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Characterization of Fatty Acids and Nutritional Health Indicators of *Ghee* (Butteroil) Manufactured from Bovine Colostrum and Sweet Cream

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Abstract: Large-scale bovine colostrum production yields a significant byproduct called colostrum cream (CC). Colostrum cream is the byproduct of the industry where the colostrum is separated, and the colostrum milk is processed and converted into a colostrum formula and immunoglobulin colostrum powder. However, the disposal of CC poses sustainability challenges. CC composition differs significantly from milk fat and can be a valuable source of fatty acids (FAs) in the human diet. Ghee or butter oil manufactured from cream or butter is a product with almost 99.8% fat, with longer shelf life and a unique flavor. The study was planned to see the effect of FA profile and nutritional health indicators derived from FA profiles, such as the atherogenicity index (AI) and thrombogenicity index (TI) of CC, colostrum butter oil (CBO) samples, butter oil (BO), and sweet cream (SC), as they can significantly influence cardiovascular health. Three SC samples from a dairy plant and six CC samples were collected from a private company. BO and CBO samples were made in atmospheric conditions and analyzed for chemical composition and FA profiles in triplicates. SC and BO samples have higher levels of saturated and trans FAs. CC and CBO are richer in beneficial FAs. CBO offers a healthier profile with higher PUFA/SFAs and a lower AI and TI, which can be an essential source of FAs in the human diet and support sustainability.

Keywords: colostrum cream (CC); butter oil (BO); fatty acids (FAs)

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1. Introduction

Bovine colostrum (BC) is a natural secretion from the mammary gland and the first milk produced after the birth of a calf. It is a rich natural source of macro- and micronutrients, such as proteins and peptides, fats and lipids, immunoglobulins, and bioactive compounds [1]. These components are important for the passive immunization of calves. To support body functions and immunity, a calf only requires colostrum equivalent to 7–10% of its body weight [2]. The surplus colostrum is processed into products like freeze-dried colostrum formulas and immunoglobulin-rich colostrum powder. Large-scale milk production produces considerable volumes of colostrum, typically collected at farms, chilled, and transported to central processing facilities. At the processing facility, it undergoes pasteurization, cream separation, and lactose removal before drying. The required proteins and bioactive compounds are extracted from BC. However, during centrifugal separation, 28% of immunoglobulins are obtained in the lipid fraction [3]. The colostrum cream (CC) obtained as a by-product is high in fat, containing 40–45% fat and concentrated further

to 70–80% fat. Currently, this cream is not effectively utilized and is often disposed of, resulting in sustainability concerns and economic problems.

Converting the surplus milk fat and colostrum fat to butter oil (BO) and colostrum BO (CBO) improves shelf life by lowering moisture content, offering a more concentrated dairy fat source ideal for product formulations and industrial applications, facilitating handling and transportation. BO is prepared by melting butter at a temperature not exceeding 80 °C using a vacuum chamber for residual moisture removal. Due to the vacuum manufacturing process, BO has a bland flavor. On the other hand, ghee is manufactured by clarifying milk fat at atmospheric conditions at a high temperature (110 ± 3 °C), which is achieved by heat-induced desiccation. It is manufactured by directly heating cream or butter churned from fresh or ripened cream using selected starter cultures. Ghee has less moisture content and a more pleasing flavor than BO [4,5]. Ghee is commonly used across various Asian and African regions, especially India, Pakistan, Egypt, and East Africa. It serves as both a cooking medium and a flavor enhancer in traditional cuisine, appreciated for its distinctive aroma and potential health benefits [6].

The lipid fraction of BO and ghee comprises mainly saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Some of these components, like ω-3 and ω-6 polyunsaturated fatty acids, conjugated linoleic acid, and short-chain fatty acids, are associated with various health benefits, from immunomodulation to cardiovascular health [7,8]. Some essential FA metabolites may exert health effects, such as anti-inflammatory and neuroprotection effects. Whereas other fatty acids (FAs) like trans fatty acids are known to raise LDL cholesterol and lower HDL cholesterol, which may increase the risk of cardiovascular diseases [9]. Increasing awareness about the role of FAs in cardiovascular health is essential for promoting heart disease prevention strategies. Considering this viewpoint, assessing the FA composition is crucial for determining the nutritional and/or medicinal value, particularly in foods rich in FAs [10].

High consumption of FAs in the diet is correlated with an increased risk of cardiovascular diseases (CVD). CVD remains the leading cause of death and, by 2030, is projected to account for 25 million deaths worldwide [11]. In most cases, CVDs are often caused by atherosclerosis, a progressive thickening, and a loss of elasticity of the artery wall due to the accumulation of lipids [12,13]. Eventually, atherosclerosis could lead to arterial diseases like thrombosis (blood clots), which can block the flow to the heart or brain, causing heart attacks or strokes [14]. For instance, during the last decades, some indices of dietary fat quality, including the atherogenicity index (AI) and thrombogenicity index (TI), and the ratio between hypo-hypercholesterolemic fatty acids (h/H) in the diet are studied, which might be associated with CVD and other non-communicable diseases such as obesity [15]. The AI and TI outlined by Ulbricht and Southgate are dietary risk indices for cardiovascular disease [16].

CC and CBO composition differs significantly from milk fat and can be a valuable source of FAs in the human diet and lower the risk of CVD while addressing the sustainability issue. Currently, the CC produced during colostrum processing is not of any use and is often discarded. However, being rich in fat, CC has a value that needs to be investigated to develop better handling procedures. Thus, the aim of the study was to determine and compare the FAs profile and the lipid indices, like the AI, the TI, h/H, desirable fatty acids (DFA), hypercholesterolemic fatty acids (HFA), the saturase index (SI), and the health-promoting index (HPI), derived from the FA profile of sweet cream (SC), CC, BO, and CBO.

2. Materials and Methods

2.1. Experimental Design

Three SC samples were collected from Davis Dairy Plant, South Dakota State University (SDSU), and six CC samples were collected from (B.A.R. Food Production & distribution, Brookings, SD, USA). The CC samples were designated in two groups, CC-1 and CC-2, with three samples each collected on different dates, and the butter oil manufactured from the same was designated as CBO-1 and CBO-2, respectively. The samples were collected at different time intervals.

2.2. Manufacturing of Ghee (BO)

Ghee samples were prepared using the direct cream method from SC and CC samples [17]. Each batch of cream was thoroughly mixed and transferred to a stainless-steel pan. Each cream sample was heated at a temperature ranging from 110 ± 3 °C with continuous stirring to prevent sticking and charring at the bottom of the vessel [5]. Heating continued until all the moisture evaporated to obtain golden yellow ghee. The ghee was allowed to cool gradually at room temperature to about 25 °C, and the brown ghee residues were allowed to settle at the bottom of the container. Then, it was transferred to clean glass sampling bottles using six layers of cheesecloth (14″ × 36″ Block Wrap, Sani—Net Disposable Poly Webbing, Nelson—Jameson Inc., Marshfield, WI, USA). The glass sampling bottles were stored at room temperature in a place devoid of sunlight. These ghee samples were further analyzed for moisture, free fatty acids, fatty acids composition, instrumental color values, and refractive index in the laboratory under standard conditions.

2.3. Analysis of Samples

2.3.1. Analysis of Cream Samples

All SC and CC samples were analyzed for fat, protein, total solids content, and ash. The fat content was determined using the Mojonnier ether extraction method [18] using 1 g cream sample; protein was measured using the Kjeldahl method [19] with 1 g cream sample. Total solids of cream were determined using direct forced-air oven drying at 100 °C for 5–6 h [20], and ash was determined by placing the samples in a muffle furnace at 500 °C after evaporating residual moisture over a hot plate [21].

2.3.2. Analysis of Butteroil/Ghee Samples

Moisture in ghee was determined by putting 10 g of ghee sample in a hot air oven at 105 °C to constant mass. The dishes were weighed to obtain moisture/fat content in the samples [22]. For free fatty acids (FFAs) and acid value, 10 g ghee liquid samples were added to 50 mL neutralized alcohol (95%) and titrated against 0.1 Normal NaOH after bringing the mixture to boil [22].

FFA was calculated using Equation (1).

$$\text{FFA(percentage oleic acid)} = \frac{2.8 \times \text{T}}{\text{W}} \quad (1)$$

where T is the titer value as ml of 0.1 Normal NaOH used and W is the weight of the ghee sample.

Acid value was calculated using Equation (2).

$$\text{Acid Value} = \left(\frac{\text{T}}{\text{W}}\right) \times 5.61 \quad (2)$$

where T is the titer value as mL of 0.1 Normal NaOH used and W is the weight of ghee sample.

2.4. Instrumental Analysis

2.4.1. Color Values of Ghee

Cream and BO/Ghee samples' color values were obtained at 40 ± 3 °C using a CR400 colorimeter (Konica Minolta Hard Case Chroma Meter-A103, Ramsey, NJ, USA). Three measurements were taken for each sample, and the average of three readings was calculated for each measurement. Data obtained included lightness values (L^*), redness values (a^*), and yellowness values (b^*), and the overall color difference (ΔE) was calculated by using Equation (3).

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \quad (3)$$

where L^*_0, a^*_0, and b^*_0 were the values of the control and L^*, a^*, and b^* were the values of the treated sample.

2.4.2. Refractive Index

The refractive index of ghee samples was determined using the SPER Scientific 300058 Waterproof Digital Refractometer (Scottsdale, AZ, USA) instrument.

2.5. Fatty Acid Composition and Nutritional Indices

The fatty acid composition and various nutritional and health indices were calculated for SC, CC, BO, and CBO samples.

2.5.1. Fatty Acid Composition/Fat (Total, Saturated, and Unsaturated) by Gas Chromatographic Method

Gas chromatography was used to analyze the FA profiles of the cream and BO/ghee samples. Fatty Acid Methyl Esters (FAMEs) were made from the samples by fat extraction and methylation followed by FAME analysis using an Agilent 7890A gas chromatograph system containing autosampler and flame ionization detector [23].

2.5.2. Nutritional Indices (The Ratios of Fatty Acids and Health Lipid Indexes)

Nutritional indices for SC, CC, BO, and CBO were determined from the data obtained from fatty acid composition. These indices, like the unsaturated fatty acids to saturated fatty acids ratio (UFA/SFA), $n-3/n-6$, the atherogenic index (AI), the thrombogenic index (TI), the saturase index (SI), desirable fatty acids (DFAs), hypercholesterolemic fatty acids (HFAs), and the hypocholesterolemic/hypercholesterolemic index (h/H), were determined. The values of these indexes indicate the quality of atherogenicity, thrombogenicity, and the risk of cardiovascular issues using Equations (4)–(12) [24,25].

$$\mathrm{AI} = [(\mathrm{C12:0} + 4\,(\mathrm{C14:0}) + \mathrm{C16:0}]/[(\mathrm{n6} + \mathrm{n3})\ \mathrm{PUFA} + \mathrm{C18:1} + \Sigma\mathrm{MUFA}] \quad (4)$$

$$\mathrm{TI} = (\mathrm{C14:0} + \mathrm{C16:0} + \mathrm{C18:0})/[(0.5 \times \mathrm{C18:1}) + 0.5\ (\Sigma\mathrm{MUFA}) + 0.5\ (\mathrm{n6\ PUFA}) + 3\ (\mathrm{n3\ PUFA}) + (n-3\ \mathrm{PUFA}/n-6\ \mathrm{PUFA})] \quad (5)$$

$$SI = \frac{\mathrm{C14:0} + \mathrm{C16:0} + \mathrm{C18:0}}{MUFA + PUFA} \quad (6)$$

$$DFA = UFA + \mathrm{C18:0} \quad (7)$$

$$HFA = \mathrm{C12:0} + \mathrm{C14:0} + \mathrm{C16:0} \quad (8)$$

$$\mathrm{h/H} = (\mathrm{cis} - \mathrm{C18:1} + \Sigma\mathrm{PUFA})/(\mathrm{C12:0} + \mathrm{C14:0} + \mathrm{C16:0}) \quad (9)$$

$$\text{HPI} = \Sigma\text{UFA}/[\text{C12}:0 + (4 \times \text{C14}:0) + \text{C16}:0] \quad (10)$$

$$\frac{UFA}{SFA} \quad (11)$$

$$\frac{n-3}{n-6} \quad (12)$$

2.6. Statistical Analysis

The data collected were subjected to statistical analysis using RStudio (Version 4.4.0). One-way ANOVA was performed to obtain $p < 0.05$ values. The significant values were compared using Tukey's test, and the results were considered significant at $p < 0.05$. The fat percentage in sweet cream (SC) was adjusted to 67% for statistical analysis to correspond with the higher fat content observed in colostrum cream (CC). It is important to note that the original fat percentages of the colostrum samples were not modified, as we aimed to retain their natural composition. However, the sweet cream fat percentage was recalculated accordingly to ensure a consistent basis for comparison in the statistical analysis. The data were analyzed using one-way ANOVA, performed using R software (version 4.4.1).

3. Results and Discussions

3.1. Physicochemical Parameters and Color Values of Cream

The original fat content of the SC samples was 42.43%, 44.83%, and 45.16%, respectively. To ensure a consistent statistical analysis and compare it to CC, the fat percentage was adjusted to 67%, the average fat percentage of the CC samples. Table 1 presents the statistical analysis of the SC and CC samples' chemical composition and color values. Despite minor variations in fat, TS, protein, and ash content values among all the samples, no significant difference was observed ($p < 0.05$). The SC samples' higher L* values indicate their lightness compared to the CC samples, a difference that could be attributed to the vitamin A content in the samples [25]. The a* and b* values were significantly ($p < 0.05$) higher for the CC samples than for the SC samples. The L* (lightness) and b* (yellowness) values are associated with the high fat content of CC samples [26]. The a* (redness/greenness) values were lower and close to zero, corresponding to a lower intensity of these colors, as observed in other studies [27].

Table 1. Physicochemical parameters and color values of cream samples.

Parameters	SC	CC-1	CC-2
Fat (%)	65.86 ± 2.22	66.54 ± 3.14	67.51 ± 2.59
TS (%)	66.13 ± 6.97	72.34 ± 0.52	74.19 ± 5.13
Protein (%)	1.24 ± 0.11	1.34 ± 0.35	1.30 ± 0.03
Ash (%)	0.33 ± 0.03	0.31 ± 0.04	0.29 ± 0.01
Color Values			
L*	55.29 ± 0.81	51.87 ± 0.41	52.63 ± 2.84
a*	−1.69 ± 0.08 ^{b}	0.71 ± 0.45 ^{a}	0.87 ± 0.18 ^{a}
b*	8.88 ± 0.92 ^{b}	24.56 ± 2.14 ^{a}	23.78 ± 2.19 ^{a}

SC: Sweet cream fat adjusted to 67% for statistical comparison; CC: Colostrum cream. Values with different letters in superscripts (a,b) within the same row differ significantly ($p < 0.05$).

3.2. Physicochemical Parameters and Color Values of Butteroil

The physicochemical parameters and color values of butteroil samples are summarized in Table 2. The moisture and fat content showed significant differences, which may be due to small-scale sample preparation. All three samples had no significant difference in acid

value, free fatty acids, and refractive index. The value for the free fatty acids (as oleic acid) of samples was 0.52% for SC, 0.67%, and 0.60% for CC. The target value for free fatty acids should not be more than 1.4, and the samples' average value for free fatty acids was within the range [28]. There was no significant difference in L* values among all the samples, as the ghee obtained for both samples was golden yellow in color but slightly darker than the CC samples. The a* values were significantly ($p < 0.05$) lower for BO samples. There was a highly significant ($p < 0.05$) increase in the yellowness (b*) values of CBO–1 and CBO–2 than for BO because of an increase in the fat content. An increase in L* and b* values and a decrease in a* values are generally observed in colostrum and the products made from it [27,29]. The overall color difference (ΔE) significantly ($p < 0.05$) differs in all three samples. The ΔE values were highest for CBO-2, followed by CBO-1, and lowest for BO. These differences indicate a pronounced variation in color attributes, with the darker color of CBO-2 making it ideal for applications in bakery and confectionery products. High ΔE values often correlate with distinct visual appeal, a critical factor influencing consumer preference and product functionality in food applications [30,31].

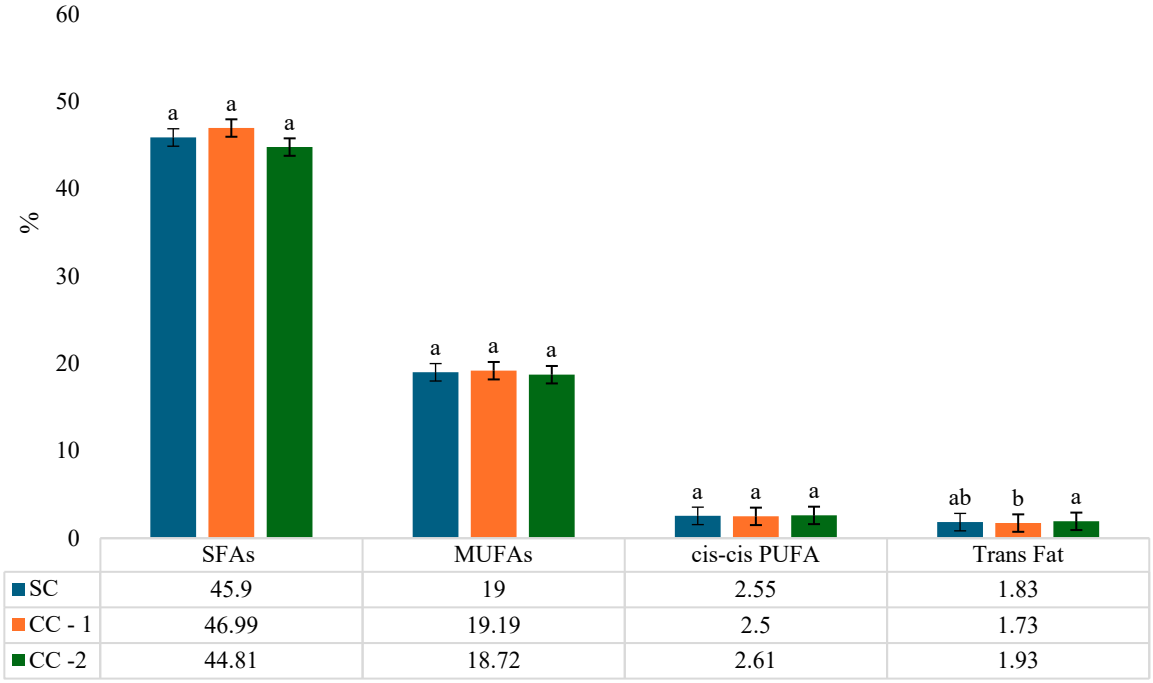
Table 2. Physicochemical parameters and color values of butteroil samples.

Parameters	BO	CBO-1	CBO-2
Moisture (%)	0.13 ± 0.05^{b}	0.21 ± 0.01^{a}	0.19 ± 0.02^{a}
Fat (%)	99.89 ± 0.05^{a}	99.80 ± 0.02^{b}	99.78 ± 0.01^{b}
Acid Value (%)	1.04 ± 0.50	1.34 ± 0.20	1.20 ± 0.23
Free fatty Acids (% oleic acid)	0.52 ± 0.25	0.67 ± 0.10	0.60 ± 0.11
Refractive Index	1.416 ± 0.00	1.416 ± 0.00	1.415 ± 0.00
Color Values			
L*	53.28 ± 1.00	50.58 ± 2.04	52.70 ± 1.00
a*	-1.44 ± 0.66^{a}	-2.70 ± 1.21^{ab}	-3.92 ± 0.85^{b}
b*	8.61 ± 2.33^{b}	30.78 ± 2.73^{a}	34.87 ± 1.13^{a}
ΔE	2.56 ± 1.06^{b}	7.71 ± 3.81^{ab}	12.28 ± 0.95^{a}

BO: Butter oil, CBO: Colostrum butter oil. Values with different letters in superscripts (a,b) within the same row differ significantly ($p < 0.05$).

3.3. Fatty Acids Profile of Cream Samples

Figure 1 represents the percentage composition of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), cis-cis polyunsaturated fatty acids (cis-cis PUFAs), and trans fats for three different samples: SC, CC-1, and CC-2. The statistical analysis indicates no significant differences in the percentages of SFAs, MUFAs, and cis-cis PUFAs among the three samples. However, there is a significant difference in the trans fat percentages among the SC, CC-1, and CC-2 samples, with CC-1 showing the lowest percentage of trans fats. Trans fats adversely affect the cardiovascular health. A 2% increase in energy from trans fats is associated with a 23% increase in the risk of CVD [32]. The Food and Drugs Administration (FDA) has made it mandatory to include trans fats on the nutrition facts label. Thus, the National Cholesterol Education Program [33] recommends that the daily intake of trans fats be kept to a minimum. Trans fats lower the HDL cholesterol [34] and increase LDL cholesterol [35]. The detailed profile of fatty acids for cream samples is presented in Table 3, and that for butter oil samples is presented in Table 4. Trans fats generated from the partial hydrogenation of vegetable fat are directly associated with CVD; however, no such association was observed for dairy products [36].



	SFAs	MUFAs	cis-cis PUFA	Trans Fat
SC	45.9	19	2.55	1.83
CC - 1	46.99	19.19	2.5	1.73
CC -2	44.81	18.72	2.61	1.93

Figure 1. Fatty acid contribution of SC, CC-1, and CC-2 using gas chromatography: SC (Sweet cream), CC (Colostrum cream), SFAs (Saturated fat), MUFAs (Monounsaturated fat), and cis-cis PUFA (Cis-cis polyunsaturated fat). Bars that do not share common letters (a–b) are considered significantly different ($p < 0.05$).

Table 3. Fatty acids profile of cream samples (mg 100 gm^{-1}).

Parameters	SC	CC-1	CC-2
Saturated fatty acids (SFA)			
C4:0 Butyric	2.749 ± 0.049 ^{b}	2.768 ± 0.069 ^{b}	2.985 ± 0.052 ^{a}
C6:0 Caproic	1.654 ± 0.025 ^{a}	1.383 ± 0.035 ^{b}	1.427 ± 0.022 ^{b}
C8:0 Caprylic	0.953 ± 0.009 ^{a}	0.729 ± 0.019 ^{b}	0.736 ± 0.012 ^{b}
C10:0 Capric	2.254 ± 0.010 ^{a}	1.635 ± 0.032 ^{b}	1.567 ± 0.032 ^{b}
C12:0 Lauric	2.651 ± 0.041 ^{a}	2.087 ± 0.054 ^{b}	2.013 ± 0.050 ^{b}
C13:0 Tridecanoic	0.102 ± 0.007 ^{a}	0.042 ± 0.00 ^{b}	0.045 ± 0.006 ^{b}
C14:0 Myristic	7.973 ± 0.078 ^{a}	7.761 ± 0.289 ^{ab}	7.370 ± 0.209 ^{b}
C15:0 Pentadecanoic	0.854 ± 0.025 ^{a}	0.501 ± 0.021 ^{b}	0.533 ± 0.015 ^{b}
C16:0 Palmitic	23.44 ± 0.544 ^{ab}	25.52 ± 1.247 ^{a}	23.01 ± 0.583 ^{b}
C17:0 Margaric	0.501 ± 0.008	0.513 ± 0.027	0.537 ± 0.016
C18:0 Stearic	7.412 ± 0.261 ^{a}	6.508 ± 0.371 ^{b}	6.946 ± 0.172 ^{ab}
C20:0 Arachidic	0.094 ± 0.006	0.097 ± 0.006	0.097 ± 0.005
C21:0 Heneicosanoic	0.143 ± 0.007 ^{a}	0.114 ± 0.010 ^{b}	0.128 ± 0.005 ^{ab}
C24:0 Lignoceric	0.011 ± 0.009	0.021 ± 0.001	0.021 ± 0.001
Monounsaturated fatty acids (MUFA)			
C14:1 trans-Tetradecanoic	0.281 ± 0.005 ^{a}	0.179 ± 0.010 ^{c}	0.207 ± 0.005 ^{b}
C14:1 Myristoleic	0.619 ± 0.021 ^{a}	0.559 ± 0.018 ^{b}	0.454 ± 0.010 ^{c}
C16:1 trans-Hexadecenoic	0.325 ± 0.048 ^{a}	0.178 ± 0.010 ^{b}	0.181 ± 0.006 ^{b}
C16:1 Palmitoleic	1.109 ± 0.013 ^{c}	1.984 ± 0.082 ^{a}	1.739 ± 0.042 ^{b}
C17:1 Margaroleic	0.184 ± 0.023 ^{b}	0.290 ± 0.012 ^{a}	0.304 ± 0.001 ^{a}
C18:1 trans-Elaidic	1.340 ± 0.028 ^{a}	1.038 ± 0.064 ^{c}	1.180 ± 0.038 ^{b}
C18:1 Oleic	14.99 ± 0.380 ^{b}	17.19 ± 0.932 ^{a}	17.03 ± 0.401 ^{a}
C20:1 Gadoleic	0.018 ± 0.003 ^{b}	0.045 ± 0.005 ^{a}	0.052 ± 0.001 ^{a}
Polyunsaturated fatty acids (PUFA)			
C18:2 trans-Octadecadienoic	0.506 ± 0.009 ^{a}	0.324 ± 0.017 ^{b}	0.352 ± 0.012 ^{b}

Table 3. *Cont.*

Parameters	SC	CC-1	CC-2
C18:2 Linoleic	1.932 ± 0.042 ^{a}	1.634 ± 0.088 ^{b}	1.637 ± 0.030 ^{b}
C18:3 γ-Linolenic	0.039 ± 0.007 ^{ab}	0.042 ± 0.001 ^{a}	0.031 ± 0.002 ^{b}
C18:3 Linolenic	0.298 ± 0.006 ^{a}	0.181 ± 0.005 ^{c}	0.223 ± 0.005 ^{b}
C20:3 γ-Eicosatrienoic	0.113 ± 0.007 ^{b}	0.170 ± 0.015 ^{a}	0.163 ± 0.006 ^{a}
C20:4 Arachiodonic	0.151 ± 0.009 ^{b}	0.319 ± 0.021 ^{a}	0.323 ± 0.010 ^{a}
C22:2 Docasadienoic	0.000 ± 0.000 ^{b}	0.013 ± 0.006 ^{a}	0.021 ± 0.001 ^{a}

SC: Sweet cream fat adjusted to 67%, CC: Colostrum cream. Values with different letters in superscripts (a–c) within the same row differ significantly ($p < 0.05$).

Table 4. Fatty acids profile of butteroil samples (mg 100 gm^{-1}).

Parameters	BO	CBO-1	CBO-2
Saturated fatty acids (SFA)			
C4:0 Butyric	3.721 ± 0.145 ^{b}	3.855 ± 0.030 ^{b}	4.171 ± 0.046 ^{a}
C6:0 Caproic	2.262 ± 0.076 ^{a}	1.922 ± 0.006 ^{b}	2.022 ± 0.023 ^{b}
C8:0 Caprylic	1.276 ± 0.043 ^{a}	0.975 ± 0.006 ^{b}	1.037 ± 0.016 ^{b}
C10:0 Capric	2.959 ± 0.109 ^{a}	2.136 ± 0.010 ^{b}	2.136 ± 0.021 ^{b}
C12:0 Lauric	3.434 ± 0.138 ^{a}	2.686 ± 0.006 ^{b}	2.690 ± 0.018 ^{b}
C13:0 Tridecanoic	0.123 ± 0.005 ^{a}	0.053 ± 0.001 ^{c}	0.064 ± 0.001 ^{b}
C14:0 Myristic	10.21 ± 0.192 ^{a}	9.992 ± 0.036 ^{ab}	9.841 ± 0.040 ^{b}
C15:0 Pentadecanoic	1.098 ± 0.052 ^{a}	0.627 ± 0.005 ^{b}	0.684 ± 0.001 ^{b}
C16:0 Palmitic	29.76 ± 0.711 ^{b}	32.47 ± 0.073 ^{a}	30.38 ± 0.103 ^{b}
C17:0 Margaric	0.635 ± 0.006 ^{b}	0.642 ± 0.005 ^{b}	0.691 ± 0.00 ^{a}
C18:0 Stearic	9.398 ± 0.328 ^{a}	8.123 ± 0.015 ^{b}	9.025 ± 0.100 ^{a}
C20:0 Arachidic	0.117 ± 0.006 ^{ab}	0.114 ± 0.00 ^{b}	0.125 ± 0.000 ^{a}
C21:0 Heneicosanoic	0.149 ± 0.012 ^{a}	0.097 ± 0.012 ^{b}	0.107 ± 0.005 ^{b}
C24:0 Lignoceric	0.157 ± 0.002 ^{a}	0.136 ± 0.002 ^{b}	0.142 ± 0.005 ^{b}
Monounsaturated fatty acids (MUFA)			
C14:1 trans-Tetradecanoic	0.356 ± 0.012 ^{a}	0.211 ± 0.002 ^{c}	0.253 ± 0.001 ^{b}
C14:1 Myristoleic	0.806 ± 0.047 ^{a}	0.729 ± 0.001 ^{b}	0.598 ± 0.006 ^{c}
C16:1 trans-Hexadecenoic	0.339 ± 0.176 ^{b}	0.531 ± 0.005 ^{ab}	0.612 ± 0.012 ^{a}
C16:1 Palmitoleic	1.522 ± 0.200 ^{c}	2.229 ± 0.005 ^{a}	1.903 ± 0.015 ^{b}
C17:1 Margaroleic	0.199 ± 0.002	0.359 ± 0.006	0.268 ± 0.187
C18:1 trans-Elaidic	2.560 ± 0.058 ^{a}	1.672 ± 0.00 ^{c}	1.964 ± 0.027 ^{b}
C18:1 Oleic	18.23 ± 0.429 ^{b}	21.63 ± 0.063 ^{a}	21.73 ± 0.342 ^{a}
C20:1 Gadoleic	0.031 ± 0.002	0.062 ± 0.002	0.062 ± 0.003
Polyunsaturated fatty acids (PUFA)			
C18:2 trans-Octadecadienoic	0.638 ± 0.021 ^{a}	0.400 ± 0.015 ^{c}	0.460 ± 0.027 ^{b}
C18:2 Linoleic	2.480 ± 0.033 ^{a}	2.066 ± 0.033 ^{b}	2.111 ± 0.037 ^{b}
C18:3 γ-Linolenic	0.048 ± 0.005	0.055 ± 0.006	0.052 ± 0.002
C18:3 Linolenic	0.397 ± 0.010 ^{a}	0.223 ± 0.005 ^{c}	0.278 ± 0.005 ^{b}
C20:3 γ-Eicosatrienoic	0.131 ± 0.005 ^{b}	0.167 ± 0.00 ^{a}	0.173 ± 0.005 ^{a}
C20:4 Arachiodonic	0.198 ± 0.010 ^{b}	0.417 ± 0.00 ^{a}	0.420 ± 0.005 ^{a}
C22:2 Docasadienoic	0.017 ± 0.006 ^{b}	0.021 ± 0.00 ^{b}	0.031 ± 0.00 ^{a}

BO: Butter oil, CBO: Colostrum butter oil. Values with different letters in superscripts (a–c) within the same row differ significantly ($p < 0.05$).

In the case of SFAs, SC had significantly higher amounts of C4:0 butyric, C6:0 caproic, C8:0 caprylic, C10:0 capric, C12:0 lauric, C13:0 tridecanoic, C14:0 myristic, C15:0 pentadecanoic, C18:0 stearic, and C21:0 heneicosanoic acid. The increase in LDL cholesterol in the blood is attributed to the intake of C12:0, C14:0, and C16:0 [37,38]. Most of the C12:0 and C14:0 in the diet is obtained from milk fat. Some other SFAs tend to decrease this effect by increasing HDL cholesterol [39]. However, researchers still suggest reducing SFAs as they are directly associated with atherosclerosis [40]. This is also reflected in the results

obtained for lipid indices in Table 5, where a higher AI was observed for SC and BO. CC-1 had significantly higher amounts of C16:0 palmitic acid. Even though palmitic acid is the most abundant saturated fatty acid in milk fat quantitatively [41], the dietary intake of palmitic acid becomes crucial, with an optimal ratio of UFAs for cellular functions [42], and it also plays a significant role in early-life nutrition [43,44]. No significant differences were observed for C17:0 margaroleic, C20:0 arachidic, and C24:0 lignoceric acid.

Table 5. Nutritional indices of cream and butteroil samples.

Parameters	SC	CC-1	CC-2	BO	CBO-1	CBO-2
UFA/SFA	0.709 ± 0.007 ^{c}	0.753 ± 0.002 ^{a}	0.722 ± 0.003 ^{b}	0.447 ± 0.011 ^{b}	0.496 ± 0.00 ^{a}	0.505 ± 0.003 ^{a}
n3/n6	0.154 ± 0.016 ^{a}	0.111 ± 0.003 ^{c}	0.136 ± 0.001 ^{b}	0.160 ± 0.006 ^{a}	0.108 ± 0.002 ^{b}	0.132 ± 0.00 ^{c}
DFA	41.48 ± 0.449	41.88 ± 1.954	39.34 ± 0.988	36.92 ± 0.574 ^{c}	37.99 ± 0.102 ^{b}	39.12 ± 0.459 ^{a}
HFA	34.07 ± 0.658 ^{ab}	35.37 ± 1.583 ^{a}	32.39 ± 0.838 ^{b}	43.41 ± 1.039 ^{b}	45.15 ± 0.105 ^{a}	42.92 ± 0.161 ^{b}
AI	1.713 ± 0.065 ^{a}	1.525 ± 0.019 ^{b}	1.435 ± 0.024 ^{b}	1.768 ± 0.064 ^{a}	1.554 ± 0.003 ^{b}	1.501 ± 0.014 ^{b}
TI	2.214 ± 0.070 ^{a}	2.024 ± 0.010 ^{b}	1.917 ± 0.020 ^{b}	2.202 ± 0.05 ^{a}	2.053 ± 0.00 ^{b}	1.999 ± 0.01 ^{b}
SI	0.773 ± 0.003 ^{a}	0.729 ± 0.001 ^{b}	0.730 ± 0.002 ^{b}	1.042 ± 0.022 ^{a}	0.940 ± 0.00 ^{b}	0.919 ± 0.006 ^{b}
h/H	0.516 ± 0.022 ^{c}	0.556 ± 0.005 ^{b}	0.606 ± 0.008 ^{a}	0.497 ± 0.022 ^{c}	0.548 ± 0.00 ^{b}	0.583 ± 0.007 ^{a}
HPI	0.365 ± 0.013 ^{c}	0.399 ± 0.004 ^{b}	0.427 ± 0.006 ^{a}	0.372 ± 0.011 ^{c}	0.397 ± 0.00 ^{b}	0.415 ± 0.003 ^{a}

SC: Sweet cream fat adjusted to 67%, CC: Colostrum cream, BO: Butter oil, CBO: Colostrum butter oil, UFA: Unsaturated fat, SFAs: Saturated fat, n3: gamma-linolenic acid, n6: Linoleic acid, DFAs: Desirable fatty acids, HFAs: Hypercholesterolemic fatty acids, AI: Atherogenic index, TI: Thrombogenic index, SI: Saturase index, h/H: Hypocholesteolemic/hypercholesterolemic, HPI: Health-promoting index. Values with different letters in superscripts (a–c) within the same row differ significantly ($p < 0.05$).

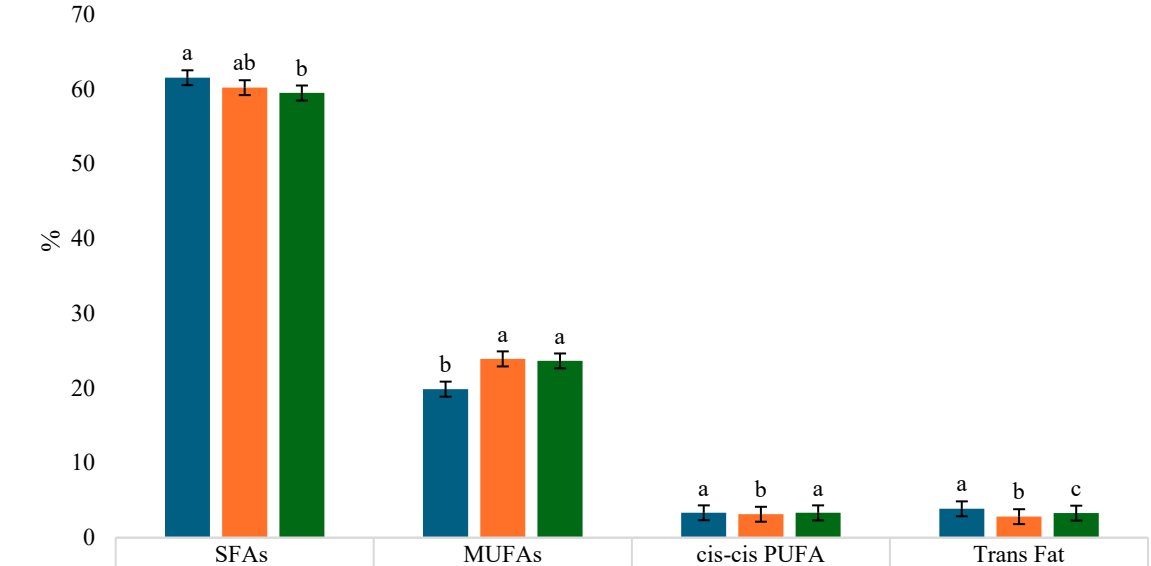
For MUFAs, SC had significantly higher amounts of C14:1 t-tetradecanoic, C14:1 myristoleic, C16:1 trans-hexadecanoic, and C18:1 trans-elaidic acid. The MUFAs do not reduce the total cholesterol content as they tend to decrease the LDL cholesterol but increase HDL cholesterol. However, MUFAs decrease LDL susceptibility to oxidation [45]. High MUFA intakes of up to 29% are associated with a lower prevalence of CVD [46]. CC had significantly higher amounts of C16:1 palmitoleic, C17:1 margaroleic, C18:1 oleic, and C20:1 gadoleic acid. C-18:1 is highly nutritional and associated with immunomodulation and cardiovascular health. [8,47,48]

PUFAs have been widely recognized due to their beneficial role in cardiovascular health [49]. They can be classified as omega-3 and omega-6 PUFAs. SC had significantly higher amounts of C18:2 t-octadecanoic acid, C18:2 linoleic acid, and C18:3 γ-linolenic acid. Linoleic acid and α-linolenic acid are considered the essential fatty acids which are indispensable. These are further converted into long-chain PUFAs, like arachidonic acid, EPA, and DHA. However, linoleic acid (LA)-enriched diets may induce the oxidation of LDL, increasing the risk of CVD [50,51]. CC-1 had significantly higher amounts of C18:3 g-linolenic and C20:3 γ-eicosatrienoic acid, and CC-2 had significantly higher amounts of C20:4 arachidonic and C22:2 docasadienoic acid. The intake of dietary C18:3 γ-linolenic acid has been shown to slow down the development of atherosclerosis in transgenic mouse models. It also possesses a unique ability to suppress tumor growth. Human milk is also an abundant source of γ-linolenic acid [52]. CC can be a good source of C20:4 due to its importance in developing the central nervous system, especially the brain and cognitive functions. It is an inherent component of breast milk [53] and is also added to infant formulas [54].

3.4. Fatty Acids Profile of Butteroil Samples

The percentage composition of SFAs, MUFAs, cis-cis PUFAs, and trans fats for three different samples, BO, CBO-1, and CBO-2, is illustrated in Figure 2. The fatty acid profile of butter oil samples is presented in Table 4. SFA percentages are significantly ($p < 0.05$) higher in the BO (61.55%) sample as compared to CBO-1 (60.21%) and CBO-2 (59.5%) samples. The

MUFA content in BO (19.87%) was significantly ($p < 0.05$) lower than the CBO-1 (23.92%) and CBO-2 (23.66%), which are not significantly different from each other. The cis-cis PUFA percentage in CBO-1 (3.13%) is significantly lower than in BO (3.33%) and CBO-2 (3.32%) samples. Trans fat percentages were significantly different among all three samples, with BO (3.87%) being the highest, followed by CBO-2 (3.28%) and CBO-1 (2.82%). When cream is converted into BO, the percentage of FAs increases as moisture is removed. Hence, the fatty acid composition trends obtained for the BO samples were similar to those observed for cream samples.



	SFAs	MUFAs	cis-cis PUFA	Trans Fat
BO	61.55	19.87	3.33	3.87
CBO -1	60.21	23.92	3.13	2.82
CBO - 2	59.5	23.66	3.32	3.28

Figure 2. Fatty acids contribution of BO, CBO-1, and CBO-2 using gas chromatography: BO: Butter oil, CBO: Colostrum butter oil, SFAs (Saturated fat), MUFAs (Monounsaturated fat), cis-cis PUFA (Cis-cis polyunsaturated fat). $^{a–c}$ Bars that do not share common letters are considered significantly different ($p < 0.05$).

In the case of SFAs, BO had significantly higher amounts of C6:0 caproic, C8:0 caprylic, C10:0 capric, C12:0 lauric, C13:0 tridecanoic, C14:0 myristic, C15:0 pentadecanoic, C18:0 stearic, C21:0 heneicosanoic, and C24:0 lignoceric acid. CBO-1 was significantly higher in C16:0 palmitic acid, and CBO-2 was significantly higher in C4:0 butyric acid, C17:0 margaric, and C20:0 arachidic acid. The excess of palmitic acid is directly associated with an increased risk of cholesterol and CVD [55]. However, in infant formulas, palmitate at the sn-2 position is known to improve the absorption of calcium and fat in infants [43].

For MUFAs, BO was significantly higher in C14:1 t-tetradecanoic acid, C14:1 myristoleic acid, and C18:1 t-elaidic acid. Trans elaidic acid is a trans isomer of oleic acid and is an important component of hydrogenated fats [56]. Diets rich in trans-elaidic acid can promote atherosclerotic plaque formation without increasing plasma cholesterol. However, supplementation with omega-3 fatty acids like α-linolenic acid can counteract these effects [57]. CBO-1 was significantly higher in C16:1 palmitoleic acid and C17:1 margaroleic acid. Only the trans isomer of palmitoleic acid is associated with the risk of CVD, whereas the cis isomer is known to raise HDL cholesterol [58]. CBO-2 was significantly higher in C16:1 t-hexadecanoic acid and C18:1 oleic acid. Oleic acid is an important antiatherogenic factor. It also lowers cholesterol and maintains the HDL to LDL cholesterol ratio [8]. No significant differences were observed for C20:1 gadoleic acid.

For PUFAs, BO was significantly higher in C18:2 t-octadecadienoic, C18:2 linoleic, and C18:3 linolenic acid. The presence of C18:3 linolenic acid shows low to moderately beneficial effects on cardiovascular health due to a limited number of clinical trials [59,60]. CBO-1 was significantly higher in C18:3 γ-linolenic acid. CBO-2 was significantly higher in C20:3 γ- eicosatrienoic acid, C20:4 arachidonic acid, and C22:2 docosadienoic acid.

3.5. Nutritional Indices of Cream and Butteroil Samples

In this study, nutritional indices of cream and butteroil samples were investigated, and the values are shown in Table 5. The higher the ratio of UFAs to SFAs, the more favorable the effect on cholesterol levels [10]. The unsaturated to saturated (UFAs/SFAs) fatty acids ratio of all the cream samples was significantly different, whereas the CBO–1 and CBO–2 samples showed significantly ($p < 0.05$) higher values than the BO sample. These values suggest that consuming a diet rich in UFAs can reduce LDL-C and decrease overall serum cholesterol levels. The Expert Committee of the World Health Organization and the Food and Agriculture Organization recommended maintaining the n-6/n-3 fatty acid ratio below 4, as this proportion is associated with a significant reduction (70%) in cardiovascular disease-related deaths [61,62]. Desirable fatty acids (DFAs) include all the unsaturated FAs and oleic acid. There was no significant difference in DFAs among all cream samples. However, the BO sample showed a significantly lower value since it had lower amounts of UFAs and oleic acid. The AI gives the relationship between the sum of SFAs and the UFAs, where the SFAs, including C12:0, C14:0, and C16:0, are considered pro-atherogenic FAs. The AI indicates the relationship between FAs with pro-atherogenic properties and those with anti-atherogenic properties, reflecting the inhibition of plaque aggregation and the levels of esterified FAs, cholesterol, and phospholipids [17]. On the other hand, the UFAs are responsible for inhibiting plaque accumulation and reducing phospholipids, cholesterol, and esterified FAs [63]. Consuming dairy products with a lower AI can reduce cholesterol levels in the blood [64]. The AI of CC and CBO samples were significantly lower, which suggests that the CBO is a suitable option for the diet compared to the BO sample. The saturase index (SI) was significantly lower for CC and CBO samples. The TI describes the thrombogenic potential of FAs, indicating their tendency to form clots in blood vessels. It shows the relationship between pro-thrombogenic FAs (C12:0, C14:0, and C16:0) and anti-thrombogenic FAs (MUFAs and the n3 and n6) [16]. The TI was significantly lower in the CC and CBO samples. The hypocholesterolemic to hypercholesterolemic (h/H) ratio describes the relationship between hypocholesterolemic fatty acids (cis-C18:1 and PUFAs) and hypercholesterolemic fatty acids. It is associated with the functional role of fatty acids in cardiovascular metabolism for plasma cholesterol transport and risk of developing CVD [65]. Therefore, the h/H ratio more accurately reflects the effect of the composition of fatty acids (FAs) on heart-related diseases rather than the UFAs/SFAs ratio. The h/H was significantly higher in the CC and CBO samples. The health-promoting index (HPI) is the inverse of AI, and a higher HPI suggests the presence of beneficial fatty acids. The HPI values were significantly higher for CC and CBO samples. Similar values were found in the traditional ghee derived from Arunachali yaks (0.48 ± 0.01), yak–cow hybrids (0.71 ± 0.01), and cows (0.55) [25].

4. Conclusions

Based on the limited number of samples, the study's findings demonstrate the distinct impact of sweet and colostrum cream samples on the quality characteristics of BO and CBO samples. Even though the sample size is small, it is statistically relevant, and the trends and essential information relevant to colostrum cream (CC) and colostrum butter oil (CBO) are reported. The total saturated fatty acids (SFAs) and trans fatty acids are significantly higher

in the sweet cream (SC) and butter oil (BO) samples than in the CC and CBO samples. The SC and BO samples are significantly higher in SFAs like caproic, caprylic, capric, lauric, and myristic acid, etc., than trans MUFAs like trans-tetradecanoic and trans-elaidic acids, which are known to be associated with cardiovascular risk. However, it also has beneficial PUFAs like linolenic and linoleic acid. CC and CBO were significantly higher in some SFAs, such as palmitic acid, MUFAs, oleic, and palmitoleic acid, and PUFAs, such as γ-linolenic and arachidonic acid. Palmitic acid and arachidonic acid are beneficial FAs for infant growth and are also added to infant formulas, which may be associated with their higher amounts of colostrum fat. It is important to evaluate health indices such as PUFAs/SFAs, AI, TI, h/H, and HPI because they are related to cardiovascular health. CC and CBO samples showed higher values of PUFAs/SFAs and HPI indices and lower values of AI, TI, and SI, indicating that colostrum butter oil is a better option for consumption. Also, the research findings indicate that using colostrum fat, a by-product of the colostrum processing industry which currently has little use, can be converted into a value-added product, helping the industry's sustainability efforts. These findings are important for milk processors and product manufacturers looking to sell fatty products or use these ingredients as raw materials for recipe formulations.

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