ORIGINAL ARTICLE

Cholesterol-Lowering Efficacy of Plant Sterol-Enriched Flavored Milk, Yogurt, Fruit Bar, and Soya Milk in Mild Hypercholesterolemic Indian Subjects

M. Penchalaraju[1], Aparna Kuna[2], P. S. S. Shailaja[1], K. Vinay Kumar[1], P. Uma Devi[1], T. Supraja[1], Vanu Ramprasath[3], Peter J. H. Jones[3]

[1]Post Graduate and Research Centre, Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India, [2]MFPI, Quality Control Laboratory, Prof. Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India, [3]Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada

ABSTRACT

Objective: The study was designed to measure the relative efficacy of four phytosterol (PS) ester-enriched low-fat foods (Flavoured milk, Yoghurt, Fruit bar and Soya milk) on serum lipids in Indian subjects with mild hypercholesterolemia. **Research Methodology:** Forty-eight mild hypercholesterolemic men and women were recruited (6 subjects for control group and 6 subjects for experimental group for all products. Experimental group were supplemented with phytosterol enriched (2%) products and control group were supplemented with placebo (product without addition of phytosterol) for 30days. Results: The results confirmed that serum total and LDL cholesterol levels were significantly lowered by consumption of phytosterol-enriched foods: soya milk (8.7 and 12.6%) and fruit bar (5.0 and 9.1%), yoghurt (4.3 and 5.3%), flavoured milk (2.5 and 2.6%) respectively. Compared to phytosterol enriched soy milk and fruit bar, Serum LDL cholesterol levels decreased significantly by 5.3% with yoghurt and 2.6% with Flavoured milk. Similarly serum total cholesterol levels decreased significantly (P<.005) by 4.3% with yoghurt and 2.5% with flavoured milk. They were both significantly (P<.005) less efficacious than sterol-enriched soya milk and fruit bar. **Conclusion:** These results indicated that cholesterol-lowering effects of plant sterol esters may differ according to the food matrix. Soya milk and fruit bar seem to be better vehicles for enrichment with phytosterol esters for reducing the serum total cholesterol and LDL cholesterol than yoghurt and flavoured milk.

Key words: Hypercholesterolemic subjects, low density lipoprotein cholesterol, phytosterol

INTRODUCTION

Plant sterols are minor constituents of vegetable oils (0.1–0.9 g/100 g) that have a chemical structure similar to that of cholesterol.[1] There are extensive data confirming the effectiveness of esterified phytosterols (PS) in different foods with low-density lipoprotein cholesterol (LDL-C) lowering of 10–20% with a dose of 1.6–3 g/day of sterol.[2] Normal dietary intake of plant sterols is 160–360 mg/d, with a typical composition of 65% as β-sitosterol, 30% as campesterol, and 5% as stigmasterol.[3] When consumed at levels 5–10 times higher than the normal intake, plant sterols have been shown to lower blood LDL-C levels. PSs decrease cholesterol absorption rate, by displacing cholesterol from intestinal micelles and thus preventing cholesterol absorption. PSs can enter the enterocyte but are immediately released to the intestinal lumen through the ABCG5/G8 molecular system.[4] The western diet contains about 150–400 mg of PSs and stanols per day.[5] At least 1 g of PSs per day is necessary to obtain a significant 5–8% LDL-C

Address for correspondence:
Aparna Kuna, MFPI - Quality Control Laboratory, Prof. Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India. E-mail: aparnakuna@gmail.com

reduction.[6] This effect is dosage dependent until reaching a plateau of around 2 g per day.[5] Dietary PS supplements are usually prescribed to moderately hypercholesterolemic patients; therefore, it may be better to incorporate them in low-fat products and diets.[7-9] Results from various studies conducted by Morton *et al*., Ras *et al*., Ras *et al*. and Jones *et al.,*[3,7-9] reported an age dependent reduction in serum LDL-C between 0.33 and 0.54 mmol/l or 9–14%, at a dose of ≥2 g/d of plant sterols or stanols. A more recent-analysis including 41 trails confirmed these findings and concluded that 2 g/d of plant sterols or stanols lower LDL-C by 10%.[10] There are two studies showing that 1–2 g/day of sterols or stanols in low-fat yogurts are effective at lowering LDL-C in patients with moderate primary hypercholesterolemia,[10,11] but no studies have used low-fat milk. It is possible that the milk fat globule membrane which has been altered by acid and/or microbial action in yogurts may adsorb sterols differently to a native membrane. Very few studies directly compared different food products to determine the effectiveness of PSs; however, no studies compared different food products to see the effectiveness of PSs in the Indian context. As Indians consume considerable amounts of dairy products such as milk, curd, lassi, buttermilk, and flavored milk, introducing PSs, as functional ingredients, into most commonly consumed dairy products will increase consumption and help maintain healthy cholesterol levels in the Indian population.[12] The main objective of this study is to compare the relative efficacy of each of four PS ester-enriched low-fat foods (flavored milk, yogurt, fruit bar, and soya milk) on serum lipids.

MATERIALS AND METHODS

For the present investigation, toned milk and skim milk powder were purchased from Heritage Foods India Ltd., (Hyderabad, India), Cocoa powder obtained from Morde Foods Private Ltd., (Mumbai, India), stabilizer (CEKOL® cellulose gum 30000A) procured from CP kelco A Huber Company (USA), matured papaya *(Carica papaya*) fruits were purchased from local market of Hyderabad, India, and analysis kits for triglycerides (TG), cholesterol, and fluoride-treated tubes were procured from Medsourse ozone Biomedicals Pvt. Ltd., (Delhi, India). All other chemicals were purchased from Qualigens Fine Chemicals (Mumbai, India) or Molychem India Pvt. Ltd. (Mumbai, India). PS powder was procured from Reducol™ original Powder (Forbes medi-Tech Inc.) USA-based company. Unless otherwise mentioned, all chemicals used were of analytical grade.

PS supplementation methodology

Four products (flavored milk, yogurt, fruit bar, and soya milk) were developed and standardized with 2% PS powder for supplementation. Products added with 2% PS served as experimental, and the same products without PS served as control. The subjects were informed about the products

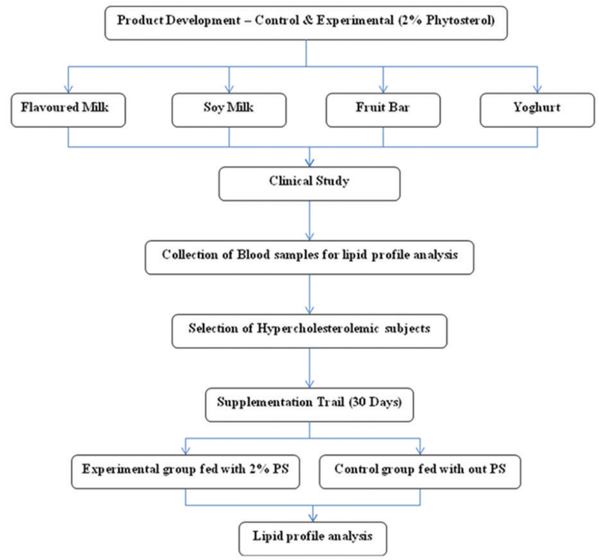

Figure 1: Design of the study for product development and lipid profile analysis

they were served with, but unaware of whether they were served with control or experimental sample. All the products prepared were coded using random three-digit numbers and served to the subjects. Table 1 and Figure 1 give detailed information about the study.

Forty-eight modestly hypercholesterolemic men and women were recruited (6 subjects each for both control and experimental group) based on initial screening. Healthy male or females, aged between 25 and 45 years, were screened for the determination of fasting circulating total cholesterol (TC) and TG levels. Subjects with fasting TC between 192.0 and 230 mg/dl and TG levels below 250 mg/dl were accepted into the study. Subjects after giving written consent to participate in the study also demonstrated an ability to understand dietary procedures and were judged as compliant and motivated by the investigators. None of the subjects had any comorbid condition in the past 6 months, and none of them were on any kind of medication. All subjects were advised to consume their regular meal pattern. All the subjects recruited in the study were staying in a hostel consuming same meal.

Participants were asked to maintain their usual dietary habits throughout the study. The control or experimental products were provided to all the subjects during lunch time. The selected subjects received 100 mL serving of either plain (control) low-fat or PS-enriched soy milk/flavored milk/200 g yogurt/50 g of fruit bar per day along with the main meal (Lunch) for 30 days.

Fasting blood samples were drawn from the subjects on the 0 day, after 30 days with all aseptic precautions, and after a 12-h overnight fast using disposable syringes. 5 mL of venous blood sample was collected into fluoride-treated tubes. Serum was separated immediately by centrifugation, transferred to storage vial, and stored at −80°C until analysis. Lipid profile (TC, TG, high-density lipoprotein [HDL], and LDL) was determined in collected samples on 0 day and 30th day in both the groups using CHOD-PAP, GPO-PAP, and PEG-CHOD-PAP methods.[13] Analysis of variance has been used to find the significance of study parameters between the groups of subjects. Significance was assessed at 5% level of significance.[14]

RESULTS AND DISCUSSION

Effect of the supplementation of PSs on lipid profile (hypocholesterolemic effect)

The effect of supplementation of PSs on hypercholesterolemia was assessed in human subjects for 30 days, and the mean TC values of the study are given in Table 2. The results of the serum lipids show that there was a reduction in the TC, TG, and LDL-C and slight increase in HDL-cholesterol (HDL-C) levels in all the control and experimental subjects supplemented with papaya fruit bar. However, the reduction was significant ($P < 0.05$) only in the experimental group. This could be due to PSs and also the due to the antioxidant activity of the fruit bar, which could be due to the presence of concentrated carotenoids and lycopene in the papaya fruit bar. A study by Krishna *et al.*[14] indicated that the consumption of guava and papaya fruits reduced oxidative stress and altered lipid profiles, thus reducing the risk of diseases caused by

Table 1: Supplementation for experimental and control group

Productst	Experimental group		Control group	
	PS%	30 days Supplementation	PS%	30 days Supplementation
Flavored milk	2	100 ml/day	-	100 ml/day
Papaya fruit bar	2	50 g/day	-	50 g/day
Soya milk	2	100 ml/day	-	100 ml/day
Yogurt	2	200 g/day	-	200 g/day

Table 2: Effect of phytosterol-enriched foods on lipid profile

Lipid parameters	Control group		Experimental group	
	Initial	Final	Initial	Final
Effect of PS-enriched fruit bar on the lipid profile of the subjects (mg/dL)				
Cholesterol	224.16±2.78	223.84^{NS}±2.67	224.36±5.28	210.61*±7.45
Triglycerides	198.29±16.83	196.49^{NS}±16.86	185.46±10.48	173.93*±9.94
LDL-C	129.78±6.51	126.78^{NS}±6.75	144.13±3.68	131.08*±4.54
HDL-C	45.56±8.12	45.72^{NS}±8.25	52.84±4.53	52.62^{NS}±3.83
Effect of PS-enriched soy milk on the lipid profile of the subjects (mg/dL)				
Cholesterol	211.16±6.84	210.84^{NS}±7.94	218.47±6.80	199.82*±7.23
Triglycerides	167.56±4.81	164.62^{NS}±4.51	172.21±3.11	156.88*±4.67
LDL-C	120.91±3.40	117.48*±5.03	141.46±3.04	123.65*±4.87
HDL-C	39.53±1.73	39.78^{NS}±1.63	40.11±1.25	41.73^{NS}±1.64
Effect of PS-enriched yogurt on the lipid profile of the (mg/dL)				
Cholesterol	216.06±14.92	214^{NS}±14.86	220.66±7.63	212.33*±16.62
Triglycerides	187.89±27.79	185.53^{NS}±27.83	215.33±16.62	207.33^{NS}±14.69
LDL-C	138.20±10.47	136.78^{NS}±11.18	138.73±3.91	131.36*±3.15
HDL-C	40.28±2.63	40.27^{NS}±2.60	38.86±1.53	39.50^{NS}±1.75
Effect of PS-enriched flavored milk on lipid profile of the subjects (mg/dL)				
Cholesterol	214.59±13.77	215.8^{NS}±16.00	222.29±7.32	216.66*±6.21
Triglycerides	187.89±28.00	186.46*±28.06	190.05±4.02	187.95^{NS}±4.18
LDL-C	132.1±8.62	133.94^{NS}±8.68	136.82±5.09	133.23*±6.14
HDL-C	44.90±8.37	44.44^{NS}±7.20	47.33±6.03	46.75^{NS}±8.45

*Significance at P<0.05; NS: Not significant at 5% level. PS: Phytosterol, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol free radical activities such as cancer and cardiovascular disease.[15,16]

Consumption of soy milk significantly ($P < 0.05$) reduced the TC, TG, and LDL-C levels in all the experimental subjects. Reduction of TC, TG, and LDL-C levels was also seen in the control subjects, though not significant. It was also observed that the HDL-C levels increased in both control and experimental groups indicating that soy milk could be a very good vehicle for PS enrichment for reduction of the TC, TG, and LDL-C and increase in HDL-C. The reduction was only 8.7 and 12.6% in the TC and LDL-C levels in 30 days of consuming the soy milk. This could be more if the supplementation was increased beyond 30 days. Similar results were seen in a study by Rideout *et al*.,[13,17] who reported that compared to 1% dairy milk, consumption of low- and moderate-fat PS-enriched soy beverages represents an effective dietary strategy to reduce circulating lipid concentrations in normal to hypercholesterolemic individuals by reducing intestinal cholesterol absorption.

PS-enriched yogurt and flavored milk showed a significant ($P < 0.05$) decrease in the TC and LDL-C levels in the experimental group. Volpe *et al.*[11] reported that low-fat yogurt-based drink moderately enriched with plant sterols may lower TC and LDL-C effectively in patients with primary moderate hypercholesterolemia. Doornbos *et al.*[18] demonstrated that a low-fat single-dose drink significantly lowered LDL-C both when taken with or without a meal.

Intake of the PS-fortified soy milk with lunch/dinner[19] or in combination with a (main) meal[18] might result in greater cholesterol-lowering effects and may be helpful to ensure a maximum effect for the consumers. The results of our study show that since PS was consumed along with the main meal through various vehicles, there was a reduction in the TC and LDL-C levels in varying amounts among all the experimental subjects. As plant sterols reduce the intestinal cholesterol absorption by competing with cholesterol for micellar solubilization, it seems plausible that factors affecting gastrointestinal transit time and stimulating bile flow may influence the cholesterol-lowering efficacy of plant sterols when consumed with lunch.

Results of our study showed that serum total and LDL-C levels were significantly lowered by the consumption of PS-enriched foods: Soya milk (8.7 and 12.6%), fruit bar (5.0 and 9.1%), yogurt (4.3 and 5.3%), and flavored milk (2.5 and 2.6%), respectively. While plant sterols reduce the intestinal absorption of cholesterol, soy protein is thought to lower blood cholesterol concentrations through a decrease in hepatic cholesterol synthesis[20] or through an increase of plasma cholesterol clearance.[21] Serum LDL-C levels decreased significantly by 5.3% with yogurt and 2.6% with flavored milk. Similarly, serum TC levels decreased significantly by 4.3% with yogurt and 2.5% with flavored milk. It indicates that the yogurt and flavored milk were significantly ($P < 0.005$) less efficacious than sterol-enriched soya milk and fruit bar. These results clearly indicate that cholesterol-lowering effects of plant sterol esters may differ according to the food matrix. Similar effect of PSs on lipid profile was observed by Clifton *et al*.,[22] in various food matrices (milk, yogurt, bread, and cereals).

The effect of plant sterols/stanols on LDL-C is influenced by the food carrier to which plant sterols/stanols are incorporated. Plant sterols/stanols incorporated into fat spreads, mayonnaise and salad dressing, or milk and yogurt reduced LDL-C levels to a greater extent than plant sterols/stanols incorporated into other food products. Compared to control, LDL levels were reduced by 0.33, 0.32, 0.34, and 0.20 mmol/L in the fat spreads, mayonnaise and salad dressing, milk and yogurt, and other food products, respectively.[23] Clifton *et al.*[22] have concluded that serum total and LDL-C were significantly lowered when plant sterols were added to milk (8.7% and 15.9%) and yogurt (5.6% and 8.6%) but significantly less when added to bread (6.5%) and cereal (5.4%), while other investigators have made similar observations using yogurt[11,10] and milk.[24] However, our observations in Indian context showed that PS-enriched soymilk and fruit bar were better vehicles for the reduction of TC and LDL-C compared to yogurt and flavored milk.

There are no studies reported on the addition of PS to fruit bars, and our study showed a significant decrease in the TC and LDL-C levels on consumption of PS-enriched fruit bars, indicating a new possible vehicle for PS fortification with positive effects. Apart from reduction in the TC and LDL-C levels, papaya fruit bars can improve the Vitamin A status in the individual due to a possible synergistic effect. It may be possible that a combination of natural ingredients has additive effects and may, therefore, modify the blood lipid profile more favorably than a single ingredient.[25] However, very few studies have investigated a possible synergistic effect yet.

The results showed a marked decline of 9.5% in serum TC levels, 10% decline in TG levels, and 13% decline in LDL levels in the subjects supplemented with PS enriched soy milk as compared to control group [Figures 2 and 3]. This change was significant ($P < 0.05$) in the experimental group who were supplemented with 2 g of PSs-incorporated soy milk. In the control group, there was a decrease of 0.5% in serum cholesterol levels, 1.8% decrease in TG levels, with no changes in HDL levels, and 2.5% decline in LDL levels. These results show that consumption of PSs may help prevent the onset of cardiovascular diseases or bring down the hypocholesterolemic condition. 5% increase in HDL levels was also observed in subjects consuming soy milk. There was a slight increase in the HDL-C levels in subjects

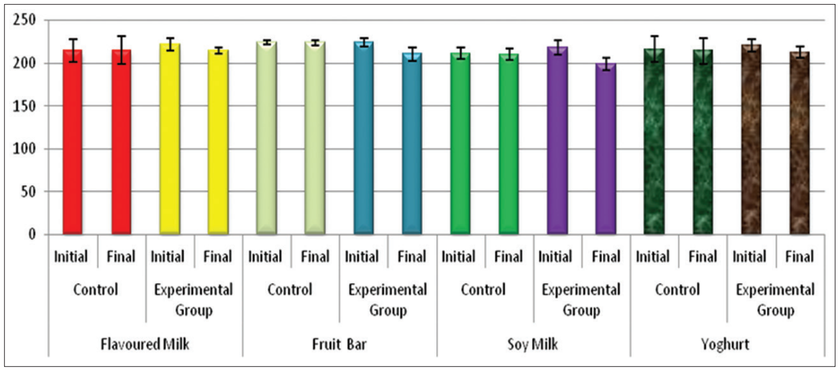

Figure 2: Changes in total cholesterol levels among control and experimental groups using phytosterol-enriched products

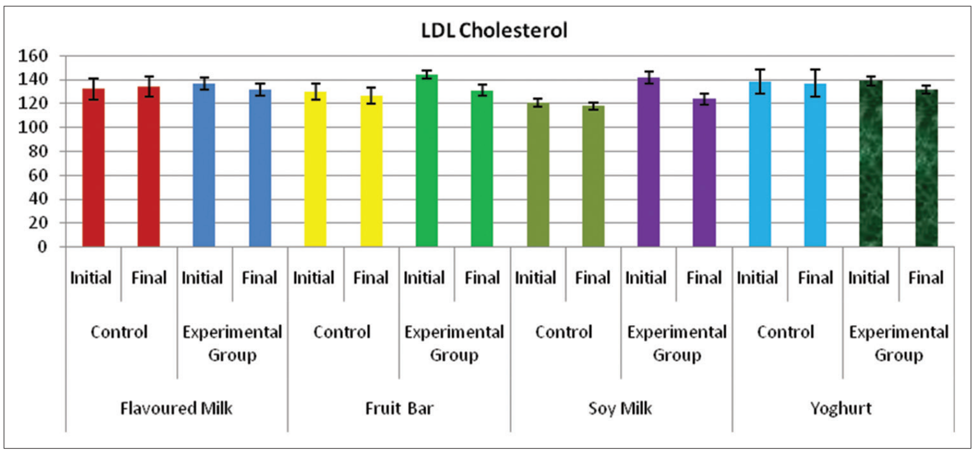

Figure 3: Changes in low-density lipoproteins cholesterol levels among control and experimental groups using PS-enriched products

consuming the fruit bars and yogurt indicating a positive scavenging activity. This is the first study in India to directly compare the efficacy of individual foods fortified with plant sterol. Although all PS-enriched products (soy milk, fruit bar, yogurt, and flavored milk) significantly lowered LDL and TC, low-fat soy milk was found to the best which had 12.6% reduction in the LDL-C followed by papaya fruit bar. This is possibly due to the nature of the vehicle.

CONCLUSION

Addition of PS can lower serum TC and LDL-C with low fat soy milk being the most effective vehicle with 8.7 and 12.6% lowering with 2 g/day of PSs. The inclusion of plant sterols in a variety of foods provides consumers and clinicians with alternatives in the management of hypercholesterolemia. Used alone in the diet or as an adjuvant to drug therapy or in combination with other functional food components, plant sterol-enriched products are effective at reducing serum total and LDL-C. There are no risks associated with their use, and widespread acceptance has the potential to reduce the costs associated with an overdependence on pharmacological approaches to cholesterol management.

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