintained at 200°C for 5 min until emergence of the peak of linolenic acid on chromatograph. By using the above specifications, chromatographs of different fatty acids profile were obtained and the percentage of each fatty acid was calculated and

**Table 1** Fatty acid profile (percentage of weight) of pure ghee and palm oil and adulterated ghee

Fatty acids	Type of	fat/oil**		Level of		Adulteration **	
	Pure ghee	Palm oil	5% PO	10% PO	15% PO	20% PO	
C <sub>4:0</sub>	2.43 ± 0.22	0.00	1.52 ± 0.008	1.28 ± 0.002	1.03 ± 0.01	1.01 ± 0.006	
C <sub>6:0</sub>	0.73 ± 0.001	0.00	0.64 ± 0.017	0.41 ± 0.004	0.39 ± 0.001	0.36 ± 0.001	
C <sub>8:0</sub>	0.62 ± 0.007	0.00	0.61 ± 0.003	0.45 ± 0.0006	0.43 ± 0.003	0.39 ± 0.01	
C <sub>10:0</sub>	1.59 ± 0.05	0.00	1.39 ± 0.001	1.29 ± 0.001	1.16 ± 0.001	1.01 ± 0.004	
C <sub>12:0</sub>	1.95 ± 0.01	0.00	1.37 ± 0.014	1.12 ± 0.0007	1.10 ± 0.005	1.08 ± 0.01	
C <sub>14:0</sub>	9.67 ± 0.03	0.96 ± 0.005	8.77 ± 0.024	8.37 ± 0.002	7.98 ± 0.01	7.47 ± 0.15	
C <sub>16:0</sub>	29.9 ± 1.64	39.68 ± 0.015	30.42 ± 0.044	31.36 ± 0.02	32.04 ± 0.21	32.12 ± 0.81	
C <sub>18:0</sub>	18.24 ± 0.31	4.01 ± 0.017	17.14 ± 0.78	14.43 ± 0.19	12.34 ± 2.25	11.89 ± 7.76	
C <sub>18:1</sub>	30.94 ± 0.53	43.55 ± 0.04	34.17 ± 0.84	35.39 ± 0.02	36.29 ± 0.27	36.51 ± 1.13	
C <sub>18:2</sub>	1.91 ± 0.037	11.18 ± 0.007	2.22 ± 0.027	4.18 ± 0.16	5.68 ± 1.59	6.02 ± 6.01	
C <sub>18:3</sub>	1.57 ± 0.019	0.246 ± 0.001	1.36 ± 0.011	1.22 ± 0.003	1.15 ± 0.02	1.12 ± 0.06	
C <sub>20:0</sub>	0.46 ± 0.009	0.37 ± 0.001	0.43 ± 0.011	0.41 ± 0.014	0.40 ± 0.05	0.39 ± 0.11	
Total saturated fatty acids	65.59 ± 0.25	45.02 ± 0.009	62.29 ± 0.1	59.12 ± 0.026	56.87 ± 0.282	55.76 ± 0.984	
Total unsaturated fatty acids	34.42 ± 0.195	54.976 ± 0.016	37.75 ± 0.292	40.79 ± 0.061	43.12 ± 0.626	43.65 ± 2.4	
Total saturated fatty acids/ Total unsaturated fatty acids	1.90	0.81	1.65	1.44	1.31	1.27	
Sum of C <sub>4:0</sub> to C <sub>16:0</sub> fatty acids	16.99 ± 0.05	0.96 ± 0.005	14.30 ± 0.01	12.92 ± 0.001	12.09 ± 0.005	11.32 ± 0.03	
Sum of C <sub>18:0</sub> to C <sub>20:0</sub> fatty acids	83.02 ± 0.42	99.03 ± 0.01	85.74 ± 0.28	86.99 ± 0.06	87.90 ± 0.73	88.09 ± 2.64	

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

compared. The identical conditions as mentioned above and the retention time of these fatty acids were used to identify the fatty acids in the ghee (pure and adulterated) samples.

Pure ghee and palm oil revealed a wide difference in their fatty acid composition. Fatty acids composition of both palm oil and pure ghee is given in table 1. There were wide differences between the fatty acids composition of milk fat (ghee) and palm oil. It was observed from table 1 that in pure ghee short chain fatty acids like  $C_{4:0}$ ,  $C_{6:0}$ ,  $C_{8:0}$ ,  $C_{10:0}$  and  $C_{12:0}$ ; 2.43, 0.73, 0.62, 1.59 and 1.95 %, respectively; however, in case of palm oil no short chain fatty acids were determined. However, even myristic acid ( $C_{14:0}$ ) was also found to be very nominal in the palm oil (0.96%) as compared to pure ghee (9.69%). The four main fatty acids in palm oil were identified as palmitic ( $C_{16:0}$ ), stearic ( $C_{18:0}$ ), oleic ( $C_{18:1}$ ) and linoleic ( $C_{18:2}$ ) acids at 39.68, 4.01, 43.55 and 11.18 %, respectively. Earlier Rebecchi et al. (2015) reported that every oil or fat has different unique signature fatty acids make up; hence, ghee or pure milk fat content mostly short or medium chain fatty acids. However, among unsaturated fatty acids oleic acids were predominated in pure milk fat or ghee.

Comparing the palm oil to ghee, it was observed in table 1 that  $C_{16:0}$ ,  $C_{18:1}$  and  $C_{18:2}$  were higher in case of palm oil than ghee. Though the  $C_{18:3}$  and  $C_{20:0}$  were 1.57 % and 0.46 %, those were higher in ghee as compared to palm oil. It was also observed in table 1 that mono-unsaturated fatty acids (oleic) in palm oil were higher as compare to ghee. However, total saturated fatty acids were higher in ghee as compared to palm oil. Our results were in line with the earlier observation of Kumar et al. (2015) who reported that short chain fatty acids were predominated in ghee, however, there were no short chain fatty acids identified in vegetable oil. Mehta (2013) analyzed different market samples of ghee and concluded that saturated fatty acids were predominated than unsaturated fatty acids in ghee. Same type of observation was earlier recorded by Upadhyay et al. (2018).

Earlier workers also suggested that unsaturated fatty acids were predominated in vegetable oil (Kumar et al. 2015; Upadhyay et al. 2018). Both ghee and vegetable oil contained very high amount of oleic acid; although the concentration of oleic acid was higher in the case of palm oil as compared to pure ghee. In a different study Upadhyay et al. (2018) reported that total unsaturated fatty acids were higher both in depot fat and vegetable oil.

The effect of palm oil adulteration was also significant on some selected fatty acids like butyric acid ( $C_{4:0}$ ) that was found to be decreased when adulterated with palm oil (Table 1). However, oleic and linoleic acids were increased during adulteration of palm oil and this significance difference even visible after 5 % level of palm oil adulteration in pure ghee (Table 1).

From table 1, it was also revealed that during palm oil adulteration in ghee; the sum of saturated fatty acids decreased; however,

sum of un-saturated fatty acids increased quantitatively. It was also observed that change of the fatty acids in ghee depends on the percentage of added palm oil in ghee. It was recorded from table 1 that total saturated fatty acid in pure ghee, pure ghee + 5 % palm oil, pure ghee + 10 % palm oil, pure ghee + 15 % palm oil and pure ghee + 20 % palm oil were 62.29, 59.12, 56.87 and 55.76 %, respectively; however, the total un-saturated fatty acids were 34.42, 37.75, 40.79, 43.12 and 43.65, respectively. This effect was clearly identified even in 5 % level of palm oil adulteration in ghee. Recently, FSSAI has implemented to detect vegetable oil adulteration in ghee using HPLC based method but that said method unable to detect palm oil in ghee (Ramani et al. 2019). However, our study suggested that palm oil could be efficiently detected by fatty acid analysis; especially the ratio of the sum of saturated fatty acids to sum of unsaturated fatty acids could be effectively use as a potent markers to detect palm oil as an adulterant in ghee. Rebecchi et al. (2015) and Kumar et al. (2015) reported that during adulteration of both vegetable oil and depot fat in milk fat or ghee; fatty acids used to be changed and this change of fatty acid even visible very low level of foreign fat adulteration. So, from the current study it could be concluded that fatty acids study able to detect foreign fat in milk fat but this study is not fruitful to confirm type of foreign fat in milk fat.

## Conclusions

The present study revealed that the palm oil could be easily detected at 5 % level in ghee/milk fat when butyric, oleic and linoleic acids were used as a marker. On the other side, ratio of sum of the total of saturated to unsaturated were found useful marker for detecting the different levels of adulteration in ghee. It can be concluded that fatty acids analysis could be very effective for the detection of palm oil adulteration in ghee to the tune of 5 %; but change of fatty acid could even observed during other types of foreign fat adulteration in milk fat or ghee.

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