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Dr. Kamal Gandhi
Scientist Dairy Chemistry Division, NDRI, Karnal, Haryana, India

Dr. Seema Rana
Senior Research FellowDairy Chemistry Division NDRI, Karnal, Haryana, India

Dr. Harish Kumar
Amity Institute of Biotechnology Amity University Jaipur, Rajasthan, India

Correspondence
Dr. Seema Rana
Senior Research Fellow Dairy Chemistry Division NDRI, Karnal, Haryana, India

Solvent fractionation technique paired with complete liquefaction time (CLT) test to detect bland of palm olein and sheep body fat in ghee

Dr. Kamal Gandhi, Dr. Seema Rana and Dr. Harish Kumar

Abstract
The CLT value (min: sec) for pure cow ghee ranged from 2:13 to 2:53 with an average of 2:32, while that for pure buffalo ghee ranged from 2:22 to 3:08 with an average of 2:43. The addition of palm olein could not be detected at any of the level studied, other than at 15 percent level in case of cow ghee. On the other side, sheep body fat added separately in cow as well as in buffalo ghee samples could conveniently be detected at all the levels studied with the exception of 5 % level. The mixture of palm olein and sheep body fat was detectable at 6+14 (20) and 9+21 (30) percent level, but not at 3+7 (10) percent level. After fractionation, the even lower level of 3+7 (10) percent was found to be noticeable.

Keywords: adulteration, complete liquefaction time, ghee (clarified milk fat), palm olein, sheep body fat

Introduction
Ghee (the clarified milk fat) is the most widely used milk product in the Indian subcontinent and is considered as the supreme cooking and frying medium (Gandhi *et al.* 2013) [1]. The unique flavour, pleasent aroma and essential fatty acid content of ghee along with high calorific value and valuable source of fat soluble vitamins assign it superiority than other fats. Therefore ghee has an important place in human diet. From ancient times ghee is used as a base material for the preparation of many ayurvedic and unani medicines. The ghee has been claimed to improved digestibility by virtue of short-chain fatty acids content. It can promote the growth of bifidobacteria, the proven beneficial bacterium in the intestine and thus can provide protection against colon cancer. Ghee is well known to boost memory, to improve physical appearances, and capable to cure many physiological disorders like ulcers. Ghee is an expensive product, costing three times as much as edible vegetable oils (Gosewade *et al*. 2017) [2] and thus prone to adulteration. Furthermore the variability in cow and buffalo species, season, feed and habitat are an incentive to adulteration practices. The limited production of milk especially during summer season cut down the supply of expensive milk fat which may lead to its adulteration. Expensive oils and fats such as ghee are often subjected to adulteration with cheaper fats in order to have an economic advantage (Kumar *et al*. 2013a; Kumar *et al*. 2013b) [3, 4]. The cheaper alternatives used for adulteration in the ghee are animal body fat, vegetable oils, mineral oils or mixture of these adulterants especially in India. The detection of milk fat adulteration has assumed a very critical dimension in today's preview especially in the regime of global competitiveness, where the quality of milk and milk products is not an alternative, but an obligation. However, setting up the purity of milk fat is a very complex phenomenon (Kumar *et al*., 2002; Boghra *et al*., 2004; Lal *et al*., 2005) [5-7].
The past literature reviewed revealed few detection methods and techniques, when adulterant fats like body fats or vegetable oils are added individually. The detection method involves methylene blue reduction test, halphen's test and baudin test etc, for detection of cottonseed oil and hydrogenated vegetable oil, respectively. The techniques used for adulterant fat detection were normal/reversed phase-thin layer chromatography, fluorescence spectroscopy, real time ionization etc. However, the complication arises, when a mixture of body fats and vegetable oils is added to milk fat. These adulterants differ in their physical and chemical characteristics. Thus they counteract the effect of each other on the changes brought in the physicochemical properties of milk fat.
On the basis of crystallization, pure and adulterated milk fat can be partitioned into solid and liquid fractions, containing body fat and vegetable oil, respectively.

Further analysis of solid and liquid fraction for various parameters can be a novel approach for establishing the purity of milk fat. The complete liquification time (CLT) test was established which uses the melting behaviour of milk fat in terms of the time taken by the solidified fat to become completely liquefied at a given temperature (Kumar, 2009) [8]. Both pure cow ghee and pure buffalo ghee had a particular range of CLT value. Any deviation from the range would give an indication of adulteration of milk fat.

CLT value of fat samples vary according to the type and amount of constituent fatty acids. Higher the amount of short chain and unsaturated fatty acid, lower is the time required for liquefying the solidified fat. A number of tests on the basis of melting or solidification behaviour have been employed to tackle with the problem of adulteration such as apparent solidification time (Kumar *et al*. 2009; 2010) [9, 10], opacity test (Panda and Bindal, 1998) [11], etc. The study of liquification behaviour of milk fat after separation of pure and adulterated ghee into respective solid and liquid fractions may help in resolving the issue of adulteration with bland of animal body fat and vegetable oil. Different types of fractionation processes have been developed, which include melt, solvent, and detergent fractionation; supercritical fluid extraction and short path distillation (German and Dillard, 1998) [12]. Reduced viscosity of the liquid, easier heat transfer, faster nucleation and growth, very low levels of entrained oil makes solvent fractionation superior over dry fractionation (Timms, 2005) [13]. Such kind of approach based on the partitioning of milk fat by using fractional crystallization, either dry or solvent has not been much explored in the past as a mean to detect adulteration.

Sheep body fat and palm olein both being inexpensive are supposed to be used as adulterants in milk fat (Gandhi et. al., 2014; Gandhi et. al., 2015) [14, 15]. Fractionation techniques have been integrated with other parameters including iodine value (Gandhi *et al*. 2015) [15], Reichert-Meissl value (Gandhi *et al*. 2014) [14], apparent solidification time (Kumar *et al*. 2010) [10], fatty acid profile using gas chromatography (Kumar *et al*. 2015) [16], Butyro-refractrometer reading (Kumar *et al*. 2017; Gandhi *et al*. 2017) [17, 18] etc. in the past for the authentication of milk fat. Therefore, keeping the above points in mind, the present study was targeted to detect sheep body fat and palm olein adulteration in milk fat using complete liquefaction time in conjunction with solvent fractionation technique.

Material and Methods

Preparation of samples

The cow and buffalo ghee samples were prepared from their respective pooled milk separately by creamery butter method (De, 2012) [19]. Refined palm olein (Ginni brand) was procured from local market and kept under refrigeration condition (4-7 °C) till further use as an adulterant. For the preparation of sheep body fat, the adipose tissues were procured from the local slaughter house (Bakra Market, Karnal). The adipose tissue was washed thoroughly under running tap water and the residual water was drained out. Then adipose tissue was heated on direct flame to 140-150 °C in a stainless steel vessel until a transparent liquefied animal body fat was obtained. The liquid fat thus obtained was filtered through 6-8 fold muslin cloth followed by vacuum filtration using Whatman No. 4 filter paper. The clear liquid fat obtained was cooled to room temperature, stored in polyethylene bottle and kept in refrigerator (5-10 ^{0}C) till further use.

Preparation of Adulterated Ghee Samples

For the preparation of adulterated ghee samples, pure ghee, palm olein, and sheep body fat were heated and maintained at 65-70 °C for 10 min before mixing. The adulterant fats/oils were added to ghee individually as well as in combinations. At individual levels, these adulterants were separately added to ghee at 5, 10 and 15% levels. In case of their combined mixtures, palm olein (3, 6 & 9%) and sheep body fat (7, 14 & 21%) were added in ghee, representing a total adulteration of 10 (3+7), 20 (6+14) & 30 (9+21)% levels, respectively, thereby maintaining a ratio of unsaturated to saturated fatty acid in adulterated ghee samples of 30:70, generally found in pure ghee. After addition of adulterant oils and fats to ghee, the samples were thoroughly mixed.

Selection of suitable temperature-time combination for solvent fractionation

First of all we have to separate sheep body fat and palm olein from pure ghee samples. At suitable temperature and time combination, the fractionation will separate the adulterant fats on the basis of their melting point. Among different fractionation techniques available, the solvent fractionation technique was selected as it combats easier heat transfer through lowered fat viscosity. Thus solvent fractionation support faster nucleation leading to crystal growth with the advantage of reduced entrapped oil in the fractions. Here in order to get first solid fraction enriched in sheep body fat and last liquid fraction enriched in palm olein by solvent fractionation, preliminary tests were conducted to obtain two successive temperature and time combination.

The thirty gram of pure ghee and other ghee samples containing mixture of adulterants were melted in 100 ml graduated glass tubes and equilibrated at 65 °C for 5 min in temperature controlled oven. Sixty ml of previously warmed acetone was added to graduated glass tube containing ghee samples to have a final ratio of 1:2 (w/v).Then samples were mixed thoroughly and again equilibrated at 50 ˚C for 5 min in temperature controlled oven.

To select the temperature-time combination for first solid fraction, the fractionation was carried out at the temperatures of 18, 15, 12 ^{0}C in a refrigerated water bath. At each temperature, time was noted till maximum amount of sheep body fat solidifies. The pure cow and buffalo ghee either do not solidify or solidify to the least extent. Similarly to get palm olein rich liquid fraction, temperature-time combination for the successive fractionation step was standardized. For this purpose, preliminary trials were conducted at the temperatures of 8, 6 and 4 ^{0}C. The time was noted at each temperature till all the palm olein and minimum of low melting triglycerides fraction of pure cow and buffalo ghee appear in the last liquid fraction. From these trials the temperature-time combination of 15^{0}C/15 min and 4^{0}C/3 hr was selected for obtaining the first solid fraction and last liquid fraction.

Complete Liquefaction Time (CLT) Test

The time taken by the solidified fat samples to melt completely at 45 ^{0}C was recorded and notified as complete liquefaction time (CLT) by the method of Kumar *et al*., (2008) [20] with some modifications. In brief, three grams of the completely melted fat sample was taken into a test tube (length 10 ± 0.1 cm, internal diameter- 1.1 ± 0.02 cm, external diameter- 1.2 ± 0.02 cm) and kept in an oven maintained at 60°C for a period of 5 minutes. Afterwards, the test tube, containing fat sample, was kept in a refrigerator (6-8^{0}C) for

45 min for solidification. Then solidified sample was subjected to liquefaction process at 45^0C for complete melting of the sample. The time for the sample to liquefy completely was recorded as CLT using stop watch. As the liquid fraction would not solidify at refrigerated temperature completely, CLT was not performed for particular fraction.

As mentioned above, pure and adulterated ghee samples were prepared at two-monthly intervals over the period of one year followed by fractionation of pure ghee samples and the samples containing mixture of adulterants. Complete liquification times were recorded at 45 °C for unfractionated samples (CLT_{45}), 46 °C for fractionated samples (CLT48) respectively. Thus a range of CLT values was established by performing CLT at various time periods, at two month interval over a period of one year for pure cow and buffalo ghee samples. Similarly the CLT range observed for samples which fall beyond the established range were considered to be adulterated.

Statistical Analysis

The results were expressed as average value ± standard error for three replicates of each sample collected at one month interval for a period of one year. P-value < 0.05 was used to denote significant differences among mean values determined by analysis of variance (ANOVA).

Results and Discussion

CLT test before fractionation of pure and adulterated ghee samples

Palm olein did not solidify completely at refrigeration temperature (a pre-condition before performing CLT test) for 45 min as described above in the procedure of test. Hence we were unable to detect their CLT value properly. But at refrigerated temperature, palm olein developed some turbidity which get cleared at the CLT temperature of 45 °C. On the other hand sheep body fat did not liquefy completely even upto a period of 25 minutes at 45 °C while at refrigerated temperature it get solidified easily.

The CLT 45 value of cow and buffalo ghee samples taken throughout the year at an interval of two months was significantly different from each other. For cow ghee as well as buffalo ghee, CLT 45 value was least in May. The highest CLT value in the month of January may reflect the effect of different animal feed in the different seasons which directly affect the milk fat composition by varying the fatty acid level. The similar trend of variation in CLT values of cow and buffalo ghee was also observed by earlier workers (Amit Kumar, 2009; Akash Patel, 2011; Anil Kumar, 2013) [8, 21, 22]. However among all samples, CLT value observed for cow ghee samples was lower as compared to buffalo ghee samples. Lower CLT value of cow ghee may be due to higher unsaturated fatty acid content of cow ghee as compared to buffalo ghee.

Further addition of palm olein individually at 5, 10 and 15 percent levels decreased the CLT value. The observed decrease was dependent upon the amount of adulterant added to the pure cow and buffalo ghee. Higher unsaturated fatty acid content of palm olein may be the reason for decrease in CLT value depending on the level of adulteration. On the other hand the sheep body fat added to both cow and buffalo ghee at the same levels (5, 10 and 15%) increased the CLT value. This increase could be explained by higher saturated fatty acid content in sheep body fat. Higher the level of sheep body fat, greater was the effect on the CLT value of ghee samples. However, addition of palm olein and sheep body fat at 3+7 (10), 6+14 (20) and 9+21 (30) percent levels in both types of pure ghee caused an increase in the CLT values. It could be due to higher proportion of sheep body fat used in adulteration.

The overall range of CLT 45 value include lowest as 2 min13sec to highest as 3 min 8sec of pure cow and pure buffalo ghee samples (Table 1). The comparison of the results with the average values of pure ghee shows that the adulteration of cow and buffalo ghee with palm olein separately could be detected at the level of 15% (w/w) only in case of cow ghee. However, statistically the average values of CLT 45 for pure cow ghee and cow ghee samples adulterated with palm olein were significantly different at 10 and 15% level of adulteration. Palm olein could not be detected at any level of study in buffalo ghee. But statistically, the average values of CLT 45 for pure buffalo ghee and buffalo ghee samples adulterated with palm olein were significantly different only at 15% level of adulteration. On the other hand, sheep body fat added individually in cow as well as in buffalo ghee samples could conveniently be detected at all the levels studied except at 5% level (Table 2). Additionally, perusal of the results of average values (Table 2) of ghee samples added with the mixture of adulterants at 3+7(10), 6+14(20) and 9+21(30) percent levels, revealed that palm olein along with sheep body fat was detectable at 9+21(30) percent levels in case of cow ghee and 6+14(20), 9+21(30) percent levels in case of buffalo ghee. On contrary, statistically the average values of cow ghee and buffalo ghee adulterated with palm olein and sheep body fats in combination were significantly different at 20 and 30% level of adulteration.

As market ghee is a mixture of cow and buffalo ghee, CLT value of which can be obtained through pooling of available data of respective pure cow and buffalo ghee and adulterated cow and buffalo ghee. Pooling of the data revealed that the addition of palm olein added individually could not be detected at any of the level studied, while sheep body fat could easily be detected at all the levels higher than 5 %. But in case of palm olein adulteration, the average value of CLT was significantly different at 15% level. The CLT value (3 min 17 second and 3 min 50sec) of sheep body fat at 10% level and 15% of adulteration in ghee respectively deviated from the range (2min 13sec to 3 min 8 sec). However statistically the average value of CLT 45 for pooled ghee and that of adulterated with sheep body fat were significantly different at all level of adulteration studied.

On contrary, the mixture of palm olein and sheep body fat was not detectable at 3+7 (10) percent level, but was detectable at higher levels i.e. 6+14 (20) and 9+21 (30)%. These finding were obtained by comparison of overall range of pure cow and buffalo ghee from 2min 13 sec to 3 min 08 sec with average values of adulterated ghee with the combination of palm olein and sheep body fat. The significant differences were observed among average value of pooled cow and buffalo ghee and that of sample adulterated with different combination of adulterants at 20% and 30% level.

Till now above described part of study on the unfractionated samples revealed that palm olein could be detected only in cow ghee at 15% level while sheep body fat could conveniently be detected in cow as well as in buffalo ghee samples and pooled samples at the adulteration levels of 10% and 15%. Palm olein could not be detected in buffalo ghee and pooled samples of cow and buffalo ghee at any level studied. However, in combination with sheep body fat it can be detected at 30% level in cow ghee and at 20% and 30% level in buffalo ghee as well as in pooled ghee.

Upadhyay *et al*., (2016) [23] reported that the addition of groundnut oil individually to cow and buffalo ghee was detectable only in cow ghee at 15% level of adulteration using CLT test at 44°C. However animal body fat (Goat body fat) was detectable in buffalo ghee at 10 and 15% level but not detectable in cow ghee. Detection level of animal body fat in adulterated ghee varies from animal to animal. Kumar *et al*., (2015) [16] reported that pig body fat was detectable in cow and buffalo ghee a level of 15% while buffalo body fat was detectable at 10% level. A similar study was conducted by Kumar *et al*., (2013) [3] to detect soyabean oil and buffalo body fat added individually or in combination in cow and buffalo ghee. Soyabean oil was detectable in cow ghee at 15% only while buffalo body fat was detectable at all the levels studied. In a similar study reported by Upadhyay *et al*., (2016) [23] goat body fat and groundnut oil used in combination at a ratio of 3:7 was detectable in buffalo ghee, pooled cow and buffalo ghee at 20% level and higher and in cow ghee at 30% level only. The findings of the present study are in agreement with the earlier reports of workers (upadhyay *et al*., 2016; Kumar *et al*., 2013) [23, 4].

CLT Test on pure and adulterated samples after fractionation

As explained earlier, CLT test for unfractionated samples was carried out at 44 °C. The fractionation of pure ghee samples and the samples adulterated with mixture of adulterants was carried out at 15 °C for enrichment of sheep body fat in first solid fraction (S15) which was then analyzed for CLT value at 45 °C. The CLT test of pure ghee samples and samples added with palm olein and sheep body fat, individually and in their combination was done at 45 ^{0}C. However, for first solid (S_{15}) fraction, it was observed that the fractions could not melt completely at 45 ^{o}C and opaqueness (haziness) persisted for a substantially long time. For that reason, the next higher temperature of 46 ^{o}C was used for recording the CLT values of fractions. Earlier reports about the non-suitability of temperature at 45 °C are in agreement with the present study. Some triglyceride fraction starts melting at 46 °C. Earlier, Kumar (2009) [8] had also recorded CLT values at 46 ^{o}C for first solid (S16) fractions obtained at 16 °C through solvent fractionation. Kumar *et al*., (2013) [3] also used 46 ^{0}C for recording CLT for the first solid (S_{15}) fraction through dry fractionation at 15 °C.

CLT test on sheep body fat enriched fraction at 46 °C

The overall average CLT values, recorded at 46 °C for the first solid fractions of pure cow ghee and buffalo ghee are presented in table 3. The CLT value for first solid (S_{15}) fraction of pure cow ghee was lower (ranged from 3:39 to 4:45 with an average of 4:12) as compared to similar S_{15} fraction of pure buffalo ghee (ranged from 3:52 to 4:55 with an average of 4:28). It was observed from the results that the CLT value of the first solid (S_{15}) fractions of both cow and buffalo ghee were found to be higher than the corresponding (unfractionated) pure cow and buffalo ghee samples from which the fractions were obtained. The higher CLT values for S_{15} of pure ghee samples as compared to CLT values of unfractionated pure ghee may be due to the reason that the fractionation process led to the separation of triglycerides containing long chain saturated fatty acids in first solid (S_{15}) fraction on the basis of their melting points.

Additionally, it was also observed that the CLT value of first solid (S15) fractions of ghee samples adulterated with mixture of palm olein and sheep body fat (3+7%, 6+14% and 9+21% levels) increased with the level of adulteration from 10% and 30% (w/w) studied. It was may be because of the sheep body fat enrichment in fraction as the sheep body fat possessed high CLT value as compared to pure ghee samples.

To check the adulteration, the overall range of 3:39 to 4:55 of the CLT value (min:sec) of first solid (S_{15}) fraction of both pure cow and buffalo ghee samples was compared with the average CLT values for the respective solid (S_{15}) fraction of cow and buffalo ghee samples adulterated with mixture of palm olein and sheep body fat at 3+7(10), 6+14(20) and 9+21(30) percent level. The samples for which CLT values falls beyond the upper and lower range were considered as adulterated. The results revealed that the adulteration of cow and buffalo ghee with palm olein and sheep body fat could be detected at 6+14(20) and 9+21(30) percent level for cow ghee, while for buffalo ghee adulteration was detectable even at 3+7(10) percent level. As observed from the table 3, the adulteration could be detected at the lowest level of adulteration. The comparison of CLT value of first solid (S_{15}) fractions of adulterated ghee samples with the pooled data of solid (S_{15}) fraction of pure and adulterated cow and buffalo ghee samples revealed the similar finding. It was observed that all the levels of adulteration [3+7(10), 6+14(20) and 9+21(30) percent] could be detected.

At first, the CLT test for fractions was done at 45 ^{o}C, but at this temperature many samples particularly those which were having high adulterant levels did not melt completely and showed some opaqueness (haziness) for a long time. Therefore, the next higher temperature of 46 ^{o}C was used for recording the CLT values of fractions in which no such problem of opaqueness was noticed.

The comparison of CLT value of fractionated ghee samples with unfractionated ghee samples, for the pooled cow and buffalo ghee together, revealed that the fractionation technique had offered advantage in lowering down the detection limit using CLT value. The level of adulteration (3+7) percent of palm olein and sheep body fat which could not be detected in case of unfractionated ghee samples, was found to be noticeable in the first solid (S_{15}) fractions of pure ghee. Upadhyay *et al*., (2016) [24] had also reported that fractionation using solvent fractionation technique has helped in lowering the detection limit of goat body fat and groundnut oil in combination. Different fractionation techniques have been reported by workers which lowered the detection limit like dry fractionation (Kumar, 2013) [23]; Difference in time temperature combination (Kumar, 2009) [8] etc.

Table 1: CLT values of pure cow and buffalo ghee samples and adulterants fat/oil in different months of year

Type of sample	CLT value (min: sec)						Average ±SE
	January	March	May	July	September	November	
Cow ghee	2:53	2:18	2:13	2:24	2:41	2:47	2:32±0:06[a]
Buffalo ghee	3:08	2:32	2:22	2:35	2:53	2:58	2:43±0:06[a]
Palm* olein	ND	ND	ND	ND	ND	ND	ND
Sheep** body fat	---------- Did not liquefy till 25 min----------						

ND- Not determined

*Palm olein did not solidify at the pre-condition temperature of refrigeration
**Sheep body fat did not liquefy till 25 min observed
*Data represent average of six determinations in alternate month over the period of 1 year.

Table 2: CLT values (CLT 45) of cow and buffalo pure ghee and ghee adulterated with individual adulterants and mixture at 45°C thereof

Adulterant	Level (78575%)	CLT value (min:sec)					
		Cow ghee		Buffalo ghee		Pooled (Cow+ Buffalo) ghee	
		Range*	Average± SE	Range*	Average± SE	Range**	Average± SE
Control	0	2:13 to 2:53	2:32±0:06^{a,l,x}	2:22 to 3:08	2:43±0:06^{a,l,x}	2:13 to 3:08	2:38±0:06^{a,l,x}
PO	5	2:01 to 2:38	2:20±0:05^{a}	2:18 to 2:56	2:39±0:06^{a}	2:01 to 2:56	2:30±0:05^{a}
	10	2:04 to 2:34	2:16±0:04^{b}	2:13 to 2:49	2:32±0:05^{a}	2:04 to 2:49	2:24±0:04^{a}
	15	2:01 to 2:27	2:10±0:03^{c}	2:09 to 2:41	2:27±0:04^{b}	2:01 to 2:41	2:19±0:03^{b}
SBF	5	2:37 to 3:27	3:03±0:06^{m}	2:49 to 3:19	3:07±0:04^{m}	2:37 to 3:27	3:05±0:05^{m}
	10	2:56 to 3:39	3:15±0:06^{m}	2:57 to 3:35	3:19±0:05^{m}	2:56 to 3:39	3:17±0:05^{m}
	15	3:01 to 4:25	3:46±0:12^{n}	3:20 to 4:28	3:54±0:10^{n}	3:01 to 4:28	3:50±0:11^{n}
SBF+PO	3+7	2:15 to 2:57	2:37±0:13^{x}	2:27 to 3:37	2:55±0:12^{x}	2:15 to 3:37	2:46±0:10^{x}
	6+14	2:29 to 3:52	3:05±0:14^{y}	2:41 to 3:57	3:18±0:11^{y}	2:29 to 3:57	3:12±0:12^{y}
	9+21	2:33 to 3:59	3:15±0:13^{z}	3:25 to 4:02	3:41±0:05^{z}	2:33 to 4:02	3:32±0:09^{z}

PO: Palm olein SBF: Sheep body fat
*Data represent mean± SE of six determinations:
**Data represent mean± SE of twelve determinations
Different superscripts (alphabetic) represent the significant difference within the column (for different levels of similar type of adulterant) from the control ghee samples at $P< 0.05$.

Table 3: CLT values (CLT 46) of first solid (S_{15}) fractions of pure ghee samples and ghee sample added with combination of adulterants at 46 °C.

Type of ghee	Type of adulterant fat/oil	Level of adulteration (%)	CLT value (min: sec)	
			Range	Averages ±SE
			S_{15}	S_{15}
Cow Ghee	Control	0	3:39 to 4:45	4:12±0:09^{a}
	PO+ SBF	3+7	4:40 to 5:19	4:54±0:06^{b}
	PO+ SBF	6+14	4:57 to 5:25	5:06±0:04^{b}
	PO+ SBF	9+21	5:16 to 5:56	5:36±0:06^{c}
Buffalo Ghee	Control	0	3:52 to 4:55	4:28±0:09^{a}
	PO+ SBF	3+7	4:46 to 5:28	5:01±0:06^{b}
	PO+ SBF	6+14	5:02 to 5:32	5:15±0:04^{b}
	PO+ SBF	9+21	5:29 to 5:58	5:47±0:04^{c}
Pooled (cow+buffalo) Ghee	Control	0	3:39 to 4:55	4:20±0:09^{a}
	PO+ SBF	3+7	4:40 to 5:28	4:57±0:06^{b}
	PO+ SBF	6+14	4:57 to 5:32	5:10±0:04^{b}
	PO+ SBF	9+21	5:16 to 5:58	5:41±0:05^{c}

PO: Palm olein SBF: Sheep body fat S_{15} First solid fraction
Different superscripts (alphabetic) represent the significant difference within the column (for different levels of similar type of adulterant) from the control ghee samples at $P< 0.05$.

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