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Detection of physico-chemical attributes of ghee adulterated with palm oil using dry fractionation techniques

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Abstract
Ghee is a high-priced product that costs three times as much as edible vegetable oil. Ghee is frequently adulterated with low-cost vegetable oil (Palm oil). The most cost-effective method of modifying the physical properties of milk fat is fractionation. Dry fractionation and solvent fractionation are methods for differentiating triacylglycerol based on melting temperature. Dry fractionation without solvents is preferred as a more neutral technique it is simplest and most cost-effective separation technique in the present study ghee blended with palm oil at the level 0, 5, 10 and 20% using physico-chemical characterization could be detected using the Reichert-Meissl value (R.M.) at 20% palm oil. The dry fractionation could be used to detect adulteration with palm oil at 20% by using the R.M. value (solid fraction), Butyro-refractometer reading (liquid fraction) and Iodine value (liquid and solid fraction).

Keywords: Dry fractionation, ghee, palm oil, butyro-refractometer reading, iodine value, reichert-meissl value

Introduction
Ghee (clarified butterfat) is the most important indigenous milk product and has contributed to the Indian diet since ancient times [1]. Ghee adulteration is harmful to consumer health as well as the dairy industry. Fraudulent practices lead to unfair competition. These result in market distortions, which can have an adverse influence on domestic or international trade. As a result, authentication of milk and milk products such as ghee through quality testing is essential for both consumers and processors [2]. The most cost-effective method of modifying the physical properties of milk fat is fractionation [3, 4]. Fat is separated into fractions based on differences in melting temperature, solidification temperature, and volatility of triacylglycerol, as well as differences in the solubility of fat components, during this process [5, 6]. Dry fractionation, solvent fractionation, and molecular distillation based on molecular mass, melting temperature, volatility, and intermolecular interactions between triacylglycerol are all intriguing techniques that can be used in fat production [7, 4]. Supercritical CO_2 extraction is also an alternative for obtaining high-purity short, medium and long-chain triacylglycerol fractions [8]. Dry fractionation without solvents is preferred as a more neutral technique. Throughout the process, the target crystallization temperature and cooling rates are monitored. Temperature changes and a wide range of melting and solidification temperatures allow for the extraction of fractions with different compositions and properties [3, 7, 9, 10]. B.R. reading is one of the quality parameter covered under legal standards for ghee. 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 [11, 12]. Polenske value is the number of millilitres of 0.1N aqueous alkali solution required neutralizing the steam volatile and water insoluble fatty acids distilled from 5g of fat under specified conditions. The value for milk fat ranges from 1.2-2.4. Caprylic acid (C8:0) contributes up-to one-fourth and Capric acid (C10:0) contributes three-fourths to Polenske value. The iodine value is expressed as the grams of iodine absorbed per 100g of lipid. It gives a measure of the degree of unsaturation of a lipid. The higher the iodine value, the greater the number of C=C double bonds. The iodine value is normally used to know the degree of unsaturation of oils. Saponification value expressed as number of milligrams of KOH required to saponified one gram of fat is an indication of the average molecular weight and hence chain length of fatty acids present in oils and fats [13].

Materials and Methods

Materials

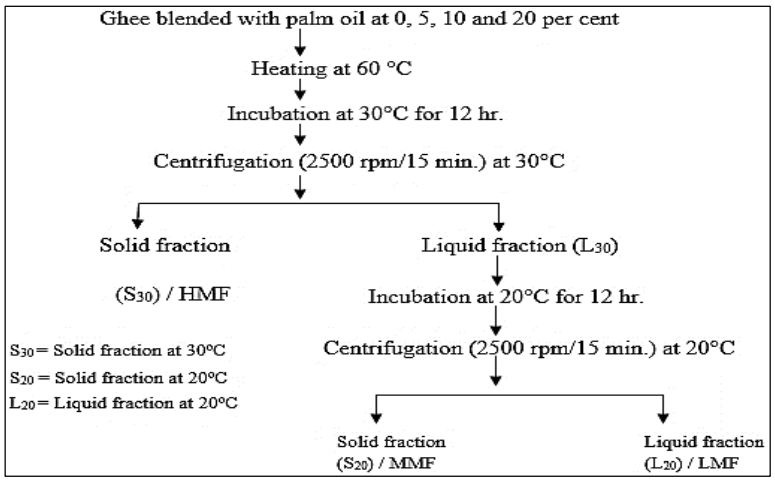
Butter was purchased from national brand from retail outlet near Hebbal, Bengaluru. Palm oil was purchased from the local Bengaluru market and used for the blended with ghee to their impact on physico-chemical characterization of ghee.

Methods

Preparation of samples

Butter was then heated on direct flame in a stainless-steel vessel and clarified into ghee with continous strring at a temperature of 115-117 °C. Ghee was then filtered through muslin cloths, cooled, filled in airtight glass bottles for futher analysis.

Dry fractionation technique



(Kankare, 1974)

Kankare, (1974) [14] with some modifications. Melting method was used to fractionate ghee. The crystal memory was removed by heating ghee to 60 °C. It was then progressively cooled to 30 °C in an incubator for 12 hr. to crystallize. After centrifugation at 2500 rpm for 15 min. in a temperature-controlled centrifuge kept at 30 °C, the liquid was separated from the crystals by decantation. At 30 °C, solid fraction obtained (S_{30}) was considered a high melting fraction. The liquid fraction collected at 30 °C was then incubated for a further 12 hr. at 20 °C. Further it was centrifugation at 2500 rpm for 15 min. in a temperature-controlled centrifuge kept at 20 °C. The produced crystals were separated. The solid portion obtained at 20 °C (S_{20}) was considered a medium melting fraction, whereas the amount that remained liquid at 20 °C was referred to as the low melting fraction (L_{20}).

Fig 1: Solid and liquid fraction

Physico-chemical characterization of palm oil, ghee and its fractions added with palm oil (S20 and L20)

The physico-chemical characterisation such as Butyro-refractometer reading, Iodine value, Reichert Meissl value, Polenske value and Saponification value was evaluated as per the methods described in ISI: SP: 18 (Part XI)-1981 [15].

Statistical analysis

Significant difference between the values was verified by one-way analysis of variance (ANOVA) and comparison between means was made by critical difference value by using R software [R. version 4.1.2 (2021-11-01), copyright © 2021, R foundation].

Results and Discussion

Physico-chemical characterization of ghee and fractions of ghee

The palm oil, control and fractions of ghee samples were analysed for physico-chemical constant namely Butyro-refractometer reading, Iodine value, Reichert Meissl value, Polenske value and Saponification value and data were presented in the (Table 1, 2, 3).

Butyro-refractometer (B.R.) Reading

The B.R. reading in ghee blended with palm oil (0, 5, 10 and 20%) were found to be 41.15, 41.79, 42.04 and 43.00, respectively (Table 1). Statistically there was a non-significant (P=.05) difference with respect to the B.R. Reading for all the sample. According to FSSAI Rules, (2021) [16] B.R. Reading of ghee was 40-44. B.R. reading of palm oil 44 (Table 1). According to FSSAI Rules, (2017) [17] B.R. Reading of palm oil was 35.5-44.0. The B.R. value is a basic value that relates to molecular weight, fatty acid, chain length, the degree of unsaturation and degree of conjugation. The B.R. value is subject to alteration to a greater extent by feed. The B.R. reading in ghee was directly proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). Our results were well correlated with the results of Sofia, (2005) [18], who also reported that B.R. reading of ghee adulterated with 5% palm oil ranged from 41.50-42.00 and with an average value 41.75. The B.R. reading of ghee adulterated with 10% palm oil 42.00. B.R. reading of ghee adulterated with 20% palm oil 43.00. Among the adulterants fat and oil (palm oil) showed the higher B.R. reading as

compared to sheep body fat. Higher the level of adulterant added, increase in the B.R. reading of ghee samples (Gandhi, 2014) [13].

The B.R. reading 44.72 in L_{20} with Palm oil (20%) liquid fraction as compared to other liquid fraction but statistically significant (*P*=.05) difference than other liquid fraction (Table 2) our results are corroborating well with the results of Gandhi, (2014) [13] reported that B.R. reading of control liquid fraction (L_4) in the range 41.10-42.40 with an average value 41.91. The B.R. reading of liquid fraction (L_4) adulterated with palm olein and sheep body fat with 10, 20 and 30% in the range 42.10-43.60, 43.10-45.10 and 43.20-45.30, respectively with an average value 42.98, 44.02 and 44.51, respectively. The B.R. reading 42.95 in S_{20} with Palm oil (20%) solid fraction as compared to other solid fraction but statistically significant (*P*=.05) difference than other solid fraction (Table 3). Similar values were reported by Gandhi, (2014) [13] B.R. reading of control solid fraction (S_{15}) in the range 40.10-42.00 with an average value 41.03. B.R. reading of solid fraction (S_{15}) adulterated with palm olein and sheep body fat with 10, 20 and 30% are 40.60-42.50, 41.60-43.80 and 42.30-44.20, respectively and with an average value 41.76, 42.66 and 43.39, respectively. B.R. reading is set in the range of 40.00 to 44.00 FSSAI Rules, (2021) [16]. However, the adulteration with palm oil even at 20% level was not detected under normal conditions. Whereas, using fractionation technique it could be detectable at 20% in liquid fraction.

Iodine value

The Iodine values in ghee blended with palm oil (0, 5, 10 and 20%) were found to be 32.40, 33.67, 34.76 and 37.90, respectively. Iodine values (I.V.) of ghee is set in the range of 25-38 FSSAI Rules, (2021) [16]. Iodine value of palm oil 54 (Table 1). According to FSSAI Rules, (2017) [17] Iodine values of palm oil was 45-56. The Iodine values in ghee were directly proportional with increasing level of adulteration with palm oil (5, 10 and` 20% respectively). Lakshminaryana and Rama Murthy, (1985) [19] and Bindal and Wadhwa, (1993) [20] reported that higher I.V. for liquid fraction than solid fractions and whole fats of cow and buffalo due to higher the content of unsaturated fatty acid in liquid fraction. Iodine value of ghee adulterated with 5% palm oil ranged for 33.15-33.41 with an average value 33.28. Ghee adulterated with 10 % palm oil ranged for 34.23-34.97 with an average value 34.60. Ghee adulterated with 20% palm oil ranged for 38.07-37.08 with an average value 37.58. Whereas, even at 20% level palm oil could not be detected (Sofia, 2005) [18].

The Iodine values 43.11 in L_{20} with Palm oil (20%) liquid fraction as compared to other liquid fraction but statistically significant (*P*=.05) difference than other liquid fraction (Table 2). Gandhi, (2014) [13] also reported that the I.V. of control liquid fraction (L_4) in the range 31.87-41.85 with an average value 36.79. Iodine value (I.V.) of liquid fraction (L_4) adulterated with palm olein and sheep body fat with 10, 20 and 30% in the range 36.12-45.15, 39.98-52.85 and 42.85-54.73, respectively with an average value 41.64, 45.90 and 49.36, respectively. The Iodine values 38.06 in S_{20} with palm oil (20%) solid fraction as compared to other solid fraction but statistically significant (*P*=.05) difference than other solid fraction (Table 3). Similar values were observed in I.V. of control solid fraction (S_{15}) in the range 30.04-40.90 with an average value of 35.46 and Iodine value (I.V.) of solid fraction (S_{15}) adulterated with palm olein and sheep body fat with 10, 20 and 30 % are 32.43-42.43, 37.84-48.93, 38.08-49.82, respectively with an average value 38.13, 42.10, 44.62, respectvely (Gandhi, 2014) [13]. The low iodine values for solid fraction and high iodine value for liquid fraction of pure ghee samples as compared to iodine values of control ghee may be possibly due to reason that the fractionation process helped in partitioning of triglycerides containing more of saturated fatty acids in to solid fraction and triglycerides containing more of unsaturated fatty acids in to liquid fraction on the basis of their melting points. According to FSSAI Rules, 2021 [16] Iodine value of ghee was 25 to 38, however, the adulteration with palm oil even at 20% level was not detected under normal conditions. Fractionation technique has offered advantage of increasing the sensitivity of Iodine value by lowering the detection limit up to 20% in liquid fraction and solid fraction of ghee sample.

Reichert-Meissl value

Reichert-Meissl values in ghee blended with palm oil (0, 5, 10 and 20%) were found to be 28.70, 27.43, 25.89 and 23.40, respectively. R.M. value of ghee minimum 24.0 as per FSSAI Rules, (2021) [6]. RM value of palm oil 0.00 (Table 1). Sofia, 2005 [18] reported that Reichert-Meissl values of palm oil 0.00. The Reichert-Meissl values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). It confines with work carried out by Sofia, (2005) [18] reported that R.M. value of ghee adulterated with 5% palm oil range from 26.50-28.05 and with an average value 27.28. The R.M. value of ghee adulterated with 10% palm oil range from 25.19-26.40 and with an average value 25.80. The R.M. value of ghee adulterated with 20% palm oil range from 22.33-24.31 with an average value 23.32. Lakshminaryana and Rama Murthy, (1985) [19] reported that both in cow and buffalo milk fats, solid fraction (high melting fractions) contained lower levels of butyric acid and other short chain fatty acids, while liquid fraction (low melting fractions) contained higher levels of these acids as compared to their respective whole fats. Palm oil added individually could be detected even at the level of 20% in ghee sample (Sofia, 2005) [18].

The Reichert-Meissl values in liquid fraction of ghee added with palm oil (0, 5, 10 and 20%) were found to be 32.50, 31.26, 30.02 and 26.09, respectively (Table 2). Statistical analysis revealed that there was a significant (*P*=.05) difference with respect to Reichert-Meissl values in liquid fraction of ghee. Sofia, (2005) [18] reported that ghee adulterated with 5, 10 and 20% palm oil after fractionation the liquid fraction (L_{25}) had 31.13, 30.03, 25.96, respectively. The R.M. values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). It confines with work carried out by Gandhi, (2014) reported that R.M. value of control liquid fraction (L_4) in the range 27.50-33.00 with an average value 29.41. R.M. value of liquid fraction (L_4) adulterated with palm olein and sheep body fat with 10, 20 and 30% in the range 24.09-32.07, 22.77-25.96 and 20.46-25.96, respectively with an average value 28.20, 25.16 and 23.27, respectively (Gandhi, 2014) [13]. The Reichert-Meissl values 20.86 in S_{20} with palm oil (20%) had statistically significant (*P*=.05) difference than other solid fraction (Table 3). Similar values were observed in ghee adulterated with 5, 10, 20% palm oil after fractionation at 25 ˚C R. M. value of solid fraction had 27.39, 26.62 and 23.65, respectively (Sofia, 2005) [18]. The Reichert-Meissl values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20% respectively). It confines with work carried out by Gandhi, (2014) [13] reported that R.M. value of control solid fraction (S_{15}) in the range 26.40-32.78 with an average value 29.23 and the R.M value of solid fraction (S_{15}) adulterated with palm olein and sheep body fat with 10, 20 and 30% are 21.01-29.37, 20.57-23.76

and 16.39-23.54, respectively with an average value 25.52, 22.18, 20.06, respectively. Reichert-Meissl value obtained in the present study comparable with standard prescribed for FSSAI rules (2021) [16] min.24, However, the adulteration with palm oil even at 20% level was detected under normal conditions. Fractionation technique has detection limit up to 20% in solid fraction of ghee sample.

Polenske value

The Polenske value in ghee blended with palm oil (0, 5, 10 and 20%) were found to be 1.47, 1.29, 1.16 and 1.01, respectively. Statistical analysis revealed that there was a significant (*P*=.05) difference with respect to Polenske value among different ghee sample. P.V. value of ghee 0.5-2.0 according to FSSAI Rules, (2021) [16]. PV value 1.0-2.0 of all the grade of ghee in AGMARK, (1983) [17] standard. Polenske value of palm oil 0.75 (Table 1) Sofia, 2005 [18] reported that Polenske value of palm oil 0.70. The Polenske values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). It confines with work carried out by Gandhi, 2014 [13] reported that P.V. not offer any advantage lowering the detection limit of adulteration on basis solid and liquid fraction. Polenske value (P.V.) of mixed (cow and buffalo) ghee ranged from 1.20-1.70 and with an average value 1.43. whereas the adulterated ghee with palm olein 5, 10 and 15% with an average value were 1.33, 1.19 and 1.05%, respectively.

The Polenske value in liquid fraction of ghee added with palm oil (0, 5, 10 and 20%) were found to be 1.23, 1.00, 0.87 and 0.72, respectively. Statistical analysis revealed that there was a significant (*P*=.05) difference with respect to Polenske value among different liquid fraction of ghee sample. The Polenske value in ghee is inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). It confines with work carried out by Gandhi, (2014) [13] described that Polenske value (P.V.) of control liquid fraction (L_4) in the range 1.00-1.30 with an average value of 1.09. Polenske value in liquid fraction (L_4) adulterated with palm olein and sheep body fat with 10, 20 and 30% in the range 0.90-1.20, 0.60-1.00 and 0.50-0.80, respectively with an average value 1.01, 0.82 and 0.68, respectively. The Polenske value in solid fraction of ghee added with palm oil (0, 5, 10 and 20%) were found to be 1.38, 1.10, 0.96 and 0.86, respectively. Statistical analysis revealed that there was a significant (*P*=.05) difference with respect to Polenske value among different solid fraction of ghee sample. The Polenske values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). It confines with work carried out by Gandhi, (2014) [13] reported that Polenske value (P.V.) of control solid fraction (S_{15}) in the range 1.10-1.50 with an average value 1.30. Polenske value (P.V.) of solid fraction (S_{15}) adulterated with palm oil and sheep body fat with 10, 20 and 30% are 1.00-1.30, 0.70-1.10 and 0.60-1.0% with an average value 1.10, 0.95, 0.83, respectively. Polenske value could be used as an indicator for checking adulteration of palm oil in ghee. However, it was observed from the study that using this parameter the fractionation process did not offer any advantage in lowering the detection limit of adulteration on the basis of both solid and liquid fractions.

Table 1: Physico-chemical characterisation of ghee added with palm oil

Sample	Butyro refractometer reading	Iodine value	Reichert-Meissl value	Polenske value	Saponification value
Palm oil	44.00^{a}	54.00^{a}	0.00^{e}	0.75^{e}	197.30^{e}
Control ghee	41.15^{a}	32.40^{e}	28.70^{a}	1.47^{a}	227.04^{b}
Ghee with PO (5%)	41.79^{a}	33.67^{d}	27.43^{b}	1.29^{b}	227.69^{a}
Ghee with PO (10%)	42.04^{a}	34.76^{c}	25.89^{c}	1.16^{c}	226.41^{c}
Ghee with PO (20%)	43.00^{a}	37.90^{b}	23.40^{d}	1.01^{d}	221.85^{d}
CD (*P*=.05)	1.81	0.63	0.55	0.01	0.55

Table 2: Physico-chemical characterization of liquid fraction of ghee added with palm oil

Sample	Butyro-Refractometer reading	Iodine value	Reichert-Meissl value	Polenske value	Saponification value
L20 (Control)	41.94^{d}	32.80^{d}	32.50^{a}	1.23^{a}	231.86^{a}
L20 with PO (5%)	42.65^{c}	34.40^{c}	31.26^{b}	1.00^{b}	227.14^{b}
L20 with PO (10%)	42.86^{b}	36.40^{b}	30.02^{c}	0.87^{c}	224.07^{c}
L20 with PO (20%)	44.72^{a}	43.11^{a}	26.09^{d}	0.72^{d}	220.18^{d}
CD (*P*=.05)	0.05	0.50	0.51	0.05	0.47

Table 3: Physico-chemical characterization of solid fraction of ghee added with palm oil

Sample	Butyro refractometer reading	Iodine value	Reichert-Meissl value	Polenske value	Saponification value
S20 (Control)	41.05^{d}	31.47^{d}	27.80^{a}	1.38^{a}	226.67^{a}
S20 with PO (5%)	41.70^{c}	33.61^{c}	26.56^{b}	1.10^{b}	225.64^{b}
S20 with PO (10%)	41.96^{b}	34.17^{b}	24.79^{c}	0.96^{c}	223.21^{c}
S20 with PO (20%)	42.95^{a}	38.06^{a}	20.86^{d}	0.86^{c}	219.45^{d}
CD (*P*=.05)	0.01	0.40	0.78	0.10	0.54

Saponification value

Saponification values in ghee blended with palm oil (0, 5, 10 and 20%) were found to be 227.04, 227.69, 226.41 and 221.85, respectively. S.V. value of ghee 205-235 according to FSSAI Rules, (2021) [16]. S.V. value of palm oil 197.30 (Table 1). S.V. value of palm oil 195-205 (FSSAI Rules, (2017) [17]. Statistical analysis revealed that there was a significant (*P*=.05) difference with respect to Saponification values among different ghee sample. The Saponification values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). It confines with work carried out by Gandhi, (2014) [13] reported that S.V. not offer any advantage lowering the detection limit of adulteration on basis solid and liquid fraction. Saponification value (S.V.) in ghee, adulterated with 5% palm oil ranged for 229.35-225.96 with an average value 227.66. Ghee adulterated with 10% palm oil ranged for 223.41-226.12 with an average value 26.31. Ghee adulterated with 20% palm

oil ranged for 223.41-226.12 with an average value 221.77 (Sofia, 2005) [18]. Saponification value (SV) of mixed (cow and buffalo) ghee ranged from 226.10-233.68 with an average value 230.42. when ghee adulterated with palm olein 5, 10 and 15% then Saponification value (SV) in the range 225.40-231.42, 224.00-229.70, 222.80-227.92 with an average value 228.78, 227.09 and 225.51, respectively (Gandhi, 2014) [13].

The Saponification values in liquid fraction of ghee added with palm oil (0, 5, 10 and 20%) were found to be 231.86, 227.14, 224.07 and 220.18, respectively. Statistical analysis revealed that there was a significant (*P=.05*) difference with respect to Saponification values among different liquid fraction of ghee sample. The Saponification values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10 and 20%, respectively). It confines with work carried out by Gandhi, (2014) [13] reported that Saponification value (S.V.) of liquid fraction (L_4) in the range 224.70-232.26 with an average value 229.00 and Saponification value (S.V.) of liquid fraction (L_4) adulterated with palm oil and sheep body fat with 10, 20 and 30% in the range 224.58-230.52, 221.06-226.42 and 217.34-224.52 with an average value 226.93, 223.90 and 219.83, respectively. The higher SV observed in liquid fraction might be due to an increase in the proportion of lower chain fatty acid or a decrease in that of higher fatty acids like palmitic and stearic acids either individually or collectively (Narayanrao, 2007) [21]. The Saponification values in solid fraction of ghee added with palm oil (0, 5, 10 and 20%) were found to be 226.67, 225.64, 223.21 and 219.45, respectively. Statistical analysis revealed that there was a significant (*P*=.05) difference with respect to Saponification values among different solid fraction of ghee sample. The Saponification values in ghee were inversely proportional with increasing level of adulteration with palm oil (5, 10, 20% respectively). It confines with work carried out by Gandhi, (2014) [13] described that Saponification value (S.V.) of control solid fraction (S_{15}) in the range 224.14-231.98 with an average value 228.57 and Saponification value (SV) of solid fraction (S_{15}) adulterated with palm olein and sheep body fat with 10, 20 and 30% are 224.30-229.16, 220.36-225.58 and 217.28-224.24, respectively and with an average value 226.54, 223.21 and 219.41, respectively. Saponification values are not a very reliable parameter for checking adulteration in milk fat. Further, using this parameter, fractionation process has not helped in lowering the detection limit of adulteration on the basis of solid and liquid fraction.

Conclusions

In the present investigation ghee blended with palm oil at the level 0, 5, 10 and 20 per cent. RM value could be used as an indicator for checking adulteration in milk fat, at 20% levels in samples. Fractionation technique has offered advantage of increasing the sensitivity of Butyro-refractometer reading and Iodine value lowering the detection limit 20% levels in ghee samples. Finally, by using dry fractionation technique the detection limit 20%, which could be till reduced by using sterol content as a tool for detection.

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