


Fatty acid profile of Greek yogurt with colostrum addition

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Abstract

The nutraceutical properties of colostrum have stimulated interest in its use in the production of functional foods and supplements that benefit human health. This study investigated the fatty acid profile of Greek yogurt formulations added with bovine colostrum. Four formulations of Greek yogurt were developed with different colostrum levels: 0, 10, 20, and 30%. Next, fat extraction and esterification were performed to determine the fatty acid profile. Then, the concentrations of fatty acid methyl esters were determined by gas chromatography. No effect was observed for the C14:0, C16:0, and C18:0 concentrations with the addition of colostrum. Although the C18:1c9, C18:2n6, C18:3n3, and C20:4n6 concentrations were not affected by colostrum, the C16:1c9, polyunsaturated fatty acids, and n-6 fatty acid contents increased, without changes in the n-6 to n-3 ratio. C18:1t9 was the only monounsaturated fatty acid that differed among formulations and it increased with colostrum addition. There was no effect of the colostrum increase on the total saturated, unsaturated, and monounsaturated fatty acid concentrations. The bovine colostrum used as an ingredient in Greek yogurt showed slight changes in the fatty acid profile. The use of colostrum is an alternative to reduce costs and maintain health benefits.

Keywords: dairy products; functional foods; nutraceutical properties; nutritional quality.

Practical Application: Adding colostrum to Greek yogurt maintains fatty acids beneficial to human health.

1. Introduction

Milk is a liquid secreted by female mammals with the unique purpose of feeding newborns (McGrath et al., 2015). Bovine colostrum is the first component secreted by lactating females for nutrition and transfer of immunity to the calf. It is usually produced in greater quantity than the daily need of the calf, and so the excess can be used for other purposes such as in the production of cosmetics, medicines, supplements, and foods (Mizelman et al., 2017).

The increased demand for nutraceutical and natural products has grown rapidly; thus, the higher content of immunoglobulins present in colostrum makes it a potential component in supplements, formulas, children's drinks, and innovative functional foods (Borad & Singh, 2018). The addition of bovine colostrum to yogurt increases its nutritional quality, which makes it an option to use in producing this food for human consumption (S. Abdel-Ghany & A. Zaki, 2018). Fatty acids present in bovine colostrum may play an essential role in physiological functions in the body, such as aiding bowel mobility,

antimicrobial activity, anti-inflammatory, and anticancer effects, by lowering cholesterol, participating in energy metabolism, and improving the immune system (Gómez-Cortés et al., 2018). Greek yogurt has a higher amount of saturated fatty acids, with the predominant ones being stearic and palmitic acids. In addition, oleic and linoleic acids are found in greater proportions regarding unsaturated fatty acids (UFA) (Sumarmono et al., 2015). Dairy products are important dietary sources of conjugated linoleic acid (CLA), and Greek yogurt has been reported to have a high CLA content (Serafeimidou et al., 2012). Therefore, this study aimed to evaluate the effects of colostrum addition on the fatty acid profile of Greek yogurt formulations.

2. Materials and methods

2.1. Ethical approval

This study was approved by the Animal Research Ethics Committee of the Universidade Federal do Rio Grande do Norte under protocol 098.023/2018, according to Law No. 11,794, 2008.

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2.2. Raw milk and colostrum

Raw milk and colostrum were obtained from Jersey cows on a commercial property. The cows were fed corn silage, soybean meal, and mineral mix. The milk and colostrum samples were obtained by mechanical milking. Colostrum was considered the secretion during the first 3 days after calving, and the colostrum of the third milking was used for yogurt manufacturing. Milk and colostrum were transported in 5-L gallons, then filtered and transferred into 1-L bottles upon arriving at the laboratory, and then stored at -5°C .

2.3. Yogurt formulations

Colostrum added to the formulations was collected 24 h after calving in the third milking. Four Greek yogurt formulations were evaluated: (C0) 0% colostrum; (C10) 10% colostrum; (C20) 20% colostrum; and (C30) 30% colostrum addition (Figure 1).

The yogurt was prepared in the Milk processing unit and Agricultural School of Jundiaí of the Universidade Federal do Rio Grande do Norte. Greek yogurt production has been reported elsewhere (Silva et al., 2021). Aliquots of 100 g of each formulation were freeze-dried for subsequent analysis of fatty acid composition.

The Greek yogurt was lyophilized at the Laboratory of Fruit and Vegetable Processing, Jundiaí Agricultural School, of the University Federal do Rio Grande do Norte. Yogurt samples were frozen and maintained at -20°C for 48 h and subsequently freeze-dried with a bench freeze dryer (MODEL Enterprise Terroni Series[®], Scientific Equipment). The product was then vacuum packaged, protected from light and moisture, and stored until fatty acid analysis.

2.4. Fatty acid analysis

The lipid extractions of the freeze-dried yogurt formulations were performed using 1 g of the powdered yogurt samples according to AOAC Official Method 989.05 (AOAC, 2012). The fat-containing supernatant was evaporated at 40°C under

nitrogen. The lipids extracted from the samples were dissolved in methyl and hexane and transesterified to fatty acid methyl esters using newly prepared sodium methoxide (Baldin et al., 2013). The mixture was neutralized with oxalic acid (1 g of oxalic acid in 30 mL diethyl ether), and calcium chloride was added to remove methanol residues. The fatty acid methyl esters were then injected (1.0 μL) into a gas chromatograph (model 7820A chromatograph, Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and a capillary column of silica CP-Sil 88 (100 m \times 0.25 mm \times 0.2 μm ; Varian, Mississauga, ON, Canada). The operating conditions were the same as described by Cruz-Hernandez et al. (2007).

The fatty acid methyl esters were identified by comparing the retention times with reference standards (Sigma-Aldrich[®], St. Louis, MO, USA; Larodan AB, Stockholm, Sweden; Fight-CLA[®] 60, BASF). The composition of acids was expressed as a percentage by weight using theoretical response factors (Wolff et al., 1995).

2.5. Statistical analysis

Linear and quadratic regression analyses were used to test the effect of colostrum addition on the fatty acid profile of Greek yogurts using the PROC REG procedure of SAS (version 9.4). Significance was declared when $p < 0.05$.

3. Results and discussion

3.1. Saturated fatty acids

The saturated fatty acid (SFA) concentration did not differ among the formulations (Table 1). Butyric acid (4:0) is the primary source of energy for intestinal cells. It is responsible for maintaining the health and integrity of the colon, has antimicrobial and anti-inflammatory activity, promotes satiety, and inhibits carcinogenesis (Gómez-Cortés et al., 2018). We also observed that the medium-chain fatty acids (MCFA) (C10:0, C12:0, and C14:0) did not change with the colostrum addition to yogurt formulations. The synthesis of short-chain fatty acid (SCFA) and MCFA is reduced during the first lactation days, which have a negative energy balance (Churakov et al., 2021). Therefore, a decrease in those fatty acids was expected with the increase in colostrum addition in the formulations. Nonetheless, all individual SCFA numerically decreased with colostrum addition.

The fatty acid concentration in cow milk varies according to the lactation stage, race, and diet, among other factors (Bainbridge et al., 2016), and the content of polyunsaturated fatty acids (PUFA) may be higher in colostrum (O'Callaghan et al., 2020).

Greek yogurt has a higher total solid content due to serum drainage. The higher amount of fat gives the yogurt a less acidic taste, and the greater amount of protein improves its texture, making it smoother (Chandan, 2017). Liposoluble vitamins may be found within the lipids present in yogurt such as A, D, E, and K. Yogurt is also a food rich in conjugating linoleic acid (CLA), providing up to 80% of daily value required (Fernandez et al., 2017).

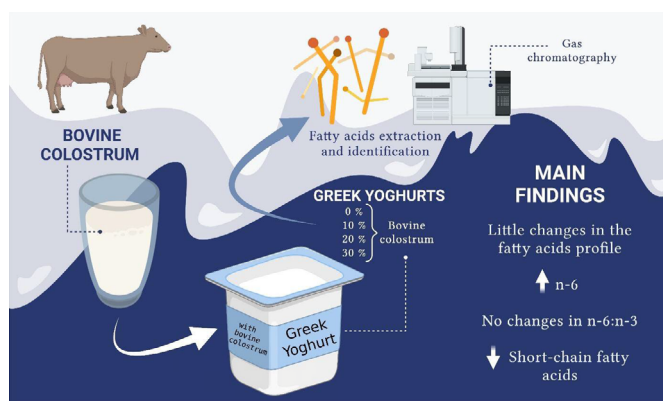


Figure 1. Experimental development and evaluation of the fatty acid profile of Greek yogurts with the addition of bovine colostrum at different concentrations: C0 (yogurt without colostrum addition), C10 (10% colostrum yogurt), C20 (20% colostrum yogurt), and C30 (30% colostrum yogurt).

Table 1. Fatty acid profile of Greek yogurt formulations with colostrum addition.

Fatty acids	Treatments ^a				SEM ^b	p-value ^c	
	0	10	20	30		L	Q
C4:0	3.235	2.912	3.120	2.734	0.105	0.316	0.9334
C6:0	2.078	1.881	1.763	1.694	0.077	0.085	0.629
C8:0	1.314	1.496	1.253	1.256	0.069	0.518	0.533
C10:0	2.437	2.446	2.398	2.380	0.021	0.304	0.736
C12:0	2.693	2.672	2.748	2.687	0.023	0.783	0.902
C13:0	0.139	0.139	0.141	0.137	0.002	0.943	0.815
C14:0	9.493	9.296	9.672	9.617	0.111	0.493	0.711
C14:0 <i>iso</i>	0.106	0.104	0.105	0.102	0.001	0.291	0.946
C14:1c9	0.694	0.695	0.739	0.716	0.011	0.346	0.829
C15:0	0.865	0.860	0.898	0.885	0.010	0.359	0.972
C15:0 <i>anteiso</i>	0.229	0.247	0.239	0.238	0.004	0.821	0.323
C16:0	27.999	26.750	28.468	28.866	0.502	0.368	0.413
C16:0 <i>iso</i>	0.228	0.219	0.217	0.204	0.004	0.007	0.860
C16:1c9	1.556	1.578	1.660	1.656	0.022	0.025	0.831
C17:0	0.520	0.512	0.574	0.540	0.012	0.301	0.868
C17:0 <i>iso</i>	0.330	0.328	0.327	0.315	0.004	0.252	0.604
C17:1c9	0.183	0.188	0.201	0.198	0.004	0.064	0.590
C18:0	13.588	12.565	12.410	12.677	0.253	0.368	0.247
C18:1t9	0.204	0.212	0.212	0.230	0.004	0.031	0.491
C18:1t10	0.345	0.307	0.353	0.363	0.012	0.357	0.272
C18:1t11	1.082	1.109	1.128	1.061	0.019	0.828	0.445
C18:1c9	20.060	19.340	20.339	20.052	0.290	0.689	0.834
C18:1c11	0.755	0.722	0.751	0.732	0.007	0.754	0.566
C18:2n6	1.681	1.682	1.817	1.803	0.032	0.078	0.918
C18:3n3	0.182	0.187	0.201	0.192	0.004	0.303	0.565
C18:3n6	0.021	0.026	0.026	0.024	0.001	0.683	0.467
CLA c9t11	0.444	0.456	0.476	0.453	0.007	0.570	0.425
CLAt 10c12	0.008	0.021	0.006	0.009	0.003	0.633	0.463
C20:4n6	0.123	0.136	0.144	0.150	0.005	0.057	0.645
C20:5n3	0.018	0.028	0.026	0.022	0.002	0.791	0.057
C22:5n3	0.046	0.057	0.058	0.052	0.003	0.672	0.325

^aSubstitution (%) of milk by colostrum in Greek yogurt formulations; ^bStandard error of the mean; ^cProbability values for the linear (L) and quadratic (Q) effects.

No effect was observed for the myristic (C14:0), palmitic (C16:0), and stearic acid (C18:0) concentrations with the addition of colostrum. Both C16:0 and C18:0 are used as energy sources by the lactating cow and are the main SFAs in cow milk. While C18:0 does not present human health issues, C14:0 may increase high-density lipoprotein (HDL) cholesterol and decrease blood triglycerides (Gómez-Cortés et al., 2018). Only excessive C16:0 presents health risks, such as increased cholesterol and increased chances of developing heart disease (Gómez-Cortés et al., 2018). Therefore, the health benefits of yogurts with colostrum addition are similar among formulations regarding the profile of long-chain SFAs.

3.2. Unsaturated fatty acids

The C18:1c9, C18:2n6, C18:3n3, and C20:4n6 concentrations were not affected by colostrum addition to Greek yogurt, although the p-values for C18:2n6 and C20:4n6 were lower than 10% (p=0.078 and p=0.057, respectively). Only C16:1c9

increased with the colostrum addition. This fatty acid is a product of stearyl-CoA desaturase, which is the enzyme responsible for adding a double bond on carbon 9. This reaction is important for controlling milk fluidity (García-Fernández et al., 2009), and oleic acid (C18:1c9) is the most important fatty acid originated by this reaction. It is the main monounsaturated fatty acid in milk fat and, thus, in Greek yogurt. However, its concentration did not change with addition of colostrum addition.

Another observed alteration among formulations was for the C18:1t9 values, as they increased with colostrum addition, being the only *trans* octadecenoic acid that differed among treatments. This *trans* fatty acid is synthesized during ruminal biohydrogenation, and it is associated with an increased risk of cardiovascular diseases (Pipoyan et al., 2021). The major C18:1 *trans*-vaccenic acid (C18:1t11) did not change with colostrum addition. It is a precursor of CLAc9t11 in mammal tissues, a known anticarcinogenic molecule. Milk and dairy products are the main sources of both fatty acids, and higher concentration in milk is related to supplemental fatty acids in ruminant diets. Therefore, this increase in 18:1t9 may be associated with cow diet after parturition. We also speculate that the significant effect may have occurred by chance or due to a dilution effect due to the low magnitude of the difference (0.026 percentage units between the lower and the higher values). The CLA (CLAc9t11 and CLAt10c12) content was not different among formulations. CLA is associated with reduced tumor growth, anti-obesity effect, and modulation of the immune system (Pipoyan et al., 2021). Therefore, the content of anticancer bioactive molecules is similar with and without colostrum addition in Greek yogurt formulations.

3.3. Microbial fatty acids

There was no effect of colostrum addition on individual odd-chain fatty acid (OCFA) concentrations. These molecules are found in the plasma membrane of ruminal microorganisms and are incorporated into milk triglycerides after digestion and absorption in the ruminant intestine. The branched-chain fatty acids (BCFA) are also of microbial origin and can also be found in milk. Both groups of fatty acids were not affected by colostrum addition in the formulation of Greek yogurts. The OCFA, C15:0, and C17:0 may have benefits to human health; they are related to a lower incidence of heart disease, while ingestion of these acids promotes decreased mortality and type 2 diabetes incidence (Venn-Watson et al., 2020).

3.4. Fatty acid categories

Changes in the concentration of specific groups of fatty acids were observed with colostrum addition. The content of PUFA and n-6 fatty acids increased as SFCA decreased (Table 2). Although no changes were observed for the individual fatty acids in those groups, numerical changes may explain these findings. For instance, C4:0, C6:0, and C8:0 decreased as colostrum was included in the formulations, although the p-values for all three fatty acids were higher than 5%. But when their sum was considered, a linear decrease in the SCFA concentration (p=0.026) was observed.

Table 2. Profile of fatty acid groups of Greek yogurt formulations with colostrum addition.

Fatty acid groups	Treatments ^a				SEM	p-value ^b	
	0	10	20	30		L	Q
SFA	62.983	60.155	61.973	62.057	0.792	0.973	0.456
UFA	37.017	39.845	38.027	37.943	0.792	0.973	0.456
MUFA	23.065	22.335	23.488	23.157	0.315	0.617	0.706
PUFA	2.071	2.115	2.272	2.242	0.040	0.044	0.700
SCFA	6.627	6.288	6.136	5.684	0.163	0.026	0.873
MCFA	14.622	14.415	14.818	14.683	0.136	0.656	0.828
n3	0.292	0.329	0.344	0.318	0.009	0.420	0.061
n6	1.825	1.843	1.987	1.977	0.036	0.043	0.988
n6/n3	6.252	5.615	5.785	6.220	0.151	0.854	0.088
OCFA	1.524	1.511	1.613	1.563	0.020	0.281	0.914
BCFA	0.942	0.942	0.936	0.908	0.009	0.265	0.548
MFA	2.466	2.453	2.549	2.470	0.022	0.615	0.775

^aSFA: saturated fatty acids (FA); UFA: unsaturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; SCFA: short-chain FA (C4:0 + C6:0 + C8:0); MCFA: medium-chain FA (C10:0 + C12:0 + C14:0); n3: sum of n-3 FA; n6: sum of n-6 FA; n6/n3: ratio of n-6 to n-3 FA; OCFA: odd-chain FA; BCFA: branched-chain FA; MFA: microbial FA (OCFA + BCFA); ^aSubstitution (%) of milk by colostrum in Greek yogurt formulations; SEM: Standard error of the mean; ^bProbability values for the linear (L) and quadratic (Q) effects.

Both SCFA and MCFA are easily digestible in the gastrointestinal tract and are a source of fast energy for the cells; however, they are poorly stored in adipose tissues. Milk fat is the main SCFA source in the human diet. They play multiple roles in the body, from participation in energy metabolism to action against pathogens (Gómez-Cortés et al., 2018). The decrease in these acids in Greek-type yogurts may represent a decrease in intake via diet, as milk and its derivatives constitute one of the main sources of SCFA (Gómez-Cortés et al., 2018).

No changes were observed for the SFA content in the yogurt formulations (Table 2). SFAs have been associated with weight control and increased lean mass (Gómez-Cortés et al., 2018). Fatty acids longer than 14 carbons are accumulated in adipose tissue. The UFAs are divided into monounsaturated and PUFA. Most of the UFAs are polyunsaturated, and many of them exert beneficial effects on humans. The essential fatty acids are UFA from the n-6 and n-3 families, and they are precursors of other necessary fatty acids for the body (Schulze et al., 2020). α -Linolenic acid (C18:3n3) is the fatty acid of the n-3 family, which is present in higher amounts in milk, while linoleic acid (C18:2n6) is the more abundant n-6 fatty acid (Figure 1). Aracuidonic acid (C20:4n6) is indispensable for the development of the fetus and child. C18:2n6 is necessary for the synthesis of other acids through the elongation process. In addition, n-6 fatty acids help some factors related to cardiovascular diseases. However, an excess of these acids can trigger harmful effects on the body (Dunbar et al., 2014).

The main fatty acid responsible for the linear increase in PUFA concentration with colostrum addition was C18:2n6. It corresponded to about 50% of all PUFA content in the yogurts. Also, the concentration of C20:4n6 numerically increased ($p=0.057$) and may have influenced the increase in PUFA. C20:4n6 assists in increasing HDL, which helps in reducing the risks of hypercholesterolemia (Hanus et al., 2018). The increased concentration of these acids in Greek yogurt may represent advantages to human

health (Dunbar et al., 2014). Thus, n-6 fatty acids increased as colostrum was added to yogurt formulations, but no differences were observed for the n-6 to n-3 ratio (Table 2).

The average n-6 to n-3 ratio observed for the Greek yogurt formulations was 6.0. For the proper functioning of human metabolism, it is necessary that the diet should contain a correct proportion of n-6 and n-3 fatty acids. The recommended intake ranges from a 2:1 to 4:1 ratio of n-6 to n-3, since these acids compete for the same enzymes. A higher n-6 to n-3 ratio may increase the chances of developing cardiovascular, allergic, and inflammatory diseases (Dunbar et al., 2014). Colostrum has high levels of n-6 fatty acids that are beneficial for the calf's development. Increased intake of n-6 and palmitic acid is not favorable to human health because they can help in the development of cardiovascular diseases and inflammatory processes (Dunbar et al., 2014; Hanus et al., 2018). Milk from the third day after delivery is the most indicated for consumption because the fatty acid profile is already more favorable, with decreased C16 and n-6 concentrations and increased UFA, oleic acid, and CLA concentrations (O'Callaghan et al., 2020). However, nutritional management may change the concentration of these fatty acids in milk.

4. Conclusion

The addition of bovine colostrum to Greek yogurt formulations may lead to small changes in the fatty acids profile. The more pronounced change is the increase in n-6 fatty acids without changes to the n-6 to n-3 ratio. Also, a decrease in the SCFA percentage is expected with colostrum addition. Overall, the use of colostrum in dairy products is an alternative that may decrease costs and maintain health benefits.

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