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RESEARCH ARTICLE

Detection of cotton seed oil in cow ghee using triglyceride profiling

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Abstract: The triglyceride profiling of ghee shows significant variations in different regions of India, influenced by factors such as feeding practices, fatty acid composition, lactation stage, and season. Our study focused on detecting and identifying ghee adulterated with cottonseed oil and ghee sourced from the cotton tract area. Triglyceride profiling was employed on pure, adulterated, and cotton tract area ghee samples. Our findings revealed consistent lower levels of triglycerides with carbon numbers 24 to 30 in cow ghee, while higher levels were observed for carbon numbers 38 to 40 and 50 to 54. However, in ghee adulterated with cottonseed oil, triglycerides with carbon numbers 34 to 38 significantly increased ($p<0.05$), while those with carbon numbers 50 to 52 decreased significantly ($p<0.05$) with increasing concentrations of cottonseed oil. To verify the authenticity of the ghee samples, we analyzed normal cow ghee from Livestock Research Centre, National Dairy Research Institute using parameters including S-total, S2, S3, S4, and S5. The observed values for these parameters (98.77 ± 0.23, 103.30 ± 0.19, 99.55 ± 0.22, 97.42 ± 1.31, and 98.34 ± 0.27, respectively) fell within the range specified by International Organization for Standardization. In conclusion, our study highlights the potential of triglyceride profiling as an effective tool for detecting and differentiating ghee adulterated with cottonseed oil and ghee sourced from the cotton tract area. This method is suitable for detecting the presence of cottonseed oil to the tune of 10% in ghee. This technique provides valuable insights into assessing the authenticity and quality of ghee, contributing to the prevention of adulteration practices in the ghee industry.

Keywords: Adulteration, cotton tract area ghee, Gas chromatography, Mass spectrometry, S-values, Quality assurance

Abbreviations:

Abbreviation	Full name
DAHD	Department of Animal Husbandry and Dairying
GC	Gas chromatography
ISO	International Organization for Standardization
IDF	International Dairy Federation
LRC	Livestock Research Centre
NDRI	National Dairy Research Institute
PHVO	Partial hydrogenated vegetable oil
CSO	Cotton seed oil
CTA	Cotton tract area
MMT	Million metric ton
TG	Triglyceride
AOAC	Association of Official Analytical Chemists

Introduction

India, as the leading milk producer globally, witnessed milk production of 209.96 MT in 2020-2021 and 221.06 MT in 2021-2022, exhibiting an annual growth rate of 5.29% (Annual report; DAHD, 2022). Approximately 27.5% of the total milk produced in India is utilized for ghee production (Atbhaiya et al. 2022), making ghee the largest segment among indigenous milk products. Ghee packaging consists of about 60% bulk packaging and 40% consumer packs (Atbhaiya et al. 2022). The production levels of ghee vary across regions, with 57% in the northern region, 9.5% in the eastern region, 23.5% in the western region, and 10% in the southern region (Ramani et al. 2019). Ghee holds a superior position among fats intended for human consumption due to its unique properties, such as better digestibility and potential anti-cancer properties attributed to short-chain fatty acids (Sharma et al. 2020).

Due to its high price and demand, ghee is vulnerable to adulteration by middlemen. Adulterants commonly found in ghee include vegetable oil, cottonseed oil, and animal body fat.

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Differentiating between ghee sourced from the cotton tract area and ghee adulterated with cottonseed oil poses a major challenge. Previous researchers have utilized physico-chemical tests to detect the presence of non-dairy fats and adulterants in butter fat (Kumar et al. 2009; Gandhi et al. 2020; Atbhaiya et al. 2022). Additionally, Joachim (2007) recommended analyzing the lipid composition of milk fat to determine its quality, while Kala (2013) analyzed the triacylglycerols (TG/TAG) composition of milk fat to detect small amounts of extraneous fat. The International Organization for Standardization (ISO) developed a reference method (ISO 17678:2010) to determine the purity of cow milk fat based on the triglyceride (TG) composition consisting of even carbon numbers (24 to 54) using gas liquid chromatography. However, this method is limited to detecting animal body fat and vegetable oils up to 2% levels (Kala et al. 2016; Hazara et al. 2017). Triglyceride profiling has been explored to distinguish cow and buffalo ghee from designer fats used to manipulate the Reichert-Meissl value of ghee (Pathania et al. 2021). Studies have observed structural similarities in the distribution and composition of TG in milk from different bovine breeds (Christie, 1983). Feeding cows diet rich in stearic and linoleic acids, which are modified by rumen microflora, resulted in increased levels of stearic, palmitic, and oleic fatty acids (Christie, 1983). Multiple regression analysis models have been developed to detect the presence of non-dairy fats in pure milk fat. The European Union (EU) has adopted an official reference method based on TG analysis using low-resolution gas chromatography coupled with capillary or packed columns to detect foreign or non-dairy fat in dairy fat. ISO and International Dairy Federation (IDF) have also documented a TG composition-based method, along with standardized formulae (referred to as standardised (S) values), for detecting specific adulterants (ISO, 2010).

Differential scanning calorimetry has been employed to detect ghee adulterated with animal body fat at higher levels (Upadhyay et al. 2016), while Fourier transform infrared (FTIR) spectroscopy has emerged as a sensitive and rapid technique for detecting various types of adulteration in milk and ghee (Gandhi et al. 2022). Recent applications of FTIR include detecting adulteration with common sugar, added urea, soymilk, synthetic milk, goat fat, and pig fat in pure ghee (Jaiswal et al. 2015; Jha et al. 2015; Upadhyay et al. 2016; Upadhyay et al. 2018).

Considering the aforementioned research gaps, this study aimed to determine the triglyceride profile of cow ghee sourced from the cotton tract area and ghee adulterated with cottonseed oil using GC-MS/MS.

Materials and methods

Ghee samples were collected from various cotton tract regions in India, including Maharashtra, Gujarat, and Haryana. Sahiwal breed cows in mid lactation were fed with cottonseed cake at different percentages (15% and 30%) along with regular feed at the Livestock Research Centre (LRC), NDRI, Karnal, and milk samples were collected at specific time intervals (0, 20, 40, 60, and 85 days). All reagents and chemicals used in the analysis were of HPLC or analytical grade and purchased from Sigma Aldrich India. Standard triglyceride mixes (Catalogue No. T7140) were purchased from Sigma Aldrich St. Louis, USA for analysis.

Sample preparation

Ghee was prepared using the creamery butter method described by De (2010), utilizing unripened cow cream. Milk samples from various cotton tract areas were collected for ghee preparation. Pure ghee was then adulterated with cottonseed oil at different concentrations (1, 5 and 10%) on a weight-to-weight basis.

Triglyceride profiling using GC-MS/MS

Samples were analyzed for triglyceride profiling according to the ISO 17678:2010/IDF 202:2010 standard. Initially, 50 grams of melted ghee were weighed, and 0.5 to 1.0 grams of previously dried sodium sulfate at 90°C for 1 hour were added to the ghee. The mixture was then filtered using filter paper. Subsequently, 0.5 milliliters of the melted and filtered ghee were combined with 5 milliliters of n-hexane in a 15-milliliter centrifuge tube. The mixture was vortexed for 1 minute. Afterwards, 1.5 milliliters of the resulting test portion were transferred to GC vials for analysis using gas chromatography-mass spectrometry (GC-MS).

The samples were analyzed using a GC-MS/MS instrument (Shimadzu O207051) equipped with an autosampler and a fused silica *SLB®-35ms* capillary column (30 meters length, 0.25 mm internal diameter, and 0.25 µm particle size, Supelco, Sigma-Aldrich, USA). The derivatized sample was injected into the column using a volume of one microliter, while keeping the injector temperature at a constant 330°C. The GC oven program started with an initial temperature of 80°C (held for 0.5 minutes) and then increased incrementally at a rate of 50°C per minute up to 190°C. Subsequently, the temperature was further increased to 330°C at a rate of 6°C per minute. The final temperature of 330°C was held constant for 10 minutes. The carrier gas (helium) flowed at a constant rate of 3 milliliters per minute in constant flow mode, with a purge flow of 3 mL/min. The interface temperature was set to 350°C, and the split ratio was maintained at 50:1.

Statistical analysis

All the experiments were conducted at least in triplicates (n=3). All the results were expressed as mean of replicates analysis. The results of triglycerides profile were statistically analyzed using SPSS 20.0 software (IBM SPSS version 20.0.NY).

Results and discussion

Triglyceride composition

Table 1 presents the triglyceride composition of cow and buffalo ghee, consisting of 16 triglycerides (C24 – C54). The triglyceride profile of ghee is influenced by factors such as fat content, lactation stage, and season (Palmquist et al. 2005). The triglyceride composition of milk also affects various physicochemical and functional properties of dairy-based products (Smiddy et al. 2012).

In cow ghee, the levels of triglycerides with carbon numbers ranging from 24 to 30 were lower, while those with carbon numbers from 38 to 40 and 50 to 54 were higher (Fig. 1). However, in ghee adulterated with cottonseed oil, there was a significant increase ($p<0.05$) in triglycerides with carbon numbers ranging from 34 to 38, while those with carbon numbers from 50 to 52 decreased significantly ($p<0.05$) with increasing concentrations of

Fig. 1 GC-MS chromatogram showing peaks of triglycerides of normal cow ghee obtained from LRC, NDRI

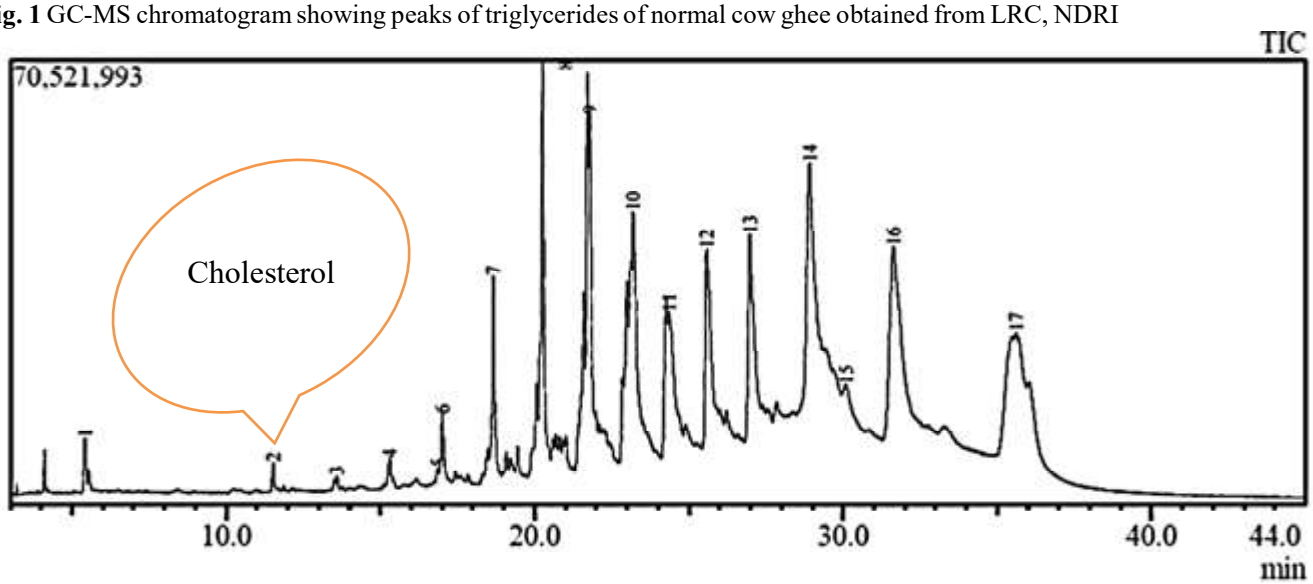

Table 1 Percentage relative triglycerides composition of the milk fat of cow and buffalo

	Pure Cow milk fat	Cow milk procured from Maharashtra	Cow milk procured from Gujarat	Cow milk procured from Haryana	Cottonseed oil 1%	Cottonseed oil 5%	Cottonseed oil 10%
C24	0.10±0.08^{a}	0.28±0.16^{c}	0.22±0.14^{c}	0.25±0.08^{c}	0.06±0.03^{a}	0.19±0.05^{bc}	0.19±0.05^{bc}
C26	0.17±0.06^{a}	0.24±0.06^{b}	0.23±0.10^{b}	0.15±0.07^{a}	0.21±0.01^{b}	0.09±0.00^{c}	0.38±0.02^{d}
C28	0.21±0.09^{a}	0.15±0.02^{c}	0.20±0.08^{a}	0.51±0.31^{d}	0.28±0.02^{e}	0.06±0.02^{f}	0.46±0.04^{g}
C30	0.64±0.48^{a}	0.76±0.09^{c}	0.51±0.10^{a}	0.74±0.06^{a}	0.65±0.48^{a}	0.38±0.03^{d}	1.48±0.03^{be}
C32	1.13±0.13^{a}	1.16±0.07^{a}	1.41±0.23^{ac}	1.45±0.09^{ac}	1.15±0.11^{a}	2.38±0.05^{d}	2.20±0.01^{de}
C34	3.72±0.32^{a}	3.31±0.24$^{a\,c}$	3.38±0.20^{ac}	3.52±0.43^{ac}	3.71±0.31^{a}	4.85±0.01^{ab}	7.45±0.05^{d}
C36	7.61±0.15^{a}	7.60±0.10^{a}	7.22±0.22^{ab}	7.36±0.05^{bc}	7.67±0.26^{ab}	11.86±0.02^{d}	8.93±0.06^{de}
C38	10.41±0.29^{a}	10.23±0.01^{a}	10.17±0.09^{a}	10.36±0.27^{a}	10.38±0.33^{a}	12.57±0.05^{c}	13.25±0.02^{d}
C40	10.44±0.14^{a}	10.56±0.27^{a}	10.19±0.10^{a}	10.15±0.11^{a}	10.40±0.10^{a}	6.93±0.03^{b}	9.46±0.05^{c}
C42	8.34±0.04^{a}	8.30±0.04^{a}	8.42±0.36^{a}	8.35±0.08^{a}	8.34±0.04^{a}	3.29±0.07^{b}	8.57±0.02^{a}
C44	7.65±0.34^{a}	7.32±0.01^{a}	7.54±0.38^{a}	7.41±0.26^{a}	7.49±0.31^{a}	7.64±0.01^{a}	7.08±0.04^{a}
C46	6.96±0.43^{a}	7.36±0.34^{b}	8.25±0.71^{c}	7.77±0.46^{b}	7.12±0.38^{a}	9.53±0.05^{d}	6.08±0.00^{ae}
C48	7.67±0.23^{a}	7.91±0.07^{a}	7.66±0.79^{a}	7.51±0.34^{a}	7.45±0.33^{a}	0.58±0.02^{b}	9.22±0.05^{c}
C50	14.55±0.11^{a}	14.79±0.12^{a}	14.59±0.20^{a}	14.48±0.13^{a}	14.45±0.20^{a}	12.36±0.02^{c}	8.79±0.03^{d}
C52	11.47±0.30^{a}	11.46±0.25^{a}	11.28±0.16^{a}	11.35±0.16^{a}	11.41±0.26^{a}	6.66±0.09^{b}	8.63±0.02^{c}
C54	8.79±0.00^{a}	8.59±0.09^{a}	8.19±0.65^{a}	8.34±0.19^{a}	9.10±0.53^{b}	19.42±0.05^{c}	7.14±0.05^{d}

Values are means ± standard deviation; different superscript letters in a row indicate significant differences (One-way ANNOVA with least significant Difference Tukey and Duncan tests, $P<0.05$). Calculation was based on triplicate measurement per milk sample, standard deviation were calculated over samples at least in triplicates.

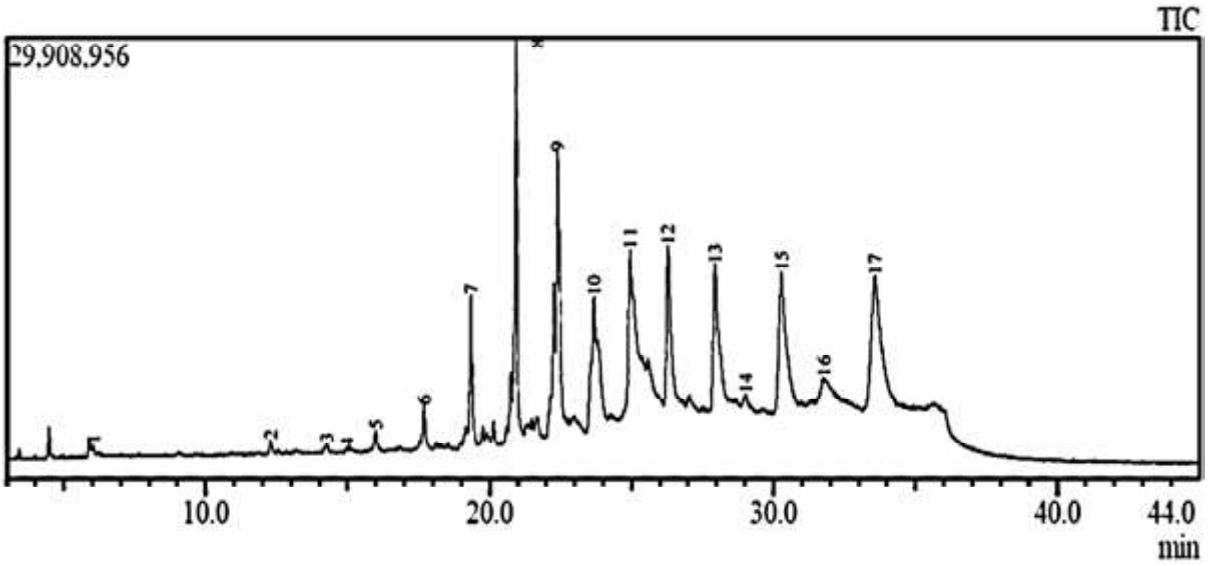

Fig. 2 GC-MS chromatogram showing peaks of triglycerides of ghee adulterated with 5% cotton seed oil.

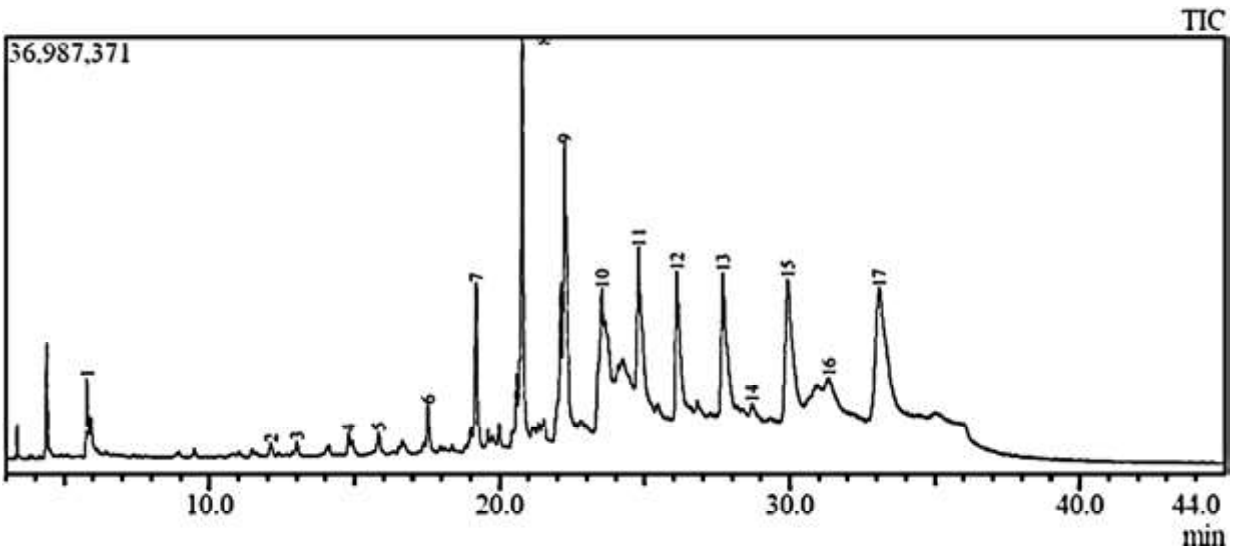

Fig. 3 GC-MS chromatogram showing peaks of triglycerides of ghee adulterated with 10% cotton seed oil

cottonseed oil (Fig. 2 and 3). Van Ruth et al. (2010) and Kala et al. (2013) reported that during the summer period, when cows were fed on grass, the triglyceride with carbon number 52 was highest, while in the winter season, when cows were fed with bran, the maximum levels were found for triglycerides with carbon number 50. Triglycerides C38, C50, and C52 were observed to be at their maximum in cow milk fat (Smiddy et al. 2012).

The triglyceride profiles of pure cow and buffalo milk fat differed, particularly with significantly ($p<0.05$) higher levels of C24 to C30 and C38 in buffalo milk fat compared to cow milk fat. However, triglycerides with carbon atoms ranging from 50 to 54 were significantly ($p<0.05$) lower in pure buffalo ghee compared to pure cow ghee. It is evident from the above discussion that the triglyceride profile of milk fat varies among different species, primarily influenced by genetic factors and feeding habits. Fontecha et al. (1998) reported that the triglyceride content of milk fat not only varies among different species but can also be observed within breeds of the same species.

A similar trend was observed in the ghee adulterated with cottonseed oil, with a significant increase ($p<0.05$) in the triglyceride profile of C34 to C38, while the profile of C50 to C52 significantly decreased ($p<0.05$) compared to other values like C40 to C48 and C54.

The relative weight percentages of triglycerides C48, C50, C52, and C54 were higher in beef tallow, lard, and partially hydrogenated vegetable oil (PHVO), while C34, C36, and C38 were higher in coconut oil compared to milk fat triglyceride profile. The reference method for triglyceride profiling does not have documented detection levels for these adulterants. Therefore, a study was conducted to investigate the triglyceride profile of ghee prepared from standardized milk (which represents mixed milk and is commonly used for ghee production in established dairy industries in India) adulterated with the aforementioned foreign fats. The triglyceride profiles of the ghee showed a decrease in C24, C26, C28, and C30 for all types of adulterants.

Table 2 Standardized (S)- limits of NDRI cow ghee, NDRI buffalo ghee, cotton tract area ghee and ghee adulterated with cotton seed oil ghee samples

S- limits for pure cow milk fats as per ISO standard		NDRI cow ghee	Cotton tract area ghee			Ghee adulterated with different concentration of cotton seed oil		
S- equations	Limits	(n=3)	Maharashtra (n=3)	Gujarat (n=3)	Haryana (n=3)	1% (n=3)	5% (n=3)	10% (n=3)
S2	98.05-105.95	103.30±0.19^{a}	104.45±0.86^{a}	103.45±0.75^{a}	102.91±0.80^{a}	102.10±0.10^{b}	100.13±0.63^{b}	89.87±0.29^{c}
S3	99.42-100.58	99.55±0.22^{a}	99.62±0.35^{a}	99.69±0.24^{a}	99.75±0.21^{a}	99.57±0.25^{a}	99.46±0.08^{a}	99.65±0.20^{a}
S4	95.9-104.10	97.42±1.31^{ab}	98.89±1.43^{bc}	97.75±1.48^{ab}	97.29±1.65^{ab}	98.82±0.33^{bc}	100.23±1.33^{c}	96.17±0.35^{a}
S5	97.96-102.04	98.34±0.27^{a}	98.90±1.52^{ab}	100.46±1.47^{bcd}	99.32±1.24^{abc}	101.06±0.08^{cd}	101.83±0.32^{d}	99.06±0.21^{ab}
S-total	95.68-104.32	98.77±0.23^{ab}	99.15±2.79^{ab}	96.81±1.52^{a}	97.50±1.68^{a}	100.40±0.44^{b}	97.49±0.54^{a}	96.85±0.24^{a}

Soy bean, sunflower, olive, rapeseed, linseed, wheat germ, maize germ, cotton seed and fish oil); S3 (Coconut and palm kernel fat); S4 (Palm oil and beef tallow) and S5 (Lard)

Table 3 Standardized (S)- limits of ghee obtained from animal fed cotton seed cake in different concentration at LRC, NDRI

S- limits for pure cow milk fats as per ISO standard		Animal fed cotton seed cake in different concentration at LRC, NDRI								
S- equations	Limits	Control	15%, 20 days (n=3)	30%, 20 days (n=3)	15%, 40 days (n=3)	30%, 40 days (n=3)	15%, 60 days (n=3)	30%, 60 days (n=3)	15%, 85 days (n=3)	30%, 85 days (n=3)
S2	98.05-105.95	103.30±0.19^{a}	102.78±0.25^{ab}	102.35±0.37^{bc}	101.81±0.36^{d}	102.19±0.45^{cd}	103.58±0.27^{a}	102.40±0.24^{cd}	103.49±0.15^{a}	104.83±0.07^{e}
S3	99.42-100.58	99.55±0.22^{a}	99.79±0.12^{a}	99.49±0.33^{a}	99.67±0.33^{a}	99.74±0.16^{a}	99.65±0.36^{a}	99.48±0.09^{a}	99.49±0.14^{a}	99.49±0.06^{a}
S4	95.9-104.10	97.42±1.31^{ab}	96.74±0.96^{b}	96.97±0.38^{b}	96.97±0.38^{b}	100.15±0.27^{c}	99.51±0.27^{c}	96.38±0.37^{a}	96.79±0.27^{a}	98.75±0.33^{bc}
S5	97.96-102.04	98.34±0.27^{a}	101.89±0.12^{b}	101.08±0.39^{c}	98.32±0.20^{a}	98.75±0.37^{a}	98.43±0.17^{a}	98.41±0.13^{a}	98.45±0.35^{a}	98.69±0.29^{a}
S-total	95.68-104.32	98.77±0.23^{a}	96.94±0.37^{bc}	97.66±0.51^{ab}	97.61±0.41^{ab}	100.60±0.25^{d}	100.50±0.42^{d}	97.53±0.29^{bc}	96.20±0.83^{c}	98.45±0.31^{a}

S2 (Soy bean, sunflower, olive, rapeseed, linseed, wheat germ, maize germ, cotton seed and fish oil); S3 (Coconut and palm kernel fat); S4 (Palm oil and beef tallow) and S5 (Lard)

The standardized milk ghee adulterated with beef tallow and lard at 6.56% and 6.27%, respectively, exhibited an increase in C50, C52, and C54 (Kala, 2013). As discussed earlier, in the present study, pure cow ghee adulterated with cottonseed oil showed an increase in C24 to C30 and a decrease in C50 to C54.

Gutiérrez et al. (2009) detected non-milk fat adulteration in milk samples, using low- resolution GC. Adulteration with PHVO, indicated the increase in C54 height and area, which could be considered as a better marker than the changes in any other TG. Application of GC analysis for TG profiling and estimation of trans fatty acid content, detected PHVO adulteration at 5% level in milk fat (Destaillats et al. 2006). Although the TG profiles and S values predict the possible adulteration, precise fingerprinting of the adulterants used to adulterate the sample cannot not be obtained. PHVO adulteration also could be judged by examining the sterol components (Alonso et al. 1997). TG profiling can also be applied to detect the presence of animal body fats, as they lack in short-chain fatty acid, and have a low amount of 24 – 28 carbon-numbered TG. The increase in peak heights of C50 and C52 compared to C38, as well as the increase in the relative weight percentages of C50, C52, and C54 compared to control ghee samples, could be considered for detecting foreign fats in ghee samples through triglyceride estimations.

Standardized (S)–limits of different ghee samples

The S-limits for different types of ghee samples were determined using the equations outlined in the ISO 17678 (2010) reference method. The S-limits for pure cow ghee were found to be within the range specified by the ISO standards, as shown in Table 2. When comparing the S-values obtained from the equations (Table 2) for various S-limits, it was observed that all corresponding values for the pure ghee samples fell within the specified limits for S-values.

However, if the calculated S-value falls outside the corresponding limits, it indicates that the fat sample is adulterated with foreign fat. For example, when analyzing milk fat adulterated with substances such as sunflower, soybean, rapeseed, olive, wheat gram, linseed, cottonseed, fish oil, and maize germ, the corresponding S-limits were found to be outside the prescribed range (i.e., S2-limit of 98.05 – 101.95) for pure cow ghee.

The S-total and S2 limit for cottonseed oil were found to be 4.13 and 15.35, respectively, which is significantly lower than the values prescribed for pure cow milk fat (S-total: 95.68 – 104.32; S2 limit: 98.05 – 101.95). When comparing the obtained S-values for ghee adulterated with cottonseed oil, the values were within the prescribed limit at 1% and 5% adulteration. However, at a higher level of adulteration (10%), the S-value decreased to 89.70. The other four S-limits (S-total, S3, S4, and S5: 96.71, 99.77, 95.97, and 98.94, respectively) were within the range specified by the ISO for pure cow ghee.

The triglyceride profiles of ghee showed an increase in C24 – C30, C36 – C38, and C48, while there was a decrease in C50 – C54 for ghee adulterated with 10% cottonseed oil. However, in the case of ghee, the S-limits value was outside the range. This discrepancy could be attributed to various factors such as different operating GC conditions and the method of extraction of milk fat.

Table 3 presents the S-values of ghee obtained from cows fed on cottonseed cake at different concentrations at LRC, NDRI, and all values were within the range specified by the ISO standard. Cows fed with 15% cottonseed cake at LRC, NDRI, after 80 days of feeding showed S-values (S-total, S2, S3, S4, and S5: 96.20±0.83, 103.49±0.15, 99.49±0.14, 96.79±0.27, and 98.45±0.35, respectively) within the ISO standard range. Similarly, cows fed with 30% cottonseed cake at LRC, NDRI, after 80 days of feeding displayed S-values (S-total, S2, S3, S4, and S5: 98.45±0.31, 104.83±0.07, 99.49±0.06, 98.75±0.33, and 98.69±0.29, respectively) within the ISO standard range.

Fontecha et al. (1998) also reported that the recommended S-value is not applicable for triglyceride profiling of cheese due to biochemical reactions during ripening and storage. Kala (2013) found that the S-value of market samples of ghee deviated from the recommended value, possibly due to storage conditions after preparation. Another study examined ghee samples containing 5% PHVO, which resulted in S-values outside the range for pure milk fat due to an increase in C50, C52, and C54 triglycerides. The detection level for beef tallow and coconut oil adulteration was found to be 2%. Vegetable fat and lard additions could be detected up to 5% and 6.3%, respectively (Kala, 2013). Furthermore, at a 2% level of adulteration of cow ghee with PHVO, an increase in C50, C52, and C54 was observed, but the S2 value (for vegetable fat adulteration) remained within the prescribed range (Kala, 2013).

The detection of adulteration of milk fat with an unknown adulterant up to a certain level can be achieved by estimating the triglyceride profile and calculating the S-total value. A standardized method can be applied to estimate the amount of foreign fat by specifying a value of 7.46 (for general unknown adulterant) and the S-total value obtained from the triglyceride profile (ISO 2010).

Conclusion

Triglyceride profile analysis of ghee samples is as an effective and reliable method to differentiate between pure ghee and ghee adulterated with cottonseed oil. This technique allows for the identification of specific triglyceride patterns that indicate the presence of adulterants. However, it may not be able to differentiate between ghee sourced from the cotton tract area and ghee adulterated with cottonseed oil especially when the adulteration level is below 5%. In such cases, the triglyceride profile may not exhibit significant variations that can be used for

accurate differentiation. Nevertheless, this method proves to be useful in detecting the presence of cottonseed oil in ghee when the adulteration level reaches 10%. This is evident from the S2 values obtained for such adulterated ghee, which were measured to be 89.87±0.29. These values fall below the prescribed limit of 98.05-105.95, indicating a clear deviation from the triglyceride profile of pure ghee. Therefore, by analyzing the triglyceride profiles of ghee samples, it is possible to identify and differentiate between pure ghee, ghee adulterated with cottonseed oil up to a certain level, and ghee originating from the cotton tract area. In summary, triglyceride profile analysis represents a significant step forward in combating ghee adulteration.

Conflicts of interest

None

Reference

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