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Mini Review

Phytosterols: An Appraisal of Present Scenario

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Abstract

Phytosterols exist as naturally occurring plant sterols that are present in the non-saponifiable fraction of plant oils. Phytosterols (plant sterols and stanols) are well known for their low density lipoprotein-cholesterol (LDL-C)–lowering effect. Phyto sterols specifically sitosterol, stigma sterol and campesterol are the major ones available among the foods. These are found in cereals, legumes, fruits and vegetables, nuts & oilseeds, unrefined vegetable oils in variable quantities in general. Specifically, the nuts and oilseeds have high amounts of phytosterols. The solvent extraction and supercritical fluid extraction can be used for lab-scale recovery of phytosterols from oilseeds apart from the industrial byproducts from oil refineries or paper industry. Different techniques such as thin layer chromatography, high performance liquid chromatography and column chromatography can be used for identification and purification/isolation of phyto sterols. The phytosterols have various functional properties such as hypo-cholesterolaemic ef-fect, anti-oxidative effect, anticancer activity and Immuno-modulatory activity, however, the cholesterol lowering effect has been the attraction for research studies. The daily doses, considered optimal for the purpose of lowering blood cholesterol levels, are 2-3g of phyto stanols and/or phyto sterols. Foods such as bread-spreads, milk shakes etc. can be developed to ensure the consumption of required dose.

Keywords: Phyto sterol; Phytostanol; Oilseeds; Nuts; Hypo-cholesterolaemic effect

Introduction

A balance diet is defined as one which contains a variety of food in appropriate quantities and proportion so that, the need for energy as well as essential vitamins & minerals are adequately met for maintaining health vitality and general well-being. Of late, consumers look for foods that provide health benefits beyond basic nutrition and strive for optimal health and longevity. Therefore, a diet with bioactive components in adequate amounts is precious for healthy life. The bioactive plant chemicals that humans eat and have significant positive effects on human metabolism are referred as phytochemicals. The phytochemicals that are frequently associated with human health are Phenolics, carotenoids, organic acids, and several miscellaneous bioactive compounds such as saponins and sterols. Phytochemicals in perfect dosage which have a therapeutic effect are referred to as Neutraceuticals [1]. Broadly, phytosterols can be described as one of the groups of Nutraceuticals exclusively from plant source and incorporation of which into the food makes it a functional food. Research and utilization of phytosterols focused on their value as precursors in the synthetic synthesis of several steroid hormones in the past [2], how-ever during last decade, there has been an unprecedented escalation of interest in phytosterols. Most of this interest has focused on the cholesterol-lowering properties (both dietary and endogenously-produced) of 4-desmethyl Phytosterols and phytostanols, which results in a decrease in serum total and low-density lipoprotein cholesterol (LDL-C) [3]. The trend of occurrence of cardiovascular disease (CVD) s has now crossed the age barriers and the humans have to be cautious about their diet in all ages. The phytosterols can help to alleviate the causes of CVDs through diet. The present article will assist researchers planning to work on phytosterol as well as health conscious readers by providing information in a nutshell while the readers will benefit.

Physical and Chemical Characteristics

Phytosterols and Phytostanols are a large group of compounds that are found exclusively in plants. They are structurally related to cholesterol but differ from cholesterol in the structure of the side chain. They contain a total of 27-30 carbon atoms. They consist of a steroid skeleton with a hydroxyl group attached to the C-3 atom of the A-ring and an aliphatic side chain attached to the C-17 atom of the D-ring. Sterols have a double bond, typically between C-5 and C-6 of the sterol moiety. They are derived from hydroxylated polycyclic isopentenoids having a 1, 2-cyclopentanophenthrene structure [4]. Phytosterols exists in different forms and available in wide sources (Table 1). The oils and nuts are the major source of phytosterols including certain seeds (Table 2). In addition, the fruits such as banana, oranges, fig and passion fruit the phytosterol content range 16-44 mg/100g while that of vegetables such as lettuce, cauliflower and broccoli was 39-43 mg/100g [5] The legumes such as kidney bean, broad bean, and pea the phytosterol range was 124-135 mg/100g while in the vegetables such as cabbage, carrot, cauliflower, onion and yam contains phytosterols in the range 10-18 mg/100g [6].

Phytostanol and phytosterol esters are chemically stable, fat-type materials, having comparable chemical and physical properties to edible fats and oils. Phytosterols are insoluble in water, but are soluble in non-polar solvents, such as hexane, iso-octane and 2-propanol. The esters are also soluble in vegetable fats and oils. Heat stability of the phytosterol esters is comparable to or even better than that of the parent vegetable oil or oil blend from which the fatty acids were derived [7]. A study by Soupas., et al. [8] stated that during shelf-life studies (long term storage), as pure material or in a product, phytosterol esters produce similar decomposition products to those of edible oils and fats as oxidation of the fatty acid moiety is the major cause of the quality deterioration and formation of off-flavors in oils and fats. The same study revealed that the phytosterol moieties are very stable at ambient temperatures and at higher temperatures some oxidation may occur. The isolated phytosterols in general will be a whitish solid and/or a pale yellow colour. These phytosterols are soluble in organic solvents but insoluble in water which reveals the hydrophobic nature of the sterols. The melting point is between -25.7 to 38.8°C.

Neutraceutical Property

Dietary sterols recently have received increased attention because they are associated with public health. Dietary cholesterol raises the serum cholesterol level and therefore increases the risk of heart diseases. On the contrary, dietary plant sterols have been demon-strated to reduce serum cholesterol levels [3]. Plant sterols also may inhibit colon cancer development [9,10]. For many years, the exis-tence and dietary effects of these minor sterols were largely ignored and poorly understood. Sterol chemists and biochemists focused their efforts on cholesterol because elevated serum cholesterol levels were shown to be a prominent risk factor for cardiovascular dis-ease (CVD). Recent strategies for lowering serum cholesterol (and risk of CVD) utilize dietary restrictions to limit cholesterol intake and/ or require the use of drugs which inhibit cholesterol biosynthesis in humans. In the prospect of lowering cholesterol levels with the re-cent knowledge about the functional foods consumption of functional foods fortified with natural phytonutrients has become attractive to many than use of drugs or dietary restrictions. As phytosterols have many neutraceutical properties (Table 3), it is considered as func-tional food component. Phytosterols have several functional use such as hypo-cholesterolaemic action [11], anti-oxidative effect [12], can protect against several cancers such as colon, breast and prostate cancer [13], and positive effects on benign prostatic hyperplasia have been reported [14]. Their actions as immune modulators and their anti-inflammatory properties have also been described [15].

Stability and Textural characteristics of Phytosterols

The major factors affecting phytosterol oxidation include temperature and heating time as well as the composition of the lipid ma-trix. Phytosterol esters were found to be more susceptible to oxidation at elevated temperatures than free phytosterols [8]. Temperature withstanding capacity of phytosterols/phytosterol esters is comparable or even better than their parent vegetable oils from which they are derived. Studies have reported that phytosterols and their fatty acid esters are very stable compounds and undergo only limited degradation during oil processing. Only under harsh conditions at temperatures > 100°C and in the presence of oxygen, oxidation of the phytosterol moiety may occur, in the same way as that for cholesterol [16]. As phytosterols contain only one double bond in the B-ring like the fatty acids but 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 sterol oxidation products are formed slowly. Salta., et al. [7] have reported that under conditions like when used for shallow frying (temperatures 160-200°C, 5-10 minutes of frying) the level of oxidation of sitosterol esters remains below 1.3%. Somewhat higher values of oxidation

products were seen (2.5 and 5.1%) using free sterols instead of esters in the same levels are respectively. Soupas., et al. [17] observed that, during a pan-frying, phytosterol oxides were formed at a very low level.

Phytosterols like cholesterol undergo oxidation during storage. The presence of the tertiary carbon atoms in the structure of phytos-terols makes them prone to this degradation and formation of variety of oxidized products may occur [18]. Commercial spreadable fats such as margarines, milk and yoghurts formulated with phytosterols are available in the market, and only a very few researchers have dealt with the evaluation of oxidative stability of sterols and factors affecting the formation of oxidized sterol derivatives [19]; Grandgi-rard., et al. [20] Johnsson and Dutta [21]; Moreau [2]; Moreau., et al. Soupas., et al. [8]. A study by Rudzińska., et al. [22] on phytosterol enriched margarines revealed that the total phytosterol level decreased from 79 mg/g to 63 mg/g during storage. It was found that dur-ing storage at higher temperature, oxidation took place 1.5 times faster than at the refrigeration temperature (4°C) (Dutta [23]; Smith [18]. A study by Conchillo., et al. [19] have detected phytosterol oxidation products in commercial vegetable spreads and low-fat spreads, both enriched in phytosterol esters. The phytosterol-enriched products exhibited four times higher amounts of phytosterol oxidation than the traditional spreads. However, a study by Garcia-Llatas., et al. [24] on ready-to-eat infant formulas observed no significant differ-ences in the total amounts of sterol oxidation products when stored at 25°C for 9 months.

The textural characteristics of products fortified with phytosterols are significantly influenced by the nature of phytosterol incor-porated. Studies have revealed that when phytosterols are added to products, the firmness of the products was reported to increase. Addition of phytosterol powders at 3 and 4% level in cheese spreads resulted in significant increase in firmness of the cheese spread [25]. They have reported that the increased firmness of the cheese spread might be due to addition of free phytosterols in powder form which occupied the free space present in the cheese spread. Work of shear measures the resistance offered by the sample throughout the probe penetration. It is the amount of energy required to perform the shearing process. The same study revealed an increase in the work of shear from 112.3 to 138.0 N s as the levels of phytosterols addition were increased from 0 to 4% in cheese spread.

Stickiness is described as a feeling that can be perceived by tongue and palate [26,27]. The degree to which the product comes loose from different parts of the mouth determines the intensity of stickiness. In the same study by Giri., et al. [25], as the levels of phytosterols addition was increased from 0 to 4%, a slight, but gradual reduction of stickiness was observed from 9 to 8.5 N; however, this reduction of stickiness was not statistically significant ($p > 0.05$). The slight decrease of stickiness might be due to increase of water activity that represents the decrease of water binding capacity of the product. Phytosterols are insoluble in water or oil. The decrease water holding capacity could be attributed to disturbance of protein matrix due to insoluble phytosterols addition that led to weak gel formation [28]. Work of adhesion is the work necessary to overcome the attractive force between the surface of the product and surface of the probe. The area under the negative peak in penetration was measured as work of adhesion. In the same study the phytosterols addition followed an inverse relationship with the work of adhesion of the product. As the levels of phytosterols addition was increased from 0 to 4%, a sharp, steady and significant ($p < 0.05$) decrease in work of adhesion from 77.0 to 40.2 N s was noticed in cheese spread. The decrease of work of adhesion in the product was reported as due to reduction of work needed to overcome the attractive force between the surface of the product and surface of the probe due to reduction of stickiness of the product and also might be due to weak gel formation due to insoluble phytosterols addition.

Extraction of Phytosterols and Regulatory Aspects of Food Use

Phytosterol extraction in large scale is done from the two main common source vegetable oil distillates and wood pulp/tall oil ir-respective of their use in health, pharmaceutical and food applications. Table 4 describes the extraction procedures. Other recovery methods (Table 5) generally used for the Lab-scale separation of phytosterols in small scale process are- Saponification and distillation method; Solvent extraction and Supercritical fluid extraction. This extracted crude phytosterol concentrates needs to be purified for fur-ther targeted application. The purification of phytosterols can be achieved by different chromatographic methods. The isolated or crude phytosterol can be incorporated in various food products ranging from milk shakes to bread spreads. However, their usage for health benefits and the claims made by the commercials needs to be clear. In United States a variety of vegetable oil and tall oil based phytos-terol ingredients are considered as GRAS and have been used in variety of food products. Their typical usage level ranges from 0.6 to 1.1 g/ serving [29]. In Canada, phytosterols are permitted as novel food ingredients [30]. Total phytosterol consumption is restricted to 3 g/day from specific food categories where amounts per serving are restricted to 1 g/

serving. Specific food uses include unstandardized spreads, mayonnaise, margarine, calorie-reduced margarine, salad dressings and unstandardized salad dressings, yogurt and yogurt drinks, and vegetable and fruit juice drinks. In the EU, there are several regulations authorizing the addition of phytosterols to a variety of foods. The safety of phytosterol enriched foods was reviewed by the Scientific Committee on Food (SCF) who issued the following report titled "the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular atten-tion to the effects on [3-carotene". The SCF concluded that the consumption of 3g of phytosterols per day was safe [31].

The FDA issued guideline specifications for phytosterols as dietary supplement [32] should meet the certain specifications such as Peroxide value < 0.5 meq/kg; sodium residues < 0.5; total aerobic count, yeast & mould count should be less than 10 CFU/g sample and nil coliforms and heavy metals such as lead, arsenic, mercury, cadmium should be less than 0.2 ppm. The total phytosterol content in the commercial product such as Cardiabeat and omega-3 phytoseterol esters should be more than 40-45% while the free phytoserol content should not be more than 4%. Under the existing FDA [33] regulation, a health claim associating diets that include plant sterol/ stanol esters with reduced risk of heart disease may be made on the labeling of specified conventional foods and dietary supplements. Such health claims must:

a. State that plant sterols/stanols should be consumed as part of a diet low in saturated fat and cholesterol.

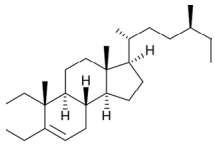
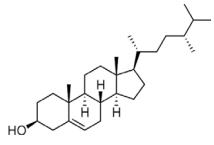
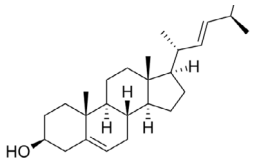
b. State that diets that include plant sterols/stanols "might" or "may" reduce the risk of heart disease, use the term "heart disease" or "coronary heart disease".

c. Use the terms "plant sterol esters" or "plant stanol esters" to refer to the substance in question.

d. Not attribute "any degree of risk reduction" for CHD to diets that include plant sterol/stanol esters.

e. Not imply that consumption of diets including plant sterols and stanols is the only method of achieving a reduced risk of CHD, and

f. Specify the intake of plant sterols/stanols that are necessary to reduce the risk of CHD and the contribution one serving of the product can make to that amount.

No	Phytosterol	Probable structure	Source	Form(s)	References
1	Sitosterol (3β- stigmast-5-en-3-ol)		Abundant in nuts walnuts, almonds, peanuts, hazelnuts and the macadamia nuts also found in cereals, legumes, fruits and vegetables, nuts & oilseeds, unrefined vegetable oils	Free alcohols or as conjugated forms, including glycosides & esters of fatty acids or ferulic acid [C10H10O4]	Laakso 2005 [34]; Ling and Jones 1995 [3]; Dutta and Appelqvist 1996 [35]; Weihrauch and Gardner 1978 [36]; Normen., *et al*. 1999[37]; Piironen., *et al*. 2000[38]; Phillips., *et al*. 2005[39]; Maguire., *et al*. 2004[40]
2	Stigmasterol (3β- stigmasta-5,22- dien-3-ol)				
3	Campesterol (24-α-methylcholesterol)				
4	Brassicasterol (3β-ergosta-5,22-dien-3-ol)				

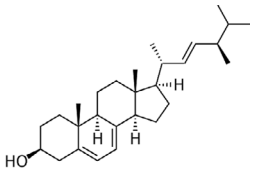
5	Ergosterol Δ7,22 (24-α-ethylcholesterol		Principal sterol of yeast also found in corn, cotton seed, peanut and linseed oils		Kritchevsky and Chen, 2005[42] Wikipedia 2015. [41]

Table 1: Different forms of phytosterol.

No.	Phytosterol food sources	Total phytosterols content (mg/100g)	
		Aparna., *et al.* 2011 [6]	**Gupta., *et al.* 2011 [5]**
	Oils and fats		
1.	Coconut oil	91	NR
2.	Corn oil	952	909
3.	Cottonseed oil	327	NR
4.	Flax seed oil	338	NR
5.	Olive oil	176	300
6.	Palm oil	49	NR
7.	Peanut oil	206	NR
8.	Rapeseed oil	NR	668
9.	Rice bran oil	1055	NR
10.	Sesame oil	NR	411
11.	Soybean oil	221	320
12.	Sunflower	NR	400
13.	Wheat germ oil	553	919
Nuts & Seeds			
14.	Almond	143	183
15.	Cashew	158	NR
16.	Hazelnut	NR	138
17	Pecan	108	NR
18.	Pistachio	108	276
19.	Pumpkin seeds	NR	265
20.	Walnut	108	113

Table 2: Major sources of phytosterol [oils, Nut and Seeds].

No	Effect	Mechanism	Reference
1	Hypo-cholestero-laemic effect	In appropriate condition phytosterols can become efficiently incorporated into the micelles in the intestinal lumen, displace the cholesterol, and lead to its precipitation with other non-solubilised phytosterols and is excreted in the faeces	Awad., *et al*. 2003; [43] Gupta., *et al*. 2011 [5]

2	Antioxidative effect	1.By decrease in the plasma catalase activity and superoxide dismutase activity which results in lower peroxide production and in turn, would indicate a lower degree of oxidative stress 2. By a significant reduction in hepatic peroxide index and in plasma and liver malondialdehyde levels an indicator of cell oxidative stress	Rafaela., *et al*. 2012; [12] Cantwell., *et al*. 1999; [44] Santos-Zago., *et al*. 2007; [45] Yamasaki., *et al*. 2000 [46]
3	Anticancer activity	The proposed mechanism can be via the immune system dysregulation which plays an important role in cancer me- tastasis i.e. by increased secretion of both interleukin 2 and interferon-γ important in preventing metastasis	Qin., *et al*. 2011; [47] Calpe-Berdiel., *et al*. 2007; [48] Bouic., *et al*. 1996; [49] Imanaka., *et al*. 2008; [50] Awad., *et al*. 2008 [51]
4	Immunomodulatory activity	Immune modulation is by increase in TH1 helper cells related cytokines, a decrease in TH2 helper cells related cytokines, increased lymphocyte proliferation, and greater natural killer cells activity	Bouic., *et al*. 1996 [52]; Bouic 1997 [52]; Myers & Bouic, 1998 [53]; Bouic and Lamprecht, 1999 [54]
5	Anti-inflammatory activity	They inhibit the action of hormones such as interlukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in a dose-dependent manner, which remains to the causative agents for inflamma- tion in disease conditions	Bouic and Lamprecht, 1999 [54]

Table 3: Neutraceutical property of Phytosterols.

No	Source	Method	Reference
1	Vegetable oil distillates	The volatiles removed in deodorization step of oil refining are recovered using a vapor condenser enriched using caustic refining and converted to esters by trans-esterification (methanolysis) process. After separation of the methanol/glycerol phase, the methyl esters are removed and the free phytosterols are removed by distillation	Coss., *et al.* 2000 [55]; Hayes et al., 2002 [56]; Quillez., *et al.* 2003 [11]; Copeland and Belcher,2001 [57]; Kamm., *et al.* 2001 [58]; Akimoto., *et al.* 2004 [59]
2	Wood pulp/ tall oil	1. Soapy lipid phase which is obtained in Kraft pulping process is subjected to solvent (methanol) extraction after which the phytosterols are purified by precipitation from the solvent. 2. Tall oil soap is acidified to produce an oily phase rich in sterols, fatty alcohols, squalene, waxes and other esters subjected to distillation, where the phytosterols are concentrated and subsequently purified by saponification with food-grade caustic soda to hydrolyze phytosterols esters and saponify the fatty acids. The mixture is then neutralized with a food grade mineral acid (such as sulfuric acid, hydrochloric acid or phosphoric acid). Thereafter the aqueous phase is removed and any remaining water is removed by flash evaporation.	Coss., *et al.* 2000[55]; Hayes., *et al.* 2002 [56]; Quillez., *et al.* 2003 [11]; Wong., *et al.* 1999 [60]; Rouskova., *et al.* 2011 [61]; Sato., *et al*. 2004 [62]; Rohr., *et al*.2005 [63]

Table 4: Extraction procedure for Phytosterols.

Method	Apparatus used	Conditions	Further separation	References
Saponification and distillation	Distillation Unit	Temperature: room temperature Solvents: ethanolic potassium hydroxide, diethyl ether, Column packing materials: anhydrous sodium sulfate, deactivated alumina	An aliquot of sample and 1M ethanolic KOH stirred over night at room temperature. Then the mixture is diluted with water & extracted with portions of diethyl ether. Process repeated till neutral pH of product obtained then dried sequentially with short columns of anhydrous sodium sulfate, deactivated alumina. Unsaponifiable residue obtained is subjected to further separation using chromatographic method for quantification of sterols	Abidi., *et al*. 1999; [64] Firestone, 1990; [65] Ibrahim., *et al*. 1990; [66] Nourooz-Zadeh and Applequist., 1992. [67]
Solvent extraction	Soxhlet apparatus	Solvent: Absolute ethanol Temperature: 100°C in steam bath	To the pooled extract water and petroleum ether is added and shaken in a separating funnel. Evaporation of the top organic layer under water aspirator pressure leaves the lipid extract which is further purified and phytosterols are separated	Abidi., *et al*. 1999 [65]
Supercritical fluid extraction and fractionation	Supercritical fluid extractor	Extracting medium: Supercritical CO_2 Flow rate: 2-2500 ml/min; Pressure: 5000-12000 psi Temperature: 40-80°C Time: 10-130 min	Repetitive extractions should be done to obtain sufficient materials. The collected extracts are pooled and dissolved in hexane and stored in freezer for later enrichment of sterols by saponification	Moreau., *et al*. 1996; [68] List., *et al*. 1989[69] Snyder., *et al*. 1999; [70] Taylor and King., 2000; [71] Eller and King, 2000; [72] King., *et al*. 1997 [73] Montanari., *et al*.1997 [74]

Table 5: Lab-scale recovery methods of Phytosterols.

Conclusion

Phytosterols being an interesting area for researchers, dieticians as well as consumers; the information summarized can be useful for the all of them. The extraction, identification and the incorporation of the concentrated phytosterols in a shelf stable food product would be the right thing to ensure its RDA to consumers. The practical utility of the phytosterols and the regulations indicates that the phytosterols have got high potential for the researchers to fill the gaps through establishing the commercial as well as clinical feasibility of use of phytosterols.

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