RESEARCH ARTICLE

# Detection of adulteration of *ghee* (clarified milk fat) with palmolein and sheep body fat using Reichert-Meissl (RM) value coupled with solvent fractionation technique

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Received : April 2014 / Accepted : September 2014

**Abstract** RM value for pure cow ghee ranged from 28.60 to 30.36 with an average of 29.50, whereas that for pure buffalo ghee ranged from 31.46 to 34.98 with an average of 33.30. Palm olein and sheep body fat added individually could be detected only at 15 per cent levels in pooled cow and buffalo ghee samples based on RM value determination. Mixture of palm olein and sheep body fat was detectable at 6+14 (20) and 9+21 (30) per cent levels. However, after fractionation, even lower level of 3+7 (10) per cent which was not detectable before fractionation, also became detectable.

**Keywords** : Ghee (clarified milk fat), RM value, adulteration, fractionation, palm olein, sheep body fat

## Introduction

Ghee is one of the valuable fats that continue to be a target of unscrupulous traders for the maximization of profits. Estimates from the latest report "Indian Dairy Market Report & Forecasts 2012-2017" suggest that during 2011 and 2017, the total sales for ghee are expected to grow at a compound annual growth rate (CAGR) of 14% (http://www.slideshare.net/imarc123/indian-ghee-market). Adulteration of ghee adversely affects the consumer health and also the dairy industry. Fraudulent practices create unfair competitiveness. These lead to market distortions, which in turn may adversely impact the local or even the international trade. Therefore, authentication of milk and milk products such as ghee through quality testing is important to both consumers as well as processors.

Reichert Meissl (RM) value is substantially a measure of the lower chain fatty acids of ghee i.e. butyric (4:0) and caproic (6:0). The value of milk fat ranges from 17-35, which is well above all other fats and oils. Butyric acid contributes about three-fourths and caproic acid one-fourth to the RM value. RM value is covered as one of the quality parameters for the ghee under FSSAI Rules (2011) and AGMARK Rules (1981).

FSSAI Rules (2011) have prescribed a minimum value of 21-28, depending upon the place (State/ Union territory) so as to ensure the quality of ghee to consumers. AGMARK (1981) also prescribes value not less than 28 for non-cotton tract areas on all India basis, and not less than 23 in winters and not less than 21 in summers for cotton tracts of Saurashtra and Madhya Pradesh. Any deviation from these values would give an indication of adulteration of milk fat.

Methods currently employed for the detection of adulteration of foreign fats in milk fat are to some extent able to help when adulterant fats like body fats or vegetable oils are added individually. However, when a mixture of body fats and vegetable oils is added to milk fat, the complication arises. Thus, partitioning the pure and adulterated milk fat into solid and liquid fraction on the basis of crystallization, which enriches the solid fraction with the body fat and the liquid fraction with vegetable oil followed by the analysis of these fractions for various parameters can be a novel approach for establishing purity of milk fat. Different types of fractionation

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processes have been developed, which include melt, solvent and detergent fractionation; supercritical fluid extraction and short path distillation (Deffense, 1993; Dimick *et al.* 1996; German and Dillard, 1998). Such kind of approach based on partitioning of milk fat by using fractional crystallization, either dry or solvent has not been much explored in the past as a means of detecting adulteration. Solvent fractionation has advantages over dry fractionation in that besides lowering viscosity of the liquid, it makes heat transfer easier, nucleation and growth faster, and there are very low levels of entrained oil. (Timms, 2005).

Sheep body fat and palm olein both being cheaper are suspected to be used as adulterants in milk fat. Therefore, keeping the above points in mind, the present study was aimed to detect sheep body fat and palm olein in milk fat using RM value coupled with solvent fractionation technique.

## Materials and Methods

Chemicals and reagents

Glycerol (98% W/W), NaOH (50% W/W), Dil. $H_2SO_4$ (Approximately 25ml of conc. sulphuric acid was diluted to one litre and adjusted until 40 ml of it neutralized 2.0 ml of 50% NaOH solution), Sodium Hydroxide solution ( N/10), Phenolphthalein indicator, Whatman No 4 filter paper 9 cm dia.

Preparation of samples

Cow and buffalo ghee samples were prepared from their respective pooled milks separately by creamery butter method (De, 2010). Refined palm olein used for the study was procured locally and kept under refrigeration condition (5-10°C) till its further use as adulterant. For the preparation of sheep body fat, the adipose tissues were procured from the local slaughter house (Bakra Market, Karnal). The adipose tissues after collection were washed thoroughly under running tap water. After draining out the residual water, the adipose tissues were heated at 140°C to 150°C on direct flame in a stainless steel vessel till 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, filled in polyethylene bottle, cooled to room temperature, capped and kept in a refrigerator (5-10$^0$C) for its further use as the adulterant fat.

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 their 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 and 9% and sheep body fat @ 7, 14 and 21% were added to ghee, representing a total adulteration of 10 (3+7), 20 (6+14) and 30 (9+21) % levels, respectively, thereby maintaining a ratio of unsaturated to saturated fatty acid in adulterated ghee samples as 30 to 70, as is generally found in pure ghee. After the addition of adulterant oils and fats to ghee, the samples were thoroughly mixed.

Selection of suitable temperature-time combination for solvent fractionation

Preliminary trials were conducted with pure ghee samples (cow and buffalo separately) and adulterant oil and fat (palm olein and sheep body fat) with a purpose of obtaining two temperatures in a successive manner so as to get two solids and one liquid fraction so that the first solid fraction is sheep body fat enriched while the last liquid fraction is palm olein enriched. For this, 30 g samples (pure ghee and adulterants) were melted and equilibrated at 65$^0$C for 5 min in 100 ml graduated glass tubes. These were then dissolved in 60 ml acetone previously warmed in a water bath followed by equilibrating the mixture at 50$^0$C for 5 min. For the selection of temperature-time combination of the first solid fraction, the fractionation was carried out at temperatures of 18, 15, 12 $^0$C in a refrigerated water-bath, noting time at each temperature so as to know at which temperature-time combination maximum amount of sheep body fat solidifies while the pure cow and pure buffalo ghee either do not solidify or solidify to the least extent. After the desired temperature-time combination, was selected for obtaining the first solid fraction, temperature-time for the successive fractionation step was standardized so as to get the last liquid fraction enriched with the palm olein. For this purpose, preliminary trials were conducted at temperatures of 8, 6 and 4$^0$C, noting time at each temperature so as to know at which temperature-time combination, all the palm olein and minimum of low melting triglycerides fraction of pure cow ghee and pure buffalo ghee appear in the last liquid fraction. From these trials, temperature-time combination of 15°C/15 min was selected for obtaining the first solid fraction, whereas a temperature-time combination of 4$^0$C/ 3 h was selected for obtaining the subsequent solid and liquid fractions.

Statistical Analysis

Results are presented as means ± standard error of six determinations. P-value < 0.05 was used to denote significant differences among mean values determined by analysis of variance (ANOVA) with a subsequent least significant difference (LSD) test was applied to test for any significant differences ($P<0.05$) in the mean values as described by Snedecor and Cochran (1994).

## Results and Discussion

The results obtained in the present study on RM values for pure cow and buffalo ghee, sheep body fat and palm olein samples collected throughout the year at an interval of two months are depicted in Figure 1. The RM value for palm olein ranged from 0.44 to 0.99 with an average of 0.72, while for sheep body fat it ranged from 0.33 to 0.55 with an average of 0.39.

The RM value for pure cow ghee ranged from 28.60 to 30.36 with an average of 29.50, whereas that for pure buffalo ghee ranged from 31.46 to 34.98 with an average of 33.37. When the data of pure cow and buffalo ghee were combined together, the RM value ranged from 28.60 to 34.98 with an average of 31.43 (Table 1).

From the results, it can be seen that the RM value for pure cow ghee was the lowest (28.60) in May and highest (30.36) in March and July. For pure buffalo ghee, the lowest RM value (31.46) was observed to be in May as observed for pure cow ghee and the highest (34.98) was observed to be in March (Figure 1). This type of variation has also been reported by previous workers (Das Gupta, 1939; Katrak *et al*, 1946; Rangappa and Achaya, 1974; Kumar, 2008; Kumar, 2010; Kumar, 2013) who have suggested temperature difference to be the genuine reason behind such variation, although they have reported different months for highest and lowest RM values.

Analysis of variance of data (Figure 2) for RM value revealed that pure ghee (cow and buffalo) and adulterant oil and fat differed significantly ($P<0.05$) from each other. Comparison among all the pure samples of ghee and adulterant fat and oil revealed that palm olein differed significantly from sheep body fat ($P<0.05$). Pure cow ghee and pure buffalo ghee also differed significantly from each other ($P<0.05$). Pure ghee (cow and buffalo) showed the higher RM values as compared to adulterants fat and oil (Figure 2). Of the two pure milk fats, the RM values were observed to be higher for buffalo ghee. Palm olein and sheep body fat showed only the negligible RM values which were much lower than that of pure cow and buffalo ghee samples.

The RM values (range and average ± SE) for cow and buffalo ghee samples adulterated with palm olein and sheep body fat at 5, 10 and 15% levels, individually and in their combination of 3+7 (10), 6+14 (20) and 9+21 (30) per cent along with their respective pooled values are presented in Table 1. The RM values were found to decrease in cow ghee as well as buffalo ghee with the addition of palm olein and sheep body fat, individually at 5, 10 and 15 per cent levels and in their combinations at 3+7(10), 6+14(20) and 9+21(30) per cent levels, and this decrease was dependent upon the amount of adulterants added to the pure cow and buffalo ghee. Higher the level of adulterant added, greater was the decrease in the RM value of ghee samples. It was also observed that addition of both vegetable oil (palm olein) and animal body fat (sheep body fat), individually, caused almost equal decrease in the RM value of ghee.

On comparison of average values of RM value obtained in

**Table 1** RM values of pure cow and buffalo ghee and ghee adulterated with individual adulterants and combination thereof

| Adulterant | Level of adulteration (%) | RM values | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cow ghee | | Buffalo ghee | | Pooled samples (Cow+Buffalo) ghee | |
| | | Range* | Average±SE | Range* | Averages' | Range** | Average±SE |
| Control | 0 | 28.60-30.36 | 29.50±0.37 | 31.46-34.98 | 33.37±0.32 | 28.60-34.98 | 31.43±0.33 |
| PO | 5 | 27.83-29.92 | 28.88±0.09 | 29.81-33.33 | 31.48±0.22 | 27.83-33.33 | 30.18±0.11 |
| | 10 | 25.96-27.72 | 26.68±0.25 | 28.38-31.46 | 29.98±0.20 | 25.96-31.46 | 28.33±0.20 |
| | 15 | 23.76-26.62 | 25.19±0.53 | 27.28-29.15 | 28.40±0.40 | 23.76-29.15 | 26.79±0.37 |
| SBF | 5 | 27.72-29.48 | 28.36±0.26 | 29.59-32.78 | 30.97±0.46 | 27.72-32.78 | 29.66±0.43 |
| | 10 | 25.19-27.61 | 26.33±0.62 | 28.38-32.01 | 30.43±0.45 | 25.19-28.93 | 28.38±0.25 |
| | 15 | 23.98-27.06 | 25.23±0.33 | 26.29-28.93 | 27.92±0.31 | 23.98-32.67 | 26.57±0.53 |
| PO+SBF | 3+7 | 26.51-28.49 | 27.59±0.61 | 30.69-32.67 | 31.70±0.56 | 26.51-32.67 | 29.65±0.65 |
| | 6+14 | 25.96-26.51 | 26.25±0.58 | 27.83-29.26 | 28.56±0.39 | 25.96-29.26 | 27.41±0.62 |
| | 9+21 | 22.99-24.64 | 23.87±0.42 | 25.63-27.06 | 26.51±0.42 | 22.99-24.64 | 25.19±0.33 |

PO-Palm olein SBF-sheep body fat

* Data represent mean±SE of six determination. **Data represent mean ± SE of twelve determinations.

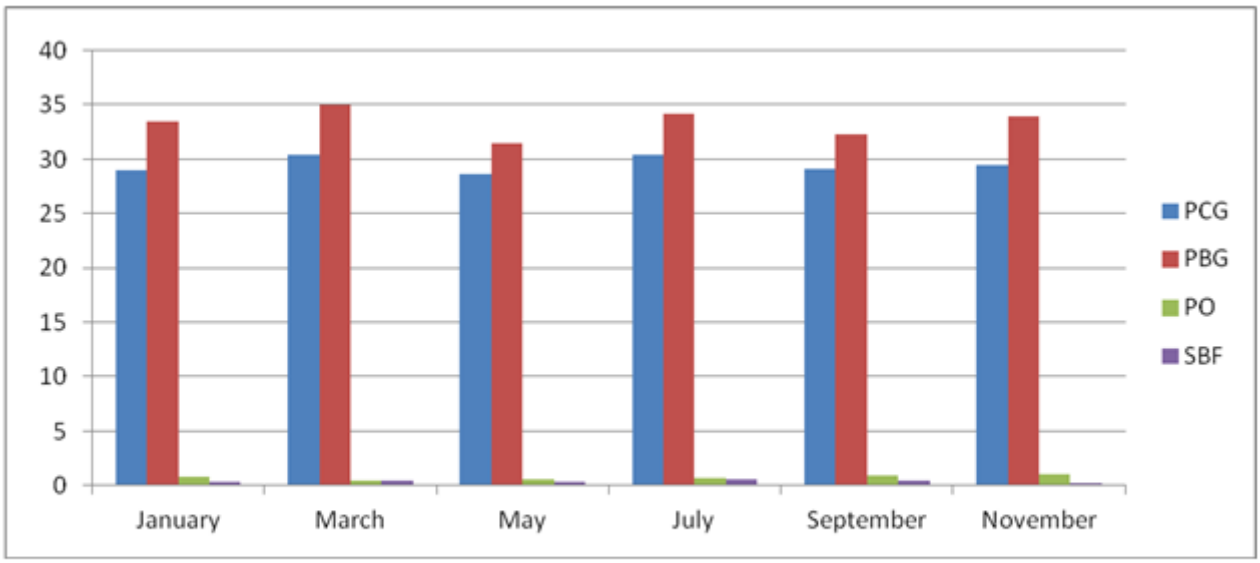


**Figure 1 . RM values of pure ghee (cow and buffalo) and adulterants (palm olein and sheep body fat) in different months of the year**

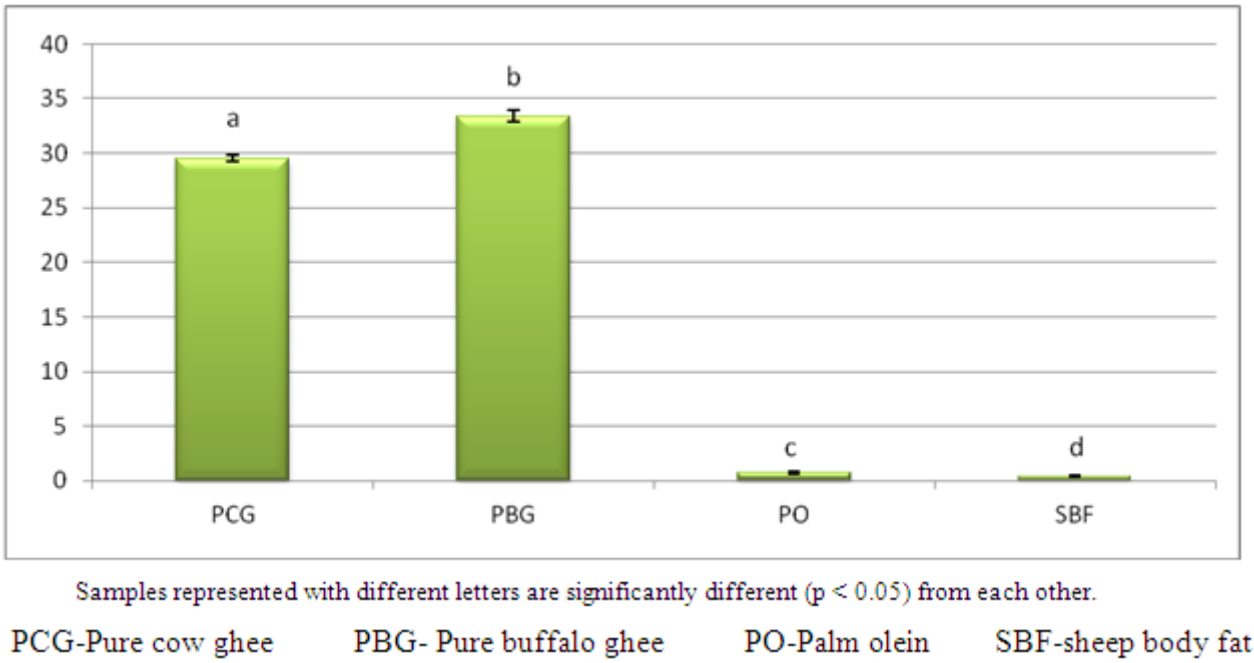


**Figure 2 Average RM values of pure ghee samples and adulterant fat and oil**

the present study (Table 1) with the standards prescribed for RM values (not less than 28) under FSSAI Rules (2011) for Haryana, it was revealed that the adulteration of cow ghee with palm olein and sheep body fat individually could easily be detected at all the levels, except 5 per cent level of both the adulterants. On the other hand, the adulteration in buffalo ghee samples added with individual adulterants could not be detected at all the levels of adulteration studied, except for sheep body fat at 15% level, where the average value was almost close to 28 .

**Table 2** RM values of first solid (S15) and last liquid (L4) fractions of pure ghee samples and ghee samples added with combination of adulterants

| Type of ghee | Type of Adulterant fat/oil | Level of adulteration (%) | RM values | | | |
|---|---|---|---|---|---|---|
| | | | Range* | | Average±SE | |
| | | | $S_{15}$ | $L_4$ | $S_{15}$ | $L_4$ |
| Cow Ghee | Control | 0 | 26.40-28.16 | 27.50-29.26 | 27.30±0.30 | 28.22±0.24 |
| | PO+ SBF | 3+7 | 21.01-24.31 | 24.09-27.61 | 22.46±0.51 | 25.39±0.56 |
| | PO+ SBF | 6+14 | 20.57-22.99 | 22.77-24.86 | 21.30±0.37 | 23.69±0.35 |
| | PO+ SBF | 9+21 | 16.39-19.14 | 20.46-22.22 | 17.82±0.37 | 21.30±0.26 |
| Buffalo Ghee | Control | 0 | 29.26-32.78 | 29.92-33.00 | 31.17±0.53 | 31.46±0.48 |
| | PO+ SBF | 3+7 | 27.39-29.37 | 29.59-32.07 | 28.58±0.34 | 31.01±0.38 |
| | PO+ SBF | 6+14 | 22.33-23.76 | 27.28-25.96 | 23.06±0.22 | 26.64±0.21 |
| | PO+ SBF | 9+21 | 21.23-23.54 | 24.42-25.96 | 22.29±0.32 | 25.23±0.26 |
| Pooled (cow+buffalo) Ghee | Control | 0 | 26.40-32.78 | 27.50-33.00 | 29.23±0.40 | 29.41±0.24 |
| | PO+ SBF | 3+7 | 21.01-29.37 | 24.09-32.07 | 25.52±0.34 | 28.20±0.44 |
| | PO+ SBF | 6+14 | 20.57-23.76 | 22.77-25.96 | 22.18±0.17 | 25.16±0.24 |
| | PO+ SBF | 9+21 | 16.39-23.54 | 20.46-25.96 | 20.06±0.24 | 23.27±0.23 |

PO- Palm olein SBF-sheep body fat * Data represent mean±SE of six determination.

Further, it was observed from the results of the average values (Table 1) of the ghee samples added with mixture of adulterants at 3+7(10), 6+14(20) and 9+21(30) per cent levels that palm olein in the presence of sheep body fat could easily be detected in case of cow ghee samples. On the other hand, in case of buffalo ghee samples, only the 9+21 (30) per cent level of adulteration of both the adulterants could be detected.

Pooling of the data of pure and adulterated cow and buffalo ghee samples revealed that palm olein and sheep added individually could be detected only at 15 per cent levels. Mixture of palm olein and sheep body fat was detectable at 6+14 (20) per cent or higher levels of addition.

As described before, the pure ghee samples and the samples adulterated with mixture of adulterants were subjected to fractionation at 15°C and 4°C for enrichment of animal body fat in first solid fraction and vegetable oil in last liquid fraction. The first and last fractions were also analyzed for RM value so as to know whether fractionation process increases the sensitivity of detection of adulteration. The results obtained on RM value of first and last fractions along with their respective pooled values are shown in Table 2

The RM values ranged from 26.40 to 28.16 with an average of 27.30 for the first solid (S15) fraction of pure cow ghee, whereas the similar values for S15 fraction of pure buffalo ghee ranged from 29.26 to 32.78 with an average of 31.17. The RM value of the last liquid (L4) fraction of pure cow ghee ranged from 27.50 to 29.26 with an average of 28.22, while that of L4 of pure buffalo ghee ranged from 29.92 to 33.00 with an average of 31.46 (Table 2). It was observed from the results that the RM value of the first solid (S15) and last liquid (L4) fractions of both cow and buffalo pure ghee was lower than that of the corresponding (unfractionated) pure cow and buffalo ghee samples from which the fraction was obtained. Of the two fractions (S15 and L4), last liquid (L4) fractions obtained from pure cow and buffalo ghee samples and samples added with individual and combinations of adulterants showed the higher RM value as compared to corresponding first solid (S15) fractions. Our findings of higher RM values in liquid fraction as compared to solid fraction are in close agreement with the reports of earlier workers for cow, buffalo and goat milk fat (Laxminarayana and Ramamurthy, 1985; Arora and Rai,1998 and Kumar, 2013).

Further, it was also observed that the RM value of the first solid (S15) and last liquid (L4) fractions of ghee samples adulterated with mixture of palm olein and sheep body fat at 3+7(10), 6+14(20) and 9+21(30) per cent levels decreased with the level of adulteration. The low RM value for S15 and L4 as compared to RM values of whole ghee may be possibly due to the reason that more of the C4:0 (butyric acid) and C6:0 (caproic acid) containing glycerides might have been retained in to the middle fractions (S4), which have not been analyzed in the present study.

Considering the overall range of the RM values of first solid

(S15) fractions (26.40 to 32.78) and last liquid (L4) fractions (27.50 to 33.00) of pure cow and buffalo ghee samples as the basis and then comparing it with the average RM values for the respective first solid (S15) fraction and last liquid (L4) fractions of cow and buffalo ghee samples added with mixture of palm olein and sheep body fat at 3+7(10), 6+14(20) and 9+21(30) per cent levels of adulterations, it was noted that the adulteration of palm olein and sheep body fat could easily be detected at all the levels studied, except at 3+7(10) per cent levels in case of buffalo ghee.

On pooling of the data obtained on first solid (S15) fraction of pure as well as adulterated cow and buffalo ghee samples and using the overall range (26.40 to 32.78) of RM values of S15 factions of both pure cow and buffalo ghee as the basis for comparison of RM values of first solid (S15) fractions of adulterated ghee samples, it was observed that even the lower level of adulteration done at 3+7(10) per cent level could easily be detected. On the other hand, when the data of last liquid (L4) fractions of pure as well as adulterated cow and buffalo ghee samples was pooled together, and the overall range (27.50 to 33.00) of RM values of L4 fractions of pure cow and buffalo ghee was used as the basis for comparison of RM values of last liquid (L4) fraction of adulterated ghee samples, it was observed that the lowest level [3+7(10)] could not be detected on the basis of RM values of last liquid (L4) fractions. However, higher levels were easily detectable. This indicated that using the RM value of first solid (S15) fractions, all the levels of adulteration studied for the mixture of palm olein and sheep body fat could easily be detected.

Based on the pooled data of cow and buffalo ghee together, a comparison of the results obtained on average RM values of the fractionated ghee samples with those of unfractionated ghee samples, revealed that fractionation technique has offered advantage in lowering the detection limit using because even that level of adulteration (3 and 7 per cent respectively of palm olein and sheep body fat) which could not be detected in case of unfractionated ghee samples, was found to be detectable on the basis of first solid (S15) fractions.

The results obtained in the present study on the RM value of various oils and fats including milk fats are in general agreement with those reported by earlier workers (Singhal, 1973; Rangappa and Achaya, 1974; Sharma and Singhal, 1995; Amit Kumar, 2008; Ashvin Kumar, 2010; Akash Patel, 2011; Anil Kumar, 2013). Vegetable oils and animal body fats showed negligible RM values as against high RM values observed for cow and buffalo milk fats. Similar observations on negligible RM values have also been reported by earlier workers for body fats (Singhal, 1973; Sharma and Singhal, 1995; Kumar, 2008;Patel, 2011; Kumar, 2013) as well as vegetable oils (Rangappa and Achaya, 1974; Sofia, 2005; Kumar, 2008; Patel, 2011; Kumar, 2013). Vegetable oils and body fats are not expected to have any RM value because this value is typical for milk fats only, and is represented by butyric (C4:0) and caproic (C6:0) acids which are synthesized in the cells of mammary glands of milch animals where de novo synthesis of fatty acids from C4:0 to C14:0 as well as part of C16:0 fatty acids takes place (Fox, 1995). Such a phenomenon is not found to occur in the vegetable cells as well as animal body cells other than mammary gland cells. The traces of RM value observed in the present study for the vegetable oils and animal body fats may be possibly because of the presence of some unidentified water soluble carboxylic acids with a similar molecular weight as that of C4:0 and C6:0 fatty acids. It is also possible that in case of vegetable oils certain breakdown products of some long chain fatty acids might have been formed during the refining process, whereas in case of body fats these might have formed during the clarification of adipose tissue to get clear fat.

The difference found in the present investigation in the RM value of cow and buffalo pure ghee may be attributed to the species characteristics, since the study was carried out under identical conditions of feeding and management. Buffalo ghee has generally a slightly higher content of butyric acid and caproic acid (Ramamurthy and Narayanan, 1971; Bector and Narayanan, 1974 ; Arumughan and Narayanan, 1979; Lal and Narayanan, 1984; Kumar, 2008; Kumar, 2010; Patel, 2011; Kumar, 2013) which could be responsible for its higher RM value than cow ghee. Results obtained in the present study may not be applicable directly to cotton tract area ghee because it differs from the non- cotton tract area ghee in terms of physico-chemical characteristics such as RM value. Therefore, suspected ghee samples should be first tested for MBRT and Halphen test to confirm whether it is cotton tract area ghee or not, before proceeding to check its purity through above said approach of testing adulteration.

## Conclusions

It may be concluded from the present study, that RM value can be used as an indicator for checking adulteration in milk fat, both for the detection of palm olein and sheep body fat addition. Both the adulterants added individually could be detected only at 15 per cent levels in pooled samples. Mixture of palm olein and sheep body fat was detectable at 6+14 (20) per cent and above levels. Fractionation technique has offered advantage of increasing the sensitivity of RM value by lowering the detection limit because that level (3+7) of adulteration with adulterants (palm olein and sheep body fat) which could not be detected in case of unfractionated ghee samples, was found to be detectable on the basis of solid (S15) fraction.

## Acknowledgements

The first author acknowledges the Institute fellowship from

ICAR and infrastructural facilities received from National Dairy Research Institute, Karnal, Haryana (India) during the course of research.

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