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RESEARCH ARTICLE

Quality attributes of ghee residue prepared using Deoni and Holstein Friesian crossbred cow milk as influenced by method of preparation

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Abstract: Ghee residue (GR), a dairy by-product, was found to be nutrient dense. The ghee residue composition could be affected by clarification temperature, method of ghee preparation and breed of the cow. So, the present study was conducted to know whether the clarification temperature (110°C and 120°C), methods of ghee preparation (direct cream and creamery butter), and breed of the cow (Indigenous Deoni and Holstein Friesian crossbred) can affect the composition of ghee residue. The moisture, protein, fat, lactose and ash content of various ghee residue samples prepared from the milk of Deoni and HF crossbred cows varied from 10.50±0.22 to 14.65±0.1, 13.49±3.37 to 34.17±3.37, 51.61±0.86 to 67.69±0.09, 9.44±0.06to14.15±0.22 and 1.89±0.03to 5.59±0.41%, respectively. Further, the phospholipid content and antioxidant activity varied from 3.18 to 15.09 and 27.04 to 67.86%, respectively. In this study, it was found that temperature of clarification had a significant ($p<0.05$) effect on moisture, lactose, ash, hydroxy methyl furfural (HMF) and browning index. The clarification temperature significantly affected the protein, fat, lactose, ash, phospholipids and antioxidant activity. The protein, fat and browning index of ghee residue were also significantly different for the breeds. Thus, it can be concluded that GR's composition was affected by breed and method of ghee preparation.

Keywords: Antioxidants, Ghee residue, Holstein Friesian crossbred, Indigenous Deoni, Phospholipids

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Introduction

Milk is either utilized directly for consumption or converted into various products which can result in by products. One of the dairy products by name ghee is manufactured by utilizing 30 to 35% (Gandhi et al. 2013) of the total milk collected which leads to production of huge quantity of ghee residue. Ghee residue accounted for more than 3MT per annum (Verma and Raju 2008). Ghee residue; a slightly brown to darker brown coloured by-product of dairy industry is obtained by the conversion of cream or butter into ghee after the process of clarification. Prahlad (1954) reported the differences in ghee residue composition obtained by different methods of ghee making such as desi method, creamery butter, direct cream. The ghee residue obtained after processing of cream/butter was found to be rich in nutrients such as lactose (2 – 14%), protein (12 – 39%), fat (32 – 70%), moisture (8 – 30%), ash (1 – 8%) (Prahlad 1954). Santha and Narayanan (1978) reported that hand pressed ghee residue obtained from direct cream method had higher fat content than the ghee residue obtained from creamery butter method. But moisture, ash, proteins were higher in the latter (Prahlad 1954, Santha and Narayanan 1978). However, it was found that the temperature and method of clarification can affect the nutrient composition of the ghee residue. Compositional variation in ghee residue was also linked to raw material (Mani 1952).

The ghee residue obtained after processing of cream/butter was found to be rich in nutrients such as protein (25.07%), fat (50.25%), moisture (13.28%), ash (8.24%) (Ranjan et al. 2010). Selvamani et al. (2017) also reported that ghee residue contains 40.69 % fat and 24.32% protein. According to Munirathnamma et al. (2017) ghee residue is composed of 35.99 % fat, 17.88 % lactose, 3.81 % ash, 25.29 % protein (dmb). Janghu et al. (2014) reported that ghee residue prepared from direct cream showed 26.64 % moisture, 33.13 % fat, 3.27 % ash, 30.91 % protein whereas the ghee residue obtained from creamery butter was found to have 17.71 % moisture, 41.83 % fat, 2.56 % ash, and 31.69 % protein. The wide variation for the amount of various constituents was observed due to variation in method of preparation.

Apart from major nutrients, the method of preparation is expected to affect the minor constituents as well. It has been reported that

heating time and temperature used for ghee clarification can also affect the phospholipid content, due to the movement of phospholipids from GR to ghee (in minute amounts due to their polar nature) when ghee is clarified for longer time (Santha and Narayanan 1979). Also, the antioxidant activity of GR is expected to be affected by ghee manufacture method as it depends on the intensity of the brown coloured pigments such as melanoidins and reductones (Kiriyaga et al. 1968).

So, these differences in composition, prompted us to undertake the work on the characterization of ghee residue, which is obtained from the processing of indigenous (Deoni) *vs* HF crossbred cow's milk concerning temperature and method of making. Also, there are limited reports on the properties of ghee residue obtained from these breeds (indigenous and HF crossbred). Thus, the present article explores the differences in the physico-chemical characteristics of ghee residue as affected by the method of preparation for both Deoni and HF crossbred cows.

Materials and Methods

Milk and Ghee residue

The pooled milk was collected from 16 Deoni cows and 11 HF crossbred cows from the Livestock research centre of Southern Regional Station, ICAR-NDRI, Bengaluru. The animals were in their 2nd, 3rd and 4thlactations. The cream obtained by separation of Deoni and HF milk was collected from the dairy plant using a cream separator (Noe Tech Int. Pvt. Ltd, Haryana, India). Ghee was prepared by direct cream and creamery butter methods. For the creamery butter method, the butter having minimum 80-85 % fat was prepared from aged cream. Ghee clarification was done at two different temperatures; 110°C and 120°C. Ghee was then filtered through muslin cloth and residual ghee was removed by gently pressing the ghee residue in muslin cloth. The ghee residue obtained in previous step was further pressed using a hydraulic press (Multipurpose machine, Milk Tech Engineers, Bangalore, India) at 4 kg/cm^2 for 5 min to remove the entrapped ghee from ghee residue. Eight different samples of ghee residue were obtained in the study *viz*. ghee residue obtained by processing of Deoni milk by direct cream and creamery butter method, and ghee residue obtained by processing of HF crossbred milk by direct cream and creamery butter method for two different clarification temperatures, respectively.

Proximate composition

The pressed ghee residue samples were pooled for three replicates for further analysis. The collected samples were evaluated for moisture, protein, fat, ash and lactose content following the AOAC (2003) methods. All the parameters were analyzed in triplicates.

Phospholipid estimation

The estimation of phospholipids was performed by the method of Sharma et al. (2007). The fat was extracted by the process of Rose Gottlieb method. The extracted fat was digested in kjeldahl flask by adding sulphuric acid (0.5%) till the colour of the sample was colourless to light yellow in colour. The 5 ml of the aliquot was taken from the digested sample, 4ml 0.44% ammonium molybdate solution was mixed and 0.4 ml of reducing agent was also added before determining the absorbance in the UV-Visible spectrophotometer (LABINDIA Analytical UV 3200XE, India) at 720nm. Phospholipids content was estimated using the equation 1.

$$\text{Phospholipid(mg/100g)} = \frac{\text{O.D of sample}}{\text{O.D of standard}} \times 0.1 \times \frac{1000}{W} \times 25.9 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, W= weight of fat extracted from ghee residue

Antioxidant activity

For determining the antioxidant activity by the method of Shimada et al. (1992), the stock solution of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) dye was prepared, from which 1 ml was taken and volume was made up to 100 ml to get working solution. Around 5000 mg of ghee residue sample was macerated using 10 ml of methanol, followed by centrifugation at 4000 rpm for 30 minutes. For measuring the absorbance using spectrophotometer (LABINDIA Analytical UV 3200XE, India) at 517 nm, 0.1 ml of supernatant and 3ml of working solution was mixed and incubated at 25 to 30°C for 30 minutes. Antioxidant activity, per cent DPPH free radical scavenging activity was measured using the formula given in equation 2.

$$\text{Antioxidant activity (\% scavenging of DPPH)} = A_0 - \frac{A_1}{A_0} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, A_o = absorbance of blank; A_1= absorbance of sample

Hydroxy methyl furfural

Hydroxy methyl furfural (HMF) was determined by Keeney and Bassette (1959) method. In this method, 1g of ghee residue was mixed with 9.5 ml of distilled water,5ml of oxalic acid and boiled in waterbath for 60 minutes. To this, 5ml of 40% tricarboxylic acid was added and filtration was done using whatman filter paper No. 42. Then, 0.5 ml of supernatant and 1ml of 0.05M of TBA was mixed, followed by heating in water bath for 40 minutes at 40°C. The absorbance was measured at 443nm using UV-Visible spectrophotometer (LABINDIA Analytical UV 3200XE, India). The standard curve for HMF was prepared using concentrations from 0 to 35 µmole/ml and the amount of HMF in ghee residue

sample was determined using the absorbance values and standard curve.

Browning index

Ghee residue samples were scanned in a scanner (Canon) in triplicates. Browning index values were analysed using the colour values L, a, and b. The observed parameters were: L* (luminosity or brightness L* = 0 black and L*= 100 white), a* = (red green component – a* = greenness and a+ = redness) and b* yellow – blue component, b* blueness, and +b* yellowness. The formulas used to calculate L* a* b*, chroma values and browning index (Yam and Papadakis 2004) are mentioned below in equations 3 to 7.

$$L^* = 100 \times \frac{L}{255} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$a^* = \frac{240 \times a}{255} - 120 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$b^* = \frac{240 \times b}{255} - 120 \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$\text{chroma} = [(a^*+b^*)]^{1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$\text{Browning index (BI)} = 100 \times \text{chroma} - \frac{0.31}{0.17} \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Statistical analysis

The entire experiments were performed in triplicates and means and standard deviations/errors were calculated. The pooled milk sample for each breed was used for ghee preparation by two different methods. For each method, ghee was prepared in triplicates and collected GR was pooled for estimation of various parameters. From this pooled GR, each parameter was further analysed in triplicates. All statistical analyses were performed using SPSS software and statistical significance was set at $p<0.05$. The least significant difference (LSD) test was used to find out significant differences between sample means. Analysis of variance (ANOVA) was used to determine differences among treatment means using the Post Hoc Test (Duncan).

Results and Discussion

The ghee residues (GR) were obtained from the Indigenous (Deoni) *vs* Holstein Friesian (HF) Crossbred cows' milk processing by direct cream and creamery butter methods. The clarification temperatures of 110°C and 120°C were used for ghee preparation and the obtained samples were subjected to proximate analysis. The results obtained are mentioned in the below sections.

Proximate composition of ghee residue prepared at 110°C clarification temperature

Among the analysed samples, highest moisture content was observed in ghee residue samples prepared by creamery butter method, i.e. CB-Deoni (14.65%) and CB- HFC (14.35%) followed by DC-Deoni (12.71%) and DC-HFC (10.91%) (Table 1). The presence of higher amount of protein in ghee residue samples obtained from creamery butter method might have the ability to hold more moisture. There were significant differences ($p<0.05$) in the samples for protein, fat, lactose and ash content. Protein (34.17%) and ash content (3.87%) of CB-Deoni was significantly highest ($p<0.05$) and this could probably be due to retention of curd particles of butter in the GR. The DC-HFC ghee residue had highest fat content (67.69%) which may be attributed to the losses of fat from ghee to ghee residue samples obtained by direct cream method. The DC-Deoni ghee residue had the highest lactose content (10.68%), which may be due to the presence of solids not fat in higher amounts (4.53%). Several authors (Relwani 1978, Santha and Narayanan 1978, Grewal 1979) have reported a wide variation in ghee residue composition *viz.* 12 to 39% protein, 1 to 8% minerals, 2 to 14% lactose, 32 to 70% fat.

Phospholipids in ghee residue samples prepared at 110°C clarification temperature

Phospholipids are the lipids which have phosphate groups in their structure. They are amphiphilic in nature with hydrophilic moiety as polar head and glycerol and fatty acid as hydrophobic tail (Krishnegowda et al. 2021). They are surface active due to their amphiphilic nature. In this study, significant differences ($p<0.05$) were observed among all the four samples (Figure 1).

Table 1 Proximate composition of ghee residue samples prepared at 110°C clarification temperature

Sample	Moisture (%)	Protein (%)	Fat (%)	Lactose (%)	Ash (%)
DC- Deoni	12.71±0.1^{b}	26.72±3.37^{a}	59.88±0.09^{b}	10.68±0.06^{a}	2.63±0.03^{b}
DC-HFC	10.91±0.1^{c}	13.49±3.37^{b}	67.69±0.09^{a}	10.23±0.06^{b}	1.89±0.03^{d}
CB-Deoni	14.65±0.1^{a}	34.17±3.37^{a}	51.99±0.09^{d}	9.97±0.06^{c}	3.87±0.03^{a}
CB-HFC	14.35± 0.1^{a}	28.89±3.37^{a}	59.33±0.09^{c}	9.44±0.06^{d}	2.33±0.03^{c}

(N=12, Results are expressed as Mean ± SE, with different small letters superscript (a,b,c) within row differ significantly ($P< 0.05$) among the samples. Where, DC- Deoni- ghee residue obtained from processing of Deoni cow's milk by direct cream method; DC- HFC – ghee residue obtained from processing of HF crossbred cow's milk by direct cream method; CB- Deoni – ghee residue obtained from processing of Deoni cow's milk by creamery butter method; CB-HFC- ghee residue obtained from processing of HF crossbred cow's milk by creamery butter method)

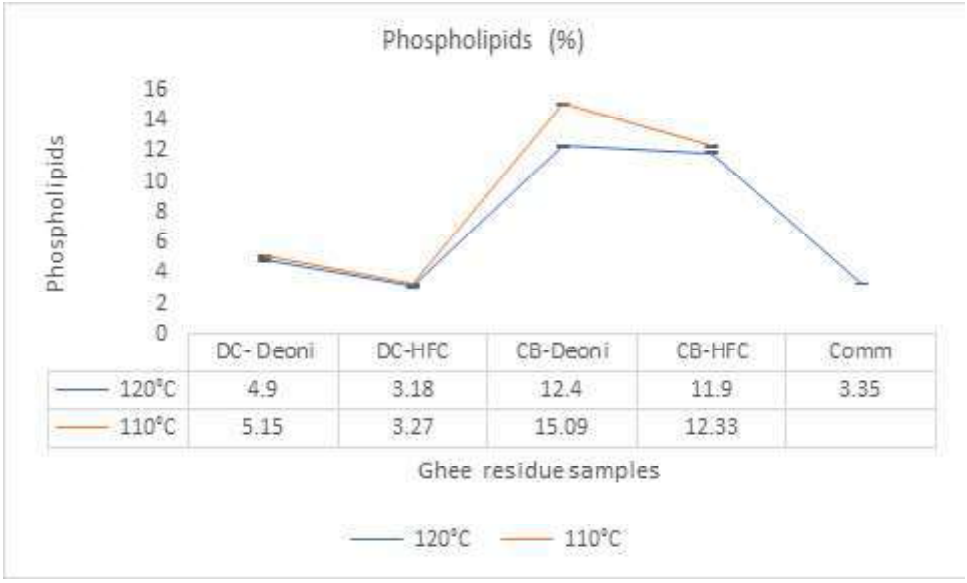


Fig. 1 Phospholipids content of ghee residue samples prepared at 110°C and 120°C clarification temperature

(n=12 for 110°C clarification, n= 15for 120°C clarification samples, results are expressed as Mean ± SE)

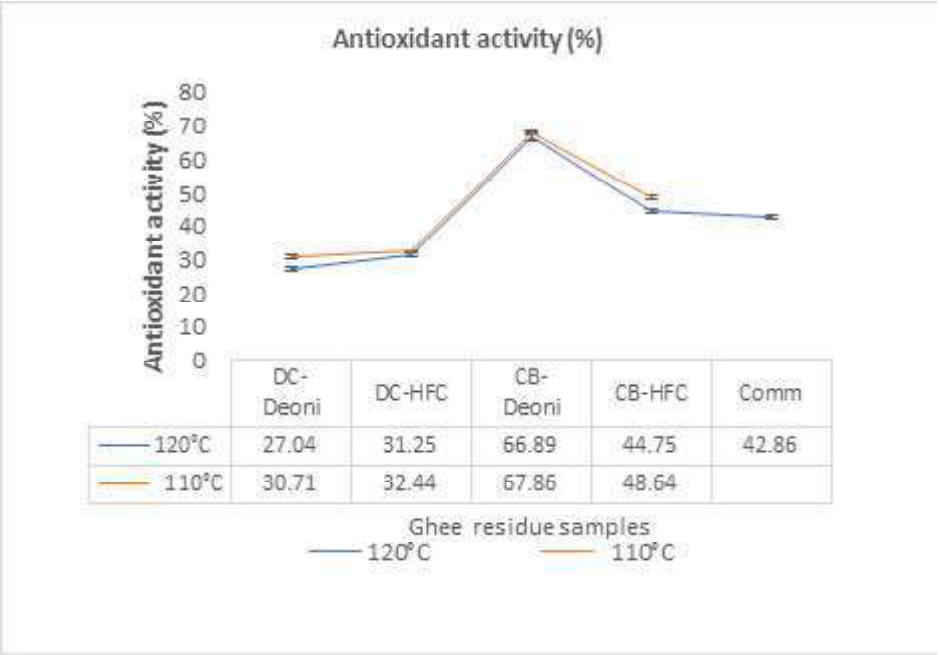


Fig. 2 Antioxidant activity of ghee residue samples prepared at 110°C and 120°C clarification temperature

(n=12 for 110°C clarification, n= 15for 120°C clarification samples, results are expressed as Mean ± SE)

CB-Deoni ghee residue sample had the highest phospholipid content (15.09%) compared to other samples such as CB- HFC (12.33%), DC-Deoni (5.15%) and DC-HFC (3.27%). This shows that ghee residue obtained by creamery butter method (13.71%) had higher content of phospholipids than ghee residues obtained by direct cream method (4.21%). Santha and Narayanan, (1978) also reported higher levels of phospholipids for the ghee residue prepared by creamery butter (17%) than direct cream method (1.5%). According to Jenness and Patton (1984), the phospholipid content in milk varies from 0.2 to 1%. The presence of phospholipids in the ghee residue might be due to the rupture of milk fat globule membranes owing to the leaching and hence, concentration of phospholipids in the ghee residue

Antioxidant activity of ghee residue samples prepared at 110°C clarification temperature

Antioxidants are substances that can delay or prevent the oxidation reactions of food product. The antioxidant activity of CB-Deoni (67.86%) sample was highest, followed by CB- HFC (48.64%), DC-HFC (32.44%), and DC-Deoni (30.71%). There were significant differences ($p<0.05$) in all the four samples (Figure 2). The antioxidant activity was significantly highest in ghee residue samples prepared from creamery butter method than direct cream method, which may be attributed to the higher presence of phospholipids in the creamery butter ghee residue (~15% phospholipids). According to Santha and Narayanan (1979), the

phospholipids also act as antioxidant. Other than phospholipids, presence of tryptophan, amino acids, cysteine hydrochloride lysine, proline etc. are also responsible for the antioxidant activity (Kiriyaga et al. 1971, Yamaguchi et al. 1981).

Hydroxy methyl furfural (HMF) content in ghee residue samples prepared at 110°C clarification temperature

The HMF compounds are those which are formed due to maillard reactions or dehydration of reducing sugars. HMF is most common in foods which contains sugars and have been exposed to high temperature during processing. The ghee residue samples such as DC-Deoni (38.1), CB- Deoni (37.47), CB-HFC (37.17) had significantly higher ($P<0.05$) HMF content (µmole/mg) than DC-HFC (34.03) (Figure 3). This shows that sugar caramelization and maillard reactions were least in DC-HFC compared to the other three samples. This could be correlated with least protein content in DC-HFC ghee residue than all other samples (Table 1), as it is well known that free amine groups are essential for forming maillard reaction products.

Browning index of ghee residue samples prepared at 110°C clarification temperature

The browning intensity of the product can be measured by browning index (BI). The browning of the product occurs due to maillard reactions and/or caramelization of sugars. CB-HFC (34.26), DC-HFC (33.75) samples had significantly ($p<0.05$) higher browning index than CB-Deoni (32.64), DC-Deoni (32.55) (Figure 4). Thus, it is evident that the BI of the ghee residue samples obtained from the processed Deoni cow's milk was lower than the HF crossbred cow's milk. This could be due to the presence of more free amine groups in the latter for maillard reaction.

Proximate composition of ghee residue prepared at 120°C clarification temperature

Ghee residues prepared in laboratory, clarified at 120° C were compared with commercial ghee residue sourced from a dairy plant (Table 2). Ghee residue samples obtained by creamery butter method i.e. CB- Deoni and CB- HFC had higher moisture content compared to the samples prepared by direct cream method which may be attributed to the presence of higher protein and their ability to hold water. But moisture content of commercial ghee residue samples was significantly ($p<0.05$) lowest. This may be due to the removal of fat from ghee residue during pressing for ghee recovery which might have resulted in oozing out of the moisture along with fat. Highest protein content (33.86%) was observed in CB- Deoni sample, which may be due to the presence of curd particles from butter. The DC- HFC had significantly higher fat (66.11%) compared to all the samples. Losses of fat occur from ghee to ghee residue samples in direct cream method which might have resulted in higher fat content. CB- Deoni and CB-HFC had significantly higher lactose content (14.15%, 13.60%) which can be due to presence of solids not fat (4.53%) in cream. The ash content (7.15%) was significantly high ($p<0.05$) in commercial ghee residue samples which can be due to the extreme treatments given to the ghee residue and low moisture content (Table 2). Several authors (Relwani 1978, Santha and Narayanan 1978, Grewal 1979) have reported on wide variation in ghee residue compositions *viz*.; 12 to 39% protein, 1 to 8% minerals, 2 to 14% lactose, 32 to 70% fat. Thus, the obtained ranges of composition of ghee residue are comparable with previous findings.

Phospholipids of ghee residue samples prepared at 120°C clarification temperature

Cow milk contains phospholipids in the range 0.2 to 1% (Jenness and Patton 1984). The concentration of phospholipids in the ghee residue may be due to the rupture of milk at globular membrane which leads to the leaching of phospholipids into the ghee residue. Significant differences were observed among all the ghee residue samples. However, highest phospholipid content ($p<0.05$) was observed in CB-Deoni (12.4%) compared to other samples *viz*; CB-HFC (11.9%), DC-Deoni (4.9%), DC-HFC (3.18%) and commercial sample (3.35%) (Figure1). Overall, ghee residue samples obtained by creamery butter and direct cream method had phospholipids content up to 12.15% and 4.04%, respectively. The results are comparable with the previous findings of Santha and Narayanan (1978) who reported 17% and 1.5% of phospholipids content in ghee residues obtained by creamery butter and direct cream method, respectively.

Table 2 Proximate composition of ghee residue samples prepared at 120°C clarification temperature

Sample	Moisture (%)	Protein (%)	Fat (%)	Lactose (%)	Ash (%)
DC- Deoni	11.92±0.22^{b}	25.73±0.21^{d}	59.41±0.86^{b}	11.92±0.22^{b}	5.16±0.41^{d}
DC- HFC	10.50±0.22^{c}	19.87±0.21^{e}	66.11±0.86^{a}	10.50±0.22^{c}	4.4±0.41^{e}
CB- Deoni	14.16±0.22^{a}	33.86±0.21^{a}	51.61±0.86^{d}	14.15±0.22^{a}	5.59±0.41^{b}
CB- HFC	13.6±0.22^{a}	27.22±0.21^{c}	58.67±0.86^{b}	13.60±0.22^{a}	5.31±0.41^{c}
Comm	8.77±0.22^{d}	29.44±0.21^{b}	55.33±0.86^{c}	8.14±0.22^{d}	7.15±0.41^{a}

(N=15, Results are expressed as Mean ± SE, with different small letters superscript (a,b,c) within rows differ significantly ($P< 0.05$) among the samples. Where, DC- Deoni- ghee residue obtained from processing of Deoni cow's milk by direct cream method; DC- HFC – ghee residue obtained from processing of HF crossbred cow's milk by direct cream method; CB- Deoni – ghee residue obtained from processing of Deoni cow's milk by creamery butter method; CB-HFC- ghee residue obtained from processing of HF crossbred cow's milk by creamery butter method; Comm- ghee residue obtained from a commercial dairy plant)

Fig. 3 Hydroxy methyl furfural (µmole/mg) in ghee residue samples prepared at 110°C and 120°C clarification temperature

(n=12 for 110°C clarification, n= 15for 120°C clarification samples, results are expressed as Mean ± SE)

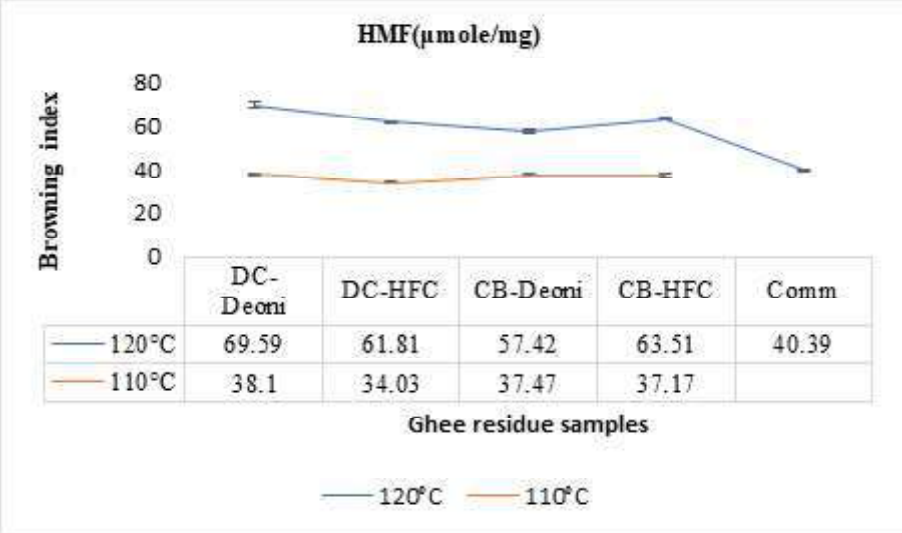
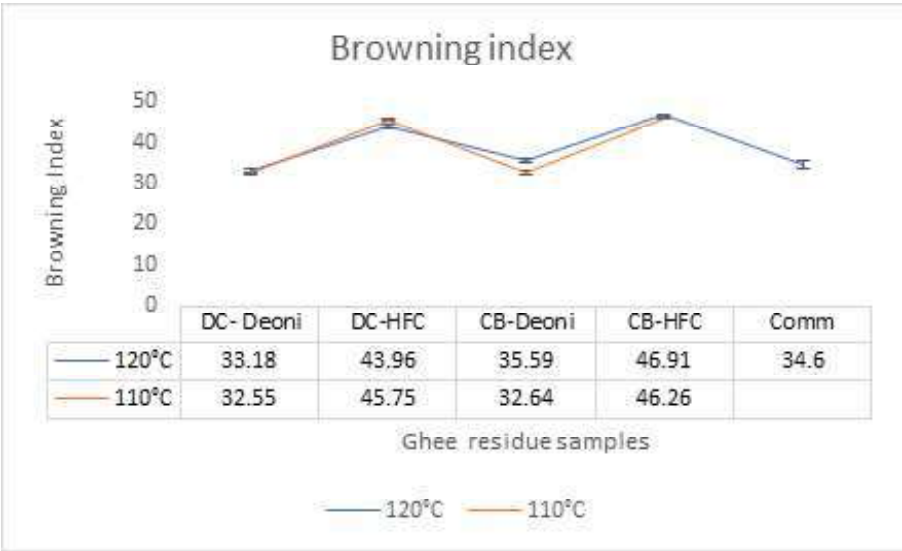


Fig.4 Browning index of ghee residue samples prepared at 110°C and 120°C clarification temperature

(n=12 for 110°C clarification, n= 15for 120°C clarification samples, results are expressed as Mean ± SE)



Antioxidant activity of ghee residue samples prepared at 120°C clarification temperature

The amino acids and condensation of sugars are one of the reasons for antioxidant activity (Kiriyaga et al. 1971, Yamaguchi et al. 1981). The CB-Deoni (66.89%) had the highest ($P<0.05$) antioxidant activity followed by CB-HFC (44.75 %), DC-HFC (31.25%), DC-Deoni (27.04%) and commercial sample (42.86%) (Figure 2). Significant ($p<0.05$) differences were observed among the five samples. Due to the higher presence of phospholipids in creamery butter ghee residue, the antioxidant activity tends to be higher than the ghee residue samples obtained by direct cream method. Santha and Nayrayanan (1979) reported that phospholipids were the main causes of antioxidant activity. Antioxidant activity decreases as temperature of clarification increases as there seems to be a movement of phospholipids from ghee residue to ghee. Since ghee residue contains both lactose and certain protein in the form of curd particles, this leads to development of brown colour pigments with antioxidant activity upon clarification of ghee. From the results obtained for the antioxidant activity, it can be inferred that the products obtained using DC-Deoni ghee residue may help in extension of shelf life owing to high antioxidant activity.

Hydroxy methyl furfural (HMF) content of ghee residue samples prepared at 120°C clarification temperature

The HMF compounds causes browning in the product, due to the reactions occurring between amino and carbonyl compounds (Keeney and Bassette 1959). The browning compound: 5-hydroxy methyl-2-furfural (HMF) also exhibits an antioxidant property. Among all the samples, the HMF content (65.59µmole/mg) in DC- Deoni was the highest (Figure3). The significant differences ($P<0.05$) were observed in all the five samples and HMF content ranged from 40.39 to 69.59 µmole/mg. The HMF content in ghee residues may be attributed to browning due to the degradation

Table 3 Quality characteristics of ghee residue samples as affected by the breed of cow, method followed for ghee preparation and clarification temperature

Parameter	Variation due to breed		Variation due to method of ghee preparation		Variation due to clarification temperature	
	Deoni (indigenous breed) cows	HF crossbred cows	Direct cream method	Creamery butter method	110°C clarification temperature	120°C clarification temperature
Moisture	13.36 ± 0.33^{a}	12.34± 0.51^{a}	11.51 ± 0.28^{a}	13.10 ± 0.53^{a}	13.15 ± 0.45^{a}	11.79± 0.44^{b}
Protein	30.12 ± 1.18^{a}	22.37± 2.34^{b}	21.45 ± 2.15^{b}	30.71 ± 1.25^{a}	25.82 ± 2.70^{a}	27.22± 1.50^{a}
Fat	57.2 ± 1.19^{b}	62.95± 1.23^{a}	63.27 ± 1.13^{a}	55.38 ± 1.27^{b}	59.72± 1.68^{a}	58.22 ± 1.29^{a}
Lactose	11.68 ± 0.47^{a}	10.94± 0.49^{a}	10.83 ± 0.22^{b}	11.06 ± 0.52^{a}	10.08± 0.13^{a}	11.66± 0.54^{b}
Ash	4.31 ± 0.34^{a}	3.48± 0.42^{a}	3.52 ± 0.39^{b}	4.85 ± 0.39^{a}	2.68 ± 0.22^{b}	5.52 ± 0.13^{a}
Phospholipids	9.38 ± 1.34^{a}	7.67± 1.34^{a}	4.12 ± 0.27^{b}	11.01 ± 1.06^{a}	8.96 ± 1.47^{a}	7.14± 1.10^{a}
scavenging activity)	48.13 ± 5.82^{a}	39.35± 2.31^{a}	30.36 ± 0.62^{b}	54.27 ± 2.90^{a}	44.91 ± 4.4^{a}	42.62 ± 3.72^{a}
Hydroxy methyl furfural (HMF) content	50.65 ± 4.10^{a}	49.13± 4.10^{a}	50.88± 4.56^{a}	47.19 ± 2.96^{a}	36.69 ± 0.49^{b}	58.54± 2.64^{a}
Browning Index (BI)	33.49 ± 2.62^{b}	39.72 ± 0.35^{a}	35.61± 1.82^{a}	39.91 ± 2.08^{a}	33.3 ± 2.02^{b}	42.26 ± 2.00^{a}

(N=12 for DC and N=15 for CB, Results are expressed as Mean ± SE, with different small letters superscript (a,b) within columns differ significantly (P< 0.05) among the samples. Where, DC- ghee residue obtained from processing of cow's milk by direct cream method; CB– ghee residue obtained from processing of cow's milk by creamery butter method)

of lactose or decomposition of sugars and proteins (Yamaguchi et al. 1981). Additionally, Sripad (1988) reported that as clarification temperature increases, the HMF content also increases. Temperature accelerates the browning reactions and it is well known that HMF is a product of high heat treatment or processing. Thus, HMF content was directly proportional to the increase in clarification temperature as the samples obtained at 120°C clarification temperature had higher HMF content than those obtained at 110°C (34.03 to 37.47 µmole/mg). Further, it is vital to note that the amount of lactose was the highest in the DC-Deoni samples (Table 2), which in turn could have contributed towards the browning reaction, thereby leading to higher HMF content in this sample.

Browning index of ghee residue samples prepared at 120°C clarification temperature

The browning in ghee residue could be either due to maillard reaction or caramelization of sugar. In the present study, the GR samples were analysed for BI and it was found that the browning index of CB-HFC (46.91) was significantly highest ($p<0.05$) followed by DC-HFC (43.96), commercial (34.6), CB-Deoni (35.59) and DC-Deoni (33.18) (Figure4).For both the methods of ghee preparation, higher browning index was observed for HF crossbred samples. The higher browning index in HF crossbred samples may be due to presence of free amine groups which might have been formed during processing of cream into ghee (Yamaguchi et al. 1981).

Properties of ghee residue samples as affected by the breed of cow

The effect of breed of cow on the properties of ghee residue has not been reported earlier, so in this study, the effect was evaluated on some properties, which are discussed here. The breed of the cow had significant effect ($p<005$) on protein, fat and browning index. There are reports in the literature, which suggest that the ghee residue composition is dependent on the raw material used for ghee processing (Santha and Narayanan 1979). On the contrary, there was no significant effect ($p>0.05$) of breed on moisture, lactose, ash, phospholipids, antioxidant activity and HMF (Table 3). However, higher fat content in ghee residue samples obtained from processing of HF crossbred cow milk (62.95 ± 1.23) may be due to the entrapment of fat moieties within the protein matrices or due to process conditions during cream separation such as speed of the bowl and rate of milk inflow. Ghee residue obtained from Deoni cream had higher protein content (30.12 ± 1.18) due to presence of higher total solids (Veeresh et al. 2019).

Browning index of ghee residue samples obtained from the HF crossbred cows was higher ($P<0.05$) than that of Deoni breed. This may be due to lactose degradation or reaction between amino acids and sugars. Further, as the HMF content and

phospholipids content were non-significantly ($p>0.05$) affected by the breed, the antioxidant activity was also found to be similar for both the breeds (Table 3). Since, antioxidant activity depends upon the phospholipids and certain processing products including HMF content, so the similar activity for both the breeds could be attributed to their (phospholipids and HMF) insignificant differences.

Properties of ghee residue samples as affected by the method of ghee preparation

The methods of ghee preparation are responsible for certain constituents in the ghee residue owing to the technological differences which results in fat globule membrane ruptures thereby leaching of certain molecules in the ghee residue from ghee. In the present study, ghee preparation method had significant effect ($p<005$) on proteins, fats, lactose, ash, phospholipids, antioxidant activity but non-significant effect ($p>0.05$) was observed for moisture, hydroxy methyl furfural, browning index (Table3). The protein content of ghee residue obtained by creamery butter method was higher (30.71 ± 1.25) than that obtained from direct cream (21.45 ± 2.15). The reason for higher protein may be due to more retention of curd particles in the butter. The fat of ghee residue obtained by direct cream method was higher than creamery butter method. This may be due to losses of butter fat into ghee residue from ghee, which is obtained from direct cream method, whereas not much fat losses occur in the creamery butter ghee residue. Higher lactose content (11.06 ± 0.52) was found in creamery butter ghee residue owing to higher solids not fat content (4.53%) in the cream itself. Another reason may be due to the higher lactose content (2.47%) in the cream. The lactose present in the raw material might have been the reason for higher lactose in direct cream ghee residue. Ghee residue obtained directly by clarifying cream showed higher fat content (63.27 ± 1.13) which may be due to losses of fat into ghee residue from ghee. Higher ash content was found in creamery butter ghee residue. According to Prahlad (1954) creamery butter ghee residues had higher moisture, ash and protein content whereas, direct cream ghee residues had higher fat and lactose content. Santha and Narayanan, (1978) reported similar results. From this, it can be inferred that the results are comparable with the previous findings.

Higher phospholipid content (11.01 ± 1.06) in creamery butter ghee residue may be due to higher phospholipid content in butter than in cream (Table 3). Similarly, Santha and Narayanan (1979) also reported higher phospholipid content of 17.39% for creamery butter ghee residue and upto 4.94% in direct cream ghee residue. Antioxidant activity increases as phospholipids content increases since phospholipids are responsible for the antioxidant property (Santha and Narayanan 1979). As far as the HMF content is considered, it's a product of maillard reaction, the samples were non-significantly different indicating independence on the method of ghee preparation. Contrary to this, the two major ingredients required for maillard reaction *i.e.*, lactose and amino acids were significantly affected by the method of ghee preparation. This could be attributed to the reaction complexity for formation of HMF, as it is controlled by various factors including type and nature of sugar and amino groups participating in the interaction, temperature, pH and any catalysts e.g., metal ions in the food substrate (Ames 1990, Van Boekel 2001).

Properties of ghee residue samples as affected by clarification temperature

Ghee residue is obtained from heating the cream or butter at temperatures above 100°C and several thermo-chemical reactions are controlled by temperature. Thus, the temperature used for clarification may also affect various ghee and ghee residue properties. With this background, the effect of two clarification temperatures (110°C and 120°C) was evaluated on the properties of GR samples. Moisture, lactose, ash, browning index and HMF content of ghee residue were significantly affected ($p<0.05$) by temperature of clarification while proteins, fats, phospholipids and, antioxidant activity were non-significantly affected ($p>0.05$) (Table 3). Ghee residue from the ghee processed at 120°C had lower moisture content ($11.79 \pm 0.44\%$) than the ghee residue obtained at 110°C ($13.15 \pm 0.45\%$) which may be due to losses of moisture due to evaporation which occurs at higher temperature of clarification. Similar trend with respect to decreasing moisture content with increasing clarification temperature was also reported by Santha and Narayanan (1979). Also, at 120°C, the ash content ($5.52 \pm 0.13\%$) of ghee residue samples were higher due to the minerals concentration resulting from moisture loss at higher clarification temperature.

The HMF content increased significantly ($p<0.05$) with the increase in temperature of clarification (Table 3). This is due to the positive correlation between temperature and the formation of browning pigments, resulting from condensation of lactose and maillard reaction between the sugar moiety and free amine groups. This also led to the changes in colour of the samples from light brown (at110°C) to darker brown (at 120°C) due to condensation of lactose, reaction between sugar and free amine groups and decomposition of sugar (Kiriyaga et al. 1971). Due to this, browning index is also affected. These findings related to HMF content are also supported by the reports of Sripad (1988), where, the amount of HMF in GR was 113.93 µg/g at 110°C clarification temperature and 460.64 µg/g at 120°C, indicating an increase in HMF content with increasing temperature. McGookin (1991) reported that lactose degradation also contributes to higher HMF content. Thus, the results obtained for HMF content and browning index of the ghee residue support the foundation of increasing maillard reaction products upon increasing the clarification temperature used for ghee manufacture.

Conclusion

It could be observed from the present study that ghee residue composition was affected by ghee preparation method. The cow's breed had significant ($p<0.05$) effect on the protein, fat and the browning index of the ghee residue, with higher protein for the ghee residue obtained from processing of Deoni cow's milk and higher fat for HF crossbred cows. The method of ghee preparation significantly ($p<0.05$) affected the protein, fat, lactose, phospholipids content and antioxidant activity with higher protein, phospholipids content and antioxidant activity; while lower fat and lactose content were found for the ghee residue obtained from creamery butter method. The moisture and lactose content of ghee residue decreased significantly ($p<0.05$) while; hydroxy methyl furfural (HMF) content and ash content increased significantly ($p<0.05$) with increasing clarification temperature for ghee preparation. Thus, the present study gave good insights for the factors affecting ghee residue composition.

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