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Effect of Phytosterols Supplemented Health Food (Papaya Fruit Bar) On Serum Cholesterol and Ldl Cholesterol Levels In Normocholesterolemic And Mildly Hypercholesterolemic Subjects – A Pilot Study

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Abstract: *Phytosterols exist as naturally occurring plant sterols that are present in the nonsaponifiable fraction of plant oils. Phytosterols are plant components that have a chemical structure similar to cholesterol except for the addition of an extra methyl or ethyl group; however, phytosterol absorption in humans is considerably less than that of cholesterol. Phytosterols (plant sterols and stanols) are well known for their total cholesterol and LDL- cholesterol lowering effect. The technologies adopted for processing of fruit and vegetables range from traditional sun-drying to sophisticated and state-of-the-art techniques of juice concentration and freeze drying. The traditional way of dehydrating pulp into leather now turned into sophisticated fruit bars. Papaya fruit bars were formulated by incorporating phytosterol powder at 1.5% level. Physico-chemical and proximate composition studies were conducted for the fruit bar. A randomized double-blind parallel study was conducted to evaluate the effect of phytosterol incorporated papaya fruit bar on the cholesterol levels in human subjects. 12 subjects were supplemented with papaya fruit bar either enriched with 1.5 g phytosterols (experimental) or without phytosterols (control) for 30 days. Significant ($P < 0.05$) decrease in serum total cholesterol (6.12%), triglycerides (6.21%) and LDL cholesterol (9.05%) was seen in experimental group compared to control group. These results indicate that phytosterol enriched food products could be a good vehicle for reducing plasma cholesterol in hypercholesterolemic subjects.*
Keywords: *Phytosterol, Papaya fruit bar, LDL cholesterol, Lipid profile*

I. Introduction

Cardiovascular diseases (CVD) are increasing throughout the developing world and cause almost 16.7 million deaths each year, 80 per cent of which occur in low and middle-income countries. Indeed, 34 per cent of annual deaths are due to CVD. It has been projected that by 2015, 41million people world-wide will die of chronic CVD, unless effective concerted action is taken now. In low and middle income countries, almost half of these deaths will occur in people below 70 years of age compared with only 27 per cent in high-income countries. Furthermore, although in recent decades age-adjusted rates for cardiovascular mortality have decreased in developed countries, rates have increased in low and middle-income countries. In Latin America, it is estimated that between 1990 and 2020, deaths from CVD, including coronary heart disease (CHD), will increase by approximately 145 per cent in both men and women compared with an increase of 28 per cent in women and an increase of 50 per cent in men in developed countries during the same period [7]. Phytosterol have been shown to inhibit the uptake of both dietary and endogenously produced (biliary) cholesterol from intestinal cells. Such inhibition results in a decrease in serum total and LDL-cholesterol levls. Levels of HDL-cholesterol and triglycerides do not appear to be affected by dietary phytosterol consumption [1]. Phytosterols may reduce cholesterol absorption by competing with cholesterol for incorporation into the bile salts micelles or for up taking of cholesterol by enterocytes through Neiman Pick C1 Like1 (NPC1L1) transporter. In addition, phytosterols may enhance cholesterol excretion back into the intestinal lumen through the adenosine triphosphate binding cassette G 5 (ABCG5) and G 8 9ABCG8) transporters. Phytosterol could also prevent esterification of the free cholesterol into cholesterol esters and thus it's assembling into the chylomicrons. As a result of reducing cholesterol absorption by phytosterols, the cholesterol synthesis rate increase, but the net effect is a reduction in LDL-cholesterol levels [11].

[5] demonstrated the efficacy of microcrystalline free phytosterols; serum total and LDL-cholesterol levels were reduced by 7.5-11.6 per cent by consuming phytosterol-enriched margarine (1.5 or 3.0 g of phytosterols per day) during a 6-week run-in and a 6-month experimental period.

[8] reported that for hypercholesterolemic patients the trial was performed using two groups: the hypercholesterolemic subjects treated with non-enriched milk (HNEM) and the hypercholesterolemic subjects

treated with phytosterol-enriched milk (HPEM). The results obtained for both groups after 15 and 30 days of milk intake, showed that in the hypercholesterolemic group treated with non-enriched milk, the lipid profile was not significantly altered during the trial. In the hypercholesterolemic group treated with phytosterol-enriched milk showed a significant reduction between the total cholesterol and LDL-C concentrations.

[13] reported that at a dose of ≥ 2 g/d of plant sterols or stanols (the saturated form), the average reduction in serum LDL-cholesterol is 0.33 to 0.54 mmol/l or 9 to 14 %, depending on the age of the participants studied. A more recent- analysis including 41 trails confirmed these findings and concluded that 2 g/d of plant sterols or stanols lower LDL-cholesterol by 10 % [12]. [18] Reported that the study was planned to utilize the preserved wood apple by preserving them as jam and fruit bar. Using wood apple preserved products like jam and fruit bar were developed, stored and quality parameters were assessed for a period of 90 days. Organoleptic evaluation and nutritive analysis showed good results except reduction in vitamin-c, calcium and phosphorus in both jam and fruit bar during 90^{th} day of storage. The microbial load of both jam and fruit bar was under the limit at the end of 90 days. Hence, the prepared jam and fruit bar was safe and fit for consumption. Functional food development has enjoyed heightened interest by commercial, academic and governmental sectors over the past decade. Food products with functional capacity are eagerly accepted by consumers and likely results in decreased morbidity, mortality and increased quality of life in the general population. Functional foods can be developed by incorporating phytosterols as they act as functional food ingredients. Fruit is a major food for mankind from time immemorial and it has immense nutritive and medicinal value. Processing is necessary as they are highly perishable in nature. Aseptically processed and packed fruit bars are emerging in the market. The fruit bars incorporated with phytosterol will enhance the health benefits of fruit based foods as the phytosterol have immense cholesterol lowering properties to cure various diseases.

Therefore an attempt was made to incorporate phytosterols into papaya fruit bars which could be used as a functional food which helps to reduce the risk of CVD by reducing the total and LDL cholesterol levels in normocholesterolemic and mildly hypercholesterolemic human subjects.

II. Materials And Methods

Materials and chemicals:

For the present investigation, matured papaya *(Carica papaya)* fruits were purchased from local market of (Hyderabad, India), and analysis kits for Triglycerides, 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). Microbiology media were obtained from Hi-Media Laboratories (Mumbai, India). Phytosterol powder was procured from ReducolTM original Powder (Forbes medi – Tech lnc) USA based company. Unless otherwise mentioned all chemicals used were of analytical grade.

Physico-chemical and nutritional characteristics:

PH, total soluble solid content, fat, protein, dietary fibre, moisture, ash of developed PS enriched Papaya fruit bar samples were determined as per the AOAC methods [3]. Vitamin-c and acidity were estimated by the method of [14].Carotenoids and Sugars were determined using approved AOAC methods [3].

Microbial analysis:

Ten gram analytical unit of each food sample [PS enriched papaya fruit bar] was homogenized with 90 mL of sterile Ringer's solution for 2 min and then 10 fold serial dilutions were prepared in sterile Ringer's solution [4].Briefly, individual serial decimal dilutions, starting with the prepared sample and each of the subsequent dilutions were prepared in 9 mL volume of sterile Ringer's solution up to 1×10-6 dilution, of the original food sample. Triplicate 1 mL inoculums of appropriate dilutions were pour plated, on the following media; for enumeration of total plate counts (TPC) on plate count agar and for enumeration of yeast and moulds on potato dextrose agar. The inoculated petri plates were incubated at 37°C for 48 h for TPC and at 25°C for 48 h for yeast and moulds, respectively. Colonies were counted and expressed as colony forming units (cfu) per gram. Standard enumeration procedures were followed [17].

Sensory analysis:

The sensory assessments were conducted at the Post Graduate & Research Centre, Acharya N.G. Ranga Agricultural University, Hyderabad. A panel of 12 members consisting of staff and students of university evaluated the products. To ensure that there was no bias towards the products, it was ensured that the panelists chosen were naive to project objectives. The control was compared with the prepared PS enriched papaya fruit bar samples were T1 (1.5 % PS), T2 (2 % PS), T3 (2.5 % PS). Prior to sensory evaluation the samples were coded using random three-digit numbers and 25g of each sample was served, with the order of presentation counter balanced. Panelists were provided with a glass of water and, instructed to rinse their palate with water and drink water between samples. They were given written instructions and asked to rate the coded samples on color, sourness, flavor, sweetness and overall acceptability, using a nine-point hedonic scale [1=like extremely to 9 =dislike extremely] [2]. The phytosterol powder incorporated at 1.5% level was identified as the best acceptable product and subjected for further studies.

Experimental design and diets:

A randomized double- blind parallel study was conducted on 12 normocholesterolemic and mildly hypercholesterolemic human subjects after the ethical committee approval. All the subjects were aged between 20 to25. No dietary modifications were made in their diet and they all had their normal food without any modifications in their meal patterns. They were divided into experimental (n = 10) and control (n = 6) groups for the purpose of the study. The experimental group (10 subjects) were supplemented with phytosterols (1.5%) incorporated papaya fruit bar (30g per day) for a period of 30 days. The control group (6 subjects) was supplemented with plain papaya fruit bar without phytosterols (30 g per day) for a period of 30 days trial. Fasting blood samples were drawn from the subjects on the 0 day and after 30 days in the morning between 8am and 9am using disposable syringes with the help of a trained laboratory technician at the university (ANGRAU) health centre. 5mL of venous blood sample was collected into fluoride treated tubes. Serum was separated immediately by centrifuging at 2000 rpm for 10-15 min and transferred to plastic storage vial. The vials were immediately covered with aluminum foil. Lipid profile (Total cholesterol, TG, HDL and LDL) was determined in samples collected before and at the end of the experimental period in both the groups using CHOD-PAP, GPO-PAP, and PEG-CHOD-PAP methods [9].

Statistical analysis:

Statistical analysis was carried out at the end of the study. The data was subjected to students paired‘t’ test, two-way analysis of variance (ANOVA), karl Pearson’s correlation coefficient and means were tested for significance by critical difference [16].

Development of PS enriched papaya fruit bar:

Fruit bars with papaya were developed using 100% fruit pulp with 20% sugar, 10% liquid glucose and 0.3% citric acid in control, whereas experimental papaya bars were prepared using 100% fruit pulp, 20% sugar, 10% liquid glucose, and 0.3% citric acid and the phytosterol powder at different concentrations i.e., (T1) 1.5g, (T2) 2g, (T3) 2.5g were used. The fruit pulp was dried in cabinet drier at 60- 65°C for 4- 5 hours. [15] developed papaya bar using 15% sugar, 15% liquid glucose and 0.5% citric acid and dried at 70°C for 4-5 hrs and produced sensorily most acceptable bars.

Preparation of papaya fruit bar (Control)

Ripe papaya
↓
Washing
↓
Peeling
↓
Cutting into pieces
↓
Pulping
↓
Boiling for 15min
↓
Sieving
↓
Addition of sugar (20%) liquid glucose (10%),
Citric acid (0.3%)
↓
Homogenizing
↓
Pouring in greased trays (250gm/sq. ft)
↓

Drying in drier at 60- 65°C for 4 hrs
↓
Piling four layers
↓
Cutting in 3x 9 cm bars
↓
Packing in flexible packaging pouches

Fig. 1 The generic method for the development of papaya bar

Preparation of papaya fruit bar (experimental)

Ripe papaya
↓
Washing
↓
Peeling
↓
Cutting into pieces
↓
Pulping
↓
Boiling for 15min, Sieving
↓
Addition of sugar (20%), liquid glucose (20%)
Citric acid (0.3%)
↓
Adding 1.5, 2, 2.5g phytosterol powder for three different formulations and mix well in the mixer.
↓
Pouring in greased trays (250gm/sq. ft)
↓
Drying in drier at60- 65°C for 4 hrs,
Piling four layers
↓
Cutting in 3x 9 cm bars
↓
Packing in flexible packaging pouches

Fig. 2 Formulation of papaya fruit bar using phytosterol as functional ingredient.

Ingredients used	**Number of combinations**			
	Control	**T1**	**T2**	**T3**
Papaya pulp	100g	100g	100g	100g
Sugar	20%	20%	20%	20%
Liquid glucose	10%	10%	10%	10%
Citric acid	0.3%	0.3%	0.3%	0.3%
Butter	For greasing	For greasing	For greasing	For greasing
Phytosterol powder	-	1.5g	2g	2.5g

Table1. Product development

III. Results And Discussion:

Table 2. Physico-chemical composition of the papaya fruit bar.

Parameters	Control	T1 (experimental)	S.Ed.	CD
pH	3.80±0.02	3.56±0.05	0.067	NS
Titrable acidity (%)	0.63±0.02	0.61±0.01	0.189	NS
Total soluble solids (°)	36.02±0.65	36.06±0.70	12.187	NS
Total sugars (g)	35.20±0.80	35.4±0.92	10.395	NS
Reducing sugars (g)	13.36±0.36	12.31±0.39	4.456	NS

*significant at 5% NS- Non significant Values expressed as Mean ± SD

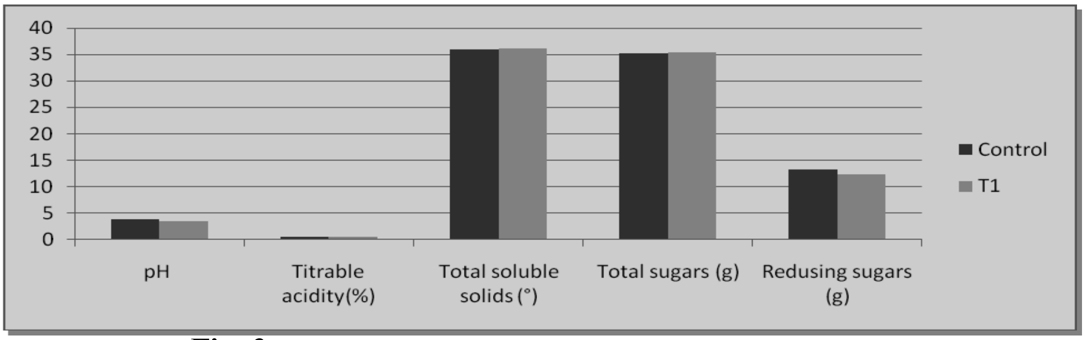

Fig. 3 Physico-chemical composition of the papaya fruit bar.

Changes in physico- chemical parameters of papaya fruit bar were analyzed and were given in Table 2 and Fig 3. On analysis, the values for various parameters of papaya fruit bar were, control (3.80±0.02) and T1 (3.56±0.05) of pH, control (0.63±0.02%) and T1 (0.61±0.01%) of titrable acidity, control (36.02±0.65°) and T1 (36.06±0.70°) of TSS, control (35.20±0.80g) and T1 (35.4±0.92g) in total sugars and control (13.36±0.36g) and T1 (12.31±0.39g) in reducing sugars were observed. No significant (P > 0.05) changes were recorded in physico- chemical parameters between the control and experimental samples.

Table 3. Proximate composition of the papaya fruit bar.

Nutrients	Control	T1 (experimental)	S.Ed.	CD
Moisture (g)	16.66±1.44	15.66±0.57	0.134	0.381
Protein (g)	0.67±0.01	0.65±0.01	0.024	0.069
Fat (g)	0.07±0.01	0.07±0.01	12.252	NS
Ash (g)	0.57±0.04	0.40±0.01	12.193	NS
Carotenoids (µg)	0.18±0.01	0.15±0.01	0.061	NS
Vitamin-c (mg)	35.90±0.97	30.38±0.46	0.186	0.529
Fibre (g)	0.86±0.01	0.85±0.02	10.055	NS

*significant at 5%   Values expressed as Mean ± SD   NS- Non significant

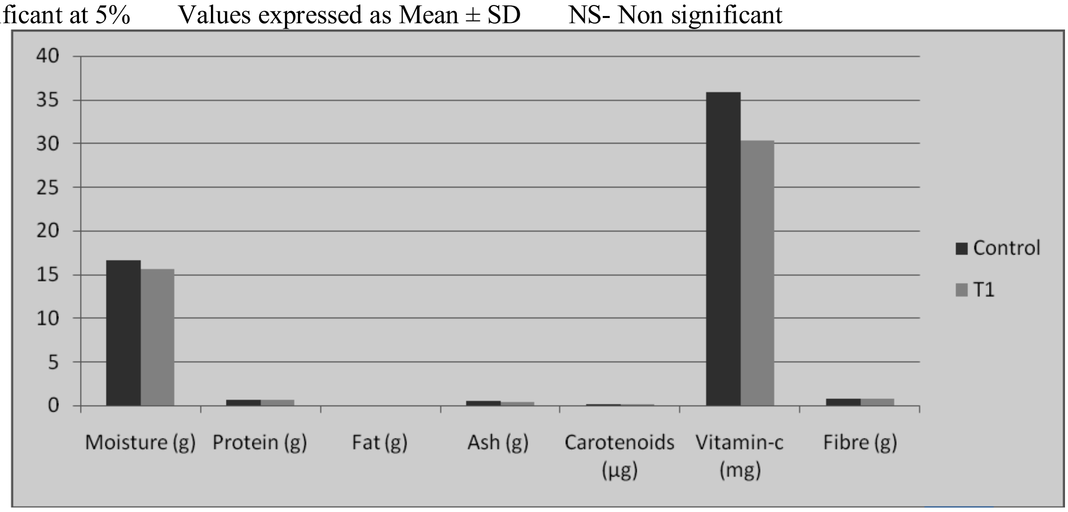

Fig. 4 Proximate composition of the papaya fruit bar.

Changes in proximate parameters of papaya fruit bar were analyzed and were given in Table 3 and Fig 4. On analysis, significant (P < 0.05) changes were recorded in Control (16.66±1.44g) and T1 (15.66±0.57g) in moisture, control (0.67±0.01g) and T1 (0.65±0.01g) in protein and control (35.90±0.97mg) and T1 (30.38±0.46mg) in Vitamin-c. Whereas no significant (P > 0.05) changes were recorded in parameters like control (0.07±0.01g) and T1 (0.07±0.01g) in fat, control (0.57±0.04g) and T1 (0.40±0.01g) in ash, control (0.18±0.01µg) and T1 (0.15±0.01µg) in carotenoids and control (0.86±0.01g) and T1 (0.85±0.02g) in fibre content was recorded.

Microbial Analysis:

Microbial analysis was conducted on 0day, 30th day and 60th day for the best acceptable phytosterol incorporated papaya fruit bar (1.5%) and control plain papaya fruit bar. The results showed showed no microbial count in both the control and experimental samples. TBC values of 0.50 cfu g/ml in control sample and 0.20 cfu g/ml experimental (T1) samples were observed on 60th day. [18] reported that there was acceptable amount of microbes present in the jam and fruit bar at the end of 90 days of storage period of the entire study

Effect of supplementation of phytosterols on lipid profile (Hypocholesterolemic Effect):
The effect of supplementation of phytosterols for a period of thirty days on serum total cholesterol and LDL cholesterol was assessed in human subjects and the mean total cholesterol values of the study are given in Table 4 and Fig 5.

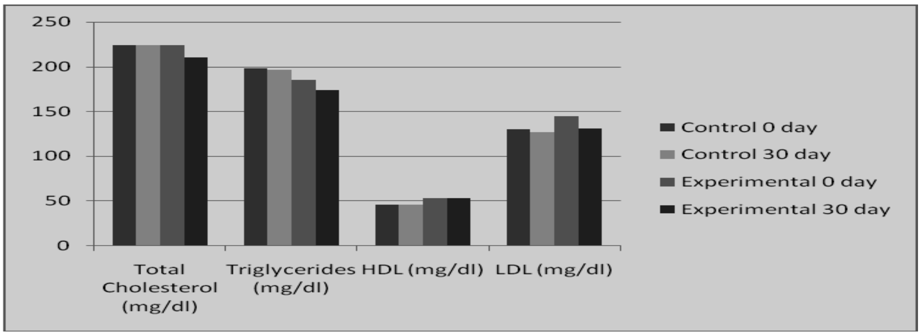

Fig.5 Effect of phytosterols supplementation on lipid profile of selected subjects.

Table 4 Effect of phytosterols supplementation on lipid profile of selected subjects

Parameters		Total Cholesterol (mg/ dl)		Triglycerides (mg/ dl)		HDLcholesterol (mg/ dl)		LDL cholesterol (mg/ dl)	
Control	0 day	224.16±2.78		198.29±16.83		45.56±8.12		129.78±6.51	
	30^{th} day	223.84±2.67		196.49±16.86		45.72±8.25		126.78±6.75	
Experi- Mental		224.36±5.28		185.46±10.48		52.84±4.53		144.13±3.68	
	30^{th} day	210.61±7.45		173.93±9.94		52.62±3.83		131.08±4.54	
		SE.d	CD (0.05)	SE.d	CD(0.05)	SE.d	CD (0.05)	SE.d	CD (0.05)
For Groups		2024	4.224*	5.688	11.869*	2.551	5.545*	2.258	4.711*
For Periods		2.024	4.224*	5.688	NS	2.551	NS	2.258	4.711*
For G* P		2.863	5.974*	8.045	NS	3.607	NS	3.193	6.663*
% increase or decrease (0^{th} – 30^{th} day)									
Control		0.14		0.90		-0.35		2.31	
Experimental		6.12		6.21		0.41		9.05	

Mean values are significantly different (P< 0.05). Values are expressed in mean ± standard deviation;

The results showed a marked decline of 9.12% in serum total cholesterol levels, 6.21% decline in triglyceride levels, 0.41% decrease in HDL levels which is non significant (P > 0.05) and 9.05% decline in LDL levels in the subjects supplemented with phytosterol enriched papaya fruit bar as compared to control group. This change was significant (P < 0.05) in the experimental group who were supplemented with 1.5gm of phytosterols enriched papaya fruit bar. In the control group there was a decrease of 0.14% in serum cholesterol levels, 0.90% decrease in triglyceride levels, 0.41% increase in HDL levels and 2.31% decline in LDL levels. These results show that consumption of phytosterols may help prevent the onset of cardiovascular diseases or bring down the hypocholesterolemic condition. Similar results were reported by John *et al.* (2006).Use of a novel phytosterols- enriched snack bar (1.5gm per day) effectively decreased total and LDL cholesterol by 4.7% and 6.0% levels in a population with hypercholesterolemia.

Devaraj *et al.* (2004) conducted a study on the cholesterol-lowering efficacy of a low-fat (2g/serving) phytosterols enriched orange juice in 72 mildly hypocholesterolemic subjects in comparison with un enriched orange juice for 8 weeks. The results showed that compared to un enriched orange juice, the Phytosterols-enriched orange juice reduced the plasma cholesterol concentrations by 7.2%, LDL cholesterol concentrations by 12.4% and high-density lipoprotein (HDL) cholesterol concentrations by 7.8%.

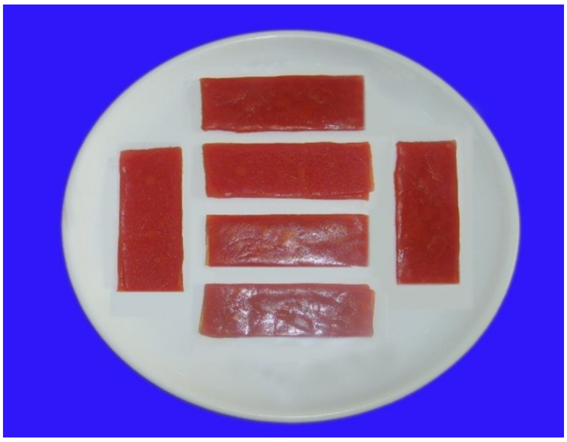

Plate 1 Plain papaya fruit bar (Control)

Plate 2 Phytosterol (PS) powder

Plate3 PS incorporated Papaya Fruit Bar

Plate 5 Estimation of lipid profile using a Spectrophotometer

IV. Conclusion:

Nutritionally the fruit bars are high calorie foods retaining the natural vitamins and minerals. Hence, fruit bars being principally made out of fruit pulps, retains most of these ingredients and therefore, form a good nutritional supplement. The above results show that the intake of phytosterols in papaya fruit bar could be efficacious for lipid profile modulation. As a large effect was not observed with phytosterol enriched papaya fruit bar at a dose of (1.5g/day) for 30 days supplementation, use of higher dose may show much greater effect.

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