A novel approach to detect highly manipulated fat adulterant as Reichert–Meissl value-adjuster in ghee (clarified butter) through signature peaks by gas chromatography of triglycerides

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Abstract Ghee, the clarified butter fat being the costliest fat among other edible oils and fats in India, prone to adulteration with highly manipulated cheaper oils/fats, especially during lean season. The present investigation carried on triglycerides profile of one of the latest components of the adulterant fat i.e. RM (Reichert–Meissl)-adjuster, has been exploited to check the adulteration of ghee with a newly emerged highly manipulated fat. Using standardized (S)-limits specified by the ISO/IDF for cow milk fat, the minimum level of detection of the adulterant fat was observed as 7.5%. However, in case of buffalo ghee, due to non-availability of the ISO/IDF limits, the detection of this adulterant fat in buffalo ghee was not possible. Gas chromatograms showed specific signature peaks of large size in the retention time region of 4.5 to 6.5 min for RM-adjuster and adulterant fat, whereas no such peaks were observed in pure cow and buffalo ghee samples. The new approach of zooming in and superimposing of selected peaks in the chromatograms of triglycerides of suspected ghee has been used as a strategy to find adulterant fat's presence. Through this approach, the addition of RM-adjusted highly manipulated foreign fat (adulterant fat) to the tune of even 0.5% could be achieved in both cow as well as buffalo ghee.

Keywords Cow and buffalo ghee · Fat adulteration · Gas liquid chromatography · Reichert–Meissl value adjuster

Introduction

Ghee (clarified butter or anhydrous milk fat) has been used in India since 1500 BC (Achaya 1997). It is the second largest dairy product (~ 28%) consumed in India after liquid milk (46%) (GAIN 2014). In India, ghee market has witnessed a substantial growth over the past few years. According to a report published by Global Agricultural Information Network, combined ghee and butter production for the year 2018 was expected to be 5.6 MMT, approximately 3.7% higher than the previous year (GAIN 2017). At present, ghee market is growing at a CAGR of more than 11% and is expected to reach a value of INR 4653 billion by 2024 (IMARC 2019). Unfortunately, some unscrupulous manufacturers and/or the middle-men involved in ghee trade, tend to adulterate high-priced ghee with cheaper fats and oils such as vegetable oils, animal body fats, hydrogenated fats and sometimes even the non-edible mineral oils for financial advantages (Upadhyay et al. 2016; Kim et al. 2016; Sharma et al. 2018; Ayari et al. 2018).

Such 'Economically Motivated Adulteration (EMA)' has become a serious threat to the human health. Recently, the law enforcing agencies have seized a huge amount of palm oil and other adulterants from the premises of unscrupulous manufacturers of ghee (Garg 2018), which led traders towards a new trend of using highly refined palm oil along with other manipulated fats/imitates to adjust the Reichert–Meissl value (RM-value) of admixed milk fat. RM-value of ghee is based on the amount of short chain fatty acid (C4: butyric acid) and used as a basis to ascertain the integrity of milk fat. RM-adjuster is a new weapon in the existing armory of unscrupulous traders of ghee. This very adulterant seems to be acetic acid esters of short or medium chain fatty acids and commonly termed as adjuster oil, cutting oil, RM-cutting oil, etc. by the

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unscrupulous traders, which are used mainly for the manipulation of RM-value of adulterant fat and its subsequent use in ghee adulteration. This very practice is done by these traders and personnel in a very precise and meticulous manner using the science based knowledge and experience. Thereby, Law enforcing agencies as well as testing laboratories find it difficult to prove the suspected samples adulterated as these adulterated samples meet all the physico-chemical constants specified for Ghee as per the standards of FSSR (2011). Some of the recent methods also failed to detect such adulteration in ghee below 5% level (Jirankalgikar and De 2014; Ramani et al. 2018; Ayari et al. 2018) and the test houses and law-enforcing agencies face a very awkward situation in fighting such cases in the courts of law.

International Organization for Standardization has specified a reference method (ISO 17678:2010) to detect cow milk fat purity based upon the profiling of triacylglycerols with even carbon numbers ranging from C24 to C54 using Gas–liquid chromatography. However, adulteration of vegetable oils and animal depot fats could not be detected below 2% level (Precht 1992; Kala 2013; Kala et al. 2016; Hazra et al. 2018) using this approach of triglycerides analysis. At the same time, limited studies are available on the detection of foreign fats using ISO (17678) method in Ghee.

It is evident from the above cited literature that none of the studies have reported such type of designed/highly manipulated fat usage in ghee as an adulterant. Thus, the objective of the present study was to detect the adulteration of most common fat adulterant (a kind of designer fat) in cow and buffalo ghee using gas chromatographic analysis of triglycerides as per ISO reference method. The ghee samples provided by State Food testing laboratory and Referral laboratory were also checked for the adulteration of foreign fats/oils and common fat adulterant using standardized (S)-limits based equations and triglyceride profiling, respectively.

Materials and methods

Pure cow and buffalo ghee samples

To prepare pure ghee, buffalo milk samples were collected from the livestock research center of ICAR-National Dairy Research Institute (NDRI), Karnal, India. Cream obtained was then heated on a direct flame in a stainless steel vessel and clarified into ghee with continuous stirring at temperature of 120 °C/flash. Ghee was then filtered through muslin cloth (sixfold to eightfold) followed by further filtration using Whatman No. 4 filter paper. Cow ghee samples were procured from the Experimental Dairy Plant of ICAR-National Dairy Research Institute, Karnal.

Suspected ghee samples

These samples were provided by the State Food testing laboratory and Referral laboratory (ICAR-NDRI), Karnal, India.

Fat adulterants

- (i) RM-adjuster was provided by the State Food testing laboratory and one of the Dairy Cooperative facing the menace of milk fat adulteration.
- (ii) Adulterant fat was also provided by the State Food testing laboratory.

Standard triglyceride mix (CRM 17811)

Standard triglyceride mix was procured from Sigma-Aldrich (St Louis, MO, USA) consisting of Tricaprylin, Tricaprin, Trilaurin, Trimyrstin, Tripalmitin.

Standard anhydrous butter fat (BCR-519)

Anhydrous butter fat was also procured from Sigma-Aldrich (St Louis, MO, USA).

Chromatographic analysis

Gas liquid chromatography

A GLC Shimadzu 2010 GC (Kyoto, Japan), with GC solution software was used.

GLC conditions

For triglyceride profiling, following conditions as specified in the ISO 17678 (2010) were standardized and used in the study.

- Carrier Gas: Nitrogen
- Column: CP-Ultimetel SimDist CP7532 (Agilent Technologies), capillary column of 5.0 m (L) × 0.53 mm (ID) × 0.80 mm (OD), film thickness 0.17 µm
- Column flow: 3.00 mL/min of nitrogen carrier gas
- Detector: Flame Ionization detector (370 °C)
- Injector: Split (370 °C)
- Column oven temperature: Initial 80 °C and then increased to 190 °C at the rate of 50 °C/min and then to 350 °C @ 6 °C/min and held at the final temperature for 7.0 min.
- TAG standard/ ghee sample (1% volume fraction in hexane): Injection volume 0.5 µL

Determination of Reichert–Meissl (RM value)

RM value of pure ghee, adulterant fat and RM-adjuster was determined by standard method as described in Indian standard (IS 1981).

Calculations of standardized (S)-limits

S-limits for the pure cow and buffalo ghee, fat adulterant and suspected ghee samples were calculated as per the equations specified in ISO 17678 (2010) reference method and given below.

S total general formula (95.68–104.32)

$$= -2.7575C26 + 6.4077C28 + 5.5437C30 \\ + 15.3247C32 + 6.2600C34 + 8.0108C40 \\ - 5.0336C42 + 0.6356C44 + 6.0117C46$$

S2 for Soya bean, Sunflower, olive, Rape

– seed, Linseed, Wheat germ,

maize germ, Cotton seed & Fish oil detection (98.05–101.95)

$$= 2.0983C30 + 0.7288C34 + 0.6927C36 \\ + 0.6353C38 + 3.7452C40 \\ - 1.2929C42 + 1.3544C44 + 1.7013C46 \\ + 2.5283C50$$

S3 for Coconut & Palm kernel fat detection

$$(99.42–100.58) = 3.7453C32 \\ + 1.1134C36 + 1.3648C38 \\ + 2.1544C42 + 0.4273C44 + 0.5809C46 \\ + 1.2926C48 + 1.0306C50 + 0.9953C52 + 1.2396C54$$

S4 for Palm oil & beef tallow detection

$$(95.90–104.10) = 3.6644C28 \\ + 5.2297C30 - 12.5073C32 \\ + 4.4285C34 - 0.2010C36 + 1.2791C38 \\ + 6.7433C40 - 4.2714C42 + 6.3739C46$$

S5 for Lard detection (97.96 – 102.04) = 6.5125C26

$$+ 1.2052C32 + 1.7336C34 \\ + 1.7557C36 + 2.2325C42 + 2.8006C46 \\ + 2.5432C52 + 0.9892C54$$

Results and discussion

RM-value

RM-value of the pure cow and buffalo ghee used in the present investigation was (29.06) and (34.30), respectively. In case of adulterant fat the RM-value was 32.34. This clearly indicated that the adulterant fat was adjusted to the RM-value of ghee, so that it goes undetected by RM-value parameter specified in Indian standards of ghee. The available RM-adjusters were also tested for its RM-value and they were found to have RM-value of 165 and 150 in RM-adjuster-1 & 2, respectively. This clearly demonstrated that the RM-adjuster is being used by the unscrupulous traders to adjust the RM-value of adulterant fats. It is also evident from the chromatograms (Fig. 1) that the adulterant fat had prominent peaks of triglycerides with carbon numbers 48, 50, 52 and 54, which in turn indicates that adulterant fat can be a concoction of Palm oil/ palm kernel oil/ soy oil etc. The appearance of two prominent signature peaks in the retention time zone of 4.5–6.5 indicated that RM-adjuster has been used to adjust the RM-value of this adulterant fat.

S-limits of pure cow and buffalo ghee, fat adulterant and suspected ghee samples

It is evident from Table 1 that S-limits in case of pure cow ghee were very much within the range specified in ISO-standard for cow milk fat. On the contrary, in case of fat adulterant, the S-limits were altogether disturbed as expected barring S2-limit (99.92) specified for pure cow ghee to detect soy bean, sunflower, olive, rapeseed, linseed, wheat germ, maize germ, cotton seed, and fish oil adulteration. This means if such fat adulterant is mixed in ghee, it should be detected, because S-limits for palm oil and beef, and S-total was 12.5 and 13.56, respectively, which were very low as compared to these limits specified for pure cow milk fat (95.9–104.10) and 95.68–104.32. Therefore, pure ghee sample was intentionally adulterated with adulterant fat at @ 0.5, 2.5, 5.0 and 7.5% levels and S-values obtained after the analysis were observed for any deviation from the standard limits. It is evident from the data that S-limits in ghee samples adulterated with fat adulterant @ 0.5%, 2.5%, 5% levels were within the range specified for pure milk fat (Table 1). However, there was a slight deviation in the value of S-total in case of ghee sample adulterated @ 7.5% level (95.40), which was marginally low than the lower limit specified in the standard (95.68) to consider milk fat as pure. Similarly, S4-limits specified in the standard to rule out the addition of Palm oil and beef was also marginally less (95.26) than the

Fig. 1 Chromatograms of CRM (certified reference material) (a), PCG (pure cow ghee) (b), PFG (pure buffalo ghee) (c), RM-adjuster-1 (d), RM-adjuster-2 (e) and fat adulterant (f)

lower limit specified in the standard (95.9). Other three S-limits i.e. S-total, S3 and S5 (100.77, 100.26 and 99.90) were within the range as given by ISO for pure cow ghee. This indicated that the fat adulterant has been designed in such a way that it is not detectable below 7.5% level. It is also evident from the data that the deviation in S-limits in ghee samples adulterated with fat adulterant @ 7.5% level is so small that in some cases it may create a problem in declaring the samples deemed to be adulterated. Conversely, in case of buffalo ghee samples, wherein all the S-limits were different than the ISO-limits specified for cow milk fat (Table 1), suggested that the equations specified in ISO method for calculating S-limits are not applicable for the detection of adulterants in buffalo ghee. It could be attributed to the differences in milk fat extraction procedure and type of milk as specified in ISO (17678) method.

On perusal of the data related to S-value of suspected ghee samples supplied by State milk product testing laboratory and referral laboratory (Table 2), it can be seen that state milk product testing laboratory ghee sample (SFL2) was failing on account of S-limits while ghee sample (SFL1) was meeting all the S-limits. Similarly, in case of referral laboratory, six (R2–R7) did not meet the S-limits

specified for cow milk fat to be qualified as pure cow ghee samples. Therefore, it can be concluded that the sample (SFL1) and referral laboratory sample (R1) were either pure or the level of adulteration was below to the detectable limit.

Effect of addition of fat adulterant on signature peaks in chromatograms

Evaluation of the S-limits data obtained for the samples analyzed in the present investigation clearly suggested that S-limits can be used to check the purity of milk fat. However, the deviations in ghee samples adulterated with adulterant fat supplied by state milk product testing laboratory was marginal and may give false negative results if, S-limits alone are considered in such cases. It is generally noticed that whenever ghee is adulterated with foreign fats, its RM-value is adjusted to meet the RM-standard specified by FSSR (2011). Therefore, RM-adjuster was also analyzed for its S-limits and chromatograms were observed for the appearance of any additional or specific peaks. The chromatograms of certified reference material (CRM), pure cow ghee (PCG), pure buffalo ghee (PBG), RM-adjuster-1 and 2 and adulterant fat are shown in Fig. 1. It is evident

Table 1 Standardized (S)-limits of pure cow ghee, pure buffalo ghee, fat adulterant and adulterated cow/buffalo ghee samples

S-limits for pure cow milk fats as per ISO standard	Fat adulterant	Pure cow ghee	Pure cow ghee + fat adulterant @ 7.5%	Pure cow ghee + fat adulterant @ 5.0%	Pure cow ghee + fat adulterant @ 2.5%	Pure cow ghee + fat adulterant @ 0.5%	Pure buffalo ghee	Pure buffalo ghee + fat adulterant @ 7.5%	Pure buffalo ghee + fat adulterant @ 5.0%	Pure buffalo ghee + fat adulterant @ 2.5%	Pure buffalo ghee + fat adulterant @ 0.5%
S- Limits equations											
S2	98.05–101.95	99.92	100.96	100.77	101.18	100.76	101.00	101.97	101.81	102.27	102.14
S3	99.42–100.58	106.04	99.62	100.26	100.12	100.12	99.63	98.58	99.00	98.69	98.54
S4	95.9–104.10	12.52	104.08	95.26	96.12	96.93	104.01	111.29	105.87	109.13	110.43
S5	97.96–102.04	104.62	99.43	99.90	99.53	99.84	99.41	97.92	98.23	97.63	97.77
S-total	95.68–104.32	13.56	104.11	95.40	96.25	97.06	104.05	111.05	105.71	108.77	110.12

S2 (soya bean, sunflower, olive, rape-seed, linseed, wheat germ, maize germ, cotton seed & fish oil detection); S3 (coconut and palm kernel fat detection) S4 (palm oil and beef tallow detection) and S5 (for lard detection)

Table 2 Calculated S-limits of suspected ghee samples obtained from state food laboratory (SFL1 and SFL2) and referral laboratory (R1–R7)

S-limits for pure milk fats as per ISO standard		Suspected ghee samples								
S-equations	S-limits	SFL1	SFL2	R1	R2	R3	R4	R5	R6	R7
S2	98.05–101.95	99.62	95.84	99.25	99.48	99.94	93.04	101.14	93.08	98.38
S3	99.42–100.58	99.93	100.23	100.01	104.83	102.80	102.96	99.44	102.01	102.30
S4	95.9–104.10	100.41	93.40	99.10	25.48	48.16	71.50	107.25	84.30	61.10
S5	97.96–102.04	99.96	103.36	101.15	108.57	111.02	106.86	98.33	104.46	108.03
S-total	95.68–104.32	99.71	93.45	98.69	26.09	48.22	69.67	105.57	82.91	60.72

S2 (soya bean, sunflower, olive, rape-seed, linseed, wheat germ, maize germ, cotton seed and fish oil detection); S3 (coconut and palm kernel fat detection) S4 (palm oil and beef tallow detection) and S5 (for lard detection)

Fig. 2 Chromatograms of certified reference material (CRM), suspected sample and RM-adjuster (a); RM-adjuster and pure ghee (b); and pure ghee and adulterated ghee sample (c)

from the chromatograms that RM-adjuster showed the appearance of large peaks in the retention time zone between 4.5 and 6.5 min and adulterant fat also showed

Fig. 3 Chromatograms of pure ghee and suspected ghee samples (SFL-1, SFL-2 and R1–R7)

Fig. 4 Chromatograms of pure cow ghee and adulterated samples (adulterated with fat adulterant at 7.5% (a), 5.0% (b), 2.5% (c) and 0.5% (d); pure buffalo ghee and adulterated samples (adulterated with fat adulterant at 7.5% (e), 5.0% (f), 2.5% (g) and 0.5% (h))

similar peaks in this zone of retention time (Fig. 1). On the contrary, certified reference material (CRM) and pure ghee did not show any such peaks in their chromatograms. Therefore, the chromatograms of CRM, pure milk fat, suspected samples (state milk product laboratory and referral laboratory samples) and pure cow ghee samples adulterated with adulterant fat were zoomed in and superimposed to observe the presence of any additional peaks before the peaks of triglyceride with C24 carbon numbers (Fig. 2). It is evident from the results that in pure milk fat there was not any appearance of additional peaks in the retention time region of 4.5 to 6.5 min. However, the chromatograms of all suspected samples (SFL1, SFL2 and R1–R7) (Fig. 3) and samples having fat adulterant at different levels (0.5–7.5%) (Fig. 4) showed the presence of additional peaks in the retention time region of 4.5 to 6.5 min, suggesting that all suspected ghee samples

obtained from State and Referral food laboratory were adulterated with highly manipulated fat adulterant. Even in case of ghee samples adulterated with fat adulterant @ 0.5% level, such additional peaks were observed in magnified chromatograms, indicating that it is possible to detect as low as 0.5% adulteration level of such fat adulterant in ghee samples irrespective of its nature (cow or buffalo ghee) (Fig. 4). If the zooming in is done too much then small rudimentary peak appears near 4.5 min also but in case of adulterated samples its amplification is too much. However, the signature peak near 6.5 min retention time is very characteristic in all the samples adulterated with fat adulterant. Similar observations were recorded in case of buffalo ghee samples adulterated with fat adulterant (Fig. 4e–h). This clearly demonstrated that in addition to S-limits as specified by ISO for pure cow milk fat, the zooming in approach of chromatograms to look for

signature peaks of RM-adjuster or fat adulterant would be very helpful in ascertaining the purity of ghee.

Conclusion

In this study, we have detected the presence of highly manipulated fat adulterant in ghee samples. This was achieved through the triglyceride profiling of the component added to adjust the RM-value of ghee. This was found to be very useful in countering the new trend of adding such a manipulated fat which goes otherwise undetected on the basis of physico-chemical standards specified for ghee.

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