

The Role of Phytosterols Enriched Foods-A Review

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Abstract: Phytosterols exist as naturally occurring plant sterols that are present in the nonsaponifiable fraction of plant oils. Phytosterols (plant sterols and stanols) are well known for their low density lipoprotein-cholesterol (LDL-C)-lowering effect. Although many studies have been conducted to resolve the mechanisms of action by which phytosterols lower serum cholesterol. Dietary Phytosterols appear to play an important role in the regulation of oxidation and serum lipids and exhibit anticancer properties. However, as the study confines to the effect of phytosterol on lipid regulation, the review is confined to phytosterol and their role in control of cardiovascular disease (CVD). The information reviewed is presented under the following sections as types of Phytosterols and their structures, mechanism of action, phytosterols in foods for cvd risk reduction, phytosterols as ingredient in functional foods, stability of phytosterols in foods, safety aspects and regulatory status of phytosterols, phytosterols content in foods and phytosterol enriched products are reviewed.

Key words: Phytosterols, LDL-Cholesterol

I. Introduction:

Phytosterols are plant derived compounds structurally related to mammalian cell- delivered cholesterol that functions as essential constituents of plant cell membranes (Lichtenstein, 2002). Berger *et al.* (2004) reported that the phytosterols can be divided into two distinct classes, the sterols and stanols, with sterols being the predominant form found in nature. Both classes of phytosterol are naturally occurring and can be found in a variety of foods, including nuts, vegetables oils, seeds and cereals.

Types Of Phytosterols:

Sterols: Δ^5 -Phytosterols found in Sitosterol, Campesterol, Stigmasterol

Stanols: 5- α -Reduced Sterols (From Soy or Wood)

Examples: Sitostanol, Campestanol (Berger *et al.* 2004).

Phytosterols exist as naturally occurring plant sterols that are present in the nonsaponifiable fraction of plant oils. Structurally, phytosterols are similar to cholesterol except for substitutions on the sterol side chain at the C24 position. Phytosterols are not synthesized in humans, are poorly absorbed, and are excreted faster from the liver than cholesterol, which explains their low presence in human tissues. The primary phytosterols in the diet are sitosterol, stigmasterol, and campesterol. Typical consumption of plant sterols in diet approximately 200-400mg/day (Aparna Kuna *et al.* 2011). Aparna Kuna and Poshadri, (2011) reported that phytosterols occurring in free and esterified forms are present ubiquitously in the unsaponifiable fraction of the extractives of most plants. The ester moiety could be fatty acid, ferulic acid or sugar acid. In edible plants, these unsaponifiables are consumed routinely as a part of the human diet. Wong, (2008) reported that appreciable quantities of phytosterols were identified in selected grain processing residues. Preliminary testing of selected samples showed the concentration was found to vary from 1.6 grams per kg of durum wheat bran to 5.7 grams per kg in spring wheat germ. Samples of selected grain processing residues were analyzed for phytosterols with the goal of developing a new source of phytosterols for nutritional health-food purposes. The samples studied were distillers' grain, brewers' grain, wheat bran, corn germ and wheat germ. Distillers' grain was considered to be a commercially interesting source of phytosterols and corn germ was considered to be a practical source of phytosterols. The structural similarity between phytosterols and mammalian cholesterol is striking; they mainly differ by a methyl or an ethyl group at the C-24 position in the side chain (Figure .1) Most phytosterols as well as cholesterol have a double bond between C-5 and C-6 in the tetracyclic ring system and thus are called Δ^5 -sterols. Another group of common phytosterols are Δ^7 -sterols, with a double bond between C-7 and C-8, and Δ^5 , 22-sterols with double bonds between C-5 and C-6 and also between C-22 and C-23. Saturated phytosterols with no double bonds in their structure are called phytostanols. The main phytosterol encountered in higher plants is sitosterol. It is often accompanied by its 22-dehydroanalog, stigmasterol. Campesterol also occurs widely in plants (Moreau *et al.*, 2002) (Figure .2).

Figure 1: Structures of common phytosterols, phytostanols, and cholesterol (Moss, 1989).

The principal phytosterols in grain processing residue samples tested were campesterol, campstanol, stigmasterol, sitosterol and sitostanol.

Figure.2 illustrates the basic structure of these major sterols. It may be noted that brassicasterol occurs only in *Brassica spp.* plant materials such as mustard oil and rapeseed oil.

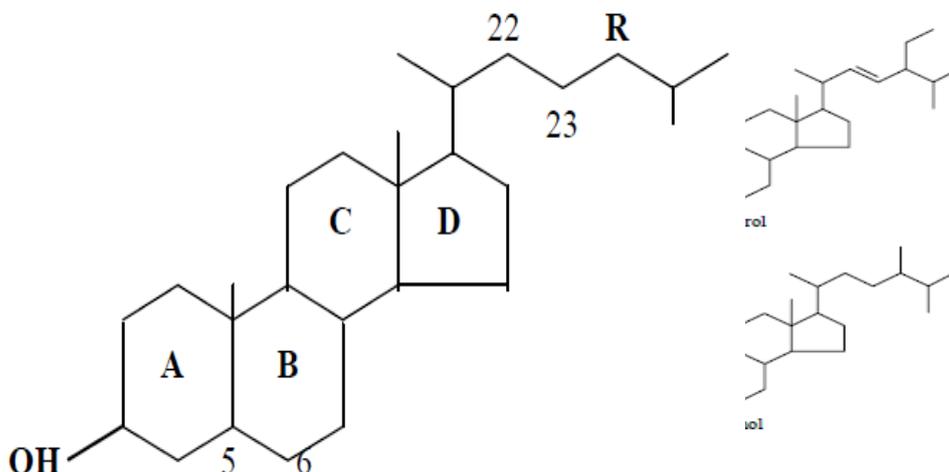


Figure 2: Chemical structure of major phytosterols found in most plant materials.

	Δ	R
Brassicasterol	5,6; 22,23	CH ₃
Campesterol	5,6	CH ₃
Campestanol	Nil	CH ₃
Stigmasterol	5,6; 22,23	C ₂ H ₅
Sitosterol	5,6	C ₂ H ₅
Sitostanol	nil	C ₂ H ₅

Source: Wong (2008).

II. Mechanism Of Action:

The exact mechanism by which phytosterols decrease serum cholesterol levels is not completely understood, but several theories have been proposed. One of them suggests that cholesterol in the intestine, already marginally soluble, is precipitated into a non-absorbable state in the presence of added phytosterols and stanols. Another theory is based on the fact that cholesterol must enter bile-salt and phospholipid-containing 'mixed micelles' in order to pass through intestinal cells and to be absorbed into the bloodstream. Moreover, phytosterols may modulate the action of key transporters involved in cholesterol absorption. Cholesterol absorption is a very important physiological mechanism that regulates cholesterol metabolism (Rozner and Garti, 2006). 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 levels. Levels of HDL-cholesterol and triglycerides do not appear to be affected by dietary phytosterol consumption (AbuMweis *et al.*, 2008). A recent trial showed that efficacy of phytosterols is not influenced by dietary cholesterol intake in hypercholesterolemic individuals. Both dietary cholesterol and recirculating biliary cholesterol mix in the intestine and are partially absorbed. Failure to reabsorb intestinal cholesterol is the principal means of cholesterol elimination from the body (kassis *et al.*, 2008). Although many studies have been conducted to resolve the mechanisms of action by which phytosterols lower serum cholesterol, the molecular actions are not fully understood. The main physiological response to ingestion of phytosterols is known to be reduced intestinal absorption of both dietary and endogenously produced cholesterol without, however, any decrease in the levels of high-density lipoprotein (HDL) -cholesterol or triglycerides (Moreau *et al.*, 2002, Ostlund *et al.*, 2002). This interference with absorption is probably related to the similarity in the chemical structures of phytosterols, stanols, and cholesterol (Salo *et al.*, 2002; Plat and Mensink, 2005). Current studies focus on the effects of phytosterols at the epithelial cell level, suggesting that there is an additional longer-lasting process in which phytosterols and stanols actively influence cellular cholesterol metabolism within intestinal enterocytes (Normen *et al.*, 2004; Plat and Mensink, 2005; Thompson and Grundy, 2005). In enterocytes, phytosterols and stanols potentially increase the expression of ABC (adenosine triphosphate (ATP) binding cassette) transporters, integral membrane proteins that use the energy generated from ATP hydrolysis for transportation of a substrate across the membrane. Of these, ABCG5, ABCG8, and

probably ABCA1 are identified as specific sterol transporters. When dietary phytosterols or stanols enter the enterocytes via micellar transport, they are assumed to be directly transported back to the intestinal lumen by the ABCG5-ABCG8 complex, thus keeping their absorption low. This complex, stimulated by phytosterols and stanols, may also promote the efflux of cholesterol, thereby regulating the total sterol absorption (Schmitz *et al.*, 2001). The suggestion of the incorporation of the ABCG5-ABCG8 complex in transport of phytosterols and stanols is based on the finding that in phytosterolemia (or sitosterolemia, a rare human genetic disorder affecting phytosterol metabolism) the high absorption of phytosterols was due to the deficiency of these transporters (Schmitz *et al.*, 2001; Yu *et al.*, 2002; Gylling and Miettinen, 2005). However, other transporters are also thought to participate in sterol absorption mechanisms, a recent finding being the Niemann-Pick C1-Like 1 transporter (von Bergmann *et al.*, 2005; Gylling and Miettinen, 2005). Mechanisms by which phytosterols and stanols affect cholesterol transportation over the enterocyte membrane are still largely open and why phytosterols are taken up by the enterocytes and then resecreted back into the intestine remains an open question (Von Bergmann *et al.*, 2005). The above-mentioned theory is supported by a recent study showing that mixed micelles enriched with sitostanol or with cholesterol and sitostanol increased intestinal expression of ABCA1 in Caco-2 cells (Plat and Mensink, 2002). In subsequent animal studies, metabolites of phytosterols have been observed to activate liver X receptors, which, in turn, activate the expression of ABCG5 and ABCG8 transporters. However, the metabolic conversion of dietary phytosterols has not been elucidated (Kaneko *et al.*, 2003). The potentially important role of ABCG5 and ABCG8 transporters in sterol trafficking has also been tested by disrupting *Abcg5* and *Abcg8* in mice. This led to a 30- fold increase in serum sitosterol and extremely low biliary cholesterol concentrations (Yu *et al.*, 2002). The mechanism of action is summarised in Figure .3 (Quilley *et al.*, 2003)

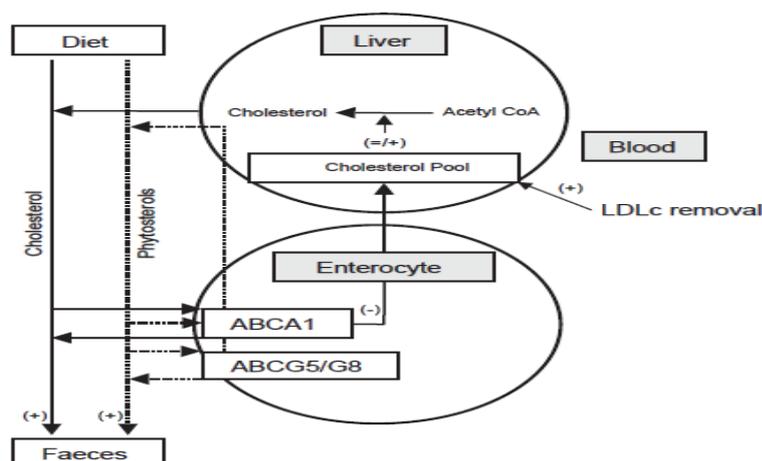


Figure.3: Simplified model of the mechanism of action of phytosterols. Signs in parentheses indicate the effect of phytosterol consumption. Diet plus biliary cholesterol is absorbed approximately by 50%, and phytosterols between 0.1% and 5% in enterocytes mediated by ABC transporters, and the rest is eliminated by faeces. Phytosterol intake in significant quantities reduces the rate of cholesterol absorption and the liver cholesterol pool is diminished. This might determine the upregulation of cholesterol biosynthesis and plasma LDL-cholesterol removal. Signs in parentheses indicate the effect of phytosterols consumption (Quilley *et al.*, 2003).

Phytosterols In Foods For Cvd Risk Reduction:

The use of phytosterols for serum cholesterol-lowering purposes in humans was studied by Heinemann *et al.* (1986). They administered sitostanol at a dose of 1.5 g/d for 4 weeks to 6 patients with hypercholesterolemia and observed that total Cholesterol was reduced by 10 per cent and 15 per cent after 3 and 4 weeks, respectively.

Christiansen *et al.* (2001) 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.

Phytosterols As Ingredient In Functional Foods:

Clifton *et al.* (2004) demonstrated that phytosterol in all food forms tested for lower serum LDL cholesterol, with low-fat milk being the most effective vehicle with a 16 per cent lowering with 1.6g/day of phytosterol.

Masana *et al.* (2008) found that plant sterol enriched fermented milk significantly reduced LDL – C and increased the number of moderately hypercholesterolemic patients achieving therapeutic targets.

Dooornbos *et al.* (2006) showed that phytosterol- enriched fermented milk had significant LDL cholesterol lowering efficacy, when used in either a multiple or single dose drinkable yoghurt, the reductions of LDL – C were in the range of 5 to 10 per cent greater where the phytosterol enriched product were taken along with a meal.

Penchala Raju *et al.* (2013) studied the effect of phytosterols (PS) enriched flavoured milk on total and LDL (Low density lipoprotein)- cholesterol levels in subjects with hypercholesterolemia and results showed that enrichment of phytosterol in low fat flavoured milk to the experimental group, brought a significant ($p < 0.05$) reduction of total cholesterol(2.53 %) and LDL- cholesterol levels(2.62 %) and concluded that the phytosterol-enriched flavoured milk can be used effectively to reduce plasma total and LDL cholesterol levels in hypercholesterolemic individuals and the data suggest that the incorporation of phytosterol enriched flavoured milk into a balanced diet represents a practical dietary strategy in the management of serum cholesterol levels.

III. Stability Of Phytosterols In Foods:

Stability at High Temperature:

Phytosterols and their fatty acid esters are quite stable compounds and undergo only limited degradation during oil processing. Only under harsh conditions, such as high temperatures ($> 1000^{\circ}\text{C}$) in the presence of oxygen, oxidation of the phytosterol moiety may occur, in the same way as for cholesterol (Soupas, 2006). Phytosterols are mono-unsaturated compounds (double bond in the β -ring), which are much more stable than the mono-unsaturated fatty acids (e.g. oleic acid), because of steric hindrance by the ring structure. Therefore, even under severe conditions, such as during shallow frying, sterol oxidation products form very slowly (Thanh *et al.*, 2006). Under conditions of use for shallow frying by consumers, (temperatures $160\text{-}200^{\circ}\text{C}$, 5-10 minutes of frying) the level of oxidation of sitosterol esters remains below 1.3%. When the matrix consists of liquid oil or liquid margarine and if free sterols are present then these levels are somewhat higher at 2.5% and 5.1%, respectively (Salta *et al.*, 2008 and Soupas *et al.*, 2005). Factors affecting phytosterol oxidation include not only temperature and heating time, but also the composition of the lipid matrix. Since Indian dairy products such as dahi, misti dahi, paneer, butter milk (chhach/chhaas), lassi, etc are processed below 100°C , it is best to opt for such dairy products as vehicles for phytosterols fortification as compared to other vehicles which involves high temperature processing. (Aparna and Poshadri, 2012).

Stability during product manufacturing and storage:

Phytosterols and phytostanols are microbiologically inert as shown by the absence of an effect during the fermentation process used to produce yoghurt (Monu *et al.*, 2008). Furthermore, the ester added to various food products show excellent stability at different pH values during long term storage (up to at least a year). Phytostanol and phytosterol esters are also stable in milk and fermented milk and products with viable bacteria like yoghurts and yoghurt drinks (Cantrill and Kawamura, 2008).

Oxidative Stability of Phytosterols in Foods:

The oxidative stability of phytosterols and stanols, i.e. their resistance to oxidation and thus to the formation of oxidation products, needs to be investigated. In terms of stability, the oxidative stability, indeed, is of major concern, since sterols, as unsaturated lipid compounds (steroid alcohols), are susceptible to oxidation (Dutta, 2004).

In a review of genotoxicity of heat-processed foods, oxidized phytosterols were classified as cooked food mutagens, along with acrylamides, heterocyclic amines, nitrosoamines, and polyaromatic hydrocarbons (Jagerstad and Skog, 2005). Research on oxidation of phytosterols has not been as systematic and extensive as with cholesterol, but based on all information available, phytosterols seem to oxidize in a similar fashion to cholesterol. This can also be assumed from their structural similarities (Dutta, 2004). A foundation for understanding sterol oxidation mechanisms has been provided by studies conducted on fatty acid oxidation (Lercker and Rodriguez-Estrada, 2002). Sterol oxidation can proceed via nonenzymic or enzymic pathways (Dutta, 2004). Nonenzymic pathways can further be divided into oxidation by a free radical mechanism, i.e. autoxidation, or oxidation by a nonradical mechanism, i.e. photooxidation (Lercker and Rodriguez- Estrada, 2002).

Safety Aspects And Regulatory Status Of Phytosterols:

Phytosterols have been used for lowering plasma cholesterol levels for half a century now, and so far, no marked adverse effects have been reported (Ling and Jones, 1995; Berger *et al.*, 2004; Kritchevsky, 2004; Gylling and Miettinen, 2005; Kritchevsky and Chen, 2005; Plat and Mensink, 2005; Salo *et al.* 2005; Penchala raju *et al.* 2013). FDA has granted GRAS status for many phytosterol/stanol ingredients (FDA, 2000a), and SCF

in the EU has thoroughly reviewed the safety of phytosterols, phytostanols, and their esters and sanctioned them for use in a variety of foods (SCF, 2000; 2003a, b,c). Noteworthy, however, is that there is no experience of the effects of long-term consumption of phytosterols and stanols as part of the daily diet. This leaves open the possibility of unsuspected effects (Katan *et al.*, 2003; St-Onge and Jones, 2003). Promotion for the use of plant sterols for LDL-C lowering is increasing, but prudence dictates that the dose of phytosterols should not exceed 2g/ day. An additional concern of uncertain significance is the interference with the absorption of certain fat soluble vitamins (Katan *et al.*, 2003, Devaraj *et al.*, 2006).

Recent studies of plant sterol and stanol esters in humans have shown; however, that maximum cholesterol-lowering benefit is achieved at doses of 2-3 g per day (Hallikainen *et al.*, 2000; Jones *et al.*, 2000; Maki *et al.*, 2001).

Phytosterols Content In Foods:

In general, the main sources of phytosterols in the human diet are vegetable oils, vegetable oil based food products, cereal grains, cereal-based foods, and nuts. According to a recent study in Finland, cereal and cereal products, especially rye, were the major sources for phytosterols, followed by margarine (Valsta *et al.*, 2004). Phytosterol content of crude vegetable oils lies between 70 and 1100 mg/100 g of oil, with rapeseed and corn oils being the richest sources, and olive and palm oils the poorest (Piironen and Lampi, 2004). Some special oils, like rice bran oil and wheat germ oil, can contain phytosterols in amounts up to 3200 mg/100 g of oil (Piironen *et al.*, 2000). The most unique oil seems to be corn fiber oil, which contains phytosterols in quantities of about 10,000 mg/100 g of oil (Moreau *et al.*, 2002). Among cereals, the richest sources of phytosterols are corn (65-120 mg/100 g) and rye (70-110 mg/100g), while wheat, barley, and oats contain 35-80 mg/100g (Piironen and Lampi, 2004). Different nuts contain phytosterols in the range of 30-220 mg/100 g (Piironen *et al.*, 2000).

Despite their high moisture content, the contribution of vegetables, fruits, and berries in phytosterols intake has also been shown to be significant (Piironen *et al.*, 2003; Valsta *et al.*, 2004). On a fresh weight basis, phytosterol contents of fresh vegetables range from 5 to 40 mg/100 g, the highest contents measured in broccoli, Brussels sprouts, cauliflower, and dill. In fresh fruits, phytosterols contents lie within the range of 10-20 mg/100 g fresh weight, and in fresh berries 6-30 mg/100 g fresh weight. Wild berries, like lingonberry and blueberry, are more phytosterol-rich than cultivated berries such as blackcurrant, redcurrant, and strawberry (Piironen *et al.*, 2003).

Phytosterol Enriched Products For The Control Of Ldl Cholesterol:

A study showed that low fat dairy based food products like milk and yoghurt enriched with plant sterol esters, were similarly effective in lowering total cholesterol (TC) and LDL- cholesterol concentrations as fat based foods like spreads and margarine (Noakes *et al.*, 2005; Hallikainen *et al.*, 2000). A study investigated the effect of phytosterols added into milk on plasma concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and LDL-C, and on biochemical, hematologic, and hemorheological profiles in healthy and hypercholesterolemic subjects in Portugal. The results obtained in this study indicate that phytosterol-enriched milk is a good vehicle for reducing plasma cholesterol in hypercholesterolemic subjects (Goncalves *et al.*, 2007).Elevated plasma total cholesterol (TC) 5 and LDL-cholesterol (LDL-C) are a major risk factor for coronary heart disease (CHD). Phytosterols (plant sterols and stanols) are among the dietary options available to lower elevated plasma TC and LDL-C concentrations. The cholesterol-lowering properties of phytosterols were observed in humans already in the early 1950s (Salo *et al.* 2005). Since then, a vast number of human trials have shown that phytosterols, mainly in the form of plant sterols or stanols esterified to vegetable oil fatty acids (mainly C18), significantly lower TC and LDL-C when incorporated into various food products (Aparna *et al.*, 2011). Dietary Phytosterols appear to play an important role in the regulation of oxidation and serum lipids and exhibit anticancer properties (Peter *et al.*, 2009). Demonty *et al.* (2010) reported that the recommended intake of 2g/d, the expected LDL-C lowering effect of phytosterols was 9 percent. A reduction in LDL-Cholesterol of 10% would reduce the incidence of coronary heart disease by 10-20 percent (Demonty *et al.*, 2010).

Table 1: Total phytosterols contents of selected foods

S.No	Phytosterol food sources	Total phytosterols content (mg/100g)
OILS		
1	Rice bran	1055
2	Corn	952
3	Wheat germ	553
4	Flax seed	338

5	Cottonseed	327
6	Soybean	221
7	Peanut	206
8	Olive	176
9	Coconut	91
10	Palm	49
Vegetables		
11	Beet root	25
12	Brussels sprout	24
13	Cauliflower	18
14	Onion	15
15	Carrot	12
16	Cabbage	11
17	Yam	10
Fruits		
18	Orange	24
19	Banana	16
20	Apple	12
21	Cherry	12
22	Peach	10
23	Pear	8
Nuts		
24	Cashew	158
25	Almond	143
26	Pecan	108
27	Pistachio	108
28	Walnut	108
Legumes		
29	Pea	135
30	Kidney bean	127
31	Broad bean	124

Source: Aparna *et al.*, (2011).

IV. Conclusion:

Phytosterols have gained a prominent position in strategies to lower CVD risk because of their serum LDLCholesterol lowering effects and it is evident from the foregoing discussion that a phytosterol- enriched products can be used effectively to reduce plasma total and LDL cholesterol levels in hypercholesterolemic individuals and the data suggest that the incorporation of phytosterol enriched products into a balanced diet represents a practical dietary strategy in the management of serum cholesterol levels. Although no direct evidence is available yet for the ability of phytosterols to lower coronary heart disease incidence, the well documented cholesterol lowering effect of phytosterols is the basis for recommendations to include phytosterols into strategies to lower LDL-C concentration. The cholesterol lowering potential of phytosterols may thus depend upon previous dispersion into a fat matrix and on the physical nature of the food.

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