Plant Sterols and Stanols as Cholesterol-Lowering Ingredients in Functional Foods

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Abstract: This article reviews developments related to the use of plant sterols and stanols as cholesterol-lowering ingredients in foods and nutraceuticals preparations. Plant sterols and stanols are extracted from the deodorizer distillates of vegetable oil refining and from tall oil, a by-product of paper pulping industry. Plant sterols/stanols inhibit cholesterol absorption possibly by competitively inhibiting its incorporation into the mixed micelles in the small intestine although other mechanisms can not be excluded. Daily consumption of 1-2 grams of plant sterols or stanols was shown to cause 10-20% reduction in low-density lipoprotein cholesterol (LDL cholesterol). Combinations of plant sterols/stanols with certain lipid-lowering ingredients were shown to potentate their cholesterol-lowering effects and, in some cases, add triacylglycerol-lowering effects. In this article, patents based information is also discussed.

Keywords: Plant sterols, plant stanols, cholesterol-lowering, health-promoting effects, functional foods.

INTRODUCTION

Currently, great efforts are focused on reducing the risk of coronary heart disease through dietary interventions. One of the major risk factors of cardiovascular diseases that can be modulated by dietary intervention is blood cholesterol. A number of dietary agents, including soluble fibres and plant sterols/stanols, were found to interfere with cholesterol absorption and to lower its levels in serum. Plant sterols and stanols, also called *phytosterols* and *phytostanols*, have chemical structures resembling that of cholesterol but are only available to humans through plant foods such as vegetable oils, nuts, seeds, cereals, legumes, fruits, and vegetables or industrial supplements from plant origin [1]. Inclusion of plant sterols/stanols in the diet was known to lower serum cholesterol in man since 1953 [2-4] and the effects of plant sterols and stanols on cholesterol and bile acid metabolism and their efficacy and safety as serum cholesterol-lowering agents have been reviewed [e.g 5-7].

Cholesterol is a tetracyclic steroid with a 3β-hydroxy group, a 5,6-double bond and an eight-carbon side chain. Cholesterol is a product of *de novo* synthesis in animals, including humans, and is an important component of cell membranes and precursor of steroidal hormones and bile acids. Unlike animals, plants can only produce insignificant amounts of cholesterol. Instead, a wide range of analogous plant sterols Fig. (**1**) are produced by plants, most predominantly sitosterol, campesterol, and stigmasterol and to less extent brassicasterol, Δ^5- and Δ^7-avenasterols, and ergosterol. Plant stanols (mainly sitostanol and campestanol) lack the 5,6-double bond and are available from a limited number of plant species including wheat and rye grains. Besides the above-mentioned plant sterols, known as 4-desmethylsterol, other sterols belonging to the 4α-mono-methyl and 4.4-dimethyl sterol classes exist in minor amounts in plants [8-10]. These methylated sterols, which are abundant in some sources such as shea nut butter and *M. alpina* fungi, do not have the cholesterol lowering properties of the desmethylsterols [11-13].

In 1953, Pollak published papers on the ability of plant sterols to lower cholesterol in the rabbit and man [2,3]. Eli Lilly Co. (USA) marketed plant sterol preparations from tall and soybean oils, called CytellinTM (mainly β-sitosterol), as a cholesterol-lowering pharmaceutical treatment. However, the cholesterol-lowering potency of plant sterols shown in studies in the 1970's [14,15] was not considered in the efficiency range required for medicinal treatments. Mattson *et al*. [16] and Pollak [17] suggested the use of plant sterols as additives in foods such as butter and margarine. The use of plant stanols as cholesterol-lowering additives in margarines was adopted by Rasio (Finland) and Unilever (The Netherlands) in the middle of 1990’s.

Several studies [18-55] have documented the cholesterol-lowering effect of plant sterols and stanols in normo- and hypercholesterolemic males and females (Table **1**). A meta-analysis of these trials concluded that daily intake of 2 grams of plant sterols or stanols similarly reduces LDL-cholesterol by 10% [56]. Food products containing plant sterol or stanol esters (including vegetable oils, margarines, mayonnaises, salad dressings, cheese, yogurt, milk, soy milk, orange juice, snack bars, and meat sausages) are sold in the markets in Europe, USA, and Asia [57]. Health claims for reduced cardiovascular risk are approved in USA and the EU for foods containing plant sterols or stanols when consumed as part of a low-fat, low-cholesterol diet [58-61]. Accordingly, FDA allowed health claims within the context "*Diets low in saturated fat and cholesterol that include at least 1.3 grams of plant sterol esters or 3.4 grams of plant stanol esters, consumed in two meals with other foods, may reduce the risk of heart disease*". Thus, to be allowed health claims, food products should meet the requirements of total fat (<13g/serving), saturated fat (≤1g/serving) and cholesterol (≤20 mg/serving) [62, 63]. The rationale for the difference

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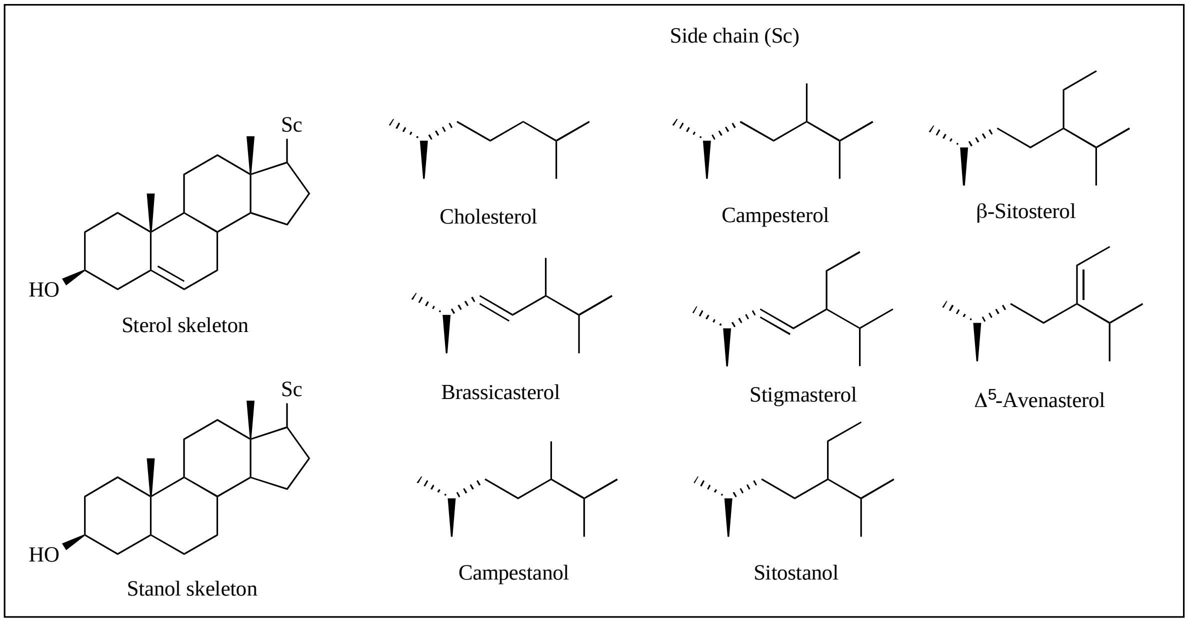

Fig. (1). Chemical structures of cholesterol and major dietary plant sterols and stanols.

Table 1. Summary of Studies and Results Documenting the Cholesterol-Lowering Effect of Dietary Plant Sterols and Stanols

Study Design*	Number of Subjects*	Mean age (year)	Dose (g/day)	Duration (week)	Placebo adjusted reduction in serum LDL cholesterol (mg/dL)	Reference
STEROLS						
Parallel	7+8	45	0.8	9	10	[18]
Cross-Over	22	35	1.6	1.4	12	[19]
Cross-Over	80	45	3.2	3.5	17	[20]
Cross-Over	80	37	0.8	3.5	8	[21]
Cross-Over	80	37	1.6	3.5	10	[21]
Cross-Over	80	37	3.2	3.5	12	[21]
Cross-Over	76	44	0.7	3	7	[22]
Cross-Over	34	49	2.1	4	17	[23]
Cross-Over	60	23	2.1	3	8	[24]
Cross-Over	39	31	2.5	4	12	[25]
Parallel	47+44	51	1.5	26	18	[26]
Parallel	21+21	45	3	8	5	[27]
Parallel	90+39	59	1.1	5	17	[28]
Cross-Over	29	52	2.5	8	20	[29]
Cross-Over	15	60	2.4	4	23	[30]
Cross-Over	53	58	1.6	8	9	[31]
Parallel	36+35	56	0.9	5	10	[32]
Parallel	36+35	56	1.9	5	14	[32]
Parallel	36+35	56	4.2	5	15	[32]

(Table 1) Contd….

Study Design*	Number of Subjects*	Mean age (year)	Dose (g/day)	Duration (week)	Placebo adjusted reduction in serum LDL cholesterol (mg/dL)	Reference
STEROLS						
Cross-Over	30	51	1	4	13	[33]
Parallel	17+17	23	2.7	4	17	[34]
Cross-Over	63	42	1.8	3	10	[35]
Cross-Over	15	52	1.9	3	22	[36]
Cross-Over	46	57	2.3	3	13	[37]
Cross-Over	35	57	2	3	15	[37]
Cross-Over	15	48	1.8	3	16	[38]
Cross-Over	15	48	1.8	3	16	[38]
Parallel	34+33	46	3.4	6	13	[39]
Cross-Over	53	45	1.8	3	9	[40
STANOLS						
Cross-Over	11	58	3	6	20	[41]
Parallel	7+8	47	0.8	6	11	[42]
Parallel	51+51	50	1.8	52	16	[43]
Cross-Over	22	51	3	7	20	[44]
Parallel	12+12	37	3	5	19	[45]
Cross-Over	80	45	2.7	3.5	16	[20]
Parallel	19+21	55	2	8	11	[46]
Cross-Over	21	53	2.4	5	17	[47]
Parallel	38+17	43	2.3	8	18	[48]
Parallel	16+16	50	1.7	4	25	[49]
Parallel	77+76	53	2	8	8	[50]
Parallel	77+76	53	3	8	17	[50]
Parallel	71+77	56	3	8	15	[51]
Cross-Over	34	49	2	4	21	[23]
Cross-Over	22	51	0.8	4	3	[23]
Cross-Over	22	51	1.6	4	10	[23]
Cross-Over	22	51	2.3	4	18	[23]
Cross-Over	22	51	3	4	20	[23]
Parallel	34+34	47	2	4	13	[52]
Cross-Over	15	52	1.8	3	10	[53]
Parallel	70+42	33	4	8	14	[54]
Parallel	30+30	36	3	4	15	[55]
Cross-Over	46	57	2.5	3	16	[37]

*Number of subjects in parallel design studies is given as the number in treatment groups+the number in placebo groups between plant sterols and stanols regarding the recommended effective level is not clear especially as a large magnitude of studies and the meta-analysis found them equivalent [55]. Although the claim specified the consumption of plant sterols or stanols in two meals, it is not well established whether meal frequency does or does not affect their cholesterol-lowering efficacy [64,65].

The current daily intake of plant sterols from conventional foods is estimated to be in the range of 160-400 mg among different populations [57]. Ostlund *et al*. [66] showed that this level of natural plant sterol level can reduce dietary cholesterol absorption and that daily consumption of wheat germ oil, delivering about 328 mg plant sterols, reduced cholesterol absorption by about 40% compared to sterol-free wheat germ oil [67]. Thus, naturally present dietary plant sterols may contribute to reduction of cholesterol absorption especially when combined with other cholesterol-lowering plant foods. Jenkins *et al*. [68-71] described and documented a portfolio diet composed of plant sterols, viscous fibers like psyllium, soy proteins, and almonds with additive or synergistic effect on the reduction of blood cholesterol. Milstein *et al.* also reported the importance of food additives containing the sterols & stanols are useful for lowering the serum cholesterol [72].

MECHANISM(S) ASSOCIATED WITH CHOLESTEROL-LOWERING BY PLANT STEROLS/ STANOLS

About 50-65% of intestinal cholesterol, coming from dietary cholesterol (300-400 mg/day) and biliary cholesterol (600-1000 mg/day), is absorbed and transported to the liver while the rest is excreted in feces [6,7,9,64]. Cholesterol absorption in the intestine occurs through "mixed micelles" that are necessary for its transport and uptake through the intestinal wall [64]. Figure **2** depicits possible mechanisms for the inhibition of the intestinal absorption of cholesterol by plant sterols/stanols. For more than a decade, the main mechanism believed to be responsible for cholesterol reduction by plant sterols was thought to involve competitive inhibition of cholesterol incorporation into the micelles leading to about 50% decreased absorption and, conesquently, increased excretion in feces [5]. Other evidence [73] and the fact that one daily dose of plant sterols/stanols can inhibit cholesterol absorption (i.e. plant sterols/stanols and dietary cholesterol do not have to be present in the intestine at the same time) questions the importance of this mechanism. According to a recent review by Calpe-Berdiel *et al*. [74], there is a need for new insights into the molecular actions of plant sterols/stanols including effects on intestinal and liver cholesterol transporters and gene expression of, for

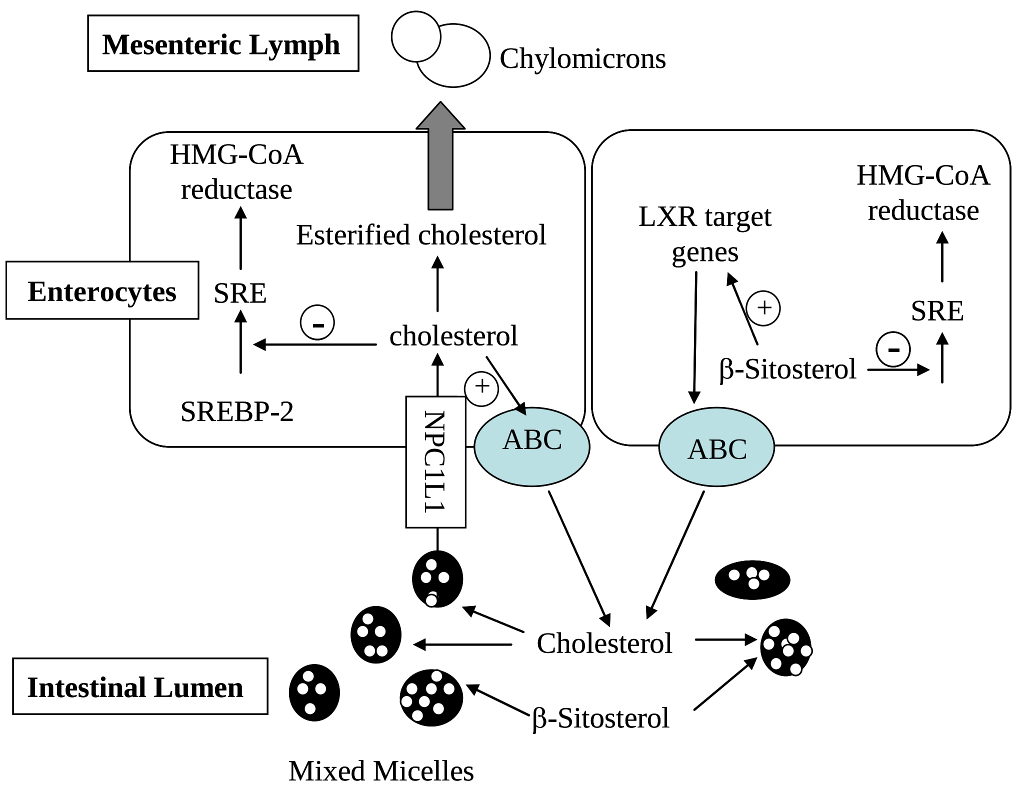

Fig. (2). Interactions of plant sterols (e.g. β-sitosterol) with cholesterol metabolism. Previously, the mechanism of cholesterol-lowering by plant sterols is believed to be the competitive inhibition of the incorporation of cholesterol into the mixed micelles. Several processes are involved in the determination of the cholesterol concentration in the enterocytes, e.g. regulation of cholesterol uptake from the lumen by Niemann-Pick C1-like 1 protein (NPC1L1) and its secretion back to the lumen by the ABC transporters located on the brush boarders of the enterocytes. In addition, both cholesterol and plant sterols may interact with liver X receptor (LXR) leading to induction of its intestinal target genes up-regulating the ABC transporters. Cholesterol and plant sterols down-regulate the sterol regulatory element binding protein-2 (SREP-2) leading to inhibition of the sterol regulatory element (SRE) and the intestinal activity of 3-hydroxy-3-methyl glutaryl coenzyme-A (HMG-CoA) reductase, which is the rate limiting enzyme in cholesterol biosynthesis [73-81].

example, liver (X) receptor (LXR) and peroxisome-proliferator-activated receptor δ (PPARδ). Several processes are involved in the determination of the cholesterol concentration in the enterocytes. These include regulation of cholesterol uptake from the lumen by Niemann-Pick C1-like 1 protein (NPC1L1) [75] and its secretion back to the lumen by the ABC transporters located on the brush boarders of the enterocytes [76].

While the absorption of dietary cholesterol is about 33%, those of plant sterols and stanols are significantly lower; being about 9.6% for campesterol, 4.2% for sitosterol, and virtually null for campestanol and sitostanol [77]. This difference is thought to be caused by differential excretion of plant sterols versus cholesterol by the adenosine triphosphate binding cassette protein transporter G5 (ABCG5) in the intestine responsible for reversed excretion of sterols into the intestinal lumen. This discriminatory action of the ABC transporters is the main reason for the lack of accumulation of plant sterols in the body except in people having the rare autosomal recessive disorder beta-sitosterolemia characterized by mutations in the tandem genes ATP binding cassettes; intestinal G5 and liver G8 [78,79]. It was suggested that the cholesterol-lowering effects of plant sterols are attributable, at least partly, to the induction of genes involved in the expression of the cholesterol transporters, e.g. intestinal *Abcg5* and liver *Abcg8*, *via* the liver X receptor (LXR) Fig. (**2**) but the involvement of the different mechanisms in the cholesterol-lowering effect of plant sterols and stanols is not yet well understood [80,81].

It is well accepted that the decreased cholesterol absorption is attenuated by increased synthesis due to feedback mechanisms leading to increased expression of hepatic 3-hydroxy-3-methyl glutaryl coenzyme-A (HMG-CoA) reductase and hepatic low-density lipoprotein (LDL) receptor. Thus, combination of plant sterols/stanols with HMG-CoA reductase inhibitors is expected to cause synergistic interactions leading to enhanced cholesterol-lowering [15], which is also described in WO08049196 patent [82]. Combination of plant sterols with statin therapy, however, has led to an additive rather than a synergistic effect [51]. Other dietary compounds, e.g. a number of phenolic derivatives, are known to have HMG-CoA reductase inhibiting activity [83]. WO/2007/137449 claims that combination of plant sterols, flavones from bamboo leaves, proanthocyanidins, and beta-glucan are able to effectively lower cholesterol and triacylglycerols. However, certain combinations might not be compatible and their absorption might be affected by the co-presence of plant sterols as is the case with sesamin whose absorption seems to be inhibited by plant sterols [84]. Combination of different cholesterol-lowering mechanisms proved effective in the portfolio diet; while plant sterols/stanols inhibit cholesterol absorption, viscous fibers increase bile acid excretion and soy proteins inhibit cholesterol synthesis [68, 69]. Sterol absorption inhibition is also discussed by Kosoglou *et al.* in EP1911462 patent [85]. Consumption of plant sterols/stanols has no effect on blood triacylglycerols but their combinations with, for example, diacylglycerols was shown to have cholesterol-lowering and anti-obesity effects [86]. A more effective cholesterol-lowering effect was obtained when plant sterols were dispersed in diacylglycerols than triacylglycerols (600 mg/day in contrast to >1000 mg/day) [87].

The reduction of cholesterol absorption is also accompanied by about 10% decrease in β-carotene levels in plasma but with no reduction in vitamins A, D, E, or K [21,47]. In nutritional supplements/functional foods containing plant sterols and stanols, this decrease in the absorption of carotenoids can be corrected by adding some β-carotene to the diets/supplements [37]. Apart from this effect, scientific literature suggests that it is safe to consume foods supplemented with plant sterols/stanols [88, 89], which are generally recognized as safe (GRAS) by the FDA [90] and the Scientific Committee on Foods of the EU [60]. However, there is some research indicating that supplementation with plant sterols imposes unfavorable vascular effects including impairment of endothelial function, aggravation of ischemic injuries, enhances atherogenesis [91-95]. The EU Committee recommended that the intake of plant sterols/stanols from foods should not exceed 3 grams per day.

PATENTS ON EXTRACTION OF PLANT STEROLS & THEIR INCORPORATION INTO FUNCTIONAL FOOD PRODUCTS

In fact, a large number of patents have been published on methods to extract and purify plant sterol/stanol esters from deodorizer distillates and tall oil and on the preparation different derivatives and forms that can be incorporated into cholesterol-lowering foods Table **2**. 4-Desmethyl sterols are available from edible vegetable oils and their refining distillates and from tall oil [96]. Vegetable oils are refined either by chemical refining including water degumming, alkali neutralization, acid-clay bleaching, and vacuum deodorization or by physical refining including water degumming, acid-clay bleaching, and steam-refining. Plant sterols, tocopherols, and other minor unsaponifiables are removed from refined oils during the deodorization step and can be recovered from the deodorizer sludge. The major refined vegetable oil sources of plant sterols include soybean oil, corn oil, sunflower oil, rapeseed oil and wheat germ oil. The other major source of plant sterols is tall oil, a byproduct in the black liquor of the pulp industry of mainly *Pinus* spp.

An excellent description of detailed methodologies for the extraction and recovery of plant sterols from vegetable oils and tall oil are provided by Fernandes & Cabral [97]. Process for recovery of plant sterols from by-product of vegetable oil refining is also demonstrated in US2008 7368583 patent [98]. In general, the deodorizer distillates from vegetable oil refining are subjected to steps of (i) sapnification or vacuum distillation to remove the bulk of fatty acids, (ii) esterification of plant sterols and removal of tocopherols by vacuum distillation, and (iii) hydrolysis of the sterol esters to release free sterol crystals that are then removed by filtration. As a result, sterols with about 95% purity are obtained. The processes for the isolation of plant sterols from tall oil are generally similar to those described above although a step of sterol complexation with calcium chloride was sometimes used. The composition of sterols obtained from vegetable or tall oils depends on the plant species of origin [99]. The composition of plant sterols obtained from major vegetable oil sources are shown in Table **3**. Although the oils vary considerably in the relative

Table 2. Selected Patents Describing Processes, Product Compositions and Uses of Plant Sterol Preparations

Patent Number	Inventors	Title	Publication Date	Described Process
US2729655	S. E. Miller B. C. Manley	Production of sterols	January, 1956	Sterols are removed from saponified distillate by extraction with petroleum ether
US2843610	B. Winton H. W. Rawlings	Separation of sterols from deodorizer sludges	July, 1958	Sterols are separated from refining sludge after saponification and acidification to release free fatty acids followed by precipitation of sterols from an acetone:methanol:water mixture
US3335154	F. E. Smith	Separation of tocopherols and sterols from deodorization sludge and the like	August, 1967	Sterols are first esterified, followed by distillation, release of the sterols from the esters and finally separating them by precipitation from a water solution
US3691211	D. V. Julian	Process for preparing sterols from tall oil pitch	September, 1972	Preparation of sterols from tall oil pitch and other sources by extraction in a water-alcohol-hydrocarbon mixture followed by saponification, recrystallization, and leaching
US3865939	R. J. Jandacek	Edible oils having hypocholesterolemic properties	February, 1975	Edible oil cholesterol-lowering preparations containing 2-6.9% plant sterols
US4420427	A. Hamunen	Process for the separation of sterols or mixtures of sterols	December, 1983	Extraction of sterols from the unsaponifiable fractions of vegetable oils by extraction with hot methanol or its mixture with methyl ethyl ketone followed by filtration and washing of the precipitated sterols with a suitable wash solvent (acetone or a mixture of acetone and methanol)
US5117016	T. L. Tackett, C. A. Mccombs	Method for obtaining a stigmasterol enriched product from deodorizer distillate	May, 1992	Preparation of a stigmasterol-enriched product from esterified deodorizer distillate using solvent systems containing water, a C1-C6 alcohol, and a non-polar solvent such as heptane
US5424457	C. E. Sumner Jr. S. D. Barnicki M. D. Dolfi	Process for the production of sterol and tocopherol concentrates	June, 1995	Isolation of sterol and tocopherol concentrates from a deodorizer distillate by treatment with methanol to liberate free sterols and convert the fatty acids and fatty acid esters into fatty acid methyl esters, which are stripped to give a concentrate that allows the isolation of sterols in high yield and high purity after the isolation of tocopherols by molecular distillation
US5487817	C. Fizet	Process for tocopherols and sterols from natural sources	January, 1996	Separation of tocopherols and sterols from deodorizer sludges by esterification of sterols with existing fatty acids, distillation to remove fatty acids, and subsequently to obtain tocopherols leaving the sterol esters in the residue of the distillation. Finally, the sterols are recovered after cleavage of their esters
US5502045	T. Miettinen H. Vanhanen I. Wester	Use of a stanol fatty acid ester for reducing serum cholesterol level	March, 1996	Preparation and use of β-sitostanol fatty acid ester or fatty acid ester mixture to lower cholesterol levels in serum
US5770749	J. P. Kutney E. Novak P. J. Jones	Process of isolating a phytosterol composition from pulping soap	June, 1998	Isolation of sterols by extracting a creamy precipitate from a pulping soap using a mixture of water, ketone, and hydrocarbon followed by purification
US5843499	R. A. Moreau K. B. Hicks R. J. Nicolosi R. A. Norton	Corn fiber oil its preparation and use	December, 1998	Oil extraction from corn fiber containing ferulate esters having cholesterol-lowering activity. The corn fiber oil extract can be used as a dietary supplement for cholesterol-lowering

(Table 2) Contd….

Patent Number	Inventors	Title	Publication Date	Described Process
WO056558	I. Wester, J. Ekblom	Phytosterol compositions	November, 1999	Preparation of plant sterol and stanol esters with a specified fatty acid composition
US5932562 and US20006063776	Ostlund. Jr.	Sitostanol formulation with emulsifier to reduce cholesterol absorption and method for preparing and use of same	August, 1999 and May. 2000	The composition of an aqueous based homogeneous micellar mixture of finely-divided plant sterols and lecithin, which is dried to be used in capsule or tablet form, or by adding it as liquid or dry powder to foods and beverages.
WO063841	D. J. Stewart R. K. Milanova J. Zawistowski S. H. Wallis	Compositions comprising phytosterol and/or phytostanol having enhanced solubility and dispersability	December, 1999	Composition of plant sterols and stanols treated to enhance the solubility and dispersability for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like
US20006031118	M. P. van Amerongen L. C. Lievense	Stanol ester composition and production thereof	February, 2000	Preparation of stanol fatty acid esters by hardening plant osterol fatty acid or a mixture thereof, or by esterification of plant sterols followed by hardening of the obtained plant sterol fatty acid esters
US20006087353	D. J. Stewart R. Milanova J. Zawistowski S. H. Wallis	Phytosterol compositions and use thereof in foods, beverages, pharmaceuticals, nutraceuticals and the like	July, 2000	Methods for the esterification and subsequent hydrogenation of plant sterols to provide preparations of enhanced solubility and dispersability, increased potency and enhanced stability for incorporation into foods, beverages, pharmaceuticals, nutraceuticals, etc.
WO00045648	J. Zawistowski	Method of preparing microparticles of phytosterols or phytostanols	August, 2000	Preparation of microparticles of plant sterols and stanols dispersed or suspended in a semi-fluid, fluid or viscous vehicle and exposed to impact forces
WO00052029	S.K. Noh D. W. Chung	Water-soluble sterol derivative for inhibiting cholesterol absorption and process for preparing the same	September, 2000	Preparation of water-soluble sterol derivatives, for use in water-based foodstuffs and beverages aiming to inhibit cholesterol absorption, through intermediate compounds formed by reacting plant sterols and succinic or glutaric anhydride in a non-polar solvent in the presence of a basic catalyst followed by coupling the intermediate compounds with hydrophilic polymers in a non-polar organic solvent in the presence of a basic catalyst and a coupling agent
WO00061694	D.W. Chung S. K. Noh K.S. Kim	Method for manufacturing fat-soluble phytosterol or phytostanol ester of unsaturated fatty acid	October, 2000	Preparation of fat-soluble plant sterol or stanol esters of unsaturated fatty acids by esterification of the sterols/stanols with unsaturated fatty acid in a nonpolar organic solvent, a basic catalyst, and a carboxyl group-activating agent followed by precipitation of the esterified product in methanol or a mixture of methanol and acetone.
US2000 6162483	I. Wester	Fat compositions for use in food	December, 2000	Preparation of plant sterols-fatty acid esters containing unsaturated fatty acids to reduce the absorption of both dietary and biliary cholesterol from the digestive tract and lower the blood cholesterol levels
US20016197357	C. W. Lawton R. Nicolosi S. McCarthy	Refined vegetable oils and extracts thereof	March, 2001	Refining of crude vegetable oils with a weak acid salt (e.g. a carbonate salt) to produce a soap stock and a refined vegetable oil that is further refined with a strong base to provide an unsaponifiable-rich concentrate

(Table 2) Contd….

Patent number	Inventors	Title	Publication Date	Described Process
WO01037681	T. V. Gottemoller	Phytosterol and phytostanol compositions	May, 2001	Preparation of an edible composition comprising plant sterols or stanols, a water soluble protein, and optionally an emulsifier
WO02028204	W.T. Yoon K.S. Kim B.C. Kim J.H. Han H.P. Hong	Method for dispersing plant sterol for beverage and a plant sterol-dispersed beverage, of which particle size is nanometer-scale in dispersed beverage	April, 2002	Preparation of plant sterols with particle size in the nanometer-scale for dispersion in beverages. The dispersion starts admixing the plant sterol with at least one emulsifier (selected from sucrose fatty acid ester. sorbitan fatty acid ester and polyglycerine fatty acid ester) followed by melting the admixture by heating and mixing with the aqueous beverage or an emulsifier-containing aqueous beverage
US20026410758	A. Roden J. L. Williams R. Bruce F. Detrano M. H. Boyer	Preparation of sterol and stanol-esters	June, 2002	Direct esterification of sterols/stanols with fatty acids to form food-grade stanol/sterol esters free of organic solvents and mineral acids
US20026413571	L. Liu	Sterol esters of conjugated linoleic acids and process for their production	July, 2002	Preparation of sterol/stanol esters with conjugated linoleic acid for food and nutraceutical uses
WO02060916	B. D. Flickinger, R. Grabiel, G. Poppe	Methods for producing sterol ester-rich compositions	August, 2002	Preparation of a sterol ester-enriched food ingredients using base-catalyzed tranesterification of free sterol with fatty acyl glyceride from vegetable oils in the presence of an alkali catalyst
US20026147236	J. D. Higgins III	Preparation of sterol and stanol esters	November, 2002	A method for the direct esterification of stanols and sterols with fatty acids to form stanol/sterol esters
US2003003131	M. Dyer, B. Flickinger, T. Gottemoller, B. Y. Brian	Method for manufacture of free-flowing powder containing water-dispersible sterols	January, 2003	A process for producing a free-flowing powder comprising water dispersible sterols using lecithin as emulsifier, and a spray drying adjunct for spray drying of the homogenized product
US20036589588	I. Wester, J. Ekblom	Phytosterol compositions	July, 2003	Preparation of plant sterol and stanol fatty acid esters with a specified fatty acid composition.
US20036623780	L. A. Stevens, W. N. Schmelzer	Aqueous dispersible sterol product	September, 2003	Preparation of a water dispersible sterol product for use in dietary supplements and foods particularly beverage applications
US20046677327	T. V. Gottemoller	Phytosterol and phytostanol compositions	January, 2004	Preparation of an edible composition comprising a plant sterol or stanol, an isolated water soluble protein, and optionally an emulsifier
WO05072761	A. Khare	Compositions and methods for reducing cholesterol comprising guggul and beta-glucan and/or plant sterols	August, 2005	A mixture of sterols/stanols, beta-glucan and guggul, an extract from resins of mukul myrrh tree (*Commiphora mukul*) used in the treatment of obesity and lipid disorders
US20070042102	S. J. Furcich	Methods of forming phytosterol-fortified cocoa powder and the product formed therefrom	February, 2007	Preparation of a cocoa powder fortified with plant sterols

(Table 2) Contd….

Patent Number	Inventors	Title	Publication Date	Described Process
EP1749520	N. Auriou	Water-dispersible encapsulated sterols	February, 2007	Production of water-dispersible powder containing plant sterols by encapsulation of the sterols with starch and a modified starch, which is a starch ester, and preferably a surfactant. Suitable starch esters are starch alkenyl esters especially starch alkenyl succinates
US20070141221	D. K. Nakhasi R. L. Daniels	Composition with health and nutrition promoting characteristics, containing interestified lipids and phytosterol, and related methods	June, 2007	Compositions containing medium chain triglyceride oils interesterified with long chain domestic oils in combination with plant sterols to provide total cholesterol and LDL lowering as well as adipose tissue reduction
WO07067884	D. K. Nakhasi R. L. Daniels J. W. Eartly	Phytosterol containing deep-fried foods and methods with health promoting characteristics	June, 2007	Addition of plant sterols/stanols at 5-30% level in frying oils to provide fried food products having health promoting effects
WO007137449	J. Liu S. Wu	Compositions for treating and preventing hyperlipidemia	June, 2007	Preparation of plant sterols and stanols, flavones from bamboo leaf, procyanidins, and β-glucan for the prevention and treatment of hyperlipidemia
US20070154557	Y. S. J. Veldhuizen R. T. Weisbecker	Particulate comprising phytosterols and food compositions comprising said creamer	July, 2007	Process for preparing particulate compositions comprising plant sterols for use as a creamer and/or whitener
US20070218113	D. L. Miller X. Wang J. J. Watterson P. C. Ward	Health bars and compositions for improving cardiovascular risk factors	September, 2007	Methods for making nutritional health bars comprising plant sterols, β-glucan, soy protein, and omega-3 fatty acids (EPA or DHA).
US20070231447	M. J. Fleckenstein T. V. Gottemoller S. J. O'Brien	Sterol compositions and methods of making same	October, 2007	Preparation of sterol and monoacylglycerol mixture for application in foods such as beverages
US20077285297	D. H. Waggle S. M. Potter E. C. Henley	Method of reducing low density lipoprotein cholesterol concentration	October, 2007	Composition comprising plant sterols, soy protein, and isoflavones (genistein, daidzein, glycitein, biochanin A, formononetein or their glycosides) to lower LDL cholesterol and decrease the risk of atherosclerosis
WO07112572	P. L. Orchansky J. P. Kutney	Sterols/stanols chemically linked to nitrogen releasing compounds and use thereof in treating or preventing cardiovascular disease, its underlying conditions and other disorders	October, 2007	Use of nitrate esters of sterols/stanols bearing a benzoic acid moiety as linking group for the prevention and treatment of cardiovascular disease
WO007124598	D. J. Stewart	Softgel capsules with phytosterols and/or phytostanols and optionally omega polyunsaturated fatty acids	November, 2007	Combination of esterified plant sterols/stanols and edible oils containing omega fatty acids (omega-6 and/or omega-3) in a softgel capsule

(Table 2) Contd….

Patent Number	Inventors	Title	Publication Date	Described Process
WO07137449	J. Liu W. U. Shengmin	Compositions for treating and preventing hyperlipidemia	December, 2007	Use of a combination of plant sterols/stanols (30-50%), flavones from bamboo leaves (20-40%), procyanidins (10-25%), and beta-glucan (5-20%) for the treatment of hyperlipidemia (to lower blood cholesterol and triacylglycerols)
US20087368138	C. Abbas A.M. Rammelsberg K. Beery	Extraction of phytosterols from corn fiber using green solvents	January, 2008	Use of "green" solvents (e.g. ethanol, isopropyl alcohol, or butanol and their mixtures with water, ethyl lactate, ethyl acetate, acetone, or isoamyl alcohol) to extract sterols from wet corn fiber. The resulting oil product contains free plant sterols and free fatty acids
US20080015374	D. B. Wiley, T. A. Dobbins	Method for the synthesis and isolation of phytosterol esters	January, 2008	Methods for synthesis and isolation of plant sterol esters of bland taste and light color involving the use of an alkali metal borohydride to reductively bleach tocoquinones and other chromophores in plant sterol ester transesterification reaction mixtures.
US20080015367	T. A. Dobbins D. B. Wiley D. C. Dobbins	Process for isolating phytosterols and tocopherols from deodorizer distillate	January, 2008	Recovery of pure plant sterols and tocopherols from the deodorizer distillates of the refining of edible oils after saponification with potassium hydroxide in methanol/water followed by distillation of the tocopherols
US20087335389	E. P. Lerchenfeld, D. E. Striegel	Beverages containing plant sterols	February, 2008	Preparation of a stable dispersion of hydrophobic plant sterols and an aqueous material wherein the size of the plant sterol particles ranges 0.1-30 microns with the majority of particles in the range 0.2-10 microns to enhance solubility in beverages
US20087329429	M. J. Chimel J. F. Hammerstone, J. C. Johnson, M. E. Meyers, R. M. Snyder, E. J. Eric	Bars and confectionaries containing cocoa solids having a high cocoa polyphenol content and sterol/stanol esters and processes for their preparation	Fenruary, 2008	Processes for the preparation of healthy bars of dark and white chocolate with cocoa procyanidins and sterol/stanol esters are described
US20080102111	I. Hiromichi	Anticancer composition for oral use comprising liposome containing phytosterols and prevention or treatment for cancer using the liposome	May, 2008	Use of liposomes containing plant sterols (e.g. β-sitosterol, campesterol, stigmasterol, brassicasterol, ergosterol, ergostadienol, etc.) to inhibit cancer metastasis
US20087368583	T. Czuppon Z. Kemeny E. Kovari K. Recseg	Process for recovery of plant sterols from by-product of vegetable oil refining	May, 2008	Crystallization of plant sterols from deodorization distillates from chemical or physical refining of vegetable oils by distillation and saponification of the other components present
US20080103118	K. Clement M. A. Heuer M. Thomas E. Mason	Composition for improving blood cholesterol levels	May, 2008	A composition of plant sterols. stanols or derivatives thereof plus procyanidins. policosanol and niacin or derivatives of niacin to inhibit cholesterol absorption, decrease blood LDL levels, increase blood HDL levels, and inhibit HMG-CoA reductase activity
US20087387786	N. Milstein M. Biermann P. Leidl R. Von Kries	Food additive compositions containing sterol esters, solubilising agents, dispersants and antioxidants	June, 2008	Food additive combination including plant sterols/stanols and, for example, oxides, hydroxides, and carboxylic acid salts of calcium or magnesium are described

Table 3. Relative Sterol Composition (%) of Vegetable Oils Used in the Preparation of Plant Sterols and Stanols

Sterol	Soybean Oil (*Glycine max*)	Palm Oil (*Elaeis guineensis*)	Rapeseed Oil (*Brassica napus*)	Sunflower Oil (*Helianthus annuus*)	Corn Oil (*Zea mays*)	Wheat Germ Oil (*Triticum aestivum*)	Tall Oil (*Pinus spp.*)
Brassicasterol	-	-	10	-	-	-	-
Campesterol	20	14	25	8	23	22	10
Campestanol	-	-	-	-	-	-	1
Stigmasterol	20	8	-	8	6	-	1
β-Sitosterol	53	75	58	60	66	67	78
Sitostanol	-	-	-	-	-	-	8
Δ5-avenasterol	3	2	2	4	4	6	1
Other	4	-	5	20	1	5	1

Source: Data from Kamal-Eldin [99] except for tal oil [100].

proportions of different plant sterols, the contribution of the different molecular species of 4-desmethyl sterols to cholesterol-lowering is not yet known. Nevertheless, the lack of differences between plant sterols and stanols suggest that any differences are not expected to be significant.

CURRENT & FUTURE DEVELOPMENTS

Initially, esterified plant sterols/stanols solubilized in fat-containing foods were used to provide cholesterol-lowering effects [56]. The first food products containing plant sterols/ stanols were foods of relatively high lipid content, namely margarines, spreads, mayonnaise and salad dressings. Afterwards, innovative food processing methods were developed for the preparation of different forms of plant sterols/stanols for incorporation of lipid-rich as well as aqueous food products. Incorporation of plant sterols/stanols, at about 9% level, in margarines was eased by esterification that increases their lipid solubility [100]. Subsequently, plant sterol/stanol esters incorporated in a new range of low-fat food products, including bread and cereals, low-fat milk, and low-fat yogurt, were found to be as effective in lowering serum cholesterol as when incorporated in margarines [101]. It was found that non-esterified plant sterols/stanols can also be effective as part of low-nonfat foods, e.g. juices and yogurts, provided that they are adequately solubilized either as fine powder or in emulsified forms as documented in a number of patents Table **2**. Beverages also containing the plant sterol is reported by Lerchenfeld *et al.* in US20087335389 patent [102]. Bars and confectioneries incorporating high content of plant sterol or stanol esters described by Chimel *et al.* in US20087329429 patent [103]. In the future, further development of innovative products incorporating esterified and non-esterified plant sterols/ stanols and processes for producing them and search for synergistic functional ingredients are expected.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFRENCES

[1] Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi A-M. Plant Sterols: biosynthesis. Biological function and their importance to human nutrition. J Sci Food Agric 2000; 80(7): 939-966.

[2] Pollak OJ. Successful prevention of experimental hypercholes-terolemia and cholesterol atherosclerosis in the rabbit. Circulation 1953; 7(5): 696-701.

[3] Pollak OJ. reduction of blood cholesterol in man. Circulation 1953; 7(5): 702-706.

[4] Best MM, Duncan CH, Van Loon EJ, Wathen JD. Lowering of serum cholesterol by the administration of a plant sterol. Circulation 1954; 10(8): 201-206.

[5] Miettinen TA, Gylling H. Regulation of cholesterol metabolism by dietary plant sterols. Curr Opin Lipidol 1999; 10(1): 9-14.

[6] Yanni AE. Novel plant sterol and stanol derivatives with beneficial properties: Efficacy and safety. Recent Patents Endocr Metabol Immune Drug Discov 2008; 2(1): 16-23 .

[7] Calpe-Berdiel L, Escolà-Gil JC, Blanco-Vaca F. New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism. Atherosclerosis 2008, Epub ahead of print.

[8] Moreau R, Whitaker B, Hicks K. Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res 2002; 41(6): 457-500.

[9] Ostlund RE Jr. Phytosterols in human nutrition. Ann Rev Nutr 2002; 22(4): 533–549.

[10] Phillips KM, Ruggio DM, Toivo JI, Swank MA. Simpkins AH. Free and esterified sterol composition of edible oils and fats. J Food Compost Anal 2002; 15(2): 123-142.

[11] Ikeda I, Nakashima-Yoshida K, Sugano M. Effects of Cycloartenol on absorption and serum levels of cholesterol in rats. J Nutr Sci Vitaminol 1985; 31(3): 375-384.

[12] Kritchevsky D, Tepper SA, Czarnecki SK, Kyle DJ. Effects of 4-methylsterols from algae and of β-sitosterol on cholesterol metabolism in rats. Nutr Res 1999; 19(11): 1649-1654.

[13] Vissers MN, Zock PL, Meijer GW, Katan MB. Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in humans. Am J Clin Nutr 2000; 72(6): 1510-1515.

[14] Kucchodkar BJ, Horlick L, Sodhi HS. Effect of plant sterols on cholesterol metabolism. Atherosclerosis 1976; 23: 239-248.

[15] Lees AM, Mok HYI, Lees RS, McCluskey MA, Grundy SM. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 1977; 28(3): 325-338.

[16] Mattson FH, Grundy SM, Crouse JR. Optimizing the Effect of plant sterols on cholesterol absorption in man. Am J Clin Nutr 1982; 35(4): 697-700.

[17] Pollack OJ. Effect of Plant sterols on lipids and atherosclerosis. Pharmac Ther 1985; 31(3): 177–208.

[18] Miettinen TA, Vanhanen H. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein e phenotypes. Atherosclerosis 1994; 105(2): 217-226.

[19] Jones PJ, Howell T, MacDougall DE, Feng JY, Parsons W. Short-term administration of tall oil phytosterols improves plasma lipid profiles in subjects with different cholesterol levels. Metabolism 1998; 47(6): 751-756.

[20] Weststrate JA, Meijer GW. Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1998; 52(5): 334–343.

[21] Hendriks HF, Weststrate JA, van Vliet T, Meijer GW. 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 1999; 53(4): 319-327.

[22] Sierksma A, Weststrate JA, Meijer GW. Spreads enriched with plant sterols, either esterified 4,4-dimethylsterols or free 4-desmethylsterols, and plasma total- and LDL-cholesterol concentrations. Br J Nutr 1999; 82(4): 273-282.

[23] Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uusitupa MI. Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur J Clin Nutr 2000; 54(9): 715-725.

[24] Vissers MN, Zock PL, Meijer GW, Katan MB. Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in humans. Am J Clin Nutr 2000; 72(6): 1510-1515.

[25] Plat J, van Onselen EN, van Heugten MM, Mensink RP. Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters. Eur J Clin Nutr 2000; 54(9): 671-677.

[26] Christiansen LI, Lähteenmäki PL, Mannelin MR, Seppänen-Laakso TE, Hiltunen RV, Yliruusi JK. Cholesterol-lowering effect of spreads enriched with microcrystalline plant sterols in hypercholesterolemic subjects. Eur J Nutr 2001; 40(2): 66-73.

[27] Davidson MH, Maki KC, Umporowics DM, *et al*. Safety and tolerability of esterifed phytosterols administered in reduced-fat spread and salad dressing to healthy adult men and women. J Am Coll Nutr 2001; 20(4): 307-319.

[28] Maki KC, Davidson MH, Umporowicz DM, *et al*. Lipid responses to plant-sterol-enriched reduced-fat spreads incorporated into a national cholesterol education program step I diet. Am J Clin Nutr 2001; 74(1): 33–43.

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

[30] Nestel P, Cehun M, Pomeroy S, Abbey M, Weldon G. Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods. Eur J Clin Nutr 2001; 55(12): 1084-1090.

[31] Nigon F, Serfaty-Lacrosnière C, Beucler I, *et al*. Plant sterol-enriched margarine lowers plasma LDL in hyperlipidemic subjects with low cholesterol intake: Effect of fibrate treatment. Clin Chem Lab Med 2001; 39(7): 634-640.

[32] Tikkanen MJ, Högström P, Tuomilehto J, Keinänen-Kiukaanniemi S, Sundvall J, Karppanen H. Effect of a diet based on low-fat foods enriched with non-esterified plant sterols and mineral nutrients on serum cholesterol. Am J Cardiol 2001; 88(10): 1157-1162.

[33] Volpe R, Niittynen L, Korpela R, *et al*. Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hypercholesterolaemia. Br J Nutr 2001; 86(2): 233-239.

[34] Matvienko OA, Lewis DS, Swanson M, *et al*. A single daily dose of soybean phytosterols in ground beef decreases serum total cholesterol and LDL cholesterol in young, mildly hypercholesterolemic men. Am J Clin Nutr 2002; 76(1): 57-64.

[35] Mussner MJ, Parhofer KG, von Bergmann K, Schwandt P, Broedl U, Otto C. Effects of phytosterol ester-enriched margarine on plasma lipoproteins in mild to moderate hypercholesterolemia are related to basal cholesterol and fat intake. Metabolism 2002; 51(2): 189-194.

[36] Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Jones PJ. No changes in serum fat-soluble vitamin and carotenoid concentrations with the intake of plant sterol/stanol esters in the context of a controlled diet. Metabolism 2002; 51: 652-656.

[37] Noakes M, Clifton P, Ntanios F, Shrapnel W, Record I, McInerney J. An increase in dietary carotenoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations. Am J Clin Nutr 2002; 75(1): 79-86.

[38] Vanstone CA, Raeini-Sarjaz M, Parsons WE, Jones PJ. Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. Am J Clin Nutr 2002; 76(6): 1272-1278.

[39] Vanhanen HT, Blomqvist S, Ehnholm C, *et al*. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different ApoE phenotypes during dietary sitostanol ester treatment. J Lipid Res 1993; 34(9): 1535-1544.

[40] Ntanios FY, Homma Y, Ushiro S. A A spread enriched with plant sterol esters lowers plasma cholesterol and lipoproteins without affecting vitamins A and E in normal and hypercholesterolemic Japanese men and women, J Nutr 2002, 132: 3650-3655.

[41] Gylling H, Miettinen TA. Serum cholesterol and cholesterol and lipoprotein metabolism in hypercholesterolaemic NIDDM patients before and during sitostanol ester-margarine treatment. Diabetologia 1994; 37(8): 773-780.

[42] Vanhanen HT, Kajander J, Lehtovirta H, Miettinen TA. Serum levels, absorption efficiency, faecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolaemic subjects. Clin Sci (London) 1994; 87(1): 61–67.

[43] Miettinen TA, Puska P, Gylling H, Vanhanen HT, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 1995; 333(20): 1308-1312.

[44] Gylling H, Radhakrishnan R, Miettinen TA. Reduction of serum cholesterol in post-menopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine. Circulation 1997; 96(12): 4226-4231.

[45] Niinikoski H, Viikari J, Palmu T. Cholesterol-lowering effect and sensory properties of sitostanol ester margarine in normocholesterolemic adults. Scand J Nutr 1997; 41(1): 9-12.

[46] Andersson A, Karlström B, Mohsen R, Vessby B. Cholesterol-lowering effects of a stanol ester-containing low-fat margarine used in conjunction with a strict lipid-lowering diet. Eur Heart J 1999; 1(suppl.): S80-S90.

[47] Gylling H, Miettinen TA. Cholesterol reduction by different plant stanol mixtures and with variable fat intake. Metabolism 1999; 48(5): 575–580.

[48] Hallikainen MA, Uusitupa MI. Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am J Clin Nutr 1999; 69(3): 403-410.

[49] Jones PJ, Ntanios FY, Raeini-Sarjaz M, Vanstone CA. Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am J Clin Nutr 1999; 69(6): 1144-1150.

[50] Nguyen TT, Dale LC, von Bergmann K, Croghan IT. Cholesterol-lowering effect of stanol ester in a us population of mildly hypercholesterolemic men and women: A randomized controlled trial. Mayo Clin Proc 1999; 74(12): 1198-1206.

[51] Blair SN, Capuzzi DM, Gottlieb SO, Nguyen T, Morgan JM, Cater NB. Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am J Cardiol 2000; 86(1): 46-52.

[52] Homma Y, Ishikawa T, Tateno M, Mitaniyama A, Sugano M. Cholesterol and apolipoprotein-lowering effect of plant stanol ester in healthy Japanese men and women. J Jpn Soc Nutr Food Sci 2000; 53(2): 155-162.

[53] Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res 2000; 41(5): 697-705.

[54] Plat J, Mensink RP. Vegetable oil-based versus wood-based stanol ester mixtures: effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. Atherosclerosis 2000; 148(1): 101-112.

[55] Mensink RP, Ebbing S, Lindhout M, Plat J, van Heugten MM. Effects of plant stanol esters supplied in a low-fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations. Atherosclerosis 2002; 160(1): 205-213.

[56] Katan MB , Grundy SM, Jones P, Law M, Mittinen T, Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clinic Proceedings 2003; 78(8): 965-978.

[57] Berger A, Jones PJH, Abumweis SS. Plant sterols: Factors affecting their efficacy and safety as functional food ingredients. Lipids Health Dis 2004; 3: 5.

[58] Taylor CL. FDA letter regarding enforcement discretion with respect to expanded use of an interim final rule about sterol/stanol esters and reduced risk of coronary heart disease. Available at: http://www.cfsan.fda.gov/~dms/ds-ltr30.html Accessed June 17, 2008.

[59] FAD, A food labeling guide-Appendix A: Definition of nutrient content claims. Available at: http://www.cfsan.fda.gov/~dms/flg-6a.html Accessed June 17, 2008.

[60] EU, Scientific committee on food. Opinion on Applications for approval of a variety of plant sterol-enriched foods. 2003; Available at: http://europa.eu.int/comm/food/fs/sc/scf/out174_en.pdf.Accessed June 17, 2008.

[61] Lichtenstein AH, Appel LJ, Brands M, *et al*. Diet and lifestyle recommendations revision 2006: A scientific statement from the american heart association nutrition committee. Circulation 2006; 114(1): 82-96.

[62] FDA, FDA authorizes new coronary heart disease health claim for plant sterol and plant stanol esters. FDA Talk Paper. Available at: http://www.cfsan.fda.gov/~lrd/tpsterol.html Accessed June 17, 2008.

[63] FDA, A food labeling guide-appendix A: Definition of Nutrient content claims. Available at: http://www.cfsan.fda.gov/~dms/flg-6a.html. Accessed June 17, 2008.

[64] Plat J, Mensink RP. Plant stanol and sterol esters in the control of blood cholesterol levels: Mechanism and safety aspects. Am J Cardiol 2005; 96(1A): 15D-22D.

[65] Doornbos AME, Meynen EM, Duchateau GSMJE, van der Knaap HCM, Trautwein EA. Intake occasion affects the serum cholesterol lowering of a plant sterol-enriched single-dose yoghurt drink in mildly hypercholesterolemic subjects. Eur J Clin Nutr 2006; 60(10): 325-333.

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

[67] Ostlund RE, Racette SB, Stenson WF. Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. Am J Clin Nutr 2003; 77(6): 1385–1389.

[68] Jenkins DJ, Kendall CW, Faulkner D, *et al*. A dietary portfolio approach to cholesterol reduction: combined effects of plant sterols, vegetable proteins, and viscous fibers in hypercholesterolemia. Metabolism 2002; 51(12): 1596-1604.

[69] Jenkins DJ, Kendall CW, Faulkner DA, *et al*. Long-term effects of a plant-based dietary portfolio of cholesterol-lowering foods on blood pressure. Eur J Clin Nutr 2008; 62(6): 781-788.

[70] Jenkins DJ, Kendall CW, Nguyen TH, *et al*. Effect of plant sterols in combination with other cholesterol-lowering foods. Metabolism 2008; 57(1): 130-139.

[71] Jenkins DJ, Josse AR, Wong JM, Nguyen TH, Kendall CW. The portfolio diet for cardiovascular risk reduction. Curr Atherosclerosis Rep 2007; 9(6): 501-507.

[72] Milstein, N., Biermann, M., Leidl, P., Von Kries, R.: US20087387786 (**2008**).

[73] Mel'nikov SM, Seijen ten Hoorn JW, Bertrand B. Can cholesterol absorption be reduced by phytosterols ans phytostanols via a cocrystallization mechanism? Chem Phys Lipids 2004; 127: 15-33.

[74] Calpe-Berdiel L, Escolà-Gil JC, Blanco-Vaca F. New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism. Atherosclerosis 2008; doi:10.1016 (online).

[75] Altmann SW, Davis Jr HR, Zhu LJ, *et al*. Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. Science 2004; 303: 1201-1204.

[76] Yu L, Li-Hawkins J, Hammer RE, *et al*. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J Clin Invest 2002; 110: 671-680.

[77] Heinmann T, Axtmann G, von Bergmann K. Comparison of intestinal absorption of cholesterol with different plant sterols in man. Eur J Clin Invest 1993; 23(12): 827-831.

[78] Berge KEH, Tian GA, Graf L, *et al*. Accumulation of Dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000; 290(5497): 1771-1775.

[79] Lu KMH, Lee S, Hazard A, *et al*. Two genes that map to the STSL locus cause sitosterolemia: Genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8, respectively. Am J Hum Genet 2001; 69(2): 278–290.

[80] Plösch T, Kruit JK, Bloks VW, *et al*. Reduction of Cholesterol absorption by dietary plant sterols and stanols in mice is independent of the Abcg5/8 transporter. J Nutr 2006; 136(8): 2135-2140.

[81] Field FJ, Born E, Mathur SN. Stanol esters decrease plasma cholesterol independently of intestinal ABC sterol transporters and niemann-pick C1-like 1 protein gene expression. J Lipid Res 2004; 45(12): 2252-2259.

[82] Clement, K., Heuer, M.A., Thomas, M., Mason, E.: WO08049196 (**2008**).

[83] Lee SH, Park YB, Bae KH, *et al*. Cholesterol-lowering activity of naringenin via inhibition of 3-hydroxy-3-methyl-glutaryl coenzyme a reductase and acyl coenzyme A: Cholesterol acyltransferase in rats. Ann Nutr Metab 1999; 43(3): 173-180.

[84] Moazzami A, Frank J, Lundh T, Vessby B, Kamal-Eldin A. Phytosterols inhibit the lipid modulating effects of sesamin in rats. Curr Topics Nutraceut Res 2007; 5(2/3): 93–98.

[85] Kosoglou, T., Davis, H.R., Picard, G.J.B.: EP1911462 (**2008**).

[86] Takeshita M, Saito S, Moriwaki J, *et al*. Effects of dietary diacylglycerol oil containing phytosterols in mayonnaise on abdominal fat and blood cholesterol levels in japanese men. Jpn Pharmacol Therapeut 2007; 35(9): 973-987.

[87] Meguro S, Higashi K, Hase T, *et al*. Solubilization of phytosterols in diacylglycerol versus triacylglycerol improves the serum cholesterol-lowering effect. Eur J Clin Nutr 2001; 55: 513-517.

[88] Korpela R, Tuomilehto J, Högström P, *et al*. Safety aspects and cholesterol-lowering efficacy of low fat dairy products containing plant sterols. Eur J Clin Nutr 2006; 60(5): 633-642.

[89] Tuomilehto J, Tikkanen MJ, Högström P, *et al*. Safety assessment of common foods enriched with natural nonesterified plant sterols. Eur J Clin Nutr 2008; Epub ahead of print.

[90] Rulis AM. GRAS notice no. GRN 000112. 2003. Available at: http://www.cfsan.fda.gov/~rdb/opa-g112.html. Accessed on June 17, 2008.

[91] Bhattacharyya AK, Connor WE. β-Sitosterolemia and xanthomatosis. J Clin Invest 1974; 53: 1033-1043.

[92] Gluek CJ, Speirs J, Tracy T, Streicher P, Illig E, Vandegrift J. Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol, and premature coronary heart disease in hypercholesterolemic probands and their first-degree relatives. Metabolism 1991; 40: 842-848.

[93] Sudhop T, Gottwald BM, von Bergmann K. Serum plant sterols as a potential risk factor for coronary heart disease. Metabolism 2002; 51: 1519-1521.

[94] Assmann G, Cullen P, Erbey J, Ramey DR, Kannenberg F, Schulte H. Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: Results of a nested case-control analysis of the Prospective Cardiovascular Munster (PROCAM) study. Nutr Metab Cardiovasc Dis 2006; 16: 13-21.

[95] Weingärtner O, Lüjohann D, Ji S, *et al*. Vascular effects of diet supplentation with plant sterols. J Am Coll Cardiol 2008; 51(16): 1553-1561.

[96] Quilez J, Garcia-Lorda P, Salas-Salvado J. Potential uses and benefits of phytosterols in diet: Present situation and future directions. Clin Nutr 2003; 22(4): 343-351.

[97] Fernandes P, Cabral JMS. Phytosterols: Application and recovery methods. Bioresource Technol 2007; 98(12): 2335-2350.
[98] Czuppon, T., Kemeny, Z., Kovari, E., Recseg, K.: US20087368583 (**2008**).
[99] Kamal-Eldin A. Minor components in vegetable oils., In: Baileys Industrial Fats and Oils, Volume 3, Chapter 12. Edible Oil and Fat Products: Specialty Oils and Oil Products, Fereidoon Shahidi (Editor), John Wiley & Sons, Sussex, UK 2005; 319-359.
[100] Clifton PM, Mano M, Duchateau GSMJE, van der Knaap HCM, Trautwein EA. Dose-response effects of different plant sterol sources in fat spreads on serum lipids and C-reactive protein and on the kinetic behavior of serum plant sterols. Eur J Clin Nutr 2007; ahead of print.
[101] Clifton PM, Noakes M, Sullivan D, *et al*. Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. Eur J Clin Nutr 2004; 58(3): 503-509.
[102] Lerchenfeld, E.P., Striegel, D.E.: US20087335389 (**2008**).
[103] Chimel, M.J., Hammerstone, J.F.Jr., Johnson, J.C., Myers, M.E., Snyder, R.M., Whitacre, E.J.: US20087329429 (**2008**).