

Probiotic culture activity, fatty acids composition and lipid health quality indices of bio-Greek yogurt made from cow, buffalo, goat, sheep or camel milk using transglutaminase

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Abstract

The effects of dairy product fatty acids on human health, especially cardiovascular diseases, have come to light in recent decades. On the hand, as is well known, fermented milk products such as yoghurt offer several nutritional and health benefits, which has led to an increase in their consumption worldwide. Therefore, this work was established to study the starter activity, fatty acids composition and lipid health quality indices of Greek yogurt made from different types of milk using a probiotic starter culture and transglutaminase enzyme. Five Greek yoghurt treatments were prepared using milk from cows, buffalo, camels, goats, and sheep that had been fortified with 8% milk protein concentrate. ABT-5 culture (*S. thermophiles*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) was used as starter and transglutaminase (0.04%) was added to improve the body and texture of yogurt. Although the starter activity in camel milk was the slowest compared to the other samples, camel milk yogurt gained the best fatty acid composition and the most favorable lipid health indices. Greek yogurt manufactured from camel milk was rich in monounsaturated and polyunsaturated fatty acids and had the lowest amounts of saturated fatty acids (SFAs). Furthermore, camel milk yogurt possessed the greatest levels of unsaturated fatty acids/SFAs, desirable fatty acids, $\Delta 9 - (16)$ desaturase, health promoting and hypocholesterolaemic/hypercholesterolemic ratios and the lowest values of saturation, atherogenic, hypercholesterolemic and thrombogenicity indices, suggesting it aligns with new health dietary recommendations specially cardiovascular diseases. Based on fatty acids composition and lipid quality indices, camel milk yogurt has demonstrated superior health properties whereas goat and cow milk Greek yogurt ranked second followed by buffalo and sheep milk yogurt.

Introduction

Greek yogurt is a type of yogurt that is characterized by its high content of total solids and protein compared to regular yogurt. This is achieved either by straining and removing part of the whey or by adding milk proteins or hydrocolloids to raise the thickness. In a related context, the transglutaminase enzyme (Tgase) is a transferase that is capable of creating inter- and intra-molecular crosslinks in and between varieties of proteins. By creating e-(c-glutamyl)-lysine bonds, transglutaminase can alter the functional characteristics of foods. An excellent substrate for Tgase is milk casein ^[1].

However, the formulation of milk's fatty acids (FAs) is crucial for improving yogurt's physical characteristics and aroma. Also, the fatty acid profile of milk has a great effect on the nutritional value (e.g., effects on human health) of dairy products. Short-chain fatty acids (SCFAs), for instance, are known to modulate gene function and may also help prevent cancer to some extent. The most common monounsaturated fatty acid (MUFA) in milk fat, oleic acid (C18:1), has been demonstrated to increase low-density lipoprotein receptor activation and reduce serum cholesterol levels ^[2] whereas linolenic acid (C18:3) is said to display anti-carcinogenic and anti-atherogenic qualities ^[3]. Some important metabolites of essential fatty acids may have anti-inflammatory and neuroprotective properties. However, some FAs, such as trans fatty acids, have been shown to increase the risk of cardiovascular diseases by lowering HDL cholesterol and raising LDL cholesterol ^[4]. Promoting heart disease prevention

strategies requires increasing knowledge of the function of FAs in cardiovascular health. In light of this viewpoint, evaluating the composition of FAs is essential for figuring out their nutritional and/or therapeutic value, especially in food products rich in FAs [2].

The fatty acid composition of raw milk and the production process play a major role in variations in the quantity and profiles of fatty acids in the finished dairy products. In turn, the fatty acid composition of raw milk affected by number of factors, such as species, breed, genotype, stage of lactation, feeding type and ration and season [5, 6]. For example, feeding cows diets rich in unsaturated fatty acids (such linseed, sunflower, or fish oil) has been demonstrated to lower the mammary gland's *de novo* synthesis of saturated fatty acids [7]. As a result, milk fat has reduced levels of C12:0, C14:0, and C16:0. Furthermore, it is crucial to consider how the fatty acid profile is impacted by the technological procedures of dairy product processing, including heat treatment, homogenization, standardization, fermentation, starter culture type, and storage conditions [8, 9]. Khan et al. [8] explained that the high milk temperature pasteurization resulted in a decrease in long-chain fatty acids (LCFAs) and an increase in SCFAs and medium-chain fatty acids (MCFAs). As a common, all of the aforementioned factors might fluctuate significantly between countries; hence, the composition of the same product made in one nation may differ from that of another.

The proportions of individual fatty acids, such as desirable fatty acids (DFA), the saturation index (SI), the hypercholesterolemic index (OFA), the health promoting index (HPI), the hypocholesterolemia/hypercholesterolemic index (H/H), the atherogenicity index (AI) and thrombogenicity index (TI), and the (n-6)/(n-3) ratio, are usually analyzed in order to determine the nutritional impact on consumer health [10]. Specifically, these indices shed light on the possible effects of dairy lipids on metabolic and cardiovascular health [11]. For instance, the AI and the TI are great resources for determining the potential health effects of the composition of FAs. AI exhibits the relationship between the total amount of primary saturated fatty acids and the main classes of unsaturated fatty acids. While the latter is anti-atherogenic (inhibiting plaque aggregation and lowering levels of esterified fatty acids, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases), the former is believed to be pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory system). In contrast, TI has a potential to form blood vessel clots. It also explains how pro-thrombogenic (saturated) and anti-thrombogenic fatty acids (MUFAs, (n-6) PUFAs, and (n-3) PUFAs) are related [12]. Foods with a low AI and TI fatty acid profile may reduce the incidence of coronary heart disease [2]. However, there is limited information in the literature about lipid health quality indices of Greek yogurt made from various type of milk, more data relating to these indices and fatty acids profile of Greek yogurt would be useful for consumer. Therefore, the main objectives of the current study were to assess fatty acids composition and their health indices for bio-Greek yogurt made from cow, buffalo, goat, sheep or camel milk using transglutaminase.

Materials and Methods

Materials

Fresh cow, buffalo, goat, sheep and camel milk was obtained from Animal Production Research Institute, Agricultural Research center, Egypt. *S. thermophiles*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* comprise the ABT-5 culture (Chr. Hansen's Lab A/S Copenhagen, Denmark). Before being used, a starter cultures were kept at -18°C in freeze-dried direct-to-vat set form. Transglutaminase (Tgase, Activa TG-1, with activity of 100 units /g powder) was bought from Ajinomoto Europe Sales GmbH, Hamburg, Germany. Milk protein concentrate (MPC, 70% protein) was purchased from Fonterra Company, New Zealand.

Methods

Greek yogurt Preparation

Five treatments of Greek yogurt were made as follows:

A: Greek yogurt made from cow milk + 8% MPC + 0.04% Tgase

B: Greek yogurt made from buffalo milk + 8% MPC + 0.04% Tgase

C: Greek yogurt made from camel milk + 8% MPC + 0.04% Tgase

D: Greek yogurt made from goat milk + 8% MPC + 0.04% Tgase

E: Greek yogurt made from sheep milk + 8% MPC + 0.04% Tgase

After pasteurization, milk of various treatments were cooled to 40°C, mixed with MPC, stirred well and left for 30 minutes then Tgase was added, followed by inoculation with the starter culture (0.1 g/L of milk mix), incubated at 40°C for fully coagulation. After the incubation period, yogurt samples were stored at 4°C and the free fatty acids were determined the next day.

Chemical analyses

Titratable acidity and fat content of milk and Greek yogurt were determined according to AOAC ^[13].

Fatty acid analysis

Sample preparation

Free fatty acid identification and quantification were carried out using the technique previously described by Jahreis et al. ^[14]. 15 milliliters of Fokh's reagent (chloroform/methanol = 2:1 (v/v)) were used to

extract fat from 4 grams of Greek yoghurt. Anhydrous Na₂SO₄ was used to filter the purified lipids. Potassium methylate was transesterified to produce fatty acid methyl esters (FAMES). The fat solution in the Pyrex® tube was supplemented with 0.5 ml of potassium methylate (5% wt/wt in methanol). The tube was vortexed, completely tightly closed, and heated to 60°C for 15 minutes in a drying cabinet. Following cooling, 1.5 ml of sulphuric acid (2% wt/wt in aqueous dest.) was added, and the tube was once again vortexed. The gas chromatograph was filled with 1 µl of the clear organic phase.

Gas chromatography (GC) analysis

Free fatty acid identification and quantification were carried out in accordance with the procedure described by Jahreis et al. ^[14]. A ZB-5 fused silica capillary column was used for gas-liquid chromatography to determine the fatty acids. One microliter of FAMES was injected into a GC-MS auto sampler (7890 A GC System Agilent) equipped with an MSD detector. The internal standard, methyl thiocyanate (23:0) from Sigma, was used to quantify fatty acids in mg g⁻¹ total lipids and identify them. All samples were given 1.00 mL of internal standard solution (1 mg mL⁻¹) before the transesterification, and the solvent was evaporated under N₂ flow.

Lipid health quality indices

The nutritional or health index of the fatty acids profile was determined using previously published equations.

USFA/SFA: Unsaturated Fatty Acid (USFAs) / Saturated Fatty Acid (SFAs)

DFA: Desirable Fatty Acids = Unsaturated Fatty Acids (USFAs) + C18:0 ^[15]

SI: The Saturation Index = C14:0 + C16:0 + C18:0 / Monounsaturated Fatty Acids (MUFAs) + Polyunsaturated Fatty Acids (PUFAs) ^[15]

Δ9 – DI (18): Δ9 – (18) desaturase index = 100 x C18:1 / (C18:1 + C18:0) ^[16]

Δ9 – DI (16): Δ9 – (16) desaturase index = 100 x C16:1 / (C16:1 + C16:0) ^[16]

AI: Atherogenic Index = AI = [(C12:0 + (4 × C14:0) + C16:0)] / (n-3 PUFAs + n-6 PUFAs + MUFAs) ^[17]

n-6 / n-3 = total n-6 PUFAs fatty acids / total n-3 PUFAs fatty acids

OFA: Hypercholesterolemic Index = C12:0 + C14:0 + C16:0 ^[18]

HPI: Health Promoting Index = ΣUSFA / [C12:0 + (4 × C14:0) + C16:0] ^[18]

H/H: Hypocholesterolaemic/Hypercholesterolemic ratio = $(C18:1n-9 + C18:2n-6 + C18:3n-3)/(C12:0 + C14:0 + C16:0)$ ^[19]

TI: Thrombogenicity Index = $C14:0 + C16:0 + C18:0/[(0.5 \times \text{MUFAs}) + (0.5 \times \text{PUFAs}) + (\text{PUFAs } \omega 3/ \text{PUFAs } \omega 6)]$ ^[11]

Statistical analysis:

A software program ^[20] based on analysis of variance was used to statistically analyze the collected results. According to Duncan ^[21], the least significant difference (LSD) for the comparison of means was determined when the F-test was significant. The tables display the mean ($\pm$ standard deviation) of three experiments.

Results

Fat contents of milk and Greek yogurt

Figure 1 clears the values of fat content in cow, buffalo, camel, goat and sheep milk and Greek yogurt. As expected, sheep and buffalo milk and yogurt made from them contained the highest levels of fat, while camel milk and yogurt had the lowest. Goat milk and yogurt possessed higher fat ratios as compared with those of cow milk and yogurt.

Activity of ABT culture in cow, buffalo, camel, goat and sheep milk

Activity of ABT starter which contained *S. thermophiles*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in cow, buffalo, camel, goat and sheep milk fortified with 8% MPC and 0.04% Tgase was estimated by tracking changes in the pH values of milk every 30 minutes. Because a pH range of 4.1–4.6 is appropriate for casein particle aggregation, coagulum production that prevents syneresis, prevention of the growth of undesired microbes, and promotion of the desired flavor, the measurements ended at pH 4.4 ^[22]. Overall, with progressive of fermentation time, pH values gradually lowered in all milk samples (Fig. 1).

Buffalo milk recorded the shortest fermentation period, reaching a pH of 4.48 in 240 minutes. Thirty minutes longer than buffalo milk, sheep milk fermented in 270 minutes, reaching a pH of 4.49. Cow milk came in third, requiring 300 minutes to achieve a pH of 4.46. As is clear from Fig. 1, the starter activity was slower and the fermentation period was longer in goat and camel milk. Goat milk recorded a pH of 4.42, and camel milk reached a pH of 4.49, with a fermentation period of 330 minutes.

Fatty acid content of Greek yogurt

Based on the carbon chain length or degree of saturation, saturated fatty acids (SFAs) are classified into short chain fatty acids (SCFAs, C4 – C12), medium chain fatty acids (MCFAs, C13 – C16) and long chain

fatty acids (LCFAs, > C16), while unsaturated fatty acids (USFAs) divided into monounsaturated fatty acid (MUFAs, C14,16,18,20:1) and polyunsaturated fatty acids (PUFAs, C18:2,3,20:2,4) which were measured in fresh Greek yogurt (Tables 1 and 2).

Table 2
Effect of using different types of milk on free fatty acid and nutrition/health indexes of fresh bio-Greek yogurt

Indexes	Treatments				
	A	B	C	D	E
SFAs	68.51 ^A ±1.05	69.26 ^A ±1.80	59.70 ^B ±3.33	68.18 ^A ±3.90	71.07 ^A ±2.80
SCFAs	9.44 ^F ±1.23	9.24 ^E ±0.55	2.86 ^F ±0.55	16.32 ^D ±1.81	18.17 ^E ±1.00
MCFAs	44.97 ^B ±0.98	45.26 ^B ±3.8	41.16 ^C ±5.01	38.77 ^B ±1.22	40.54 ^C ±7.22
LCFAs	14.10 ^E ±1.12	14.76 ^D ±1.10	15.68 ^E ±1.80	13.09 ^D ±2.90	12.36 ^E ±2.20
USFAs	31.65 ^C ±2.10	30.73 ^C ±2.50	40.13 ^C ±3.23	31.05 ^B ±1.05	28.93 ^D ±2.66
MUFAs	28.76 ^D ±0.90	27.81 ^C ±2.1	36.20 ^C ±2.10	27.46 ^C ±1.50	25.74 ^D ±1.90
PUFAs	2.89 ^H ±0.07	2.92 ^F ±0.44	3.93 ^F ±0.90	3.59 ^G ±0.88	3.19 ^F ±1.05
USFAs /SFAs	0.462 ^J ±0.05	0.444 ^H ±0.22	0.672 ^H ±0.01	0.455 ^I ±0.04	0.407 ^H ±0.01
DFA	44.66 ^B ±3.21	43.54 ^B ±0.90	53.97 ^B ±2.90	42.92 ^B ±6.02	40.29 ^C ±3.29
SI	1.774 ^I ±0.79	1.827 ^G ±0.05	1.279 ^G ±0.05	1.577 ^H ±0.05	1.764 ^J ±0.50
Δ9 – DI (18)	65.93 ^A ±1.45	66.35 ^A ±2.55	64.41 ^A ±5.80	66.89 ^A ±3.59	67.17 ^B ±3.33
Δ9 – DI (16)	6.29 ^G ±0.50	3.77 ^F ±0.01	27.96 ^D ±2.20	6.12 ^E ±1.02	4.72 ^F ±1.20
AI	2.716 ^H ±0.22	2.467 ^F ±0.05	1.720 ^G ±0.05	2.135 ^G ±1.10	3.014 ^F ±1.01
n-6/n-3	2.568 ^H ±0.08	2.459 ^F ±0.20	2.970 ^F ±0.88	3.075 ^G ±1.00	3.090 ^F ±1.11
OFA	46.31 ^B ±4.50	46.16 ^B ±4.10	38.86 ^C ±3.02	39.61 ^B ±5.05	45.13 ^C ±5.56

SFAs: saturated fatty acids; SCFAs: short chain fatty acids (C4 to C12); MCFAs: medium chain fatty acids (C13 to C16); LCFAs: long chain fatty acids (> C16); USFAs: unsaturated fatty acids; MUFAs: monounsaturated fatty acids (C14,16,18,20:1); PUFAs: polyunsaturated fatty acids (C18:2,3,20:2,4); DFA: Desirable Fatty Acids = Unsaturated Fatty Acids (USFAs) +C18:0; SI: The Saturation Index = C14:0 + C16:0 + C18:0 / MUFAs + PUFAs; Δ9 – DI (18): Δ9 – (18) desaturase index = 100 x C18:1/ (C18:1 + C18:0); Δ9 – DI (16): Δ9 – (16) desaturase index = 100 x C16:1/ (C16:1 + C16:0); AI: atherogenicity Index = (C12:0 + (4 × C14:0) + C16:0)/(n-3 PUFA s + n-6 PUFA + MUFAs); n-6/n-3 = total n-6 PUFAs fatty acids/total n-3 PUFAs fatty acids; OFA: Hypercholesterolemic Index = C12:0 + C14:0 + C16:0; HPI: Health Promoting Index = ΣUSFAs/ [C12:0+ (4× C14:0) + C16:0]; H/H: Hypocholesterolaemic/hypercholesterolemic ratio = (C18:1n-9 + C18:2n-6 + C18:3n-3)/(C12:0 + C14:0 + C16:0); TI: Thrombogenicity Index = C14:0 + C16:0 + C18:0/[(0.5x MUFAs) + (0.5x PUFAs) + (PUFAs ω3/ PUFAs ω6)]

Indexes	Treatments				
	A	B	C	D	E
HPI	0.370 ^J ±0.03	0.410 ^H ±0.01	0.583 ^H ±0.04	0.479 ^I ±0.04	0.336 ^H ±0.03
H/H	0.602 ^J ±0.02	0.606 ^H ±0.04	0.710 ^H ±0.01	0.679 ^I ±0.01	0.577 ^H ±0.01
TI	3.455 ^H ±0.40	3.550 ^F ±0.33	2.514 ^F ±0.5	3.080 ^G ±0.05	3.437 ^F ±0.55
SFAs: saturated fatty acids; SCFAs: short chain fatty acids (C4 to C12); MCFAs: medium chain fatty acids (C13 to C16); LCFAs: long chain fatty acids (> C16); USFAs: unsaturated fatty acids; MUFAs: monounsaturated fatty acids (C14,16,18,20:1); PUFAs: polyunsaturated fatty acids (C18:2,3,20:2,4); DFA: Desirable Fatty Acids = Unsaturated Fatty Acids (USFAs) +C18:0; SI: The Saturation Index = C14:0 + C16:0 + C18:0 / MUFAs + PUFAs; Δ9 – DI (18): Δ9 – (18) desaturase index = 100 x C18:1/ (C18:1 + C18:0); Δ9 – DI (16): Δ9 – (16) desaturase index = 100 x C16:1/ (C16:1 + C16:0); AI: atherogenicity Index = (C12:0 + (4 × C14:0) + C16:0)/(n-3 PUFA s + n-6 PUFA + MUFAs); n-6/n-3 = total n-6 PUFAs fatty acids/total n-3 PUFAs fatty acids; OFA: Hypercholesterolemic Index = C12:0 + C14:0 + C16:0; HPI: Health Promoting Index = ΣUSFAs/ [C12:0+ (4× C14:0) + C16:0]; H/H: Hypocholesterolaemic/hypercholesterolemic ratio = (C18:1n-9 + C18:2n-6 + C18:3n-3)/(C12:0 + C14:0 + C16:0); TI: Thrombogenicity Index = C14:0 + C16:0 + C18:0/[(0.5x MUFAs) + (0.5x PUFAs) + (PUFAs ω3/ PUFAs ω6)]					

Saturated and unsaturated fatty acids

Data recognized in Tables 1 and 2 explain the effect of using cow, buffalo, camel, goat and sheep milk on fatty acids profile of fresh Greek yogurt. The greatest level of SFAs was detected in sheep milk yogurt (71.07%), while buffalo milk yogurt had slightly higher amount (69.26%) compared to cow or goat milk yogurt (68.51 and 68.18% respectively), which contained similar SFAs contents. Surprisingly, using camel milk in Greek yogurt preparation resulted in a sharp decrease in SFAs levels (59.70%). Regarding USFAs, camel milk yogurt had the highest concentration of USFAs (40.13%), while sheep and buffalo milk yogurt had the lowest (28.93 and 30.73% respectively). The amounts of these fatty acids were also similar in cow and goat milk yogurt (31.65 and 31.05% respectively).

Short chain fatty acids (C4 – C12)

The sum SCFAs concentrations varied markedly between Greek yogurt samples. The levels of SCFAs in sheep and goat milk Greek yogurt were almost double those in buffalo and cow milk one. Camel milk yogurt contained very low ratio of these acids. The SCFAs account for 9.44, 9.24, 2.86, 16.32 and 18.17% of the total FAs and 13.78, 13.34, 4.79, 23.94 and 25.57% of total SFAs for samples A. B, C, D and E respectively.

Medium chain fatty acids (C13 – C16)

Buffalo and cow milk yogurt had elevated MCFAs levels compared to those found in sheep and goat milk yogurt (Table 2). Camel milk yogurt recorded intermediate MCFAs values between the previous treatments. MCFAs represented 44.97, 45.26, 41.16, 38.77 and 40.54% of the total FAs and 65.64, 65.35, 68.94, 56.86 and 57.04% of the total SFAs for treatments A. B, C, D and E respectively. The values of MCFAs were higher than SCFAs and LCFAs in all yogurt samples.

Long chain fatty acids (> C16)

The LCFAs contents of various Greek yoghurt samples were in the following order: camel yogurt > buffalo yogurt > cow yogurt > goat yogurt > sheep yogurt (Table 2). LCFAs constituted 14.10, 14.76, 15.68, 13.09 and 12.36% of the total FAs and 20.58, 21.31, 26.26, 19.20 and 17.39% of the total SFAs for A, B, C, D and E samples respectively.

Monounsaturated fatty acids (MUFAs)

Greek yogurt samples demonstrated comparable total MUFAs values. Camel yogurt contained considerable amounts of MUFAs compared to the other samples. There was no pronounced difference in MUFAs content between cow, buffalo and goat milk yogurt samples (Table 2). However, the sheep milk yogurt sample exhibited a significantly lower value. MUFAs formed 28.76, 27.81, 36.20, 27.46 and 25.74% of the total FAs and 90.87, 90.50, 90.21, 88.44 and 88.97% of the total USFAs for A, B, C, D and E samples respectively.

Polyunsaturated fatty acids (PUFAs)

As illustrated in Table 2, the PUFAs were present in the highest amounts in camel, goat and sheep milk yogurt and in the lowest levels in buffalo and cow milk yogurt. PUFAs constituted 2.89, 2.92, 3.93, 3.59 and 3.19% of the total FAs and 9.13, 9.50, 9.79, 11.56 and 11.03% of the total USFAs for A, B, C, D and E samples respectively.

The Lipid health quality Indices

In the present study, the ratio of UFAs to SFAs, DFA, SI: the saturation index (SI), AI, n-6/n-3, hypercholesterolemic index (OFA), Health Promoting Index (HPI), H/H and TI indices of Greek yoghurt treatments were investigated, and the results are summarized in Table 2.

The unsaturated fatty acids/saturated fatty acids (USFAs /SFAs)

The USFAs /SFAs ratios of all Greek yogurt treatments were significantly different (Table 2). Camel milk yogurt exhibited significantly ($p < 0.05$) higher value than other samples which attributed to the high USFAs content. The USFAs /SFAs ratio of cow milk yogurt was slightly higher than that of goat milk yogurt which in turn was slightly higher than that of buffalo milk yogurt. The lowest USFAs /SFAs ratio was detected in sheep milk yogurt.

The desirable fatty acid index (DFA)

There was a significant difference in DFA among various Greek yogurt treatments. Camel yogurt contained significantly higher DFA value (53.97). Cow (44.66) and buffalo (43.54) milk yogurt exhibited relatively higher DFA ratios compared to goat milk yogurt (42.92). The sheep milk yogurt had the lowest DFA value (40.29).

The saturation (SI) and $\Delta 9$ -desaturase indices

The results of the present study indicate that camel milk yogurt had the lowest SI index value whereas the buffalo yogurt had the highest level (Table 2). The cow or sheep yogurts showed comparable SI index values which were relatively higher than that of goat milk Greek yogurt. Also, ovine, caprine and buffalo milk yogurts exhibited comparable $\Delta 9$ -desaturase (18) levels which slightly higher than those of bovine and camel milk yogurts. Extremely surprising, camel yogurt possessed a very high ratio of $\Delta 9$ -desaturase (16) as compared with other treatments which could indicate a high desaturase activity. The values of $\Delta 9$ -desaturase (16) were 6.29, 3.77, 27.96, 6.12 and 4.72 for A, B, C, D and E samples respectively.

The atherogenicity index (AI)

In our study, ewe, cow and buffalo milk yogurt exhibited the highest AI ratios, In contrast, the AI values of camel and goat milk yoghurt were much lower ($p < 0.05$), indicating a better lipid profile.

The n-6/n-3 index

As shown in Table 2, the sheep and goat milk yogurts had n-6/n-3 higher ratios than those of other treatments.

The Hypercholesterolemic Index (OFA)

As detailed in Table 2, the OFA ratio ranged from 38.86 in fat from camel yogurt to 46.31 in fat from cow yogurt. In buffalo, sheep and goat milk yogurt, the OFA values were 46.16, 45.13 and 39.61 respectively.

The health promoting index (HPI)

Camel, goat and buffalo milk yogurts exhibited the highest HPI ratios (0.583, 0.479 and 0.410 respectively), while cow and sheep milk yogurts had the lowest (0.370 and 0.336 respectively).

Hypocholesterolaemic/hypercholesterolemic index (H/H)

The values of H/H index differed markedly among the Greek yogurt treatments. The camel milk yogurt showed an obviously high desirable H/H ratio comparing with other yogurt samples, supporting its favorable fatty acids composition (Table 2). Goat, cow and buffalo milk Greek yogurt also demonstrated favorable H/H values. Sheep milk yogurt exhibited the lowest H/H ratio.

Thrombogenicity index (TI)

Camel and goat milk yogurt recorded the lowest TI index ratios and the buffalo yogurt showed the highest. The levels of TI index in cow and sheep milk yogurt were almost similar.

Discussion

Because of the heat treatment used in yogurt processing which caused some moisture evaporation, yogurt slightly contained higher fat concentrations than milk. Generally, the fat contents of milk or yogurt obtained in this study ranged within normal levels illustrated in literatures ^[23, 24].

Lowering pH values during fermentation is, of course, a result of the activity of the mentioned bacteria which ferment milk lactose to lactic acid. These results are in agreement with those previously found by Samet-Bali et al. ^[25] who stated that as Rayeb milk cultures grow during fermentation, they produce acid which causes a reduce in pH values.

Buffalo milk had shorter fermentation time followed by sheep, cow, goat and camel milk. Trentin et al. ^[26] cleared that the fermentation time of yogurt is influenced by the type of milk, with sheep's milk requiring the longest fermentation time (330 minutes) compared to cow's milk (240 minutes). These differences are likely due to higher levels of total solids in sheep's milk, particularly protein, which directly affects milk structure and the mechanism of action of milk starters. Confirming to our results, Bulca et al. ^[27] stated that camel milk contains high levels of antimicrobial substances such as lysozyme, lactoferrin, lactoperoxidase and immunoglobulins, which are heat-stable and cannot be inhibited by heating, and they also inhibit the growth of starter cultures in fermented milk.

Because each mammal species has a unique milk composition in terms of major and minor components like proteins, fatty acids and vitamins ^[28], so the type of milk used in making Greek yogurt has an effect on the concentrations of SFAs and USFAs, where sheep yogurt possessed the highest and camel yogurt contained the lowest SFAs. The content of USFAs took a completely opposite trend to that of SFAs. Similar trend was observed by Terzioğlu et al. ^[29] who mentioned that the highest SFAs values were determined in sheep milk yogurt and the lowest content was in cow milk yogurt while yogurt made from buffalo, goat, and camel milk contained values intermediate between the two. In different bio-Greek yogurt treatments, the dominant saturated fatty acids were palmitic, stearic and myristic acids whereas oleic, linoleic and palmitoleic acids were the most prevalent unsaturated fatty acids. According to Givens and Shingfield ^[30], the most common saturated fatty acid in the lipid composition of milk is palmitic acid. Consequently, dietary consumption of palmitic acid becomes crucial, with an optimal ratio of UFAs for cellular functioning ^[31], and it's crucial for nutrition in the early stages of life ^[32].

As is well known, lowering the amount of SFAs is still a good way to create functional dairy foods that are better for cardiovascular health. By changing the cow's diet, employing microbial cultures with high desaturase activity, or adding plant oils rich in UFAs, this can be achieved without necessarily compromising sensory attributes. UFAs are very important for human nutrition. The high values of UFAs and low levels of SFAs observed in camel milk Greek yogurt sample from our study demonstrate the high nutritional value of this yogurt. In similar lines, Farah et al. ^[33] demonstrated the great nutritional value of camel milk, demonstrating that it contains three times as much vitamin C, minerals (including K+), and essential and polyunsaturated fatty acids as cow milk. Camel milk was found to have the lowest levels of SFAs, the highest content of monounsaturated fatty acids, and the lowest concentrations of

polyunsaturated fatty acids in the study carried out by Barěska et al. ^[34] to assess the fatty acid composition of different types of milk.

Sheep and goat milk Greek yogurt contained higher levels of SCFAs than other samples. These findings are consistent with those of Boukria et al. ^[23], who found that the kind of milk had an impact on short chain fatty acids. Yoghurt made from sheep milk had a significantly higher total level of these acids (C4–C8) than yoghurt made from goat milk or a 1:1 blend of ewe and goat milk. Long-chain fatty acids levels of yogurt did not affected by milk type.

Butyric acid level was highest in fat derived from cow milk yoghurt and lowest in fat extracted from camel milk yoghurt. Goat and sheep milk yoghurts were found to contain the highest levels of caproic, caprylic and capric acids. Goat and sheep milk yoghurt had the highest amounts of caproic, caprylic, and capric acids. According to Erkaya and Suengul ^[35], cow yoghurt has the greatest content of butyric acid. Goat milk yoghurt contained the highest concentrations of caproic, caprylic and capric acids which may be due to the high contains of these acids in goats' milk. Goat milk's unique flavor is caused by short-chain fatty acids, specifically caproic, caprylic, and capric acids. In this sense, Park ^[37] reports that lipolysis in ewe milk products is faster than that made from cow milk which enhances the typical flavor by higher content of short-chain fatty acids (caproic, caprylic and capric acids).

Concerning lauric acid, it was more abundant in sheep and cow milk yogurt than in other samples. In cow and buffalo yogurt samples, lauric acid was the dominant SCFAs while in goat and sheep milk yogurt, capric acid was the dominant. Numerous factors, like as variations in animal breed, food habits, fermentation conditions, and postharvest processing, may be responsible for the differences in SCFAs values found between the yoghurt treatments. SCFAs levels in milk have been found to be significantly influenced by the geographic region ^[37]. Certain studies have shown that heat treatment of milk, such as pasteurization, can lower the amount of SCFA, particularly C4:0 and C6:0, by up to 8.3% ^[9]. Conversely, other studies indicate that long-chain fatty acids may partially hydrolyze by thermal processing, resulting in the production of MCFAs and SCFAs ^[9]. Regarding fermentation effect on SCFAs content, Annunziata et al. ^[38] revealed that fermented goat milk had higher SCFAs values than unfermented milk. Also, Rashidimehr et al. ^[11] illustrated that fermentation significantly promotes SCFAs concentrations by enhancement lipolysis and microbial transformation of milk fats.

The MCFAs amounts were higher in buffalo and cow milk yogurt than other treatments. Myristic acid was more abundant in sheep and cow milk yogurt than in other treatments. Buffalo and camel yogurts contained close proportions, while goat milk yogurt had low myristic acid content. Camel yogurt had high pentadecanoic acid amount comparing with cow, buffalo, and goat yogurt. The lowest level was found in sheep yogurt. Palmitic acid was presented in higher concentrations in buffalo and cow milk yogurt and lower level in sheep one. The major acid of MCFAs in different Greek yogurt treatments was palmitic acid followed by myristic acid. Because lipoprotein lipase and microbial enzymatic systems hydrolyze triglycerides into SCFAs and MCFAs, the fermentation process of milk may generally result in

an increase in MCFAs levels ^[39]. Concerning the health effects of SCFAs and MCFAs, Pfeuffer and Schrezenmeir ^[40] indicated the beneficial impacts of short and medium chain fatty acids on reducing the metabolic syndrome risk. In the same context, Prosser ^[41] reported that the high levels of SCFAs and MCFAs in goat milk may decrease the metabolic syndrome risk in humans, including obesity, hypertension and abnormal blood lipids, making it suitable for human health. Furthermore, the high concentrations of these acids in goat milk promote its digestibility.

Camel and buffalo yogurt had the highest levels of LCFAs. Among the Greek yogurt samples, buffalo milk yogurt contained the highest level of palmitic acid followed by cow yogurt whereas the sheep milk yogurt had the lowest. Camel and goat milk yogurts approximately had similar palmitic acid contents. The fat extracted from camel milk yogurt is characterized by the greatest stearic and arachidic acids contents. Stearic acid concentrate was found in high levels in cow and buffalo milk yogurt samples comparing with goat and sheep milk yogurts. Regardless of yogurt sample type, the abundant acid in LCFAs was palmitic acid followed by stearic acid. These results are consistent with an earlier investigation by Sumarmono and Sulistyowati ^[42], who found that the main SFA in yoghurt was palmitic acid. Unfortunately, palmitic acid may associate with the risk of cardiovascular disease (CVD) by rising low-density lipoprotein cholesterol (LDL-C) ^[43]. Fortunately, stearic acid has a unique metabolic behavior. Stearoyl-CoA desaturase (SCD1) quickly desaturates it to oleic acid, which makes it easier to incorporate into complex lipids. It is frequently regarded as having neutral influence on the metabolism of cholesterol. However, some research suggests that stearic acid might act as a bioactive lipid, influencing pathways associated with neurological diseases, diabetes, and cardiovascular disease ^[44].

Camel yogurt possessed the greatest concentrations of MUFAs which is consistent with the results of Wang et al. ^[45] who mentioned that camel milk had the highest levels of MUFAs among ruminant milk. Myristoleic acid content was lower in buffalo and sheep yogurt than other treatments. Due to its advantageous functional characteristics, palmitoleic acid, an important n-7 MUFA in milk, has gained a lot of attention ^[46]. Among the Greek yogurt samples tested, camel yogurt contained the highest concentration of palmitoleic acid followed by cow then goat then buffalo then sheep yogurts. Oleic acid is well known for its health-promoting properties, particularly its capacity to raise high-density lipoprotein (HDL-C) cholesterol while lowering total cholesterol and LDL-C ^[47]. While oleic acid levels were relatively consistent across all yoghurts made from camel, goat and sheep milk, yoghurts made from buffalo and cow milk showed higher contents suggesting a more favorable fat composition. *cis*-11-Eicosenoic acid was only identified in buffalo, goat and sheep milk yogurts. Lastly, the most abundant MUFAs in all bio-Greek yogurts was oleic acid.

Due to their positive effects on cardiovascular health, PUFAs are well known ^[48]. They fall into the categories of omega-3 and omega-6 polyunsaturated fats. The highest levels of PUFAs were detected in camel, goat and sheep milk yogurt and the lowest values in buffalo and cow milk yogurt. Camel and goat milk produced Greek yogurt with higher linoleic acid content, while the linolenic acid level was more plentiful in yogurt made using camel or buffalo milk but sheep yogurt had the lowest content.

Arachidonic acid was identified in all yogurt treatments, with concentrations ranging from 0.11% in camel yogurt to 0.37% in sheep yogurt. Mantzourani et al. ^[49] illustrated that C8:0, C10:0, C18:3, C22:5 and C22:6 were detected at higher amounts in ewe milk Greek yogurt in comparison to cow milk Greek yogurt, while C14:1 and C20:3 at higher levels in cow yogurt in comparison to sheep yogurt. In various fresh Greek yogurt treatments, the most common polyunsaturated acid was linoleic acid. Generally, the differences in USFAs or PSFAs composition of Greek yogurt made from bovine, buffalo, camel, caprine and ovine milk can be attributed to several factors such as milk type, lactation, feeding, production season, heat treatments, species and strains of culture bacteria, fermentation conditions, storage period and added additives ^[49].

Researchers investigate other indicators in addition to particular fatty acids to evaluate the lipid quality of dairy products. These include the atherogenicity index (AI), thrombogenicity index (TI), hypocholesterolemic/hypercholesterolemic (H/H) ratio, and preferred fatty acid (DFA) values ^[10]. The possible effects of dairy lipids on the risk of cardiovascular problems and metabolic function are shown by the values of these indices.

Due to the high USFA content, camel milk yoghurt had the highest USFAs/SFAs value compared to other samples. A higher ratio of UFAs to SFAs is associated with a more positive impact on cholesterol contents ^[2]. Consuming a diet rich in UFAs can reduce LDL-C and blood cholesterol levels.

The DFA index consists of MUFAs, PUFAs plus stearic acid (C18:0), which is regarded as metabolically neutral because of its quick conversion to oleic acid in the liver ^[50]. Since it possessed elevated levels of UFAs and stearic acid, the camel milk yogurt sample showed high DFA level, referring to a more favorable lipid profile. Comparable ratios of DFA were noticed between the current study and that of Paszczyk and Czarnowska-Kujawska ^[51].

In the several health studies, the saturation index (SI) is used to determine the nutritional and functional properties of lipids in dairy products such as yogurt. It refers to the ratio of SFAs (C14:0 + C16:0 + C18:0) to USFAs. A low SI values are generally preferable for better cardiovascular health. On the other hand, $\Delta 9$ -desaturase, also named stearyl coenzyme-A desaturase (SCD), converts specific medium- and long-chain SFAs into the corresponding MUFAs ^[52]. This activity is an essential role in the biosynthesis of USFAs which is also good for cardiovascular health. Camel yogurt had the lowest SI index ratio and the highest $\Delta 9$ -desaturase (16) value. As well, previous studies have reported variations in the $\Delta 9$ -desaturase enzyme activity between breeds and between animals within breed ^[53].

The ratio of pro-atherogenic SFAs, mainly C12:0, C14:0 and C16:0, to anti-atherogenic UFAs is known as the atherogenicity index (AI). Ulbricht and Southgate ^[17] state that the capacity of lipids to increase the formation of atherosclerotic plaques is influenced by the amounts of phospholipids, cholesterol, and esterified fatty acids. In terms of yogurt lipid quality, lower AI index values indicate better nutritional qualities. The milk yoghurt from sheep, cows, and buffalo had the greatest AI levels because it contained higher levels of lauric, myristic, and palmitic acids. The results of AI obtained in this research consistent

with other investigations like Sumarmono et al. ^[54] who stated that the AI values of yogurt made from cow, goat and cow and goat mixture (1:1) were 2.39, 2.20 and 2.06 respectively. As previously mentioned, consuming dairy products with a low AI values can decrease blood cholesterol content as reported by Yurchenko et al. ^[55]. The atherogenic profile of dairy products might be positively affected by fermentation and particular formulation, according to the same authors. In the same contest, Tsiplakou and Zervas ^[56] highlighted that consumption of food with high values of AI and n-6/n-3 is claimed harmful to human wellness (cardiovascular disease).

Because an adequate intake of n-6 and n-3 fatty acids is essential for good health, measuring these levels is very important. According to Paszczyk and Czarnowska-Kujawska ^[51], those fatty acids are associated with a lower risk of several illnesses, including cardiovascular disease, type 2 diabetes, hypertension, cancer, and some disruptive neurological functions. Because the n-6/n-3 fatty acid ratio is associated with a significant 70% reduction in cardiovascular disease mortality, the Food and Agriculture Organization's and the World Health Organization's Expert Committee advised maintaining this ratio below 4 ^[57]. Additionally, Simopoulos ^[58] came to the conclusion that in order to prevent "lifestyle diseases" like cancer and coronary heart disease, the diet must have a ratio of n-6/n-3 below 4.0 and a ratio of PUFA to SFA above about 0.45. While diets with higher amounts of (n-3) PUFAs and a lower (n-6)/(n-3) ratio have suppressive effects, diets with excessive levels of (n-6) PUFAs and a high (n-6)/(n-3) value are proposed to accelerate the pathogenesis of many disorders ^[59]. The n-3 PUFA are commonly recommended for prevention heart disease ^[60]. The ratios of n-6/n-3 in all analyzed Greek yogurt samples were below the internationally recommended level (4) which indicates that fatty acids profile of these samples introduces healthy support to the human body. The sheep and goat milk yogurts had the highest n-6/n-3. According to Paszczyk and Czarnowska-Kujawska's study ^[51], bio yoghurts and eco yoghurts had the lowest n-6/n-3 values (1.82–1.85 and 2.02–2.09, respectively). In contrast, the n-6/n-3 ratio in milk fat was 2.3 in Mësson's study ^[61], and in yoghurts tested by Paszczyk and Łuczyńska ^[62], it was 4.77 in natural yoghurts, 3.04 in bio-yogurts and up to 10.59 in yoghurts with fruit and cereal grains.

In the yogurt samples, OFA determines the relative abundance of hydroxylated fatty acids. A greater OFA values are linked with a lower cardiovascular disease risk, probably due to the anti-inflammatory and antioxidant characteristics of hydroxylated fatty acids ^[52]. Numerous factors, including animal species, diet, lactation stage, and food processing, affect the values of OFA in dairy products. For example, the activity of desaturating and lipolytic microbial enzymes during ripening and fermentation processes may slightly reduce the amount of OFA ^[11]. The lowest OFA values were observed in camel and goat milk yogurts. Consistent with the results of our study, a study of Paszczyk and Czarnowska-Kujawska ^[51] cleared that OFA values in natural and bio-yogurts were 49.59 and 46.92 respectively.

Chen et al. proposed the health-promoting index (HPI) in 2004 ^[63] to assess the nutritional value of dietary fat with an emphasis on the impact of fatty acid composition on CVD. It is believed that dairy products with a high HPI value are better for human health ^[2]. Camel yogurt contained higher HPI values

than other samples. The average of HPI ratio found in the current investigation is 0.44 which is comparable to 0.45 of fermented goat milk stated by Muelas et al. [64].

The relationship between hypocholesterolemic fatty acids (cis-C18:1 and PUFAs) and hypercholesterolemic fatty acids (C12:0, C14:0, and C16:0) is explained by the hypocholesterolemic to hypercholesterolemic (H/H) ratio; a high H/H ratio indicates a more positive effect on lipid profile. It is associated with the risk of causing CVD and the functional impact of the fatty acid profile in cardiovascular metabolism for plasma cholesterol transport [62]. Therefore, rather than the UFAs/SFAs ratio, the H/H value more clearly indicates the impact of fatty acid content on heart-related disorders. Given its high SFAs and AI values, the camel yoghurt had the greatest H/H ratio, whereas the sheep yoghurt had the lowest. In the study of Paszczyk and Czarnowska-Kujawska [51] the H/H index values were 0.44, 0.45, 0.41, 0.70 in natural, probiotic, bio yogurts and natural yogurts with additives respectively which are lower than H/H ratios of our study.

Fatty acids' thrombogenic potential—their capacity to cause clots in blood vessels—is made clear by the TI index. It illustrates the relationship between anti-thrombogenic fatty acids (MUFAs and the n3 and n6) and pro-thrombogenic SFAs (C12:0, C14:0, and C16:0) [17]. The lower values of TI index show more nutritional characteristics of yogurt lipid indicating high amounts of anti-atherogenic fatty acids in fat which give antiplatelet and anti-inflammatory properties recommended for a healthy food [10]. In contrast, the high levels of TI indices raise the risk of developing cardiovascular diseases [18]. The lowest TI ratio was found in camel yogurt. The average of TI value of different Greek yogurt treatment was 3.207 which is consistent with values found in other studies [46].

Conclusion

Greek yogurt is one of the most popular fermented dairy products worldwide. It is known for its high nutritional value and attractive taste to many consumers. In this study, Greek yogurt was manufactured from cow's, buffalo's, camel's, goat's, and sheep's milk using a probiotic starter culture and Tgase. The resulting camel's milk yoghurt showed the best lipid profile of all the tested samples, with the highest levels of USFAs/SFAs, DFA, $\Delta 9 - DI$ (16), HPI, and H/H and the lowest values of SI, AI, OFA, and TI indices, indicating high health effects and low cardiovascular risk, even though the starter activity was slower in camel's milk than in the other treatments. Goat and cow milk yogurt came in second place of lipid quality followed by the buffalo and sheep yogurt.

Declarations

Authors' contributions

OS provided the technical assistance and performed methodology and formal analysis. MI designed and supervised the study, wrote and reviewed the manuscript. AK carried out the methodology, formal analysis, investigation and statistical analyses. DM carried out the methodology and formal analysis. RE

prepared the product and carried out the methodology, formal analysis and statistical analyses. All authors read and approved the final manuscript.

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All data generated or analyzed during this study are included in the published article.

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

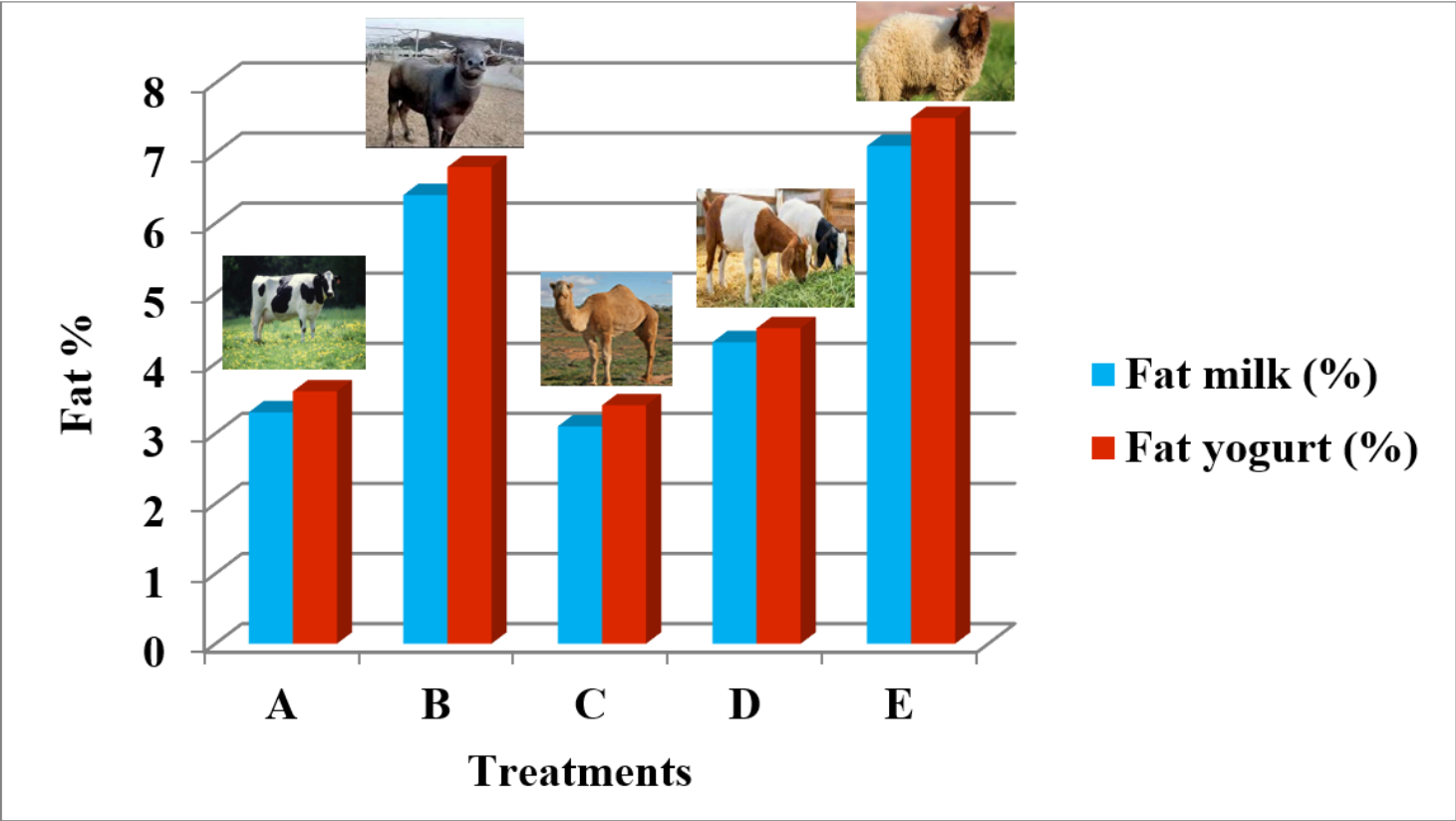


Figure 1

Fat contents of milk and Greek yogurt

A: cow milk and yogurt B: buffalo milk and yogurt; C: camel milk and yogurt D: goat milk and yogurt; E: sheep milk and yogurt

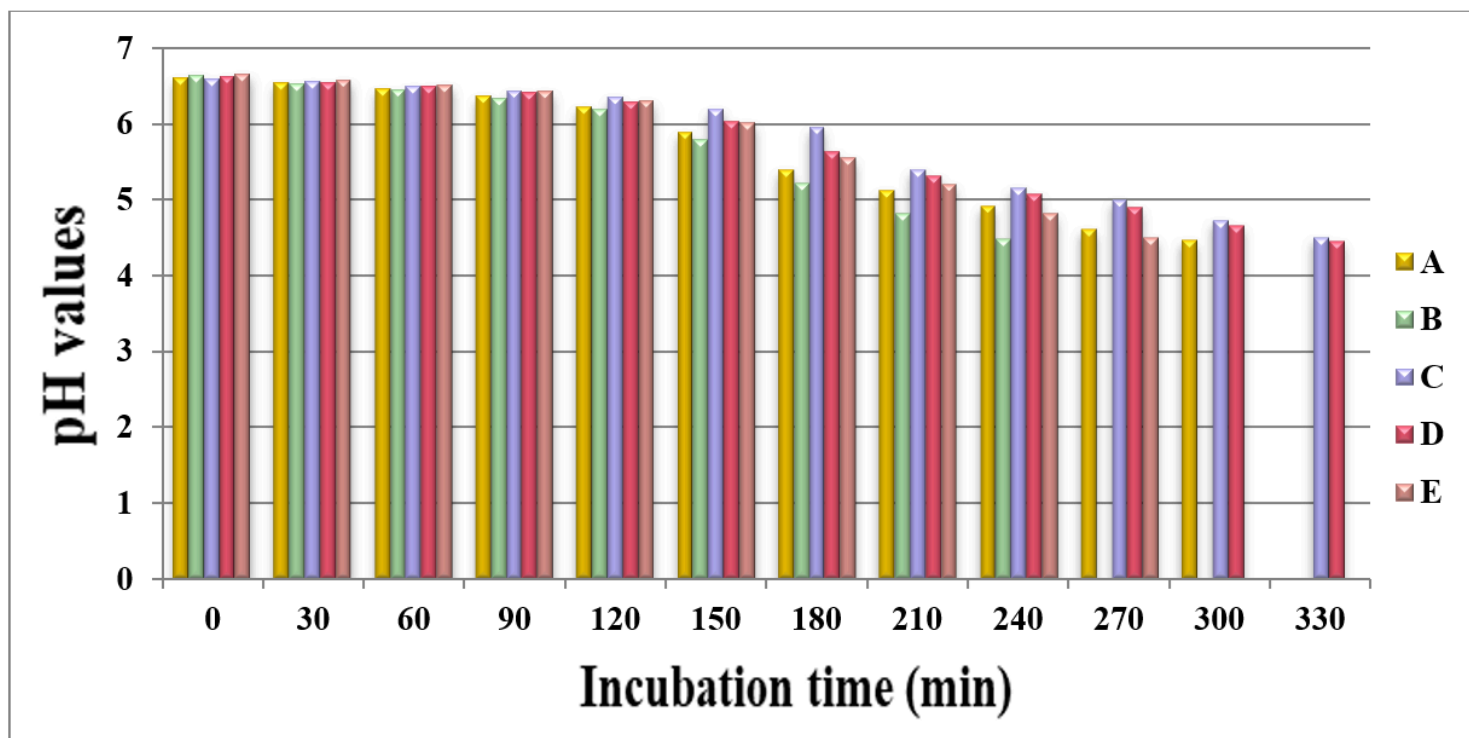


Figure 2

Changes in pH values during fermentation of cow, buffalo, camel, goat and sheep milk

A: Greek yogurt made from cow milk + 8% MPC + 0.04% Tgase; B: Greek yogurt made from buffalo milk + 8% MPC + 0.04% Tgase; C: Greek yogurt made from camel milk + 8% MPC + 0.04% Tgase; D: Greek yogurt made from goat milk + 8% MPC + 0.04% Tgase; E: Greek yogurt made from sheep milk + 8% MPC + 0.04% Tgase

Supplementary Files

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