Journal of Applied and Natural Science 7 (2): 1081 - 1087 (2015)

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Plant sterols for human health- A review

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Received: May 15, 2015; Revised received: October 12, 2015; Accepted: November 10, 2015

Abstract: The aim of the article was to review phytosterols as functional food and its significance in lowering cholesterol as well as its specific effect on human health. Phytosterols has been known for its cholesterol lowering action long time back but the uprising of phytosterols in form of functional foods gained the interest once again. Fatty food matrix provides optimal solubility but fortification of phytosterols with other food matrices like low fat fermented milk, bread, juice are showing positive results. A dose of 2 g/day of either steryl or stanyl esters has been prescribed for an optimum effect which has been confirmed by FDA and EC. A number of studies have documented the safety and the efficiency of phytosterols. But there is still a big question mark on the use of it because of their adverse effect on body in form of Phytosterol oxidation products (POPs). It needs further investigation to elucidate effect of POPs within body.

Keywords: Cholesterol lowering agent, Phytostanols, Phytosterols, Sitosterolemia

INTRODUCTION

Plant sterols are nonnutritive compounds (or phytochemicals) which regulate the membrane fluidity of plant cells (Brufaua *et al.*, 2008; Kritchevskya and Chen, 2005). The same function, cholesterol does in animals. Plant sterols include sterols and stanols and these are called phytosterols and phytostanols respectively but combinedly and generally they are called phytosterols. Structurally, both phytosterols and cholesterol have same sterol ring but the difference is in the side chain (Jong *et al.*, 2003; Fernandes and Cabral, 2007; Cantrill and Kawamura, 2008) Phytosterols have additional alkyl subtituents at C-24 and/or a double bond at C-22. More than 200 phytosterols (PS) have been identified in which ß-sitosterol (65%), campesterol (32%), and stigmasterol (3%) are most abundant (Vanmierlo *et al.*, 2013). Ergosterol is mostly found in fungal cell. In plant tissues, PS occur in five common forms: free sterols, fatty-acid esters (steryl ester), steryl glycosides, acylated steryl glycosides, and hydroxycinnamic acid steryl esters (Moreau *et al.*, 2002). Stanols do not have double bonds in the sterol ring and are called saturated sterols (Moreau *et al.*, 2002.). Stanols are less abundant in nature than sterols. Main stanols are campestanol and sitostanol. Plant stanols are also produced by hydrogenating sterols (Chen *et al.*, 2013). These phytosterols are normal constituents of the human diet.

Absorption and metabolism of phytosterols: Cholesterol absorption in humans varies from 30% to 60% but absorption of PS is rather low (Salen *et al.*, 1989; Miettinen *et al.*, 1990; Ling and Jones, 1995; Garcia-Llatasa and Rodriguez-Estradab, 2011). Absorption levels for campesterol (9.4–14.8%) are approximately 3 times higher than for ß-sitosterol (3.1–4.5%) and stigmasterol (~4%). Absorption of campestanol and sitostanol has varies (0.1–2%) (Heinemann *et al.* 1993; Miettinen *et al.*, 2000, Piironen *et al.*, 2000; Sanders *et al.*, 2000). The reason behind more cholesterol absorption is the presence of a double bond between C-5 and C-6 which increases the absorption rate. But in phytosterols, the Increase in the side chain length decreases its absorption. Phytostanols do not have double bond in sterol ring, which decreases the rate of absorption in body.

Mechanism of action of phytosterols: Phytosterols decrease absorption of cholesterol from diet which leads to suppressed feedback-regulation of enterohepatic cholesterol in circulation, resulting in increased production of cholesterol within body increasing the endogenous cholesterol synthesis. The absorbed phytosterols decrease the synthesis of cholesterol in body (Eisenberg, 2003; Shin *et al.*, 2005; Micallef and Garg, 2008; Malinowski and Gehret, 2010). This way PS decreases both exogenous and endogenous production of cholesterol (Berge *et al.*, 2000; Brufaua *et al.*, 2008). The mechanism of cholesterol lowering action of phytosterols can be easily understood by the following ways:

Inhibition of cholesterol cbsorption in intestine: There is solubilization competition between cholesterol and PS in dietary mixed micelles (Wu et al., 2009; Gupta *et al.*, 2011), in order to pass through the

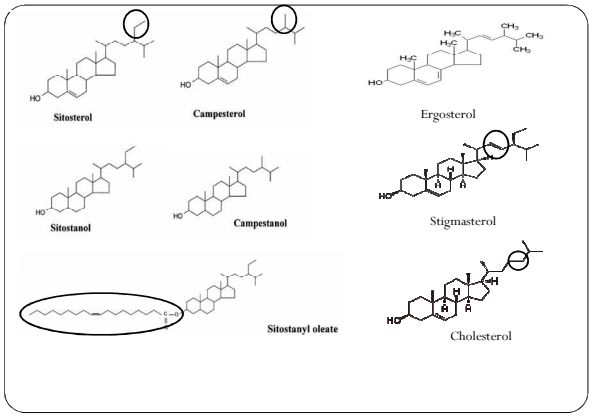

Fig 1. *Structures of different sterols* (Cantrill and Kawamura, 2008 (modified); Lavova *et al.,* 2013, (modified).

intestinal cells and to be absorbed into the blood-stream. Since cholesterol is only marginally soluble in these micelles and it is displaced by phytosterols (Plat and Mensink, 2005; Micallef and Garg, 2009). This is done by two ways:

There is competition for esterase activity between cholesterol and PS, leading to a reduction of cholesterol in its absorbable form or its transport to more distal parts of the intestine where absorption is less efficient (Brufaua *et al*., 2008).

There is competition for cholesterol transporters, such as the NPC1L1, between cholesterol and PS in the intestinal brush border membrane (Davis *et al.*, 2004; Bergmann et al., 2005; Jakulj *et al*., 2005). Since PS and cholesterol structures are very similar (Otaegui-Arrazola *et al*, 2010), PS may be transported into the enterocyte, instead of cholesterol, thus reducing the uptake of cholesterol (Brufaua *et al*., 2008).

Synthesis and excretion of cholesterol: Intestinal ACAT re-esterifies the absorbed cholesterol in the mucosal cell. Since esterification of PS occurs in slow pace than that of cholesterol which encourages suppressed activity of ACAT and reduces cholesterol uptake (Rodwell *et al*., 1976).

About 70–80% of the transported cholesterol is esterified, so it is possible that the incorporation into chylomicrones mainly depends on the esterification step (Ikeda *et al.*, 1988). There is competition between cholesterol and PS for the incorporation into chylomicrones. Since PS is esterified in a minor degree than cholesterol, thus, lowers the cholesterol level.

ABC transporters play a role in absorption of PS and cholesterol into mucosal cells. ABCG5 and ABCG8 are responsible for efflux of absorbed phytosterols and cholesterol back into intestinal lumen from enterocytes. From intestinal lumen, they are sent to large intestine where hydrolysis of cholesterol and phytosterols takes place (Berge *et al*., 2000).

Food sources: Nuts and vegetable oils can contain more than 1% of phytosterols. On the other hand, phytostanols occur in certain cereals (corn, wheat, rye, and rice), fruits and vegetables, but their concentrations are much lower than those of unsaturated PS. Oil and fat refining processes lead to a loss of PS (mainly free ones), which can vary from 10% to 70% depending on the applied conditions. PS are natural components of human diets (Weihrauch and Gardner, 1978; Phillips *et al.*, 2005). An average daily intake of PS from natural sources is estimated between 150 and 440mg in Western countries (Ostlund, 2002; Jimenez-Escrig *et al.*, 2006)

Need of fortification: Phytosterols has been known for its cholesterol lowering action since 1950s (Russel *et al.*, 2002; Demonty *et al.*, 2006; Ortega *et al.*, 2006; Acuff et al., 2007; Earnest et al. 2007; Marangoni and Poli, 2010; Rocette *et al.*, 2010). ß-sitosterol had been used as a supplement and as a drug to lower serum cholesterol for a long time. But phytosterols extracted from oil is insoluble in water, relatively insoluble in oil and soluble in alcohol. So, due to its Poor solubility and bioavailability, serum cholesterol lowering effects were not always consistent and high doses and sometimes very High doses (up to 25–50 g per day) were required to show cholesterol lowering effect. After introducing effective anti-cholesterolemic drugs like statin, the use of ß-sitosterol vanished (Pollak, 1953). But In 1990s, it regained interest as an idea of functional foods (Jones and Abumweis, 2009). An accurate PS solubilization in the food vehicle is important for the best possible action on lipid serum levels. Foods provide a vehicle (matrix) to PS to be absorbed more efficiently. Various matrices, including fatty foods, low fat foods and non-fat food have been used. Fatty food matrix provides optimal solubility of added compounds.

Safety level and frequency of consumption: Government agencies, such as the FDA and in EC has confirmed about the safety level of PS. It is considered that a dose of 2 g/day of either steryl or stanyl esters should give an optimum effect (Berger *et al.*, 2004; Jong *et al.*, 2007; Naumann *et al.*, 2008). 5–15% and 10-20% reduction in total cholesterol and LDL levels respectively can be expected by consuming 1.5–3 g of phytosterols or stanols (Nguyen, 1999; Tikkanen, M.J., 2005). Katan *et al.* (2003) reported that PS have to be present in the lumen for interference with cholesterol uptake which provided evidence of phytostanol's independence upon number of meals (Matvienko *et al.*, 2002; Wolfs *et al.*, 2006; Madsen *et al.*, 2007; Clifton *et al.*, 2008) and doses per day (Rozner and Garti, 2006). So PS showed a long-lasting cholesterol lowering effect on its metabolism.

Phytostanols vs. phytosterols: No significant difference in the serum lipids reducing properties of both sterols and stanols was found when comparable studies were conducted (Jones *et al.*, 2000; Normen *et al.*, 2000; Noakes *et al.*, 2002), The effects of sterols and stanols esters are also relatively equivalent (Law, 2000, Talati *et al.*, 2010). No-observed-adverse-effect

Table 1. Sources of phytosterols.

Food Source	Phytosterol (mg/100g)
Cereals	
Wheat bran	200
Wheat germ	345.6
Fruits and vegetable	
Banana	16
Beet	25
Broccoli	49.4
Cabbage	11.4
Carrot	15.3
Cauliflowers	18
Figs	31
Lettuce	39
Oranges	24
Soyabeans	48.3
Oils and fats	
Almond oil	265
Canola oil	657
Corn oil	909
Olive oil	157
Walnut oil	176.5
Wheat germ oil	555.56
Nuts and seeds	
Almonds	137.5
Flax seed	210
Peanut	221
Pistachio nuts	214
Sesame seeds	714
Walnut	113
Fortified Foods	**Phytosterol (g/100g or ml)**
Orange Juice	0.68
Pasta (Finland)	2.43
Snack Bar	6.8
Spread	11.33
Yogurt drinks (Ecuador)	2.83

(Piironen and Toivo, 2003, Chen and Blumberg, 2008; Cantrill and Kawamura, 2008; USDA, 2011 (US Department of Agriculture) National Nutrient Database Release)

level (NOAEL) was established at highest applied dose of 6 g/kg body weight/day of plant sterols esters.

Effect of phytosterols on different parameters:

On fat-soluble vitamins: Antioxidants studies, have shown that daily consumption of 3.8 to 4.0 g plant stanols provided as fatty acid esters during a period of eight weeks significantly lowers serum concentrations of various cartenoids and tocopherols (Plat and Mensink, 2001). Plasma retinol (vitamin A) and vitamin D concentrations were not affected because the absorption or synthesis of these fat-soluble vitamins, are not transported by lipoproteins.

On vitamin K concentrations: Effect on vitamin K concentration is not clear. Eight weeks of 3.8 g vegetable oil based plant stanols or 4.0 g wood based plant stanols had no effect on vitamin K dependent blood coagulation factors and fibrinolytic parameters (Nguyen and Dale, 1999). Study of Hendriks *et al.* (2003) contradicts the above results.

On colon and prostate cancer: There is no strong and consistent evidence that sitosterol intake affects either colon or prostate cancer. (Kennedy, 1995, Klippel *et al.*, 1997) but Woyengo *et al.* (2009) reported the inhibitory action of phytosterols on lung, stomach and ovarian cancer.

On membrane properties: Plant sterols and stanols are incorporated into cellular membranes (Child and Kuksis, 1982) and may consequently influence membrane properties. Although one of the studies (Mora *et al.*, 1999) showed no effects of sitosterol on the membrane fluidity, it has been suggested that plant sterols can incorporate in red blood cell membranes, replacing cholesterol, which made the erythrocytes less deformable and more fragile (Bruckerdorfer *et al.*, 1969).

On the immune system: Effects of plant sterols and stanols on immune function have not been studied in detail, but some studies do suggest that these compounds may have an effect. For example, plant sterol supplementation lowered serum interleukin-6 (IL-6) concentrations which assisted in lowering inflammatory response (Bouic *et al.*, 1999).

Sitosterolemia: It is a rare autosomal recessive disorder, with mutations in the ABCG5 or ABCG8 genes. In which there is Excessive accumulation of stanols/ sterols in body because of reduced ability of liver to excrete plant sterols which is known to be associated with premature coronary artery disease tendon, tuberous xanthomas (Salen *et al.*, 1992) and death.

POPs: Phytosterols are susceptible to oxidation like cholesterol because of their inherent and close molecular structure to cholesterol (Yvonne *et al*., 2014). They form hydroxy, epoxy, keto and triol derivatives which are collectively known as Phytosterol Oxidation Products (POPs). It may be enzymatic or non-enzymatic (Ryan *et al*, 2009).

Non-enzymatic oxidation: Sterol autoxidation is oxidation of the steroid nucleus through storage, processing and preparation of foodstuffs which is called non-enzymatic oxidation.

Enzymatic oxidation: it is conversion of phytosterols to their oxides on application of enzymes vivo. Formation of cholesterol oxidation products (COP) is the result of enzymatic activity of the intestinal microflora (Hwang and Kelsey 1978). It may possible with the PS also, encouraging POP formation *in vivo*.

Four individual phytosterol oxides are: 7-ketocampesterol, 7-keto-b-sitosterol, 7-b-OH-campesterol and 7-b-OH-b-sitosterol.

Effect of POP on cholesterol uptake and its metabolism and other body organs is still to be uncovered. It requires future investigation to elucidate their specific effects on human health and to reduce their production in foods and *in vivo*.

Table 2: Health benefits of phytosterols in different studies.

Author	Outcome measures	Subjects	Product	Dose	Duration	Findings
Oksana *et al.* (2002)	Plasma TC and LDL-cholesterol	Mild hypercholesterolemic young men.	Round beef with 2.7 g of phytosterols	Single dose per day	4 weeks	Lowered TC (9.3%), LDL (14.6%), TC:HDL cholesterol (9.1%) from baseline.
Quı´lez *et al.* (2003)	TC, LDL, TG lipoprotein	Normocholesterolemic subjects	PS, Croissant and muffin	two pieces daily (3.2 g of PS per day)	8 wk	Decrease in TC (8.9%) and LDL-C (14.7%) as compared to control. No changes in TG, HDL-C and lipoprotein.
Sridevi *et al.* (2004)	Plasma lipoprotein profile	Mild hypercholesterolemic healthy subjects	PS fortified orange juice	2g phytosterols per day	2 weeks, 8 weeks	Decreased total (7.2%), LDL (12.4%), and non-HDL cholesterol (7.8%) from baseline. Apolipoprotein B levels (9.5%) no changes in TG and HDL
John *et al.* (2006)	Total, LDL cholesterol, vitamin A and carotenoids	Hypercholesterolemic subjects	Cocoa flavanol-enriched snack bar containing 1.5 g phytosterol	2serving per day	6 weeks	Reduction in plasma total (4.7%), LDL cholesterol (6%), ratio of total and HDL (7.4%) as compered to control. No changes in HDL, TG, serum Vitamin A, E and serum β carotene.
Hansel *et al.* (2007)	LDL-C, HDL-C, TG, β carotene,	Moderate Hypercholesterolemic subjects	low-fat Fermented milk (FM) enriched with 0.8 g PS ester per portion	2 low-fat portions	6 weeks	Plasma LDL-cholesterol concentrations were reduced by 7.8% as compared with control. No significant changes in TG, HDL-C and β carotene.
Qianchun *et al.* (2012)	Changes in antioxidant defence capacities and lipid profile	Male Wistar rats	Diets containing phytosterol fortified flaxseed oil	2000mg/100g	4 weeks	Decreased plasma TG, TC and LDL-C, hepatic TG and TC as compared with control
Sailaja *et al.* (2014)	TC,TG and LDL-C	Normocholesterolemic and mildly hypercholesterolemic	PS incorporated (1.5%) papaya fruit bar	30 g (fruit bar) per day	30 days	Decrease in serum TC (6.12%), TG (6.21%) and LDL-C (9.05%) as compared to control.

Conclusion

Earlier, the use of phytosterols were limited as drugs for lowering cholesterol, later, it was fortified with fat matrix but now positive results have been reported for phytosterols as functional foods in other matrices also.many fortified products including spread, orange juice, pasta, yogurt, snack bars and milk drinks are available in market. No adverse effect of risk associated with decreased level of fat soluble vitamins like carotene and tocopherol was seen on consumption of phytosterols. No teratogenic effect and no toxicity were reported. So it might be a better option for patients who have fear of intolerance, side effects and cost of medicines. Natural and available form of phytosterols should be preferred for consumption. Fortification of PS in non-fat food should be encouraged. Research should be done for effectiveness of PS for general population. Role of phytosterols on colon, vessel wall and other organs is not clear. So researches are needed in this area to discover its role on colon cancer as many contradictory studies have been reported.

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