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Hypocholesterolemic effect of designer yogurts fortified with omega fatty acids and dietary fibers in hypercholesterolemic subjects

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Abstract

A cohort study of 90 days was designed to explore the hypercholesterolemic associated blood pressure with intake of extruded flaxseed powder (EFSP) fortified yogurts. The hypercholesterolemic subjects were distributed into two groups (1 and 2), then blood samples and blood pressure were taken. Descriptive statistics were used for comparison between groups. A significant increase in serum total cholesterol (TC) (6.330.48 to 6.510.04 mmol/dL) and low-density lipids-cholesterol (LDL-C) (5.170.28 to 5.370.47 mmol/dL) was observed in participants consuming plain milk yogurts for 30 days. A significant decrease in TC (6.470.95 to 6.28 ± 0.84) and LDL-C (5.350.29 to 5.130.44 mmol/dL) was observed in the group consuming EFSP fortified sheep milk yogurt. Similarly, a significant decrease in serum TC (6.381.01 to 6.200.98 mmol/dL), and LDL-C (5.301.16 to 4.980.99 mmol/dL) was noticed in the group consuming EFSP fortified cow milk yogurt. The intake of fortified yogurts reduced significantly serum cholesterol associated with blood pressure in both groups.

Keywords: milk yogurt; flaxseed; dietary fibers; omega fatty acids; fortification; hypercholesterolemia

Practical Application: Designer yogurt beneficial for hypercholesterolemic patient with high blood pressure issues.

1 Introduction

Today, consumers' knowledge regarding their health advantages and food products has been significantly improved. That's why people are more conscious to have such foods in their diet to maintain and increase the quality of life (Ahmad et al., 2018; Brouns, Vermeer, 2000). Dairy products are being used from ancient times and are believed to have various therapeutic advantages associated with high nutritional significance and better digestibility (Corbo et al., 2001). The recent studies of fatty acids and their role in health made dairy products controversial. Most of the studies demanded that cholesterol and saturated fatty acids in a high proportion of dairy fat make it's unsuitable for human health particularly in a population with lipid diseases. The fortification of dietary fiber and polyunsaturated fat, while the replacement of saturated fats in dairy products has been emphasized (Bermúdez-Aguirre & B arbosa-Cánovas, 2012; Sadeghi, 2016). The dietary management of dyslipidemia through low-fat milk products consumption exhibits improved serum lipids in humans through lactic acid bacteria. The microbial strains of yogurt ferment the milk proteins to produce bioactive peptides which suppress the expression of a lipogenic gene of enzymes and accelerate the expression of a gene involved in lipid degradation (Garg et al., 2016; Sengupta et al., 2016). Besides that, bacterial strains play a vital role in the intestinal environment by fermenting food- graded carbohydrates and producing short-chain fatty acids. The addition of fructooligosaccharides and isomalto-oligosaccharide helps in the generation of short-chain fatty acids exhibit inhibition of cholesterol synthesis and alteration of the distribution of cholesterol between blood and liver resulting in a reduction of circulatory cholesterol concentrations (Sarfraz et al., 2019). Furthermore, the ingestion of lactic acid bacteria increases the bacterial density in the large intestine and thus enhances bacterial activity. This results in an elevation of deconjugation of bile acid and reduction of absorption in the intestinal mucosa. Accordingly, the cholesterol comprising a pioneer of bile acids is mostly employed for the synthesis of *de novo* bile acid (Kobayashi et al., 2012).

Regarding this, several studies have planned to fortify polyunsaturated fatty acids in milk, cheese, and yogurt (Benito et al., 2006, Bermúdez-Aguirre & Barbosa-Cánovas, 2011, Dawczynski et al., 2013). Fish oil, flaxseed oil, Camelina sativa, raspberry, blackcurrant, and Echium plantagineum oil have been used to improve the therapeutic effect of dairy products (Bertolino et al., 2015; Goyal et al., 2016; Zhong et al., 2018). However, the addition of unsaturated fats has poor water-solubility in milk and milk products resulting in higher acidity, oxidation, and rancidity. These attributes caused negative sensorial impact and poor consumer acceptability (Divya et al., 2013; Liutkevičius et al., 2010). Another approach to make dairy

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products more health benefits for the hyperlipidemia population, dairy products were fortified with dietary fiber. The common fibers like Arabic gum, xanthan, alginates were incorporated to improve the textural properties of products and health benefits. Several recent studies show that the addition of strains with some kind of fibers like gum, resins, etc. not only leads to prebiotic and probiotic effects but also improves the sensorial characteristics and storage stability of the product (Alizadeh Khaledabad et al., 2020; Hadjimbei et al., 2020; Lucatto et al., 2020). Yogurt has various therapeutic benefits and due to this reason used for all kinds of society. Earlier studies have been reported that the hypocholesterolemic, anti-cancer, dyspepsia, and diarrhea treatment, improve the properties of thiamine synthesis, drug detoxification on yogurt consumption (Agarwal & Bhasin, 2002; Hashim et al., 2009). Yogurt intake also improves appetite, intestinal health, and digestion in lactose-intolerant people (Srinivasan, 2010). This research was intended to assess the hypercholesterolemic impact of designer yogurts double fortified with dietary fibers and omega fatty acids by the addition of extruded flaxseed powder in hypercholesterolemic subjects.

2 Material and methods

2.1 Raw materials

The sheep milk, cow milk, and golden flaxseeds were purchased from the local market of Faisalabad. The stains culture of *Lactobacillus delbrueckii sub sp bulgaricus and Streptococcus thermophilus* were obtained from Faisalabad.

2.2 Flaxseed powder preparation by extrusion

The aim of extruded flaxseed powder (EFSP) manufacturing was to enhance the sensorial, textural properties and decrease the anti-nutritional properties of end products. For extrusion, a twin-screw extruder was utilized with a 36 mm screw diameter and with a ratio of length to diameter (24:1). For thermal cooking had a temperature zone with a measuring probe (temperature) and a screw speed adjuster. Box Behnken Design, RMS was performed for optimization purposes. The optimized conditions that were used 125 rpm screw speed, 125 °C barrel exit temperature, 60 Kg/hour feed flow rate of feed, and 15 % moisture contents of the feed.

2.3 Designer yogurts preparation

Yogurts were prepared according to previously described methods with little modification (Walstra et al., 1999) as can be seen in the flowchart diagram (Figure 1). The preparation of the control sample was done by using cow milk and sheep milk with a fat content of 3.5%. EFSP was added (2%) to milk and mixed thoroughly on a magnetic stirrer for 10 min. All the samples were heated for half an hour in a water bath at 85 °C. The samples were left to cool down to 42 °C. The starter culture of *Lactobacillus delbrueckii sub sp bulgaricus* and *Streptococcus thermophilus* was added at 0.2 U/L in 450 mL of milk and further divided into 225 mL food graded plastic boxes. The samples were fermented at 42 °C until the end of the pH (4.6) was achieved. All samples were kept for further analysis at 4 °C.

2.4 Composition analysis

The estimation of crude protein, crude fat, moisture content, crude fiber, and ash content was done according to AACC 2000 methods (Latimer, 2012).

2.5 Fatty acids profile

Fatty acid methyl esters (FAME) of all samples were formed by the method stated by Liu (1994). The fatty acid measurement was carried out according to the described method of Ahmad et al. (2019). The composition and fatty acid quantitative analysis was conducted from retention time and peak area by software (Varian Chem Station).

2.6 Lipid health quality Indexes

Health lipid indexes of yogurts were assessed by calculating atherogenicity index (IA), thrombogenicity index (IT), a ratio of hypercholesterolemic and hypercholesterolemic fatty acids (HH), Δ9-desaturase (C16) index (DI (16)), Δ9-desaturase (C18) index (DI (18)), and PUFA-n-6/PUFA-n-3 ratio, by using the following Equations (1-6):

$$AI = \left(C12:0 + 4 \times C14:0 + C16:0\right) / (SMUFA + SPUFA) \tag{1}$$

(Ulbricht & Southgate, 1991)

$$TI = \left(C14:0 + C16:0 + C18:0\right) / (0.5 \times SMUFA + 0.5 \times SPUFA - n - 6 + 3 \times SPUFA - n - 3 + SPUFA - n - 3 / SPUFAn - 6) \tag{2}$$

(Santos-Silva et al., 2002)

$$HH = (C18:1n - 9 + C18:2n - 6 + C20:4n - 6 + C18:3n - 3 + C20:5n - 3 + C22:5n - 3 + C22:6n - 6) / \left(C14:0 + C16:0\right) \tag{3}$$

$$DI\ (18) = 100\left(18:1 / \left(18:1 + 18:0\right)\right) \tag{4}$$

(Siebert et al., 2003)

$$DI\ (16) = 100\left(16:1 / \left(16:1 + 16:0\right)\right) \tag{5}$$

(Siebert et al., 2003)

$$PUFA - n - 6 / PUFA - n - 3 : total\ n - 6\ PUFA\ fatty\ acids / total\ n - 3\ PUFA\ fatty\ acids \tag{6}$$

Σ = Summation, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids.

2.7 Dietary intervention studies

2.7.1 Subjects

The recruitment was done by direct interaction with patients coming to hospitals visit. Both male and females patients were recruited in this study. The mean anthropometric, biochemical,

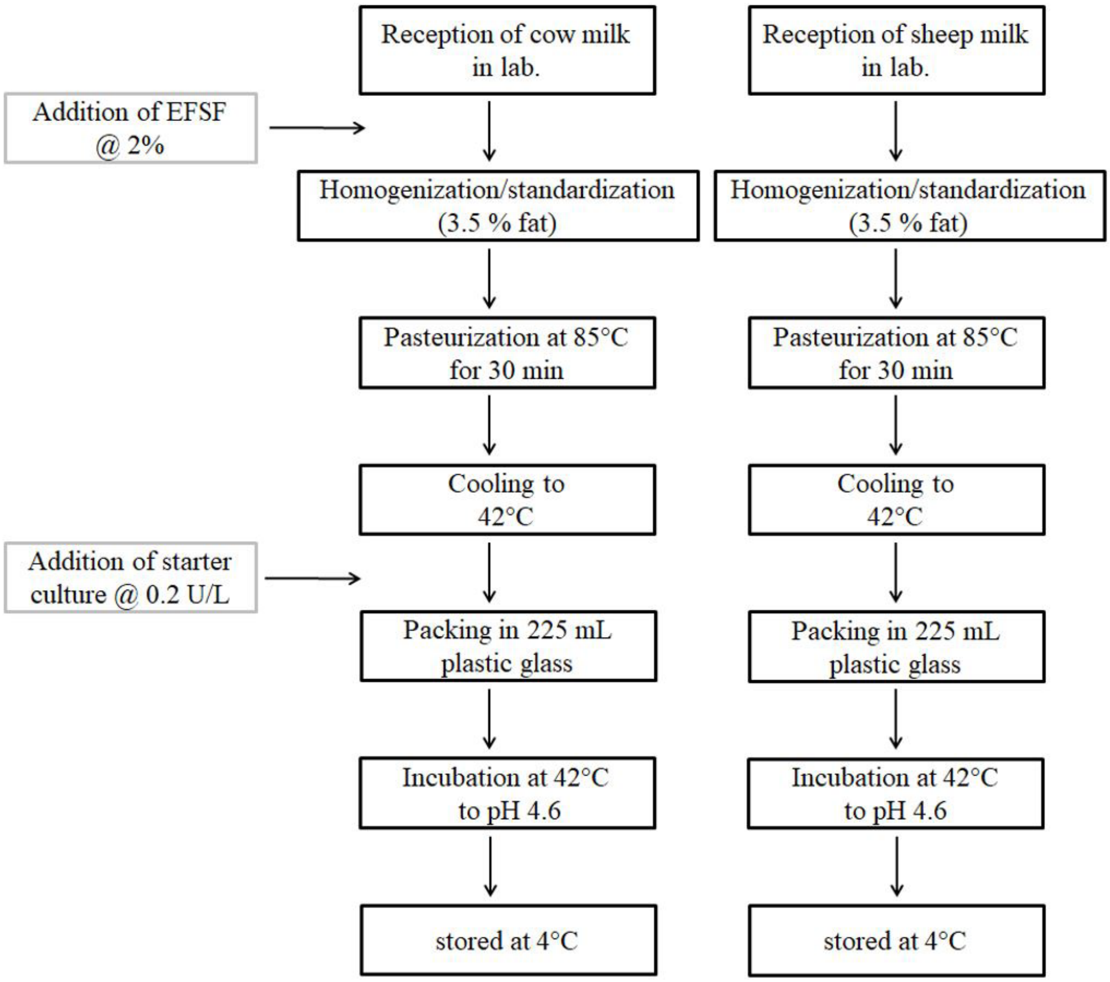

Figure 1. Experimental flow chart.

and blood pressure values of patients are presented in Table 1. The exclusion criteria included the absence of familial or secondary hypercholesterolemia, hypothyroidism, and diabetes. The patients in Pakistan do not usually use medicines of hypocholesterolemic, coagulants, immune regulators, corticosteroids, or any other hormones due to poor economic situations. The patients were only included who were not using the above medicines and observed any sign of myocardial infarction, angioplasty, or stroke in the last 4 months. Furthermore, lactose intolerant patients were excluded from the study. In the end, 57 patients were selected and taken consent to participate by signing the protocol authorized by the Institutional Review Board, GCUF (Ref# GCUF/ERC/1866) under the declaration of Helsinki.

2.7.2 Dietary Instructions

No special instructions related to dietary patterns and habits were given at the beginning or during the studies. They were instructed to continue their usual diet and activities.

2.7.3 Study design

The schematic layout of 3 months study is presented in Figure 2. All subjects were randomly divided into two groups. Group 1 (29 patients) consumed plain sheep milk yogurt and group 2 (28 patients) consumed plan cow milk yogurt for 30 days. The blood pressure and blood samples were attained at 0 and 30 days. All members were directed to keep on their normal food and actions for 30 days (wash-out period) without the provision of yogurts. At the end of the second month, the participants were again informed to participate in a study for the next 30 days. This time, group 1 consumed EFSP fortified sheep milk yogurt, and group 2 consumed EFSP fortified sheep milk yogurt for 30 days. The blood pressure was noted at baseline and blood samples were obtained end of the month. Three patients (two from group 1 and one from group 2) did not participate in second term study as they moved out of the city due to transfer or business activities.

2.8 Anthropometric measurements

The body weight and height were measured using a digital platform wireless floor balance human weight scale (Shenzhen Unique Scales Co., Ltd. China) and Stadiometer (IndoSurgicals Pvt. Ltd, India), respectively. BMI was determined by dividing height (m^2), weight (kg), and blood pressure was recorded using Blood Pressure Monitor HEM-6221 (OMRON, Singapore).

2.9 Biochemical measurements

After overnight fasting, all blood samples were obtained and sera were collected by centrifuging at 4000 rpm for 10 min. Serum TC (Allain et al., 1974), HDL-C (Finley et al., 1978), and total triacylglycerides (TG) (Bucolo & David, 1973) were determined accordingly. The level of HDL-C was measured by using the below Equation (7):

$$LDL-C = TC - HDL-C - TG/5 \tag{7}$$

Table 1. Characteristics of the hyperlipidemia patients (n = 57) at the start of the intervention study (mean ± SD).

Characters	Baseline before intervention
Age (years)	45.4 ± 15.3
Weight (kg)	85.1 ± 10.9
BMI (kg/m^2)	29.2 ± 8.1
Total cholesterol (mmol/L)	6.43 ± 0.75
HDL-C (mmol/L)	1.11 ± 0.27
LDL-C (mmol/L)	5.32 ± 0.73
Triglycerides (mmol/L)	2.97 ± 0.69
Total cholesterol/HDL-cholesterol ratio	5.59 ± 0.86
LDL-cholesterol/ HDL-cholesterol ratio	4.68 ± 0.73
Systolic blood pressure (mm Hg)	135.4 ± 15.8
Diastolic blood pressure (mm Hg)	85.7 ± 13.9

BMI: body mass index; HDL-C: high density lipoproteins-cholesterol; LDL-C: low density lipoproteins-cholesterol; mmol/L: millimoles/litre

2.10 Statistical analysis

After a triplicate analysis, the acquired results were subjected to statistical analysis. The change in lipid profile was calculated from endpoint values minus baseline values and compared using a one-way ANOVA. T-test has used to determine the difference within groups and p-value (0.05) used for statically significant.

3 Results

3.1 Serum lipid profile of subjects and proximate composition of yogurt

Lipid values were calculated in mmol/L (mean ± SD) at baseline and endpoint of the study. All patients recruited in this study have TC ranging from 6.27 to 7.01 mmol/L, LDL-C from 4.89 to 5. 59 mmol/L, HDL-C from 1.03 to 1.24 mmol/L, and TG from 2.72 to 3.17 mmol/L. The systolic and diastolic blood pressure of all patients at baseline ranged from 122 to 146 mmHg and 78.5to 91.1 mmHg, respectively. All patients were included to consume plain yogurts or designer yogurts manufactured from cow and sheep milk. Table 2 presented the nutritional composition of these yogurts. Total fat contents were ranged between 3.50-4.28%, total proteins 4.12-5.12%, moisture content 83.9-84.81%, ash content 0.91-1.18%, and total dietary fibers 24.11-24.14%. The total dietary fibers in which insoluble fibers varied from 18.71-18.73% and soluble fibers 6.35-6.36% in fortified and plain yogurts. A notable variation was observed for total dietary fiber and crude fat in EFSP fortified yogurt samples.

3.2 Fatty acid profile and health lipid indexes (HLI) of yogurts

Table 3 presented the fatty acid profile of fortified and plain yogurts. In tested yogurt samples the PUFA: 18:3n3, MUFA: C18:1n9, and SFA: C18:0, C16:0, C14:0, C10:0 are the predominant

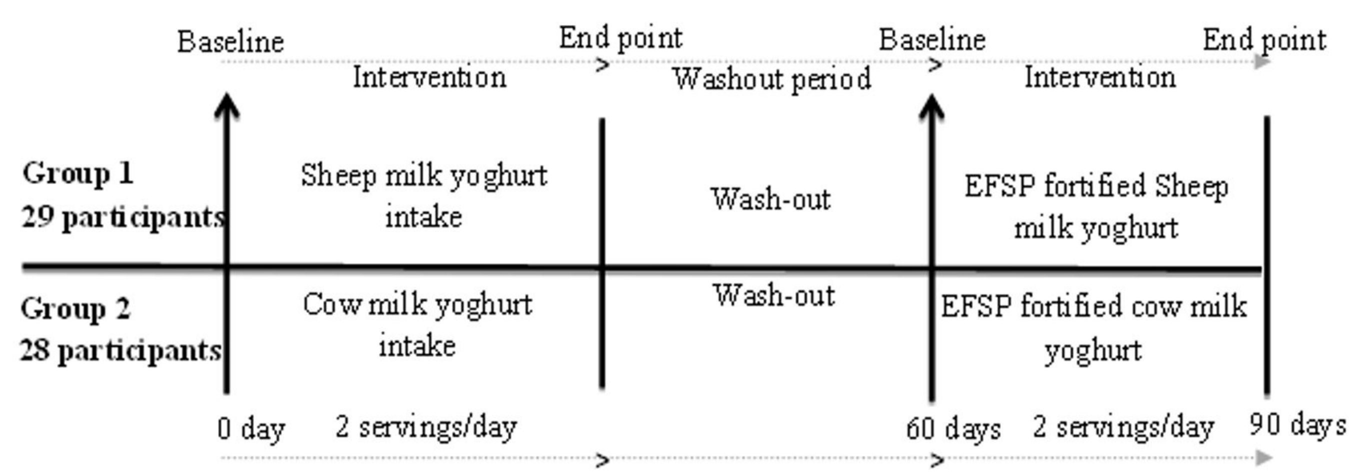

Figure 2. Study design: dietary intake of fortified yogurts and blood samples at each time point. Blood samples were collected at 0 days (baseline), after 30 days (intervention: sheep milk yogurt or cow milk yogurt intake for 30 days, 2 servings/days). Participants were allowed to eat a routine diet for a further 30 days (wash-out period) and again informed to participate in the further study period. After 30 days of wash-out periods, the blood sample was collected (baseline) and EFSP fortified sheep milk yogurt and EFSP fortified cow milk yogurt was provided to respective groups for the next 30 days (yogurt intake intervention). In the end, the blood sample was collected (endpoint). EFSP: extruded flaxseed powder.

ones. Palmitic acid (C16:0) was the most plentiful in all samples and particularly in cow milk yogurt fortified with EFSP. A highly significant improvement in fatty acid (18:3n3), while a significant rise in (C18:2n6 and C18:1n9) was recorded in fortified yogurts as EFSP contributed in high amounts. The summation ratios of health lipid indexes and fatty acids (g) of EFSP fortified and plain yogurts (450 g) are displayed in Table 4. The MUFA was ranged between 3.39-7.78%, SFA 11.59-17.21%, PUFA 0.58-3.87%, PUFA-n-3 0.33-2.99%, PUFA-n-6 0.22-0.88%, while the ratio of PUFA-n-6/PUFA-n-3 was 0.11-0.85% in yoghurt samples.PUFA-n-6/PUFA-n-3, PUFA, PUFA-n-3, and PUFA-n-6 were also observed significantly in all EFSP fortified yogurt samples. While a non-significant difference was found for SFA and MUFA among all treatments. Lipid health indexes quality was improved with fortification and fortified samples also showed a significant difference than plain yogurt samples. A significant difference was found in ESFS fortified cow milk yogurt than others for the Δ9-desaturase (18) index. HLI like HH, IT, and AI noted in sheep milk yogurt fortified with EFSP was 1.29%, 0.65%, and 1.42%; in plain sheep milk yogurt 0.82%, 1.68%, and 2.23%, respectively. While the HLI like HH, IT, and AI noted in cow milk yogurt fortified with EFSP was 1.12%, 0.39%, and 1.48%; in plain cow milk yogurt 0.54%, 1.84%, and 2.74%, respectively. Δ9-desaturase (18) index was observed lowest (62.27%) in cow milk yogurt fortified with EFSP and highest (75.67) in sheep milk yogurt fortified with EFSP. While Δ9-desaturase (16) index was observed minimum (3.93%) in sheep milk yogurt fortified with EFSP and maximum (6.21%) in cow milk yogurt fortified with EFSP.

3.3 Serum lipid profile of subjects consumed designer yogurts

In the baseline and endpoint study, the values of serum HDL-C, TG, TC, and LDL-C of groups 1 and 2 after two phases with plain and EFSP fortified sheep milk yogurt (group 1) and plain and EFSP fortified cow milk yogurt (group 2) intake are displayed in Table 5. A significant increase in serum TC (6.33-6.51) and LDL-C (5.17-5.37) was observed in participants consumed plain sheep milk yogurt for 30 days and non-significant change was observed for HDL-C and TG in the first phase of the study. After a washout period of 4 weeks, no significant change was observed in any cholesterol or TG. Group 1 consumed sheep milk yogurt fortified with EFSP but the HDL-C and TG did not vary significantly, while a significant reduction in LDL-C (5.35-5.13) and TC (6.47-6.28) was recorded. Group 2 participants consumed plain cow milk yogurt and non-significant variations were seen for serum LDL-C, HDL-C, TC, and TG after the first phase. After a washout period, group 2 consumed EFSP fortified cow milk yogurt and a significant decrease in serum TC (6.38 ± 1.01 to 6.20 ± 0.98), HDL-C (1.10 ± 0.21 to 1.27 ± 0.46), and LDL-C (5.30 ± 1.16 to 4.98 ± 0.99) were noticed while TG level did not change significantly.

3.4 Blood pressure of subjects consumed designer yogurts

Mean systolic and diastolic blood pressure after the periodical consumption of yogurts are presented in Figure 3. Group 1 had a diastolic and systolic blood pressure of 86.9 and 135.9 mmHg at baseline, non-significant change (90.3 to 140.7 mmHg; endpoint) was observed after the first interventional period. After a washout period, blood pressure (89.2 to 140.3 mmHg) was recorded and participants consumed EFSP fortified sheep milk yogurt, at the end of this interventional period, a significant decrease (81.7 to 127.6 mmHg) in blood pressure was recorded. Participants of group 2 had blood pressure 88.1 to 137.2 mmHg at baseline, non-significant change (92.5 to 141.3 mmHg) in blood pressure was recorded at the end of the first interventional period. After the washout period, at baseline, blood pressure was 90.2 to 139.9 mmHg. Participants consumed EFSP fortified cow milk yogurt 2 times/30 days, a significant decrease in blood pressure was recorded (80.8 to 123.1mmHg).

4 Discussion

Flaxseed is a valuable source of α-Linolenic acid (C18:3n3). It holds numerous health advantages such as prevention of cardiovascular disease, atherosclerosis, cancer, osteoporosis, diabetes, arthritis, autoimmune and neurological dysfunctions. Flaxseed oil also has been included in baked foods, dairy products, juices, muffins, macaroni, pasta, and meat products (Goyal et al., 2016). This study focused on yogurt from sheep's milk (plain and EFSP fortified) and the effect of its consumption in hyperlipidemic individuals' blood lipid profile, compared with the consumption of cow's milk yogurt (plain and EFSP fortified). This research was intended to assess the hypercholesterolemic impact of EFSP fortified designer yogurts. Cow and sheep milk samples were standardized at 3.5% and the addition of EFSP has improved the impact of functional yogurts. Simmons et al. (2010) declared that flaxseed has insoluble fiber and soluble fiber that are approximately 28 g/100 g. The total dietary fiber values for EFSP fortified cow and sheep milk yogurts were 24.11 and

Table 2. Nutritional composition (%) of EFSP fortified yogurt.

Indices	Sheep milk yogurt	EFSP Fortified sheep milk yogurt	Cow milk yogurt	EFSP Fortified cow milk yogurt
Crude proteins	4.68	5.12*	4.12	4.56
Crude Fat	3.50	4.28*	3.50	4.24*
Ash	0.91	0.96	1.15	1.18
Moisture	83.9	83.99	84.7	84.81
Total dietary fibers	--	24.11***	--	24.14***
Soluble Fiber	--	6.36***	--	6.35***
Insoluble Fiber	--	18.71***	--	18.73***

EFSP: extruded flaxseed powder; the superscript (*) on values shows the level of significance

Table 3. Fatty acids (g) profile of EFSP fortified yogurts (450 g).

Fatty acids (g)	Sheep Milk yogurt	EFSP fortified sheep milk yogurt	Cow milk yogurt	EFSP fortified sheep milk yogurt
C4:0	0.59 ± 0.09	0.58 ± 0.39	0.72 ± 0.08	0.73 ± 0.01
C6:0	0.37 ± 0.04	0.37 ± 0.09	0.47 ± 0.03	0.46 ± 0.39
C8:0	0.45 ± 0.09	0.45 ± 0.07	0.21 ± 0.08	0.21 ± 0.02
C10:0	1.05 ± 0.03	1.05 ± 0.08	0.58 ± 0.02	0.59 ± 0.02
C12:0	0.53 ± 0.03	0.53 ± 0.08	0.58 ± 0.05	0.60 ± 0.05
C14:0	1.58 ± 0.04	1.58 ± 0.01	1.67 ± 0.21	1.67 ± 0.51
C14:1n5	0.05 ± 0.03	0.05 ± 0.02	0.30 ± 0.06	0.29 ± 0.02
C15:0	0.12 ± 0.07	0.11 ± 0.07	0.30 ± 0.07	0.30 ± 0.02
C16:0	3.74 ± 0.99	3.97 ± 0.52	4.32 ± 0.99	4.54 ± 1.03
C16:1n7	0.15 ± 0.07	0.16 ± 0.04	0.28 ± 0.03	0.28 ± 0.01
C17:0	0.10 ± 0.07	0.10 ± 0.07	0.18 ± 0.05	0.18 ± 0.02
C18:0	1.46 ± 0.28	1.58 ± 0.09	1.77 ± 0.75	1.89 ± 0.87
C18:1n9	4.23 ± 0.55	5.08 ± 1.23*	2.93 ± 0.98	3.78 ± 0.78*
C18:2n6	0.06 ± 0.01	0.73 ± 0.05**	0.14 ± 0.01	0.80 ± 0.11**
C18:3n6	0.02 ± 0.01	0.02 ± 0.03	0.02 ± 0.01	0.03 ± 0.01
C18:3n3	0.08 ± 0.03	2.71 ± 0.33***	0.14 ± 0.03	2.77 ± 0.87***
C20:0	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02
C20:2	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C20:3n6	0.01 ± 0.01	0.01 ± 0.01	0.07 ± 0.04	0.09 ± 0.03
C20:4n6	0.04 ± 0.02	0.04 ± 0.01	0.06 ± 0.03	0.06 ± 0.02
C20:3n3	0.05 ± 0.03	0.05 ± 0.02	0.08 ± 0.01	0.08 ± 0.01
C20:5n3	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.02
C22:0	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
C22:1n9	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.03
C22:2	0.10 ± 0.07	0.10 ± 0.04	0.06 ± 0.02	0.06 ± 0.02
C22:6n3	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.07 ± 0.03
C23:0	0.05 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.02
C24:0	0.08 ± 0.03	0.08 ± 0.03	0.04 ± 0.01	0.04 ± 0.01

EFSP: extruded flaxseed powder, the superscript (*) on values shows the level of significance

Table 4. The ratios of fatty acids (g) and health lipid indexes of EFSP fortified yogurt (450 g).

Indexes	Sheep Milk yogurt	EFSP fortified sheep milk yogurt	Cow milk yogurt	EFSP fortified sheep milk yogurt
SFA	16.84 ± 1.44	17.20 ± 2.42	11.57 ± 1.43	11.90 ± 1.92
MUFA	6.90 ± 0.61	7.77 ± 1.61	3.37 ± 0.87	4.22 ± 1.18
PUFA	0.57 ± 0.23	3.86 ± 0.91***	0.61 ± 0.03	3.28 ± 0.92***
PUFA-n-6	0.21 ± 0.07	0.87 ± 0.06**	0.29 ± 0.03	0.37 ± 0.023
PUFA-n-3	0.34 ± 0.06	2.98 ± 0.37***	0.32 ± 0.12	2.94 ± 0.02***
PUFA-n-6/ PUFA-n-3	0.62 ± 0.03	0.28 ± 0.05**	0.84 ± 0.08	0.13 ± 0.06**
AI	2.22 ± 0.42	1.41 ± 0.22**	2.75 ± 0.30	1.51 ± 0.05**
IT	1.67 ± 0.93	0.64 ± 0.02***	1.83 ± 0.28	0.37 ± 0.03***
HH	0.81 ± 0.04	1.28 ± 0.45**	0.55 ± 0.06	1.14 ± 0.20**
DI (18)	74.31 ± 9.91	75.66 ± 8.03	62.25 ± 8.62	66.85 ± 9.50*
DI (16)	4.02 ± 1.62	3.91 ± 1.36	6.23 ± 1.22	5.94 ± 1.41

EFSP: extruded flaxseed powder, SFA: saturated fatty acid, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, PUFA-n-6: total n-6 PUFA fatty acids, PUFA-n-3: total n-3 PUFA fatty acids, PUFA-n-6/PUFA-n-3: total n-6 PUFA fatty acids/total n-3 PUFA fatty acids, AI: atherogenic index = (C12:0+4×C14:0+C16:0)/(∑MUFA+∑PUFA), TI: thrombogenic, Index=(C14:0+C16:0+C18:0)/(0.5×∑MUFA+0.5×∑PUFA-n-6+3×∑PUFA-n-3+∑PUFA-n-3/∑PUFA-n-6), HH: Hypocholesterolemic/ hypercholesterolemic fatty acids = (C18:1 n-9 + C18:2 n-6 + C20:4 n-6 + C18:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-6)/(C14:0 + C16:0), DI (18): Δ9-desaturase (C18) index = 100(18:1/(18:1+18:0)), DI (16): Δ9-desaturase (C16) index = 100(16:1/(16:1+16:0), The superscript (*) on values shows the level of significance

Table 5. Plasma lipid concentration on the consumption of EFSP fortified yogurts.

Supplements	Measurement period	TC	HDL-C	LDL-C	TG
Sheep milk yogurt	Baseline	6.330.48	1.130.19	5.17 ± 0.28	2.94 ± 0.64
	Endpoint	6.510.04	1.160.23	5.37 ± 0.47	2.93 ± 0.41
Change		0.180.62	0.030.44	0.20 ± 0.80	-0.01 ± 0.56
t -test	p-value	0.034	NS	0.041	NS
EFSP Fortified sheep milk yogurt	Baseline	6.470.95	1.110.16	5.35 ± 0.29	2.92 ± 0.43
	Endpoint	6.280.84	1.140.21	5.13 ± 0.44	2.93 ± 0.39
Change		-0.190.33	0.030.28	-0.12 ± 0.11	0.01 ± 0.69
t -test	p-value	0.026	NS	0.039	NS
Cow milk yogurt	Baseline	6.370.71	1.090.36	5.24 ± 1.09	2.87 ± 0.83
	Endpoint	6.400.39	1.110.41	5.27 ± 0.87	2.89 ± 0.94
Change		0.030.70	0.020.25	0.03 ± 1.03	0.02 ± 0.78
t -test	p-value	NS	NS	NS	NS
EFSP Fortified cow milk yogurt	Baseline	6.381.01	1.100.21	5.30 ± 1.16	2.88 ± 0.84
	Endpoint	6.200.98	1.27 ± 0.46	4.98 ± 0.99	2.90 ± 0.65
Change		-0.180.93	0.17 ± 0.61	-0.32 ± 1.02	0.02 ± 0.55
t -test	p-value	0.029	0.041	0.022	NS

EFSP: extruded flaxseed powder; TC: total cholesterol; HDL-C: high-density lipid-cholesterol; LDL-C: low-density lipid-cholesterol; TG: Triacylglycerides, NS: non-significant.

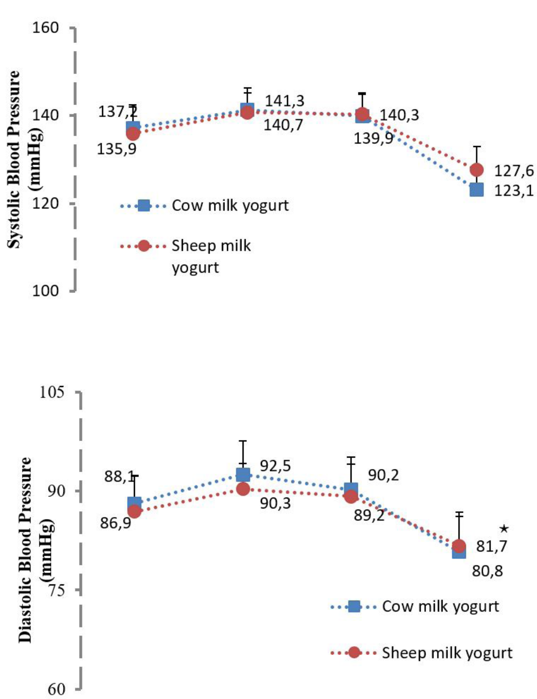

Figure 3. Mean systolic and diastolic blood pressure on the consumption of EFSP fortified yogurts. The mean values were recorded at 0 days (baseline), after 30 days (intervention: sheep milk yogurt or cow milk yogurt intake for 30 days, 2 servings/days). Participants were allowed to eat a normal diet for a further 30 days (wash-out periods) and again informed to participate for the further study period. After 30 days of wash-out periods, the mean values were recorded (baseline) and EFSP fortified sheep milk yogurt and EFSP fortified cow milk yogurt was consumed by respective groups for the next 30 days and the mean values were recorded (endpoint). EFSP: extruded flaxseed powder.

24.14% after the addition of flaxseed powder and these results are slightly varied from the findings of (Simmons et al., 2010). These verdicts might vary due to different species of flaxseed.

In flaxseed, PUFA including α-Linolenic and linoleic acids is present nearly 80%. C18:3, C18:2, and C18:1 are the most important fatty acids that are essential to support human wellness (Goh et al., 2006). They noticed a notable variation in PUFA (C18:3) between plain yogurt to EFSP fortified yogurt samples. The amount of PUFA was significantly higher in yogurt with flaxseed flour and oil; these observations are following the current study. The PUFA was significantly higher in flaxseed oil and flour fortified yogurts; these results are supporting our research. MUFA's and SFA's were significantly higher in sheep milk yogurt than cow milk yogurt, although there were no notable variations in the PUFA content in plain yogurt samples (Serafeimidou et al., 2012). The fortified samples showed ramified behavior for fatty acids upon fortification. Tsiplakou & Zervas (2008) measured AI and the n-6/n-3 ratio of milk at different stages of lactation and observed values between 4.08 to 5.13 and 3.35 to 4.27, respectively. They declared that food intake with a high n–6/n–3 ratio and AI is supposed injurious to human wellness. AI and n-6/n-3 ratio in the present study is lower than they claimed, especially in fortified yogurts (Tsiplakou & Zervas, 2008). Omri et al. (2019) measured the lipid health indexes of egg yolk of laying hens. Hens were fed on a diet that was incorporated with linseed and found the values of IT and HH (hypocholesterolemic / hypercholesterolemic fatty acids) about 0.85 to 1.16 and 1.92 to 2.41 in eggs. They stated that feeding linseed-supplemented diets significantly reduced the IT and similar findings were observed in the present study (Omri et al., 2019). Flaxseed is a good source of fiber and omega-3 fatty acids that promotes cardiovascular health (Rodriguez-Leyva et al., 2013). They suggested that patients who have peripheral artery disease may get benefit by taking flaxseed in the diet as hypertension is usually linked to this disease. After flaxseed supplementation to hypertensive patients, they observed systolic and diastolic blood pressures were ≈10 and ≈7 mmHg lower, respectively in the flaxseed group. A blood pressure reduction was also noticed in subjects fed on EFSP fortified yogurt. EFSP fortified cow milk yogurt showed better effect for hypertensive patients than sheep fortified (Rodriguez-Leyva et al., 2013). The consumption of sheep's milk (plain) and cow's milk (plain) yogurts, have ameliorated effect on the traditional biomarkers of cardiovascular diseases (TC, LDL-C, TG, and HDL-C) but did not reveal notable variations. These findings are following the study of Olmedilla-Alonso et al. (2017) but the consumption of EFSP fortified sheep milk yogurt showed significant changes for these biomarkers especially for LDL-C and TC (but not for TG and HDL-C) while the consumption of EFSP fortified cow milk yogurt significantly changed the HDL-C, TC, and LDL-C levels. This is because flaxseed contains good amounts of PUFA which help to reduce these biomarkers. EFSP fortified cow milk yogurt showed a better effect on these biomarkers because this yogurt contains more amounts of C18:3n3 (helps to reduce TC and body weight and good for the heart) as compared to EFSP fortified sheep milk yogurt. Although; the regulation of cholesterol-associated blood pressure can be attributed to PUFAs present in the EFSF but the role of complex carbohydrates cannot be neglected and required additional studies.

5 Conclusions

The current study shows the dietary effect of plain and designer yogurt from sheep and cow milk on blood pressure and lipid profile in hypercholesterolemic subjects. Our study indicates that plain yogurt with standardized fat contents of 3.5% increase TG, LCL-C, and blood pressure in hypercholesterolemic patients for 30 days consumption. The designer yogurts prepared from cow and sheep milk fortified with fibers and omega fatty acids through EFSP significantly reduced TC, LDL-C, and blood pressure in hypercholesterolemic patients. Thus designer dairy products particular yogurt can be used in the management of hypercholesterolemia on regular consumption.

Conflict of interest: No conflicts of interest exist.

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