Development of a Novel High-Performance Thin-Layer Chromatographic–Densitometric Method for the Detection of Tallow Adulteration in Cow Ghee

Subrata De*, Pankaj Nariya, and **Nikhil Jirankalgikar**

Key Words

Planar chromatography
HPTLC
Tallow adulteration
Cow ghee
Fatty acids

Summary

A new method, involving the use of high-performance thin-layer chromatography, has been developed, which detects 10–20% (*w*/*w*) of adulterant tallow in cow ghee. The method consists of high-performance thin-layer chromatographic separation of both unsaponifiable and saponified portions using *n*-hexane–diethyl ether–glacial acetic acid (4:6:0.2, *v*/*v*) (for unsaponifiable portion) and *n*-hexane–diethyl ether–glacial acetic acid (6.5:3.5:0.2, *v*/*v*) (for saponifiable portion) as mobile phases. Beef tallow was mixed with cow ghee in various proportions (5 to 90%) to obtain admixtures of beef tallow with cow ghee. The analysis of the samples of cow ghee containing different proportions of beef tallow revealed that the addition of beef tallow to cow ghee affected the chromatographic profile; the effect increased with increasing proportions of beef tallow. Ghee adulterations with tallow at levels down to 20% are clearly seen visually in the chromatographic profile.

1 Introduction

Ghee (butter fat), one of the important dairy products in India, is normally prepared by using cow milk or buffalo milk. As per Ayurvedic classics, cow ghee is purported to have a number of therapeutic attributes and is also used in a number of formulations [1, 2]. It is frequently found adulterated with vegetable oils/fats and animal body fats. Detection of animal body fat adulteration in ghee is comparatively difficult, and various techniques have been tried by different workers. The methods, based on physical or chemical characteristics such as refractive index, Reichert–Meissl, Polenske, saponification, and iodine values, fail to detect adulteration at low levels. *Ramchandra* and *Dastur* (1959 and 1960) have reported paper chromatographic methods to differentiate ghee from other fats as well as from adulterated samples containing body fats [3, 4]. Combination of preparative thin-layer chromatography (TLC) and gas chromatography (GC) method for detection of adulteration of ghee with beef tallow has been reported by *Soliman* and *Younes* (1986) [5]. Beef tallow contains a higher proportion of C18:0 and C18:1 acids than butterfat triglycerides or 2-monoglycerides. Addition of beef tallow to butterfat affects the fatty acid composition of butterfat triglycerides and 2-monoglycerides with C18:0 and C18:1 acids; the proportion is reported to increase with increasing percentages of beef tallow. *Parodi* (1973) [6] has also reported detection of adulteration of butter fat with beef tallow through gas–liquid chromatography (GLC) triglyceride analysis. *Chakrabarty* et al. [7] have reported a method based on the difference in the number of components and intensities for the detection of adulteration of butter fat (ghee) with tallow, by first separating the trisaturated glycerides (GS_3) by argentation thin-layer chromatography on silica gel G, then resolving the GS_3 into components on paraffin-impregnated Kieselguhr G plates followed by microsaponification. The fatty acids were then isolated on paraffin-coated Kieselguhr G plates to indicate the component fatty acids of particular glyceride spots. Other methods for detection of adulteration in ghee like involving the use of the effect of the random rearrangement reaction in fats [8], using ultraviolet (UV) fluorescence technique [9], and differential scanning calorimetric method [10] are also reported. Our literature search did not reveal any high-performance thin-layer chromatographic (HPTLC) method for the detection of tallow in ghee. Densitometric HPTLC has wide application as an analytical tool due to its simplicity, minimum sample-cleanup requirement, and ability to analyze a number of samples simultaneously. Hence, the present study was undertaken.

A simple, rapid, and sensitive HPTLC method for the detection of tallow adulteration in cow ghee is reported in this communication.

2 Experimental

2.1 Chemicals and Reagents

All chemicals were of analytical grade and of Merck India.

S. De, P. Nariya, and N. Jirankalgikar, RMD Research and Development Center, Waghaldhara, Valsad, India.
E-mail: subratde@gmail.com

0933-4173/$ 20.00 © Akadémiai Kiadó, Budapest
DOI: 10.1556/JPC.26.2013.6.5

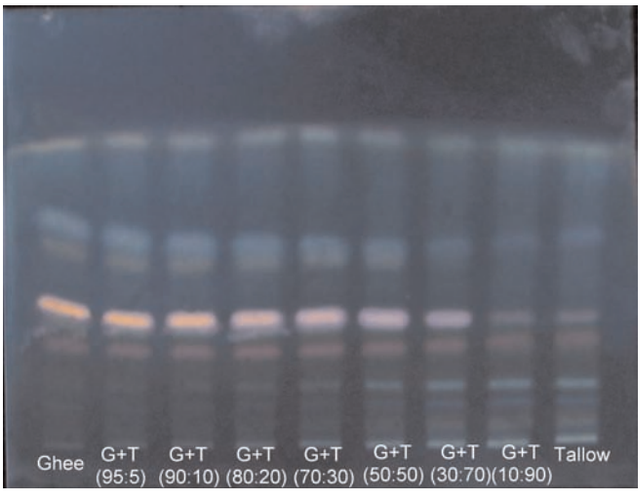

Figure 1

Chromatogram of cow ghee, tallow, and their mixtures (Fraction A).

2.2 Sample Preparation

2.2.1 Preparation of Tallow

Fat-rich beef (flesh surrounding kidneys mainly comprising of adipose tissue) was used to prepare the tallow as per prevalent practice. About 250 g of material was cut into fine pieces, mixed with about 750 mL of water, and heated with stirring, initially on mild heat and afterwards at about 80°C for 3 h. The mixture was sifted through a fine sieve while hot to separate the particulate matter. The residue was repeatedly washed with boiling water till free from fat. The filtrate and the washings were combined together and refrigerated at about 8°C for 8 h. After ensuring that the entire fatty portion has solidified, water was drained off. The tallow was mopped to remove any remaining water droplets on surface, stored in airtight container protected from moisture, and used for the present study.

2.2.2 Preparation of Cow Ghee

Cow ghee was prepared in house by conventional method from pure cow milk as described below.

The cow milk was boiled, and the cream was separated after cooling. A spoonful of curd was added to the cream and allowed to ferment overnight. Next day, it was churned, and butter was separated. The butter was heated over low heat until subsidence of frothing to obtain a clear golden coloured liquid. It was strained, and the ghee, thus obtained, was stored in an airtight, dry container.

2.2.3 Saponification of the Samples

To an accurately weighed quantity (about 1.5 g) of the sample (ghee and tallow), 50 mL 20% methanolic potassium hydroxide solution was added and kept overnight at room temperature. The mixture was refluxed for 6 h and allowed to cool to room temperature; twice its volume of distilled water was added and extracted repeatedly with ether. Combined ethereal extract was washed with distilled water until neutral to litmus paper and dried using anhydrous sodium sulphate. Ether was evaporated to obtain unsaponifiable fraction (Fraction A). The aqueous portion left after ether extraction, i.e., saponified fraction, was acidified with 5 N sulphuric acid and was extracted 3-4 times with ether. Combined ethereal layer was washed with distilled water, dried using anhydrous sodium sulphate, and evaporated (Fraction B) [11]. Both Fraction A and Fraction B were dissolved in 5 mL ether and used for further study.

2.3 Chromatographic Study

HPTLC study of both unsaponifiable fraction (Fraction A) and saponifiable fraction (Fraction B) was carried out.

2.3.1 Solvent System Designing

Various multicomponent solvent systems were tried for designing ideal mobile phase for the chromatographic separation of both Fraction A and Fraction B. The following solvent systems gave optimum separation and, hence, were used for the HPTLC study:

n-hexane–diethyl ether–glacial acetic acid (4:6:0.2, *v*/*v*) for Fraction A and *n*-hexane–diethyl ether–glacial acetic acid (6.5:3.5:0.2, *v*/*v*) for Fraction B.

The developed plates were sprayed with 10% methanolic sulphuric acid reagent followed by heating at 110°C for 5–10 min and observed in day light as well as under UV light.

Table 1

Peak area of different spots of Fraction A at 366 nm.

Sample	Peak area			
	Spot A (R_F 0.09)	Spot B (R_F 0.20)	Spot C (R_F 0.41)	Spot D (R_F 0.60)
Ghee (G)	–	96	9872	1499
G–T (95:5)	–	190	9865	1819
G–T (90:10)	–	279	9801	2017
G–T (80:20)	39	343	9780	1486
G–T (70:30)	61	371	9692	1474
G–T (50:50)	111	636	8503	1206
G–T (30:70)	211	945	5723	450
G–T (10:90)	221	1188	1342	–
Tallow (T)	245	1216	1325	–

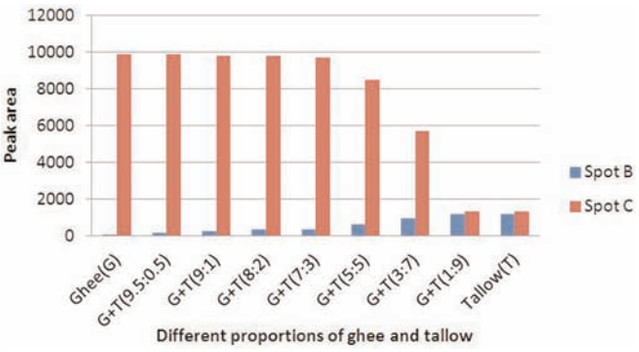

Figure 2

Comparative peak area of spot B and spot C of Fraction A.

Table 2

Peak area ratio of different spots of Fraction A.

Sample	Peak area ratio	
	Peak A/peak C	Peak C/peak B
Ghee(G)	–	102.8
G–T (95:5)	–	51
G–T (90:10)	–	35
G–T (80:20)	0.00398	28
G–T (70:30)	0.00629	26
G–T (50:50)	0.0130	13
G–T (30:70)	0.0367	6
G–T (10:90)	0.1646	1.1
Tallow (T)	0.180	1.0

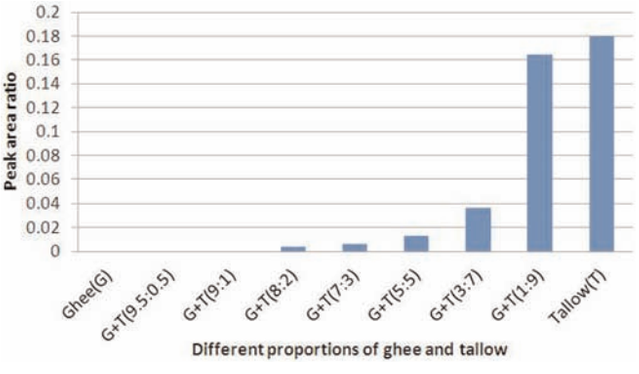

Figure 3

Comparative peak area ratio of spot A/spot C of Fraction A.

2.3.2 Instrumentation and Chromatographic Conditions

HPTLC was performed on 20 cm × 10 cm and 10 cm × 10 cm aluminum-backed plates coated with 0.2-mm layers of silica gel 60 F_{254} (Merck, Germany). The samples were applied in band with a Linomat 5 applicator (CAMAG, Muttenz, Switzerland), equipped with a 100-µL syringe (band length, 8–10 mm; track distance, 8–14 mm, as required). Plates were developed vertically, in a CAMAG twin trough chamber previously saturated with mobile phase vapor for 40 min at room temperature. After development, the plates were dried at room temperature and observed in a CAMAG UV cabinet. Densitometry scanning of the plates was performed with a TLC Scanner 4 with winCATS software (CAMAG, Muttenz, Switzerland) in reflectance–absorbance mode (scan speed, 10 mm s^{-1}).

3 Results and Discussion

3.1 Investigation of Unsaponifiable Fraction (Fraction A)

The HPTLC profile of Fraction A samples (cow ghee, tallow, and mixture of increasing proportions of tallow with cow ghee), after spraying with 10% methanolic sulphuric acid reagent (observation at 366 nm), is presented in **Figure 1.** All the samples reveal a number of spots, but the spots at R_F 0.09 (spot A), 0.20 (spot B), 0.41 (spot C), and 0.60 (spot D) are of special interest. The spot at R_F 0.09 is absent in cow ghee, but it is present in tallow and mixture of tallow with ghee. The spot can be detected visually starting from the mixture of ghee–tallow (8:2), and its intensity steadily increases with increasing concentrations of tallow in the mixture. Similarly, the intensity of the spot at R_F 0.20 steadily increases with increasing concentrations of tallow in the mixture. On the contrary, the intensities of the other two peaks at R_F 0.41 and 0.60 are maximum in cow ghee and steadily decrease with increasing concentrations of tallow in the mixture. The spot at R_F 0.60 is absent in tallow. The area of these peaks after scanning at 366 nm has been tabulated **(Table 1).** The data reveal that the peak area of the spot at R_F 0.09 is detected even in the mixture of ghee–tallow (8:2). **Figure 2** is the graphical presentation of the comparative peak area of spots B and C. The concentration of the compound at R_F 0.20 is almost negligible in cow ghee as compared to that in tallow, whereas that at R_F 0.41 is more than sevenfold in ghee than tallow (as evident from the peak area). So, the ratio of the peak area of the interested compounds was calculated **(Table 2).**

The ratio peak A/peak C is zero in pure cow ghee and remains the same up to 10% mixture of tallow in ghee, and then the value increases with increasing proportions of tallow **(Figure 3).** In contrast to this, the ratio of C/B is 102.8 in cow ghee, which decreases sharply (51) with the addition of even 5% tallow **(Figure 4).** Therefore, the presence/absence of these four spots

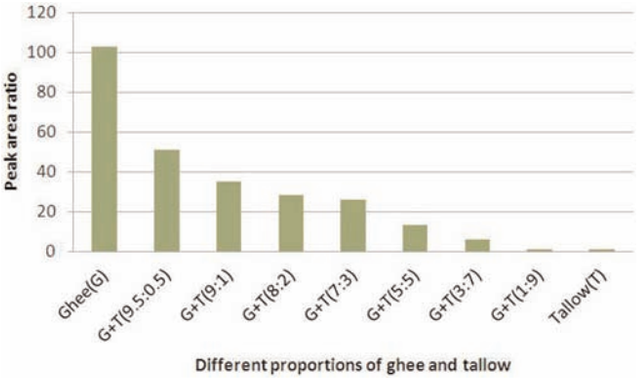

Figure 4

Comparative peak area ratio of spot C/spot B of Fraction A.

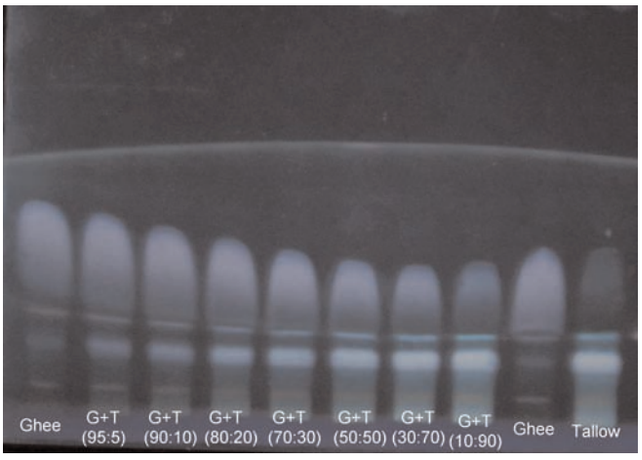

Figure 5

Chromatogram of cow ghee, tallow, and their mixtures (Fraction B).

Table 3

Peak area of the compounds at R_F 0.25 and 0.55 of Fraction B.

Sample	Peak area		Peak area ratio
	Spot A (R_F 0.25)	Spot B (R_F 0.55)	Spot B/spot A
Ghee (G)	152	3872	25.47
G–T (95:5)	525	3359	6.39
G–T (90:10)	635	3256	5.13
G–T (80:20)	975	3197	3.28
G–T (70:30)	1105	3138	2.84
G–T (50:50)	1463	2701	1.85
G–T (30:70)	1681	1934	1.15
G–T (10:90)	2019	1114	0.55
Tallow (T)	2144	268	0.125

along with their proportionate concentrations will be very helpful in the detection of tallow adulteration in cow ghee.

3.2 Investigation of Fraction B

The chromatogram of Fraction B samples after spraying with 10% methanolic sulphuric acid reagent (observation at 366 nm) is presented in **Figure 5.** The spots at R_F 0.25 and 0.55 are of special interest. The spot at R_F 0.25 is very faint in cow ghee, and its intensity increases with increasing proportions of tallow in it. On the other hand, the spot at R_F 0.55 is the major spot in cow ghee, and its concentration decreases with increasing concentrations of tallow and is very faint in tallow.

The comparative peak area of these two spots is presented in **Table 3.** It is very interesting to note that, in tallow, the peak area of the compound at R_F 0.25 is about 14 times more than that of cow ghee, while, in cow ghee, the peak area of the compound at R_F 0.55 is about 14 times more than that of tallow. The comparative graphical presentation of the area of these two spots is shown in **Figure 6.**

The concentrations of these two spots are inversely proportional in cow ghee and tallow. The ratio of peak area of these two spots

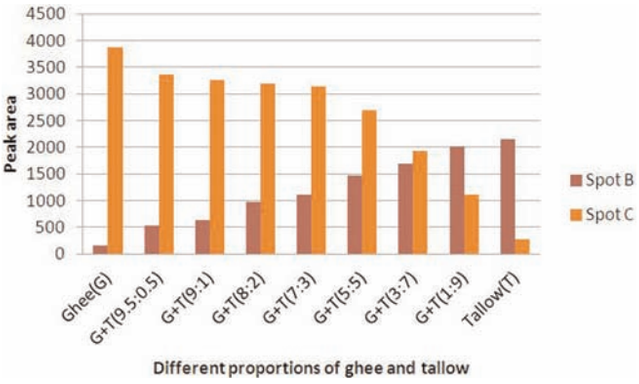

Figure 6

Comparative peak area of spot A and spot B of Fraction B.

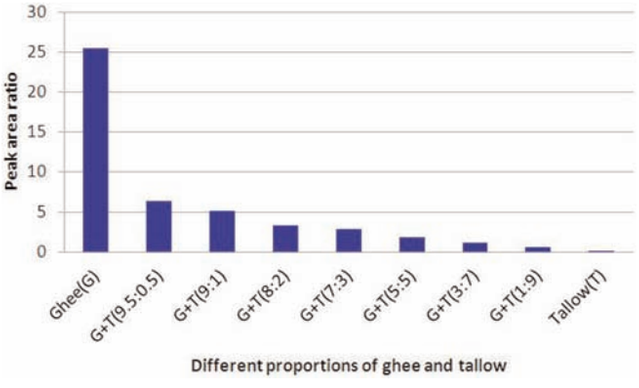

Figure 7

Comparative peak area ratio of spot B/spot A of Fraction B.

(spot B/spot A) was calculated (Table 3). In pure cow ghee, the value is 25.47 that comes down drastically to 6.39 when 5% tallow is added to cow ghee, and then the value steadily decreases and reaches 0.125 in the case of tallow **(Figure 7).** Moreover, the decrease in this ratio is quite linear (R^2 = 0.9623), i.e., proportionate to the concentration of adulteration.

The evolved data clearly reveal that the HPTLC profiles of cow ghee and tallow, under the present experimental conditions, are different. It is possible to detect the adulteration of cow ghee with tallow even at low levels with the help of the HPTLC data based on the differences in the compositions of chemical moieties such as steroids, terpenoids, and fatty acids in both ghee and tallow. The present method is simple, sensitive, and rapid as compared to many other reported methods. It can be used for routine quality control and for analysis of a large number of samples at a time.

4 Conclusion

A new HPTLC method for the detection of tallow adulteration in cow ghee has been developed. The method is sensitive, rapid, and cost-effective. It will be useful for routine quality control, and a large number of samples can be analyzed at a time by this method.

References

[1] The Ayurvedic Pharmacopoeia of India, Part II, Vol. I, Controller of Publications, Government of India, New Delhi, 2007.

[2] The Ayurvedic Formulary of India, Part I, Controller of Publications, Government of India, New Delhi, 1978.

[3] *B. Ramchandra, N. Dastur,* Indian J. Dairy Sci. **12** (1959) 139–148.

[4] *B. Ramchandra, N. Dastur,* Indian J. Dairy Sci. **13** (1960) 29–44.

[5] *M. Soliman, N. Younes,* J. Am. Oil Chem. Soc. **63** (1986) 248–250.

[6] *P. Parodi,* Aust. J. Dairy Technol. **28** (1973) 38.

[7] *M. Chakrabarty, C. Bandyopadhyay, D. Bhattacharyya, A. Gayen,* J. Chromatogr. A **36** (1968) 84–90.

[8] *M. Chakrabarty, D. Bhattacharyya, A. Gayen,* J. Chromatogr. A **44** (1969) 116–127.

[9] *M. Muthanna, B. Mukherji,* Curr. Sci. **9** (1940) 120–122.

[10] *P. Lambelet, N. Ganguli,* J. Am. Oil Chem. Soc. **60** (1983) 1005–1008.

[11] Indian Pharmacopoeia, Vol. I, The Indian Pharmacopoeia Commission, Ghaziabad, 2010.

Ms received: June 16, 2013
Accepted: September 12, 2013