Phytosterols And Its Effect On Human Health

Dr. Rekha Chawla (Corresponding Author)
Asst. Professor, College of Dairy Science & Technology, Guru Angad Dev Vet. and Animal Sciences University,
Email: rekhachawla@gadvasu.in

Nitika Goel
College of Dairy Science and Technology
Guru Angad Dev Vet. and Animal Sciences University
Ludhiana- 141004, Punjab.

Abstract

Phytosterols or stanols are large group of compounds found exclusively in plants. These are naturally present in plants and are structurally similar to cholesterol. A daily intake of 3 g of phytosterol (or their reduced form stanols) is associated with consistent and reproducible reduction in LDL cholesterol concentrations upto 10% and reduces the risk of coronary heart disease by 20% over a lifetime. Studies have concluded that the effective doses for reduction of cholesterol are between 1.5 and 3g/day, leading to decrease in 8% and 15% of LDL-cholesterol. The principal mechanism of action is based on interference with the solubilization of the cholesterol in the intestinal mucosa. Several studies have validated the cholesterol lowering effect of phytosterols and stanols along with its role in prevention of coronary heart diseases. Phytosterols and stanols fortified foods and formulations should pave the greater use of such ingredients for promotion in human health.

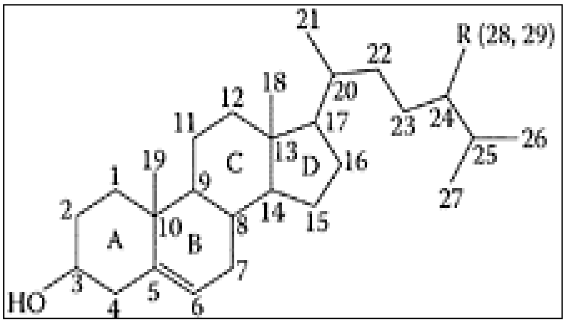
Keywords: Phytosterol, LDL cholesterol, coronary heart diseases mechanism of action, functional foods

Introduction

The term phytosterols is generally used to describe plant sterols and plant stanols collectively. Phytosterols are natural components of plant origin forming cell membrane and occur in small quantities in many fruits, vegetables, nuts, seeds, cereals, legumes, vegetable oils and other plants. Plant stanols occur in even smaller quantities in many of the same sources. It is estimated that 2500 tonnes of vegetable oil needs to be refined to produce 1 tonne of plant sterols (IFST 2005). All sterols are waxy colorless solids, soluble in most organic solvents and insoluble in water (Anon 2011[a]).

Structure of phytosterols:

Chemically, the phytosterols are classified as 4-desmethylsterols of the cholestane series. Structurally, plant sterols fall into one of three categories: 4-desmethylsterols (no methyl groups); 4-monomethysterols (one methyl group) and 4, 4-dimethylsterols (two methyl groups). However, most of the major phytosterols such as β-sitosterol, campesterol, stigmasterol, avenasterols and plant stanols belong to the group of 4-desmethylsterols (Christie 2011; Wasowicz 2002). Phytosterols differs very slightly in structure from the cholesterol by the presence of an ethyl or methyl group at C-24 position in the side chain (Scheme 1). There are over 40 phytosterols known today, but β-sitosterol is the most abundant one, comprising about 50 per cent of dietary phytosterols (Anon 2012[a]). The next most abundant phytosterols is campesterol (about 33%) and stigmasterol (about 2 to 5%). Other phytosterols found in the diet include brassicasterol, α-7-stigmasterol and α-7-avenasterol. Structurally, β-sitosterol differs from cholesterol by the presence of an ethyl group at the 24th carbon position of the side chain, whereas in the case of campesterol, a methyl group occupies the same position. The fully saturated form of phytosterols (containing no double bond at the 5,6 position) are the phytostanols, which are present in only trace amounts but can be formed by hydrogenation of phytosterols.



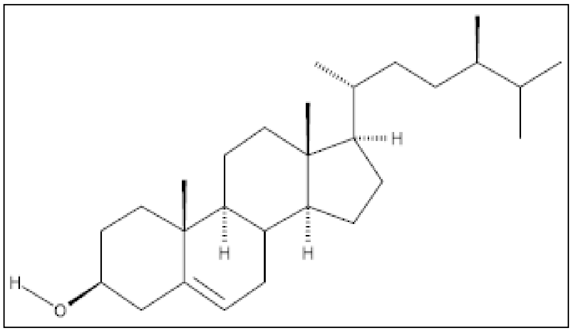
SCHEME 1. Structure of cholesterol and phytosterols.

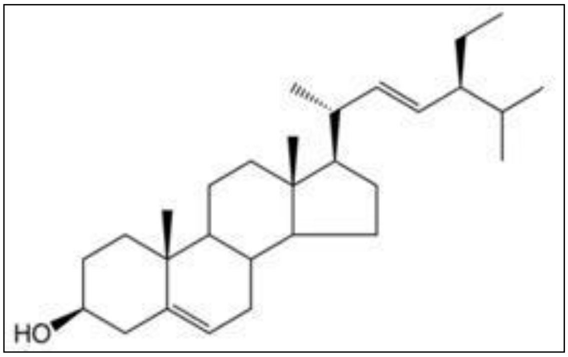
R=H : Cholesterol

$R=CH_3$: Campesterol

$R=C_2H_5$: β-sitosterol

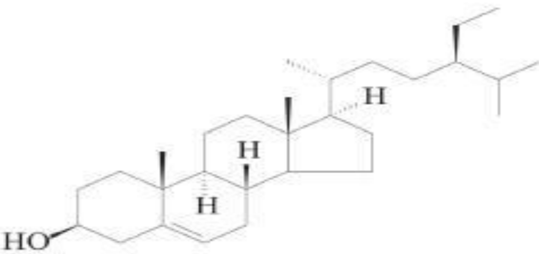
$R=C_2H_5, \Delta 22$: Stigmasterol

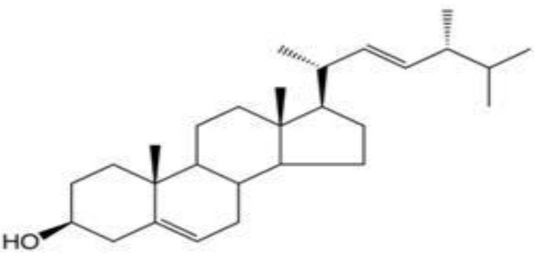




Campesterol

Stigmasterol





Sitosterol

Brassicasterol

Sources Of Phytosterols And Its Intake:

Phytosterol is abundantly present in the fat soluble fractions of all plants and foods containing plant based raw materials including principally oils, cereals, pulses and dried fruits (Piironen et al. 2000). Phytosterols may exist as free sterols (FS's), esterified with fatty acids (SE's) or phenolic acids (SPHE's) or as glycosides (SG's) and acylated glycosides **(**Moreau et al. 2002; Piironen and Lampi 2003)**.** Some packaged foods, such as specially formulated orange juices, low fat yogurts and milks are available in the market being fortified with phytosterol. However, major sources of phytosterols for fortification in current foods and for the preparation of dietary supplements include tall oil and vegetable oil deodorizer distillate.

Vegetable oils are richest source of free phytosterols and their fatty acid esters. These, in crude forms, contain 1-5 g/kg of phytosterols (Weihrauch and Gardner 1978; Johansson 1979; Piironen et al. 2000; Gunstone 2005). However, fewer oils such as corn oil (7.8-11.1 g/kg) and rapeseed oil (6.8-8.8 g/kg) are exceptions of this category. Tall oil contains a higher proportion of plant stanols than do vegetable oils (IFST 2005). The amount of available phytosterols in different vegetable oils is shown in Table 1. Refining of oils leads to significant lowering of phytosterol content (Ferrari et al. 1997) and therefore, it is very essential to develop industrial methods to minimize these losses (Quilez et al. 2003).

Distribution of phytosterols to free sterols and steryl fatty acid esters account for different proportion in different oils (Johansson 1979; Ferrari et al. 1996; Verleyen et al. 2002; Phillips et al. 2002; Worthington and Hitchcock 1984; Gordon and Griffith 1992) for example- free sterols accounts for nearly 54-85% of total phytosterols in soybean, sesame, olive, cotton seed, coconut, safflower, and peanut oil whereas the proportion varies for 32-44% in canola, rapeseed, corn, avocado and sunflower oil (Phillips et al. 2002).

Cereals such as rye, barley, wheat and oats are generally considered as good source of phytosterol wherein germ and bran fractions are known as the best reservoirs among other fractions. The total phytosterol content of various cereals varies from about 350-1200 mg/Kg (on wet basis). In cereals, sitosterol dominates among all phytosterols and its proportion ranges from 49-64% in wheat, rye, barley and oats (Piironen et al. 2003). Table 2 shows the composition of major phytosterol in different cereal grains.

Vegetables, fruits and berries are not as good source of plant sterols as vegetable oils and cereals, on a fresh weight basis. However, the variation in their sterol contents is remarkable when the results are calculated on dry weight basis. Piironen et al. (2000) have analyzed various vegetables for total sterols and reported a range of 5-37mg/100 g (on fresh weight) and 25-410 mg/100g (on dry weight) available in them.

Absorption Of Phytosterols

Initially, it was demonstrated that phytosterols are unabsorbable in rodents and rabbits. However, Salen et al. (1989) estimated 1.5-5% absorption of sitosterol in human beings. Ostlund et al. (2002) reported about 0.5% of sitosterol and 1.89% of campesterol absorption in human beings which reduced by 91% when the saturated form i.e. stanols was used. With the development of newer analytical techniques, various animal species showed phytosterols absorption ranging from 0-4% in rabbit & rats and 6% in human beings (Pollak and Kritchevsky 1981). Reported data also shows that some phytosterols get metabolized into acidic products (Kritchevsky and Chen 2005).

Cholesterol lowering effect of phytosterols and coronary heart diseases:

The cholesterol-lowering effect of phytosterol/stanol enriched foods has been well documented (Law 2000 & Plat et al. 2000). Systematic reviews studying the efficacy of phytosterols have shown that phytosterol/stanol enriched foods can significantly lower LDL cholesterol (Law 2000; Normen et al. 2005). Daily intake of phytosterols helps to prevent heart disease by lowering HDL cholesterol levels by as much as 14% (Anon 2011[b]).

A number of studies using phytosterols have been carried out and showed significant lowering of blood cholesterol levels. A summary of 52 studies revealed that an average of 13±1 g of phytosterol intake daily for 3-5 weeks, showed a 20% decrease in blood cholesterol level (Pollak and Kritchevsky 1981). Miettinen et al. (1995) prepared an esterified sitosterol (i.e. sitostanol) based spread which exhibited lowering of serum cholesterol in subjects with hypercholesterolemia. Unesterified sterols and stanols also exhibited a similar degree of low-density lipoprotein (LDL) cholesterol lowering effects (Vanstone et al. 2002). The efficacy of phytosterol esters as a hypocholesterolemic agent has recently been affirmed in a review by Katan et al (2003). In a meta-analysis of 41 trials with various enriched food products, these authors found that the optimal daily dosage of sterols or stanols is 2g/day can result in a 10% reduction in LDL cholesterol, whereas higher doses provide only a small additional effect.

Phytosterols compete with cholesterol absorption and uptake in the small intestine thereby reducing the supply of cholesterol in the blood stream. Since high blood total cholesterol and low-density lipoprotein (LDL) cholesterol levels are the main risk factors for coronary heart disease (CHD) and other diseases related to atherosclerosis, thus reducing cholesterol levels reduces the risk of CHD as well. According to recent data, cardiovascular disease (CVD) is a leading cause of death and a major cause of disability in Australia. One recent data reports that nearly 47,000 Australians died from CVD in 2007 (Australian Bureau of Statistics 2009) and in India according to a report generated by India Today, CVD is at the top position among the top ten causes of deaths in India with a percentage of 24.8 (Anon 2012[b]). In this context phytosterols have no effect on the levels of triacylglycerol or HDL cholesterol. Studies showed daily intake of 2-3 g sterols and stanols lowers LDL level by 10% and likely lowers CHD by 12-20% in the first 5 years and by 20% over a life time (Weststrate, and Meijer 1998). Phytosterols are absorbed from the blood into the body at a rate of 5-10%, whereas cholesterol is absorbed at a rate of 50% (Awad et al. 2000[b]).

Studies have also been conducted to investigate the effect of spreads containing phytostanol and sterol esters when used in conjunction with cholesterol lowering drugs i.e. Statins and Fiberates. These studies showed that phytosterol and stanol esters can be used safely, to provide an additional cholesterol lowering effect to that of the medication alone (Neil et al. 2001; Nigon et al. 2001). Since then, a large number of clinical studies with phytosterols have been conducted. Studies have shown that without adherence to a controlled diet, the consumption of 20 g phytosterol/-stanol enriched margarine has been shown to have a lipid lowering effect (Hendriks et al. 2003 and Geelen et al. 2002) and to augment the LDL cholesterol-lowering effect of other cholesterol-lowering strategies (Nestel et al. 2001). Phytosterols lower serum cholesterol by decreasing cholesterol absorption in the small intestine with a consequential increase in fecal excretion of cholesterol.

In an efficacy study on plant stanols, a daily consumption of 24 g of spread, containing 2-3 g of plant stanol esters resulted in lowering of total serum cholesterol and LDL cholesterol by 6.4 per cent and 10.1 per cent respectively (Nguyen et al. 1999; St-Onge et al. 2003). A dose dependent decrease in total and LDL cholesterol was observed with increasing levels of plant stanol esters up to a level of 1.6 g per day. However, increasing the dose from 2.4 g to 3.2 g did not provide any clinically important additional effect (Hallikainen et al. 2000a; 2000b).

To examine the effect of a food enriched with phytosterols on blood levels of cholesterol and nutrients, researchers randomly assigned 67 people with high cholesterol to eat two snack bars containing 1.5 grams of phytosterols each every day for six weeks or two bars that did not contain phytosterol. The subjects who had enriched bar showed 5 percent reduction in total cholesterol and 6 percent reduction in LDL cholesterol after six weeks. Also, an increase in the amount of HDL or "good" cholesterol in relation to total cholesterol was observed. However, the phytosterol enriched bars did not affect vitamins A or E levels in blood but at the same time reduction in β-carotene levels had also been reported (Anon 2006). Similar observations were recorded by Katan et al (2003) and Hallikainen and Uusitupa (1999).

***Phytosterols and Cancer**:*

As plant components, phytosterols (PS) may offer protection against cancer by several different means (Rao and Koratkar 1997; Awad and Fink 2000). These include inhibiting cell division, stimulating tumor cell death and modifying some of the hormones that are essential to tumor growth (Awad et al. 2000[b]). Long-term studies showed an association between the amount of plant sterol consumed in the diet and developing cancer. It is estimated that about 9 million new cancer cases are diagnosed every year and over 4.5 million people die from cancer each year in the world (Reddy 2012). However, the estimated number of new cancer patients in India per year is about 7 lakhs and over 3.5 lakhs people die of cancer each year.

Phytosterols have been found useful in treating other conditions, including rheumatoid arthritis, but their widest application is in protecting the heart. However, reports also suggest that excessive intake of dietary phytosterols and stanols in plasma and tissues may contribute to the increased blood pressure (Chen et al. 2010).

Safety of phytosterols:

Based upon results of clinical studies, plant sterols appear to be safe (Katan et al. 2003; Hendriks et al. 2003; Hepburn et al. 1999) and non-toxic (Christiansen et al. 2001; Hendriks et al. 1999) and no adverse effect has been found on the reproductive system (Baker et al. 1999; Waalkens et al. 1999). The U.S. Food and Drug Administration (FDA) granted GRAS "Generally Recognized as Safe" status for plant sterols/stanols, and the European Union Scientific Committee on Foods has concluded margarines and dairy products containing phytosterol esters are safe for human consumption. The FDA has also approved a health claim that foods containing plant sterols/stanols esters may reduce the risk of coronary heart disease (European food safety agency 2003) by reducing blood cholesterol levels as part of a diet low in saturated fat and cholesterol. However, the only drawback with phytosterols is that these require fat based medium to get solublize and the problem can be overcome by emulsification with lecithin and delivering in non-fat or low-fat foods and beverages. This can reduce the amount of fat in fat-based preparations substantially with retention of bioactivity and reduction in cholesterol (Ostlund 2004). Table 3 depicts the potential uses of phytosterol along with its future applications in non-fat dairy products.

Phytosterols in products:

Due to increasing consumer knowledge in functionality and health benefits, market of phytosterols containing products is growing continuously and various ready to eat products are available in market containing the phytosterols and stanols as novel ingredients as in yellow fat spreads (Wolfs et al. 2006). Also, their effectiveness in lowering both total and LDL-cholesterol in human subjects when incorporated to other foods matrices (yoghurt, milk, salad dressing and lean ground beef) has also been successfully demonstrated and indicated no side effects (Volpe et al. 2001; Davidson et al. 2001). These results have led to the market introduction of several foods enriched or fortified with phytosterols. Since fats are necessary to solublize sterols, hence foods with majority of the fat phase eg. Butter or margarines are an ideal vehicle for them, although cream cheese, salad dressing, and yogurt can also be used.

Conclusion

The availability of phytosterol containing products is gradually becoming more widespread due to awaken awareness and education of mass. Also, regulatory hurdles governing their use in foods are steadily overcoming. However, the only dark side effect of such products is with the interference with the absorption of carotenoids, which can be compensated by adding these compounds in appropriate carriers. Phytosterols also has anti-cancerous properties and act as immune system modulators. There are several possible future lines of research, as finding raw materials containing phytosterol content in abundance, minimization of losses during recovery processes and addition of purest form in ready bio-available product in the market and its easy availability. These ingredients should be incorporated in diet in sufficient amounts to reap the potential benefits of phytosterols. At the same time, the genetic bases of their action must be elucidated, synergic effects with other compounds must be studied along with its minimal side effects and the effects of long-term treatment must be defined precisely.

Table: 1

Phytosterol Content In Various Vegetable Oils

Sr. no.	Source	Amount present (g/kg)	Reference
1	Crude vegetable oil	1-5	Weihrauch and Gardner, 1978; Piironen et al., 2000.
2	Corn oil and rapeseed oil	8-22 and 5-11	Gunstone, 2005
3	Wheat germ and corn germ oil	17-26 and 10.7	Homberg and Bielefeld, 1989
4	Palm oil and coconut oil	0.7-0.8 and 0.7	Verleyn et al. 2002
5	Crude soybean oil	3-4.4	Moreau et al., 2002

Table: 2

Composition Of Phytosterols In Different Cereals (Mg/Kg)

Sample	Campesterol	Sitosterol	Stigmasterol	Avenasterol	Stanols	Total
Barley	150-192	437-484	24-36	56-69	17-19	720-801
Buck wheat	93	775	Tr	40	23	963
Corn	--	--	--	--	--	662-1205
Millet	112	371	18	87	ND	770
Oats	32-46	237-321	11-21	15-56	8-9	350-491
Rice	146	375	104	20	32	723
Rye	128-210	358-607	22-37	5-42	122-220	707-1134
Wheat	108-150	288-486	15-24	ND-22	151-171	447-830

(Source: Piironen and Lampi, 2003)

ND: Not Detected; Tr= Traces, -- Not reported

Table: 3

Phytosterol: Applications And Opportunities

Current uses	Potential uses	Novel forms
Margarines, fat spreads, soft spreadable cheeses, mayonnaise, salad dressings, low fat dairy products, milk, yogurt and cheeses, snack and energy bars, frying oils, breakfast cereals etc.	Baked pastry products, egg noodles and pasta, custard, ice cream, frozen desserts, muesli bars and soups, meat products, rice beverages, cereal grains and flours, food flavourings etc.	Encapsulation with egg proteins to increase bioavailability in foods Water soluble powders for inclusion in beverages (orange juice) and non fat foods.

(Source: Anon, 2008)

References

Anon (2011[a]) Phytosterols, phytostanols and their esters. Accessed on 22 May' 2009. http://www.fao.org/ag/agn/jecfadditives/specs/monograph5/additive-509-m5.pdf

Anon (2011[b]) Cholesterol. http://en.wikipedia.org/wiki/Cholesterol

Anon (2012[a]) Lower your serum cholesterol with plant sterols by Dr Harold Gunatillake. http://www.thesundayleader.lk/2012/02/26/lower-your-serum-cholesterol-with-plant-sterols/

Anon (2012[b]) India's no. 1 killer: Heart disease. (http://indiatoday.intoday.in/ story/ India's +no.1+killer:+Heart+disease/1/92422.html. Assessed on 08/06/2012.

Australian Bureau of Statistics (2009) Causes of Death 2007 (3303.0). March.

Awad AB, Downie A, Fink CS, Kim U (2000[a]) Dietary Phytosterol Inhibits the Growth and Metastasis of MDA-MB-231 Human Breast Cancer Cells Grown In SCID Mice. Anticancer Res 20:821-824

Awad AB, Fink CS (2000). Phytosterols as anticancer dietary components: evidence and mechanism of action. J Nutr 130:2127-2130

Awad AB, Karen CC, Downie AC, Fink CS (2000[b]) Peanuts as a Source of B-sitosterol, a Sterol with Anticancer Properties. Nutr and Cancer 36(2):238-241

Baker VA, Hepburn PA, Kennedy SJ, Jones PA, Lea LJ, Sumpter JP, Ashby J (1999) Safety evaluation of phytosterol esters Part 1. Assessment of oestrogenicity using a combination of in vivo and in vitro methods. Food Chem Toxicol 37:13-22

Chen Q, Gruber H, Swist E, Coville K, Pakenham C, Ratnayake WMN, Scoggan KA (2010) Dietary phytosterols and phytostanols decrease cholesterol levels but increase blood pressure in WKY inbred rats in the absence of salt-loading. Nutr & Metabol 7:11-20.

Christiansen LI, Lahteenmaki PLA, Mannelin MR, Seppanen-Laakso TE, Hiltunen RVK, Yliruusi JK (2001) Cholesterol-lowering effect of spreads enriched with microcrystal line plant sterols in hypercholesterolemic subjects. Eur J Nutr 40:66-73

Christie WW (2011) Sterols and their conjugates from plants and lower organisms: Structure, Occurrence, Biochemistry and Analysis. Cited on: 2[nd] Feb'2011. http://lipidlibrary.aocs.org/lipids/plant_st/index.htm

Davidson MH, Maki KC, Umporowicz DM, Ingram KA, Dicklin MR, Schaefer E, Lane RW, McNamara JR, Ribaya-Mercado JD, Perrone G, Robins SJ, Franke WC (2001) Safety and tolerability of esterified phytosterols administered in reduced-fat spread and salad dressing to healthy adult men and women. J Am Coll Nutr 20:307-319

European Food Safety Agency (2003) Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the Commission related to a novel food application from Forbes Medi-Tech for approval of plant sterol-containing milk-based beverages. Request No. EFSA-Q-2003-075. EFSA J. 15:1-12.

Ferrari RAP Schulte E, Esteves W, Bruhl L, Mukherjee KD (1996) Minor constituents of vegetable oils during industrial processing. J Am Oil Chem Soc 73:587-592

Ferrari RAP, Esteves W, Mukherjee KD, Schulte E (1997) Alteration of sterols and steryl esters in vegetable oils during refining. J Agric Food Chem 45:4753-4757

Geelen A, Zock PL, de Vries JH, Katan MB (2002) Apolipoprotein E polymorphism and serum lipid response to plant sterols in humans. Eur J Clin Invest. 32: 738–742

Gordon MH, Griffith RE (1992) A comparison of steryl esters of coconut and palm kernel oils. Fat Sci Technol 94:218-221

Gunstone, FD (2005) Vegetable Oils. In: Bailey's Industrial Oil and Fat Products, Sixth Edition, Volume 1. Edited by Fereidoon Shahidi., Wiley Interscience, A John Wiley & Sons, Inc., Publication pp: 213-242

Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uustiupa MIJ (2000[a]) Comparison of effects of plant sterol esters and plant stanol esters – enriched margarines in lowering serum cholesterol concentration in hypocholesterolemic subjects on a low fat diet. Eur J Clin Nutr 54:715-725

Hallikainen MA, Sarkkinen ES, Uusitupa MIJ (2000[b]) Plant sterol esters affects serum cholesterol concentration of hypocholesterolemic men and women in a dose dependent manner. J Nutr 130(4):767-776

Hallikainen MA, Uustiupa MIJ (1999). Effects of 2 low fat stanols sterol conataing margarines on serum cholesterol concentrations as part of a low fat diet in hypercholesterolemic subjects. Am J Clin Nutr.41: 403-410.

Hendriks HF, Brink EJ, Meijer GW, Princen HM, Ntanios FY (2003) Safety of long-term consumption of plant sterol esters-enriched spread. Eur J Clin Nutr 57: 681–692

Hendriks HF, Weststrate JA, Vliet VT, Meijer GW (1999) Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 53: 319–327

Hepburn PA, Horner SA, Smith M (1999) Safety evaluation of phytosterol esters. Part 2. Subchronic 90-day oral toxicity study on phytosterol esters- a novel functional food. Food Chem Toxicol 37:521-532

IFST (2005) Current hot topics: phytosterol esters (plant sterol and stanols esters). Institute of Food Science and Technology, 5 Cambridge Court, 210 Shepherd's Bush Road, London W6 7NJ, UK

Johansson K (1979) Sterols in vegetable oils: aspects of biological variation, influence of technological treatment, and the use of sterols to identify vegetable oils. Ph.D Thesis. Uppsala: Swedish University of Agricultural Sciences.

Katan MB, Grundy SM, Jones P (2003) Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 78:965–978

Kritchevsky D, Chen SC (2005) Phytosterols-health benefits and potential concerns: a review, Nutr Res 25: 413–428

Law M (2000) Plant sterol and stanol margarines and health. *Bri. Med. J.*, 320: 861–864.

Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E (1995) Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 333: 1308–1312

Moreau RA, Whitaker BD, Hicks Kevin B (2002) Phytosterols, Phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Progress in Lipid Res 41(6): 457-500

Neil HA, Meijer GW, Roe LS (2001) Randomised controlled trial of use by hypercholesterolaemic patients of a vegetable oil sterol-enriched fat spread. Atherosclerosis 156(2): 329-337

Nestel P, Cehun M, Pomeroy S, Abbey M, Weldon G (2001) Cholesterol-lowering effects of plantsterol esters and non-esterified stanols in margarine, butter and low-fat foods. Eur J Clin Nutr 55:1084–1090

Nguyen TT, Dale LC, Von Bergmann K, Croghan IT (1999) Cholesterol lowering effect of stanols ester in a US population of mildly hypercholesterolemic men and women: A randomized controlled trial. Mayo Clin Proc 74(12):1198-1206

Nigon F, Serfaty-Lacrosnière C, Beucler I, Chauvois D, Neveu C, Giral P, Chapman MJ, Bruckert E (2001) Plant sterolenriched margarine lowers plasma LDL in hyperlipidemic subjects with low cholesterol intake: Effect of fibrate treatment. Clin Chem Lab Med 39(7): 634-640

Normen L, Holmes D, Frohlich J (2005) Plant sterols and their role in combined use with statins for lipid lowering. Curr Opin Investig Drugs 307–316

Ostlund RE, Racette SB, Okeke A, Stenson WF (2002) Phytosterols that are naturally present in commercial corn oil significantly reduces cholesterol absorption in humans. Am J Clin Nutr 75(6):1000-1004

Ostlund RJ (2004) Phytosterols and cholesterol metabolism. Curr opin lipidpl 15(1): 37-41

Phillips KM, Ruggio DM, Toivo JI, Swank MA, Simpkins AH (2002) Free and esterified sterol composition of edible oils and fats, J Food Compos Anal 15:123–142

Piironen V, Lampi AM (2003) Occurrence and levels of phytosterols in foods: phytosterols as functional food components and neutraceuticals. Edited by Paresh C . Dutta CRC press. Pp:1-32.

Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. J Sci Food and Agric 80 (7):939-966

Plat J, Kerckhoffs D, Mensink R (2000) Therapeutic potential of plant sterols and stanols. Curr Opin Lipidol 11: 571–576

Pollak 0, Kritchevsky D (1981) Sistosterol. Monographs on atherosclerosis. Basel; S. Karger

Quilez JP, Garcia-Lorda, P, Salas-Salvado J (2003) Potential uses and benefits of phytosterols in diet: present situation and future directions, Clin Nutr 22**:** 343–351

Rao AV, Koratkar R (1997) Anticarcinogeniceffects of saponins and phytosterols. ACS Symposium Series 662:313-324

Reddy KR (2012) Kidwai memorial institute of oncology. **C**ited on: 2nd Feb'2012. http://kidwai.kar.nic.in/statistics.html

Salen G, Shore V, Tint GS, Forte T, Shefer S, Horak I, Dayal B, Nguyen L, Batta AK, Lindgren FT, Kwiterovich PO Jr (1989) Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. J Lipid Res 30:131 9-1 330.

St-Onge MP, Benoit L, Mauger JF, Jones Peter JH (2003) Consumption of a functional oil rich in phytosterols and medium-chain triglyceride oil improves plasma lipid profiles in men. J Nutr 133:1815–1820

Vanstone CA, Raeini-Sarjaz M, Parsons WE, Jones PJ (2002) Unesterified Plant Sterols and stanols lower LDL-Cholesterol Concentrations Equivalently in Hypercholesterolemic Persons. Am J Clin Nutr 76:1272-1278

Verleyen T, Forcades M, Verhe R, Dewettinck K, Huyghebaert A, De Greyt W (2002) Analysis of free and esterified sterols in vegetable oils. J Am Oil Chem Soc 79:117-122

Volpe R, Niittyen L, Carpel R, Sitori C, Bucc IA, Fraone N, Pazzucconi F (2001) Effects of yoghurt enriched with plant sterols on serum lipids I patients with moderate hypercholesterolemia. Br J Nut, 86:233-239

Waalkens-Berendsen HD, Wolterbeek APM, Wijnands MVW (1999) Safety evaluation of phytosterol sters. Part 3. Two generation reproduction study in rats with phytosterol esters- a novel functional food. Food Chem Toxicol 37:683-696

Wasowicz E (2002) Cholesterol and phytosterols. In: chemical and functional properties of food lipids. Zdzislaw E. Sikorski and Anna Kolakowska (Editors). CRC Press

Weihrauch JL and Gardner JM (1978) Sterol content of foods of plant origin. J Am Dietet Assoc 73: 39-47

Weststrate J, Meijer G (1998) Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 52: 334-343

Wolfs M, de Jong N, Ockéa MC, Verhagen H, Verschuren Monique WM (2006) Effectiveness of customary use of phytosterol/-stanol enriched margarines on blood cholesterol lowering. Food Chem Toxicol 44 (10):1682-1688

Worthington RE, Hitchcock HL (1984) A method for the separation of seed oil steryl esters and free sterols: application to peanut and corn oils. J Am Oil Chem Soc 61:1085-1088