# Phytosterol: A Functional Ingredient in Food

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Sterols are organic compounds, widely distributed in nature. Sterols are essential structural components of eukaryotic cell membranes, where they regulate membrane fluidity and permeability as well as membrane-associated metabolic processes. They occur naturally in animals, plants and fungi. Sterols of animals are called zoosterols. The most familiar type of animal sterol is cholesterol. The major sterol in yeasts is known as ergosterol. Plants have a variety of well-studied sterols, which are collectively termed as 'phytosterols'. This term is derived from Greek word '*phyton*' means plant and '*stereos*' means solid (Bartnikowska, 2009). More than 250 different phytosterols and related compounds have been identified in various plant and marine materials (Brufaua *et al*., 2008). Phytosterols are typically plant origin and cannot be synthesized by humans. Therefore, their source in human body originates only from diet (Tasan *et al*., 2006). The phytosterols have several bioactive qualities with possible implications for human health (Awad and Fink, 2000). Epidemiological evidences indicate reduced incidences of various diseases in populations consuming diets rich in vegetables and fruits. The studies were directed on protective effects of minerals, trace elements and vitamins. However, health benefits of phytosterols has been focused only in recent years and attempts yielded positive correlations in terms of chronic disease risk reduction (Jones and AbuMweisb, 2009; Rafia, 2013).

## Chemistry

Sterols are components of non-saponifiable fraction of lipids. They are unique among lipid components in that they have multiple-ring structure. The common structural precursor for all the sterols is cyclopentanoperhydrophenanthrene, which comprises a skeleton formed by four aromatic rings designated as A, B, C and D.

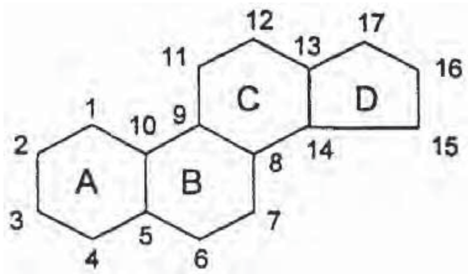


Fig. 1 Cyclopentanoperhydrophenanthrene

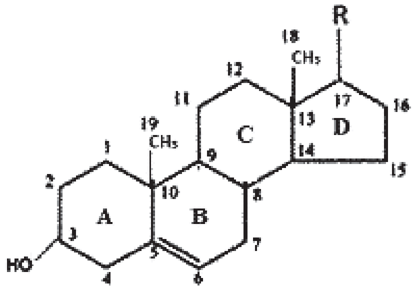


Fig. 2 General structure of sterol

All sterols have a polar hydroxyl group at carbon 3 of ring 'A', while rest of the structure is non-polar. This gives them certain amphipathic character. The sterols have a double bound between C-5 and C-6. Methyl groups are normally present at C-10 and C-13. An aliphatic side chain (R) present at C-17 of ring 'D'. The side chain consists a linear structure of 8 to 10 carbon atoms, depending on whether the sterol is from animal origin (8 carbon atoms) or from plant origin (9 or 10 carbon atoms). Thus, the major differences are found in the alkyl side chain, which can vary by the absence or presence of a methyl or ethyl group, saturation, position of a double bond and geometry of the substitutions (Trautwein and Demonty, 2007). If double bond between C-5 and C-6 in plant sterol is saturated (reduced), it leads to the formation of stanols. Often sterols and less frequently stanols, have the hydroxyl group esterified with a saturated fatty acid (usually palmitic; $C_{16:0}$) or unsaturated fatty acid (usually oleic; $C_{18:1}$). The esterification of the hydroxyl group eliminates the amphipaticity of the molecule and converts it into a structure completely non-polar. The more common phytosterols include β-sitosterol, campsterol, stigmasterol and Δ5-avenasterol.these plant sterol have one- or two-carbon substituent in the side chain and variable stereochemistry at C-24. Occasionally there is a double bond in this chain that can be of the *cis* or *trans* configuration.

## Occurrence

Phytosterols are found in fat-soluble fractions of seeds, roots, stems, branches, leaves and blossoms. They are constituents of both edible and ornamental plants, including herbs, shrubs and trees (Clifton, 2002). Phytosterols are present in all plants and in foods containing plant-based raw materials. In normal diets vegetable oils and products containing these oils are rich in free phytosterols and their fatty acid esters. The most of the crude vegetable oils contain 1–5 g of total phytosterols per kg. Cereals are generally regarded as good sources of phytosterols (350 to 1200 mg per kg fresh weight). However, phytosterols are localized mostly within the kernel. Therefore, role of cereal products in the total sterol intake depends on the dietary pattern. The total phytosterol concentrations are markedly lower in vegetables (300 mg/kg) and fruits (13 to 440 mg/kg) given on a fresh weight basis. Various nuts like peanuts and almonds are rich in phytosterols (220 to 1580 mg/kg) (Piironen and Lampi, 2004).

## Health Benefits

Phytosterols and phytostanols do not provide any energy to the body (Cantrill and Kawamura, 2008). Phytosterol has same basic functions in plants as cholesterol in animals (Brufaua *et al*., 2008). Plant sterols are known to have several bioactive qualities with possible implications for human health (Awad and Fink, 2000). Their health benefits are very well recognized, therefore, foods are being fortified with phytosterols. Now it has become the most well-known and scientifically proven benefit of phytosterols ( Rafia, 2013; Raju *et al*., 2013; Trautwein and Demonty, 2007). In addition to cholesterol reduction phytosterols have several health benefits such as prevention against certain types of cancer like colon, breast and prostate (Bruce and Grattan, 2013) stimulation of immunity and protection of skin (Awad and Fink, 2000; Bouic, 2001).

## Preparation

In preparation of Phytosterols on industrial scale, the two major sources are vegetable oil and tall oil (by product of paper industry) (Quilez *et al.*, 2003). Crude vegetable oils are refined to remove impurities. The last step in the refining process volatiles are removed and recovered in distillate. This distillate contains significant amount of phytosterols (8-20%) along with free fatty acids and tocopherols. After transesterification (methanolysis) process of distillate, phytosterols and tocopherols are distilled out. The phytosterols are separated from the tocopherols by solvent crystallization and filtration using food grade solvent (Fernandes and Cabral, 2007). Other methods used for extraction of phytosterols are pressurized fluid extraction (Pascal and Segal, 2006) and supercritical fluid extraction (Sajfrtová *et al*., 2010). Pure saturated phytostanols can be obtained by hydrogenation of phytosterol. In this process the double-bond in the sterol molecule is saturated by the addition of hydrogen. This reaction is carried out in a suitable solvent under high hydrogen pressure, generally using a noble-metal based catalyst (e.g. Pd or Pt) (Cantrill and Kawamura, 2008).

Phytostanols and phytosterols esters are produced via esterification of plant stanols or sterols with fatty acids from common vegetable oils. Esterification of phytosterols or phytostanols modifies the physical properties from high-melting crystalline powders with low oil solubility into liquid or semi-liquid substances that can easily be incorporated into a variety of (fat containing) foods (Cantrill and Kawamura, 2008).

## Applications

In 1997, first plant sterol containing margarine was introduced to the European market, many more applications have been lodged to add plant sterols to dairy products, cheeses, bakery products, sausages, plant oils, and other products.While amongst functional foods dairy-based functional foods (e.g. with added probiotics, omega-3, phytosterols) account for 43% of a $16 billion market (Papademas and Bintsis, 2010).

Major problems related to enrichment of products with phytosterols are high melting temperature, chalky taste and low solubility in water phase. To overcome these problems mainly esterification and microencapsulation technique are used (Izadi *et al*., 2012). The amount of phytosterols that can be formulated into food is very limited. If too much is added it may crystallize, causing a waxy and gritty texture. Microencapsulation of bioactive ingredients could help to facilitate controlled release of compound at the appropriate target and it also help in addition of bioactive ingredients. Formulation of phytosterols into food should be achieved through dispersion system such as emulsion and liposome which may further processed by several types of encapsulation system used depending on food applied. Spray-drying of emulsions is generally recommended for the encapsulation of lipid-soluble compound (Lee and Wong, 2014).

Low-fat dairy based food products like milk and yoghurt enriched with plant sterol esters are similarly effective in lowering total cholesterol and LDL cholesterol concentrations as fat based foods like spreads and margarine. Milk is also a good vehicle for phytosterols to lower plasma levels of cholesterol (Goncalves *et al*., 2007).

In some cases phytostanol- and phytosterol esters can be used as a fat replacer because the phytostanol/sterol moiety of the ester molecule does not provide any energy to the body. Phytostanol esters are used to modify the fatty acid composition of a fat blend and replace part of the hard fat in margarines and spreads. Plant esters can provide a crispy texture (prevents sogginess) to cereal products by coating the product surface. Improve the taste of food products by masking bitterness in soy drink (Cantrill and Kawamura, 2008).

Phytosterols and their fatty acid esters are quite stable and undergo only limited degradation during oil processing. Only under harsh conditions, such as high temperatures (> 100ºCºC) in the presence of oxygen (Raju *et al*., 2013). Phytosterols and phytostanols are microbiologically inert. Phytostanol and phytosterol esters are also stable in milk and fermented milk and products with viable bacteria like yoghurts and cheese. Phytosterol and phytostanol esters give an enhanced creamy texture to low fat dairy products like yoghurt or drinking yoghurt. Furthermore, the ester added to various food products show excellent stability at different pH values during long term storage (up to 1 year) (Cantrill and Kawamura, 2008). Under conditions used for shallow frying, the level of oxidation of sitosterol ester remains below 1.3% when phytosterol enriched fat spread used as frying medium (Salta *et al*., 2008).

## Limitation

Consumption of phytosterols over than effective doses (> 3 g/day) results in measurable increases in phytosterol level in blood, or phytosterolemia, also called sitosterolemia (Katan *et al*., 2003). As a result of untreated phytosterolemia atherosclerosis and coronary heart disease may occur. Phytosterolemia is also increased by statin drugs. Phytosterolemia occurs in 1 per 1 million people (Patel and Thompson, 2006).

Since plant sterols decrease the absorption of cholesterol, they might also affect the absorption of fat soluble vitamins (Bartnikowska, 2009). Similarly dietary carotenoids are fat-soluble phytochemicals that circulate in lipoproteins. A number of studies have observed 10-20% reductions in plasma carotenoids after short-term and long-term consumption of plant sterol- or stanol-enriched foods. These reductions were related to the reduction in LDL, which carry most of these antioxidants (Katan *et al*., 2003).

The Cost is also issue in fortification with phytosterols. Based on UK data, it is evident that the cost per Kg of food products with added plants sterols can range from 1.3 to 4.0 fold higher than that of their conventional counterparts. Thus, cost may potently constitute a deterrent to the regular purchase of these products. The main reason for the high cost is the limited number sources for commercial production. It is estimated that 2500 tonne of vegetable oil or pine is required to producing 1 tonne of phytosterols (Tasan *et al*., 2006).

## Legislation

Phytosterols have been used for lowering plasma cholesterol levels for half a century now, and so far, no marked adverse effects have been reported. It has been found that plant sterols, within the range that causes desirable reduction in blood levels of total cholesterol and LDL-cholesterol, are clinically safe. Plant sterols or stanols added to foods or supplements are not recommended for pregnant or breast-feeding women because their safety has not been studied (Berger *et al*., 2004; Raju *et al*., 2013).

Plant sterol, stanols and their esters were given GRAS status in United States. On the basis of this recognition, the US Food and Drug administration (FDA) approved fat spreads containing up to 20% of either steryl or stanyl esters (Quilez *et al.*, 2003). The EU Scientific Committee on Food approved the use of phytosterol esters in yellow fat spreads (Maximum level of 8% of free phytosterols) in 2000. The European Food Safety Authority recommended that plant sterol containing foodstuffs should not be consumed in amounts resulting in total phytosterols intakes exceeding 3g/day. In 2004 the EU commission published regulation concerning labeling of foods and food ingredients with added phytosterols and/ or pytosterol esters, requiring such products to be labeled with additional information including the words "With added plant sterols/ plant stanols" (Tasan *et al*., 2006).

A communiqué from Food Safety and Standards of India (FSSAI) stated that under the fortification category of food product standards and food additives, phyto or plant stanol esters and sterols may be added to fat spreads, milk products, milk-based fruit drinks, fermented milk products, soy and rice drinks, cheese products, yoghurt products, spice sauces, salad dressings, juices and nectars. This would allow consumers to easily their consumption to a maximum of 3 g per day. The draft guidelines also stated that either one portion of 1 g each would be added under sub-regulation 48 of Regulation 2.4.5 of the Food Safety and Standards (Packaging and labeling) Regulations, 2011 (Nandita, 2014).

## Conclusion

Market of fortified food category is increasing at very fast rate. Plant sterol enriched foods in combination with a healthy lifestyle are the most effective ways of lowering cholesterol level naturally and get all benefits of this biologically active component. Plant sterol, stanols and their esters were given GRAS status by several legal authorities. Addition of Plant sterol and stanols to foods could be an important public health policy if new technology can lower the cost to enable a greater demand to be satisfied.

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