Determination of triacyl glycerol and sterol components of fat to authenticate ghee based sweets

A. L. Amrutha Kala¹ · K. Sabeena¹ · Priya Pramod Havanur¹

Revised: 27 November 2015 / Accepted: 12 January 2016 / Published online: 20 April 2016
© Association of Food Scientists & Technologists (India) 2016

Abstract Method comparison of triacyl glycerol (TAG) and sterol components of fats of ghee based sweets was carried out on dairy ghee, laboratory prepared control sample and market samples. The fat was extracted from control and market samples. Determination of TAG and sterol composition of the fats was carried out using low resolution Gas Chromatography. The quantification of cholesterol and β -sitosterol and TAG classes of dairy ghee, control and market samples fat was also determined using single short column. Adulteration at 5 % level in milk fats showed varied TAG compositions of C50, C52 and C54 as compared to control and pure ghee sample. The cholesterol content of ghee and control sample was 2.30 ± 0.8 , 2.00 ± 0.24 g/kg respectively and β -sitosterol content of control was 0.20 ± 0.11 g/kg. The adulterated samples showed varied cholesterol and β -sitosterol contents as compared to control sample fat.

Keywords Triacyl glycerol composition · Cholesterol · Ghee based sweets · β -sitosterol · Gas chromatography

Introduction

Ghee based traditional sweets like Mysore Pak, Laddu, Soan papdi, are popular in India. These sweets prepared using ghee (clarified butter/milk fat), are well known prefixed by a word ‘special’. Presence of ghee in the place of hydrogenated fat

enhances taste of the sweets, and therefore the cost of these ‘special’ sweets is also higher. Addition of cheap non-milk fats like lauric fats, beef tallow, vanaspati and pork lard in the preparation of ghee, which are in turn carried into ghee based sweets, are in practice (Amrutha Kala 2013; Arun Kumar et al. 2009; Boghra and Borkhatriya 2005; FSSA 2011; Gutierrez et al. 2008; Timms 1980).

The conventional methods of analysis of fat from sweets, like BRR (Butyro refractometer reading), RM (Reichert Meissl) value and Baudouin’s test (Sesame oil test) give an idea of the adulteration of fats in these sweets (Amrutha Kala 2013; Boghra and Borkhatriya 2005; FSSA 2011). Some of these methodologies are time consuming and require lots of extracted fat and reagents to carry out the tests. Methods involving estimation of triacyl glycerol (TAG) and sterol components of fats used in the preparation of the sweets offer improved sensitivity and accuracy (Amrutha Kala 2013; Timms 1980; Christie 1983; Alonso et al. 1997, ISO/IDF 2010).

The main objective of the study is to evaluate the purity of the fat of ghee based sweets using low resolution gas chromatography (GC) methods of TAG (based on carbon numbers) and sterol component estimation of the constituent fat. Eight number of ghee based sweets (Mysore pak, Soan papdi and Laddu) from local market were collected and their TAG and sterol components were analyzed and compared with laboratory prepared control sweet sample.

Materials and methods

Samples of sweets, ingredients for special Mysore pak and dairy ghee (mixture of cow and buffalo milk fat) were procured from local markets of Mysore City, Karnataka State, India.

✉ A. L. Amrutha Kala
amruthakala@yahoo.com

¹ Food Safety and Analytical Quality Control Laboratory,
CSIR-Central Food Technological Research institute,
Mysore, Karnataka 570020, India

Preparation of control sweet sample

Out of many kinds of desserts prepared using ghee, Mysore pak ingredients were simple and similar with three types of sweets analysed in this study. Preparation involves, 150 g sugar dissolved in 175 g water and heated till a thread consistency. Sugar syrup was added with 100 g roasted chickpea flour. Finally 100 g ghee was added by stirring until semi solid consistency was achieved. Mysore pak mixture was removed from heat, patted while hot and cut into pieces. Special Mysore pak, special soan papdi and special laddu samples were collected and their fat was evaluated in this study.

Sample preparation Anhydrous fat was extracted from sweet samples gravimetrically after the removal of added spices and nuts, and fat content was found to be 40 to 55 g from 100 g of sample (AOAC 2005). This fat was analysed for the following tests:

BRR value determination: Butyro refractometer reading (BRR) was taken using digital refractometer (Bellingham Stanley Ltd., Kent, UK) as per the AOAC 921.08 official method (AOAC 2005).

Test for sesame oil: The sesame oil test (Baudouin's test) was carried out as per the method prescribed in IS-548: 1976 (BIS 2006).

TAG analysis: Triacyl glycerol/triglyceride (TAG) analysis was performed using low-resolution Shimadzu 2010 GC (Kyoto, Japan), with GC solution software, and nitrogen was used as carrier gas. HP-5, capillary column of 2.5 m length (cut from 15 m X 0.25 mm X 0.25 μ m) was used at column flow 1.20 mL/min of nitrogen carrier gas, with a split ratio of 1:10. The chromatographic conditions were as follows: Detector- Flame Ionization detector (FID), the initial column oven temperature of 200 °C was increased to 325 °C at the rate of 5 °C/min and held at the final temperature for 10 min. The injector and detector temperatures were 330 and 360 °C, respectively. For TAG analysis, 1 μ L of 5 mg/mL of control sample and commercial dairy ghee samples prepared in hexane and Supelco TAG standard mix (5 TAG mix- 100 mg neat mixture of 99 % pure 20 % each of C8:0, C10:0, C12:0, C14:0 and C16:0 acids) from Bellefonte, PA, USA, was injected to GC. The retention times, relative retention times and response factors were determined by repeated injection of the standard mix. Correction factors were found to be close to 1 as there is a unity response factor, and five TAG mix showed linearity with unity regression coefficient. Identification and TAG concentration were calculated as per the ISO 17678/IDF 202: 2010 method. TAG analysis was accompanied with calculations using 5

equations – Stotal, S2, S3, S4 & S5, which describe the overall adulteration. Stotal is the equation derived for general adulteration whereas equations S2, S3, S4 & S5 are derived for different foreign fats present as adulterants in ghee. S2 formula is applicable when ghee is adulterated with soybean, sunflower, olive, rape-seed, linseed, wheat germ, maize germ, cotton seed and fish oils. S3 formula is for coconut and palm kernel fat, S4 for palm oil and beef tallow and S5 for lard as adulterants in ghee. If the sample is genuine, the calculated Stotal value falls within the range of 94.68–104.32 (ISO/IDF 2010; Destailats et al. 2006). Area percent of TAG components obtained by normalization of GC chromatograms, expressed as % by weight (% w/w), of pure dairy ghee and control sample fat is as shown in Table 1.

Cholesterol and β sitosterol analysis: Around 1 g of extracted fat was saponified as per method 970.51 (Alonso et al. 1997; AOAC 2005). The unsaponifiable matter of the fats was dissolved in 0.5 mL of dichloromethane, and 1 μ L was injected into Shimadzu 2010 GC equipped with the HP-5 (2.5 m X 0.25 mm X 0.25 μ m) column, detector and the operating conditions were the same as given in TAG analysis. 5 α Cholestane was added as internal standard (Sigma-Aldrich, St. Louis, MO, USA) which was prepared in dichloromethane and injected to GC. Standard solutions of cholesterol, β - sitosterol at 78.2 % and Campesterol at 7.5 % (Acros, organic chemicals) were prepared in dichloromethane and injected into GC. The retention time for cholesterol was found to be 7.7 min, and the retention time for β -sitosterol was found to be 10.2 min. Cholesterol and β -sitosterol content of fats were expressed as g/kg.

Statistical analysis

Experimental results were mean $\pm$ S.D. of three parallel measurements and differences between means were determined by Duncan's multiple range test (DMRT). Correlations were obtained by Pearson correlation coefficient in bivariate correlations. *p* values ≤ 0.05 were considered significant.

Results and discussion

Prior to TAG and sterol component analysis, the market samples and control sample fats were tested for adulteration using conventional methods of BRR and Baudouin's test (AOAC 2005; BIS 2006). Baudouin's test showed no change in colour even in fats of adulterated samples. BR readings were measured for all the sweet sample fats (Table 2). BRR value of genuine milk fat lies from 41 to 44 range (FSSA 2011).

Table 1 TAG Compositions of Ghee and Control sample

TAG	Commercial dairy ghee %composition	Control Sample % composition
C24	0.2 ± 0.3	0.2 ± 0.3
Cholesterol	0.6 ± 0.21	0.3 ± 0.08
C26	0.4 ± 0.02	0.3 ± 0.52
C28	0.7 ± 0.05	0.6 ± 0.33
C30	1.1 ± 0.02	1.1 ± 0.21
C32	2.1 ± 0.01	2.3 ± 0.05
C34	5.5 ± 0.03	5.8 ± 0.10
C36	11.2 ± 0.17	12.1 ± 0.09
C38	13.0 ± 0.2	14.5 ± 0.34
C40	8.9 ± 0.24	10.0 ± 0.31
C42	5.9 ± 0.1	6.4 ± 0.10
C44	5.9 ± 0.05	6.1 ± 0.25
C46	6.6 ± 0.25	6.8 ± 0.22
C48	9.1 ± 0.58	8.3 ± 0.34
C50	12.3 ± 0.31	10.8 ± 0.51
C52	12.2 ± 0.35	10.0 ± 0.22
C54	4.5 ± 0.20	4.3 ± 0.40
Stotal	96.31	103

%w/w composition is average of 3 estimations ± SD

Presence of extraneous fat was indicated by out of the range BRR values (Amrutha Kala 2013; Boghra and Borkhatriya 2005). TAG and sterol component analysis of samples with

BRR values close to milk fat, showed the presence of extraneous fat like lauric fats. Thus the detection of adulteration present in the fat of ghee based sweets turned out to be difficult by measurement of BRR value alone. RM value measurement offers accurate estimation of adulterant fat other than anhydrous milk fat, but the procedure is tedious.

TAG analysis of dairy ghee, control sample and all the market samples was carried out as per ISO/IDF method. As per this method for analysis of TAG, the sensitivity of the method to detect non milk fat in ghee is 5 % level, and well established by researchers (Destailats et al. 2006; Joachim 2007; Molkentin and Precht 2000). Presence of vegetable fat and animal body fat in ghee was detected by increase in C50, C52, and C54 TAG classes and out of the range Stotal value (Destailats et al. 2006; Joachim 2007; Molkentin and Precht 2000). When control sample and dairy ghee sample TAG were analysed, the control sample TAG profile was similar to dairy ghee and the equations describing overall adulteration (Stotal), based on TAG, for dairy ghee and control sample was found to be within the prescribed range (Table 1). Stotal of market samples procured from Mysore failed to conform to the laboratory prepared control sweet with market sample no. 4 and 5 being an exception. The approximate amount of foreign fat in adulterated milk fat was also determined using Stotal value (Table 2) (ISO/IDF 2010; Destailats et al. 2006).

Dairy ghee, control sample fat, a partially hydrogenated vegetable fat and fats from market samples were analysed for cholesterol and β sitosterol. Cholesterol content of control

Table 2 Stotal*, BRR values, Boudouin's test, Cholesterol and β sitosterol of Ghee, Control sample, vegetable fat and market samples

Sample	Stotal (94.68–104.32)	Approximate adulteration** (based on Stotal)	Cholesterol (g/kg)	β -sitosterol (g/kg)	BRR Values (41–44)	Boudouin's test
Ghee	96.31	-	2.30 ± 0.8	-	41.5 ± 0.07	Negative
Control	103.0	-3.17	2.00 ± 0.24	0.20 ± 0.11	41.01 ± 0.05	Negative
1	74.23	27.84	0.43 ± 0.21	0.23 ± 0.17	45.24 ± 0.21	Negative
2	81.02	20.51	0.72 ± 0.41	0.50 ± 0.50	45.06 ± 0.03	Negative
3	38.68	66.27	0.46 ± 0.05	0.54 ± 0.19	49.15 ± 0.02	Negative
4	102.7	-7.25	1.37 ± 0.26	0.04 ± 0.12	41.71 ± 0.22	Negative
5	105.7	28.64	0.90 ± 0.58	0.41 ± 0.25	41.55 ± 0.15	Negative
6	45.68	58.70	0.71 ± 0.17	0.74 ± 0.36	47.91 ± 0.01	Negative
7	79.89	21.74	0.68 ± 0.35	0.36 ± 0.44	46.30 ± 0.04	Negative
8	87.24	13.78	0.96 ± 0.25	0.34 ± 0.05	45.51 ± 0.03	Negative
Vegetable Fat	-	-	0.19 ± 0.39	2.89 ± 0.13	49.25 ± 0.04	Negative

*Equation for Stotal =

$$[-2.7575C26 + 6.4077C28 + 5.5437C30 - 15.3247C32 + 6.2600C34 + 8.0108C405.0336C42 + 0.6356C44 + 6.01171C46]$$

For pure milk fat Stotal = (94.68 to 104.32)

(ISO/IDF 2010; Destailats et al. 2006)

**Approximate adulteration of foreign fat (X) equation: $X(g/100\text{ g}) = 100 * \left| \frac{(100 - \text{Stotal})}{92.54} \right|$

sample was 2.0 g/kg which was similar to dairy ghee, 2.30 g/kg. The β sitosterol content was present in considerable amount, 0.2 g/kg, in control sample, whereas it was below detection level in dairy ghee (Table 2). This may be attributed for the presence of chickpea flour or other ingredients used in the preparation of these sweets. Sterol estimation of the fat of sweet sample no. 5 revealed the presence of high β sitosterol content of 0.41 g/kg and lower cholesterol content of 0.90 g/kg as compared to control sample and dairy ghee. This indicates the presence of considerable amount of vegetable fat in the sweet no.5 instead of ghee/milk fat. This particular sample fat on fatty acid composition analysis showed lauric acid content above 45 %, which confirmed the presence of vegetable fat. The fat of sweet sample no. 4 showed cholesterol content of 1.37 g/kg and the β sitosterol content, 0.04 g/kg, was lower compared to control sample. The BRR of this sample, 41.71, was also within the permissible range for ghee and Stotal value was close to the ISO/IDF prescribed limit (Table 2). The cholesterol content of remaining market samples ranged from 0.43–0.96 g/kg, which was considerably lower than control sample and dairy ghee. The β sitosterol content of these sweet samples ranged from 0.34–0.74 g/kg and was high compared to laboratory prepared control sample.

By calculating the approximate adulteration based on Stotal values, sample no. 4 showed –7.25 % adulteration which was close to that of control sample, –3.17 %. The estimated approximate adulteration value ranged from 13.780–66.27 % in other market samples.

Conclusions

The fat content of ghee based sweets accounts to nearly 50 % of the edible portion. In comparison with conventional methods of analysis, the gas chromatographic analysis of TAG classes, cholesterol and β sitosterol components of these sweets serves as simple and effective analytical methods in authenticating their fat purity. Analysis survey of commercial samples based on these methods showed detection potential of extraneous fat present in the sweets.

References

- Alonso J, Fontechab L, Lozadab L and Ju_arez M (1997) Determination of mixtures in vegetable oils and milk fat by analysis of sterol fraction by gas chromatography. *J Am Oil Chem Soc* 74: 131–135.
- Amrutha Kala AL (2013) Detection of possible adulteration in commercial ghee samples using low resolution gas chromatography triglyceride profiles. *Int J Dairy Technol* 66:346–351
- AOAC (2005) Association of official analytical chemists international, official methods of analysis of the association of official analytical chemists international, 18th edn. Horwitz W- Editor, Gaithersburg, ML
- Kumar A, Darshan LG, Raman S, Vivek S (2009) Apparent solidification time test for detection of foreign oils and fats adulterated in clarified milk fat, as affected by season and storage. *Int J Dairy Technol* 62:33–38
- BIS method IS 548: Part 2 1976 (2006) Baudouin's test methods of sampling and test for oils and fats part II purity tests. In: Bureau of Indian Standards. Manak Bhavan, New Delhi
- Boghra VR, Borkhatriya VN (2005) Detection of vegetable oils in milk and milk fat by a rapid method. *J Food Sci Technol* 41:461–464
- Christie WW (1983) The composition and Structure of Milk Lipids. *Developments in Dairy Chemistry-2*, pp 1–35. Fox P F, ed. London and New York: Applied Science Publishers.
- Destailats F, Wispelaere M, Joffre F, Golay PA, Hug F, Giuffrida B, Fauconnot L, Dionisi F (2006) Authenticity of milk fat by fast analysis of triacylglycerols: application to the detection of partially hydrogenated oils. *J Chromatogr* 1131:227–234
- FSSAI (2011) Food Safety and Standards Act 2006. In: Rules 2011, regulations 2011, 7th edn. Ilbco, International Law book company publications, New Delhi, India
- Gutierrez R, Vega S, Diaz G, Sanchez J, Coronado M, Ramirez A, Perez J GM, Schettino B (2008) Detection of non-milk fat in milk fat by gas chromatography and linear discriminant analysis. *J Dairy Sci* 9: 1846–1855
- ISO 17678/IDF 202: 2010 (E) (2010) Determination of milk fat purity by gas chromatographic analysis of triglycerides. International Standard Milk and Milk Products. International Standard Organization/ International Dairy Federation.
- Joachim M (2007) Detection of foreign fat in milk fat from different continents by triacylglycerol analysis. *Eur J Lipid Sci Technol* 109:505–510
- Molkentin J, Precht D (2000) Equivalence of packed and capillary GC columns for detection of foreign fat in butter by use of the triglyceride formula method. *Chromatographia* 52:791–797
- Timms RE (1980) Detection and quantification of non-milk fat in mixtures of milk and non-milk fats. *J Dairy Res* 47:295–303