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Triglyceride profiling of ghee using gas chromatography

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Abstract
A systemic study was conducted for analysis of triglyceride profile of ghee using gas chromatography. It was observed that there were 16 major peaks of even carbon number triglycerides, corresponding to triglycerides of 24 to 54 carbon number triglycerides present in both cow or buffalo ghee. In both cow as well as buffalo ghee six triglycerides having carbon numbers (C36, C38, C40, C42, and C50 & C52) were predominant representing about sixty percentage of total triglycerides present in both of the two ghee. Presence of C24 carbon number triglyceride was almost negligible in both cow or buffalo ghee. Smaller carbon number triglycerides like (C26, C28, C30, and C32) found to be very nominal, whereas triglycerides with carbon number C34- C54 were the major triglycerides in cow ghee or buffalo ghee. Small and medium carbon numbers triglycerides (C26 to C38) were higher for buffalo ghee than cow ghee. For cow ghee C40 triglyceride was higher than buffalo ghee. Higher carbon numbers TGs C44-C54 were higher in case of cow ghee as compared to buffalo ghee.

Keywords: Ghee, milk fat, triglyceride (TG), gas chromatography

1. Introduction
Ghee, the clarified milkfat prepared chiefly from cow or buffalo milk, is the most common and popular milk fat based product in the Indian sub-continent. According to FSSR (2011)[3] "Ghee" means the pure clarified fat derived solely from milk or curd or from desi (cooking) butter or from cream to which no coloring matter or preservative has been added. Ghee is one of the costliest fat (~4 times higher) among all of the edible fats and oils. According to a report of Global Agriculture Information Network (GAIN, 2011) [5], around 27.5 and 6.5 percent of the total milk produced in India, is processed into ghee and butter, respectively. In India, Ghee is not only the choice for classic dishes, but also it is an essential for many aspects of temple worship, and Ayurvedic medicine praises it for health. GAIN (2005) [4] reported that, ghee market shared by organized and unorganized sector accounted for 35 and 210 billion rupees, respectively.

Upadhyay (2014) [12] reported that for both pure cow ghee and pure buffalo ghee, the major fatty acids were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1). Menard *et al.* (2010) [10] reported that, among the long chain unsaturated fatty acids, the content of oleic acid (C18:1) was found to be higher in cow ghee, while that of linoleic acid (C18:2) and linolenic acid (C18:3) were found to be higher in buffalo ghee. In a different study Kirchnerova *et al.* (2013) [9] reported the presence of arachidonic acid (C20:0) in both pure cow ghee and pure buffalo ghee. Though there are many scientific report found regarding the fatty acid analysis of ghee or milk fat but very few scientific report found regarding the triglyceride analysis of cow or buffalo ghee or cow /buffalo milk fat. Also the problem of adulteration has assumed a very serious dimension. Unfortunately, the producers or the middle-men involved in the ghee trade, tend to adulterate ghee with cheaper oils and fats like vegetable oils, animal body fats, hydrogenated fats and sometimes even the non-edible mineral oils, especially during lean season to earn more money. Various techniques have been employed for detection of adulteration in ghee but detection of adulteration in ghee is still a challenge. Recently, IDF&ISO (2010) [6] recommended triglyceride analysis of milk fat using gas chromatography for checking the purity of milk fat, but in India till date no systemic study reported regarding triglyceride analysis of Ghee. So considering all the facts stated above, the main aim of the present study was a systemic study of triglyceride profile of ghee (cow/buffalo) using gas chromatography.

Materials and methods

Reference standards: Triglyceride (TG) mixture (C24, C30, C36, C42, C48) (Sigma- Aldrich Chemie, USA).

Gas chromatography: Model: GC 2010 PLUS, SHIAMDZU, Japan.

Collection of milk and preparation of ghee

Pure cow milk and pure buffalo milk were separately collected from their respective pooled milk from the Livestock Research Centre, NDRI, Karnal. Samples of cow/buffalo ghee were prepared by creamery butter method (De, 2005) [1].

Gas chromatography analysis of Triglycerides (TGs)

Triglycerides profile of ghee samples were analyzed by gas chromatography using a BP5, capillary column of 2.5 m length, equipped with flame ionization detector and temperature control module. Details of temperature programme is given below-

Details of temperature programmed

Ramp Rate	Temperature	Hold Time
	80 °C	0.50 min
50 °C/min	190 °C	1min
6 °C/min	335 °C	5min

The reference standards containing Triglyceride (TG) of different 5 Triglyceride (TG) mixtures (C24.C30, C36, C42, and C48) was also run under the similar conditions of the gas chromatography for identifying the particular TG (Triglyceride) by comparing their retention time with that of obtained TG (Triglyceride) profile of test sample.

Specification for running the Gas chromatography

Injector Condition

1. Temp of injector-350 °C
2. Split ratio-1:50
3. Pressure-13.4Kpa
4. Total Flow 79ml/min (N_2)
5. Column Flow 1.49ml/min
6. Linear velocity-56.4cm/s
7. Purge flow3ml/min

Column Information

For analysis of Triglyceride we used BP5, capillary column 2.5 m length (cut from 15 m X 0.25 mm X 0.25 µm).

1. Name-BP5
2. Length- 2.3m, Inner Dia-.25mm,Film Thickness-.25µm
3. Max temp-335 °C

Detector (FID) Condition

1. FID temperature-370 °C
2. Make up flow-30ml/min, H_2 flow -40ml/min, Air flow-400ml/min

GC solution software provided by the gas chromatography company was used to determine the percentage of different tri-glycerol present in different fat samples.

Statistical Analysis

Data were analyzed using program of CSAT6 and expressed as mean values with standard deviation (SD).

Result and Discussion

During initial phase of our study we used the condition mentioned by Kala (2013) [7] the initial oven temperature of 200 °C was increased to 325 °C at the rate of 5 °C/min and hold at the final temperature for 10 min, the injector and detector temperatures were 330 and 360 °C. The reference standards containing Triglycerides (TG) of different 5 Triglyceride (TG) mixtures (C24.C30, C36, C42, and C48) was run under these conditions of Gas chromatography analysis.

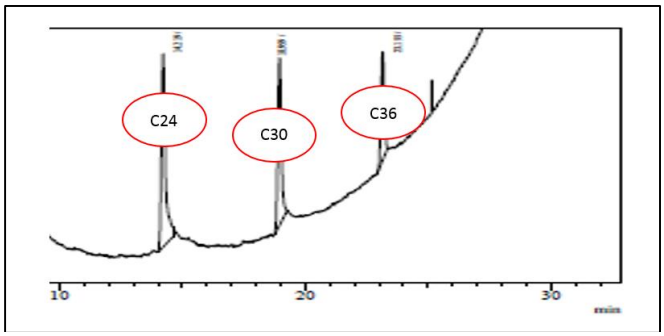

Fig 1: Chromatogram of 5 Triglycerides (C24, C30, C36, C42, C48) reference using condition specified by Kala (2013) [7]

Gas chromatography condition plays an effective role for lipid analysis. It is evident from Fig (1), that using the condition mentioned by Kala (2013) [7] only 3 TGs were clearly resolved instead of five present in the reference standard. Moreover despite of pre conditioning and repeated cleaning of the column, a stable base line could not be obtained as evident from Fig (1). This might be due to the difference in columns make as Kala (2013) [7] used a HP-5, capillary column 2.5 m length (cut from 15 m 9 0.25 mm 9 0.25 lm) but in the present investigation BP5, capillary column 2.5 m length (cut from 15 m X 0.25 mm X 0.25 µm) was used. Literature also suggested that Gas chromatography's column specifications was an important criteria for lipid analysis (Precht, 1990) [11]. Since, the resolution of triglycerides was poor using the conditions specified by Kala (2013) [7], so Gas chromatography, conditions like temperature programming, make up volume and flow rate of gas etc. were standardized so that all the five components of triglyceride in the reference standard were eluted at different retention times. It is evident from Fig (2) that on using a programme of total 32.87 min wherein sample was injected at an initial temperature of 80 °C maintained for 0.50 min at split ratio (1: 50) and then raised temperature with a ramp rate of 50 °C/min to 190 °C by holding at this temperature for 1 min, then again raised up to 335 °C with ramp rate 6 °C/min and hold at that temperature for 6min. and using. It was observed that using the specified programme all five TGs in the reference standard were clearly eluted at different retention time and base line was also stable Fig (2). To check the repeatability of the results using standardized conditions, reference TG standard was injected five times and every time retention times were recorded (Table 1).

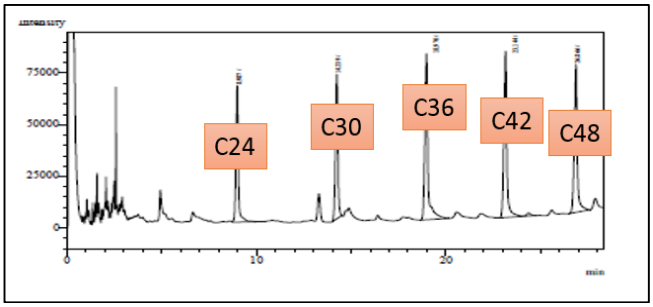

Fig 2: Chromatogram of reference triglyceride mixture using modified gas chromatography standard

Table 1: Retention time of five Triglycerides of reference standard mix

TGs	Retention time
C24	9.01±.05
C30	14.34±0.04
C36	19.23±0.1
C42	23.42±0.04
C48	27.01±0.02
Data represent average value of retention time ± SD value *(n=5)	

Triglycerides composition of pure cow and buffalo ghee

There were 16 major peaks (Fig 3&4) of even carbon number TGs, corresponding to TGs of 24 to 54 carbon number TG, were identified in both cow or buffalo milk ghee. However some unidentified peaks were also observed, which might be odd carbon number TGs, but areas of these TGs were very nominal.

In cow ghee, six TGs having carbon numbers (C36, C38, C40, C42, C50 & C52) were predominant (Table 2). Concentration of these six TGs was almost sixty percentage of total TGs present in cow ghee. Presence of C24 carbon number TG was almost negligible (Table 1) in cow ghee. Smaller carbon number TGs like (C26, C28, C30, C32) found to be very small amount ((Table 1)), whereas TGs having carbon number C34- C54 were the major TGs. It was also noted from (Table 1) that in both cow and buffalo ghee there were two clear maxima, located at C38 and C50 – C52. Almost similar observations were reported by Fontecha *et al*. (1998) [2]. It was also observed from (Table 1) that quantity of TGs C50 & C52 were almost similar. Results in the present study were in accordance with early observations of Kim *et al*. (2015) [8] and Kala *et al*. (2013) [7] who reported that the major TGs of cow milk fat were C30 to C54.

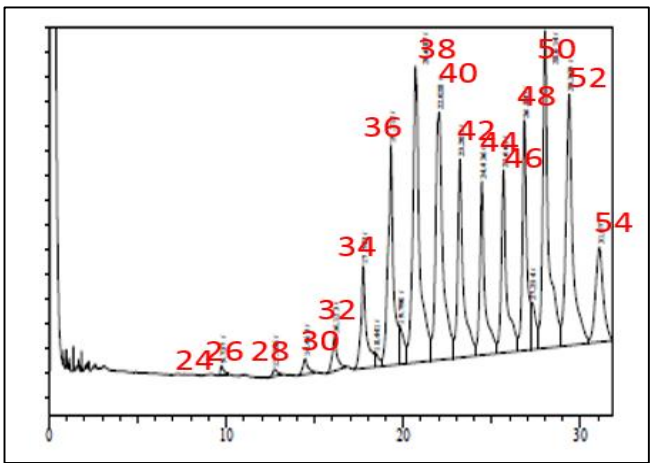

Fig 3: Chromatogram of pure cow ghee for Triglycerides profile analysis

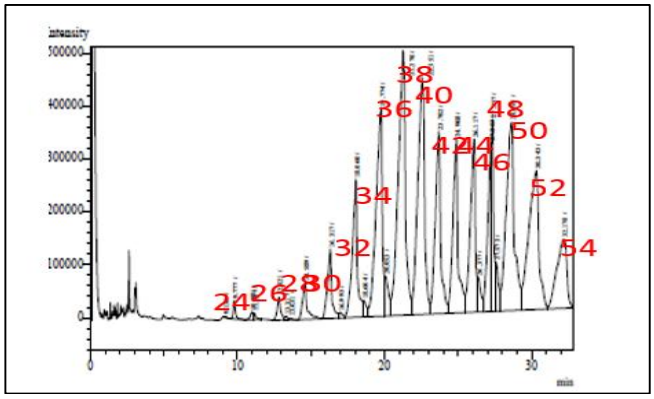

Fig 4: Chromatogram of pure buffalo ghee for Triglycerides profile analysis

Table 2: Triglycerides profile of Ghee & Buffalo ghee

TGs	Cow Ghee	Buffalo Ghee
24	0.075±0.001	0.086±0.003
26	0.33±11	0.39±0.15
28	0.35±0.1	0.64±0.1
30	0.57±0.19	1.02±0.2
32	1.41±0.18	2.51±0.32
34	3.68±1.3	4.85±0.12
36	9.25±0.8	10.64±0.9
38	12.85±0.89	13.62±1.14
40	11.92±0.36	11.44±1.36
42	6.98±0.2	7.017±0.38
44	6.15±0.3	5.81±0.86
46	6.14±0.5	6.08±0.54
48	7.85±0.8	6.97±0.48
50	11.81±1.12	10.02±1.25
52	11.61±1.1	9.88±1.1
54	6.13±0.5	4.35±.85

Data represents average value±SD value (n=6)

Similarly, in buffalo ghee, there were six predominant TGs having carbon numbers (C36, C38, C40, C42, C50 & C52) (Table 4.17). These six TGs were more than sixty percentage of total TGs present in buffalo ghee. Presence of C24 carbon number TG was almost negligible (Table 2) in buffalo ghee. Smaller carbon number TG like (C26,C28,C30,C32) found to be very nominal (Table-2), whereas TGs with carbon chain length of C34- C 54 were the major TGs identified in buffalo ghee also.

Conclusion

A systemic study was conducted for analysis of triglyceride profile of ghee using gas chromatography. It was observed that both cow and buffalo ghee contained sixteen major TGs having carbon numbers varying from 24 to 54 but TGs having carbon numbers (C36,C38,C40,C42,C50 & C52) were predominant representing about sixty percentage of total TGs present in both cow and buffalo ghee.

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References

1. De S. Outlines of Dairy Technology. Oxford University Press, New Delhi. 2005, 441.
2. Fontecha J, Diaz V, Fraga MJ, Juarez M. Triglyceride analysis by gas chromatography in assessment of authenticity of goat milk fat. J. Am. Oil Chem. Soc. 1998; 75:105-108.

3. FSSR File No.1 94/FSSAI/SP/(Labelling).Food Safety and Standards Authority of India (Ministry of Health and Family Welfare). FDA Bhavan, Kotla Road New Delhi-110002, 2011.
4. GAIN. India, Food Processing Ingredients Sector, Annual, 2005. Global Agriculture Information Network report IN5031, 2005.
5. GAIN. India, Dairy and Products, Annual, 2010. Global Agriculture Information Network report IN1112, 2011.
6. IDF & ISO. 2010. Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides. ISO 17678:2010(E). ISO copyright office International Dairy Federation Case postale56, CH-1211 Geneva.
7. Kala A. Detection of possible adulteration in commercial ghee samples using low-resolution gas chromatography triglyceride profiles. Int. J. Dairy. Tech. 2013; 3:346-351.
8. Kim J, Kim H, Jung-Min Park. Determination of Milk Fat Adulteration with Vegetable Oils and Animal Fats by Gas Chromatographic Analysis. J Food. Sci. 2015; doi: 10.1111/1750-3841.12979.
9. Kirchnerova K, Foltys V, Spicka J. Impact of lactation stage on milk fat fatty acids profile in grazing dairy cows. J. Micro, Biotech and Food Sci. 2013; 2(1):1164-1174.
10. Menard O, Ahmad S, Rousseau F, Briard-Bion V, Gaucheron F, Lopez C. Buffalo vs. cow milk fat globules: Size distribution, zeta-potential, compositions in total fatty acids and in polar lipids from the milk fat globule membrane. Food Chem. 2010; 120(2):544-551.
11. Precht D. Quantification of animal body fats and vegetable fats in milk by triglyceride analysis. *22nd* Int. Dairy Congress. 1990; 1:234.
12. Upadhyay N. Detection of Vegetable Oil and Animal Body Fat in Ghee Using Solvent Fractionation Technique. Ph.D. Thesis submitted to National Dairy Research Institute (Deemed University), Karnal, India, 2014.