PHYTOSTEROLS, PHYTOSTANOLS AND THEIR ESTERS
Chemical and Technical Assessment

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1. Summary

Phytosterols and phytostanols, also referred to as plant sterols and stanols, are common plant and vegetable constituents and are therefore normal constituents of the human diet. They are structurally related to cholesterol, but differ from cholesterol in the structure of the side chain.

Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil or corn oil, or from so-called "tall oil", a by-product of the manufacture of wood pulp. These sterols can be hydrogenated to obtain phytostanols. Both phytosterols- and stanols, which are high melting powders, can be esterified with fatty acids of vegetable (oil) origin. The resulting esters are liquid or semi-liquid materials, having comparable chemical and physical properties to edible fats and oils, enabling supplementation of various processed foods with phytosterol- and phytostanol esters.

The most common phytosterols and phytostanols are sitosterol (3β-stigmast-5-en-3ol; CAS Number 83-46-5), sitostanol (3β,5α-stigmastan-3-ol; CAS Number 83-45-4), campesterol (3β-ergost-5-en-3-ol; CAS Number 474-62-4), campestanol (3β,5α-ergostan-3-ol; CAS Number 474-60-2), stigmasterol (3β-stigmasta-5,22-dien-3-ol; CAS Number 83-48-7) and brassicasterol (3β-ergosta-5,22-dien-3-ol; CAS Number 474-67-9). Each commercial source has its own typical composition.

Dietary intake of phytosterols ranges from 150-400 mg /day in a typical western diet. Phytosterols and phytostanols, in free or esterified form, are added to foods for their properties to reduce absorption of cholesterol in the gut and thereby lower blood cholesterol levels. It is now generally accepted that sterols and stanols have the same cholesterol lowering efficacy.

The daily doses, considered optimal for the purpose of lowering blood cholesterol levels, are 2-3 g of phytostanols and/or phytosterols, which translates to 3.4-5.2 g in esterified form. This recommended daily dose is typically divided in 1-3 portions of food providing 1.7-5.2 g ester, which equals 1-3 g phytostanol and/or phytosterol equivalents.

Phytosterols, phytostanols and their esters have not been evaluated previously by the Committee. However, these substances have been evaluated and approved for use in foods in a number of countries world-wide (the European Union, Australia, Switzerland, Norway, Iceland, Brazil, South Africa, Japan, Turkey and Israel). Furthermore, in the USA a 'self-GRAS' (GRAS = Generally Recognized As Safe) procedure has been followed for both phytosterols- and phytostanols, to which the US FDA raised no objections.

2. Description

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 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, whereas this bond is saturated in phytostanols. (Figure 1).

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[1] This document is based primarily on a draft CTAs and other information provided by the following sponsors: Raisio Nutrition Ltd, Raisio, Finland; Bioresco Ltd., Basel , Switzerland, on behalf of Forbes Medi-Tech Inc., Vancouver, BC, Canada; Unilever UK, London, United Kingdom.

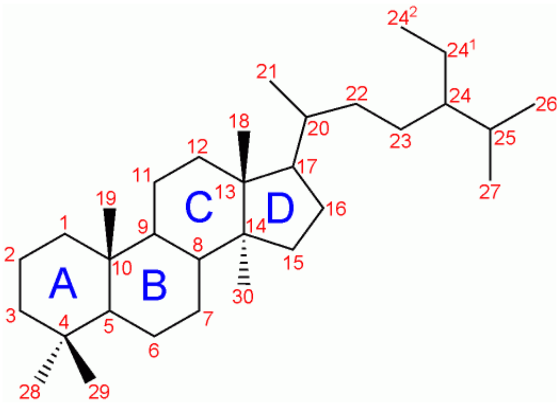

Figure 1. Steroid skeleton

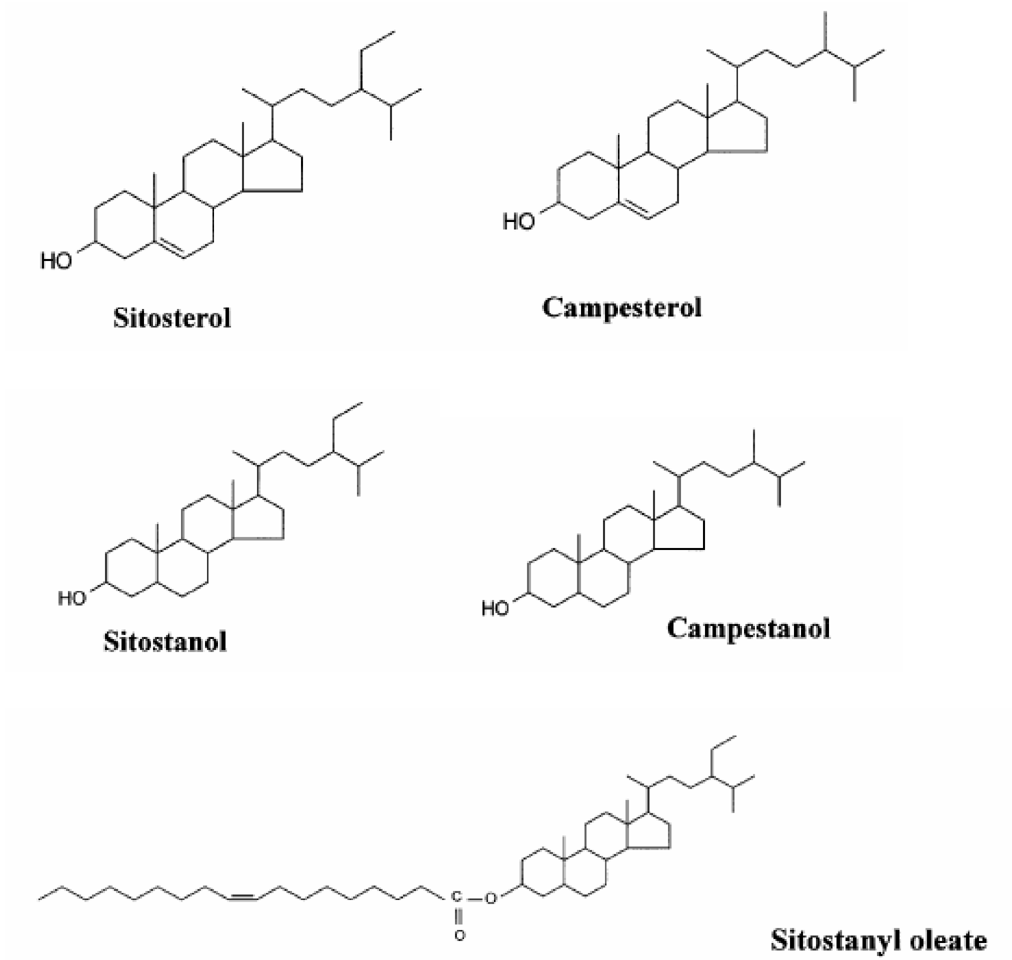

Figure 2. Molecular structure of some phytosterols, phytostanols and a fatty acid ester.

The most common phytosterols and phytostanols (examples of structures are shown in Figure 2) are sitosterol (3β-stigmast-5-en-3ol; CAS Number 83-46-5), sitostanol (3β,5α-stigmastan-3-ol; CAS Number 83-45-4), campesterol (3β-Ergost-5-en-3-ol; CAS Number 474-62-4), campestanol (3β,5α-ergostan-3-ol; CAS Number 474-60-2), stigmasterol (3β-stigmasta-5,22-dien-3-ol; CAS Number 83-48-

7) and brassicasterol (3β-ergosta-5,22-dien-3-ol; CAS Number 474-67-9). Each commercial source has its typical phytosterols composition (see further section 4, Table 2).

Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil or corn oil, or from so-called "tall oil", a by-product of the manufacture of wood pulp. Phytosterols can be hydrogenated to obtain phytostanols. Phytosterols and phytostanols are high melting powders. Phytostanol and phytosterol esters are chemically stable materials, having comparable chemical and physical properties to edible fats and oils. The substances are insoluble in water, but soluble in non-polar solvents, such as hexane, iso-octane and 2-propanol. The esters are also soluble in vegetable fats and oils.

Three separate dossiers on different commercially available materials were submitted to the committee for the assessment of the phytosterols, phytostanols and their esters.

a. Phytosterols, phytostanols and ester mixtures thereof, derived from vegetable oil distillates

b. Unesterified phytosterol and phytostanol mixtures derived from tall oil. Main constituents were sitosterol (40-65%), sitostanol (16-31%), campesterol (6-15%) and campestanol (2.5-11%).

c. Phytostanol ester mixtures derived from either tall oil (stanol composition: about 94% sitostanol and about 6% campestanol), or vegetable oil (stanol composition: about 68% sitostanol and about 32% campestanol).

3. Manufacturing

3.1 Production of sterols from vegetable oil distillates

Edible vegetable oils, extracted from oil seeds, are typically refined to remove minor oil components like phosphatides, free fatty acids, pigments and odours, with the least possible damage to the glycerides and with minimal loss of oil. The conventional or caustic refining procedure comprises degumming, neutralization, bleaching and deodorization. In physical refining, the neutralization step is omitted and the residual free fatty acids are removed in the final deodorization step.

Deodorization is the last step in the edible oil refining process in which volatiles are removed, that can cause deterioration of the oil quality during use in products (flavour, odour, colour and taste stability). This process relies on the large volatility differences between the oil itself (triglycerides) and the volatile compounds to be removed and is carried out under reduced pressure, an elevated temperature in the presence of a stripping gas. The volatiles are recovered in a vapor condenser. With caustic refining the yield of volatiles distillate is approximately 0.3 - 0.4% on the processed oil volume. This distillate mainly contains free fatty acids, but also significant levels of tocopherols (5-15%) and phytosterols (8-20%).

In a transesterification (methanolysis) step, the glycerides are converted into fatty acid methyl esters and glycerol and the phytosterol-esters into free phytosterols and fatty acid methyl esters. After removal of the methanol/glycerol phase, the methyl esters are removed and the free phytosterols and tocopherols removed by distillation. The phytosterols are separated from the tocopherols by solvent crystallization and filtration using food grade solvent. The phytosterols are further purified by re-crystallisation, mainly to remove wax-esters.

3.2 Production of sterols from wood pulp/tall oil

Commercially grown coniferous trees (*Pinus sp.*) are the usual source of wood that is chemically digested in the so-called Kraft pulping process. In this alkaline process the wood chips are digested at pH 14 (hence the term "soap") for about 18 hours at 50°C to free the wood fibers. The soapy material (black liquor pulping soap) is then separated from the cellulose pulp.

The soapy lipid phase which is obtained contains more than 2% phytosterols. One way of recovering these sterols is via solvent (methanol) extraction directly from the soap, after which the phytosterols are purified by precipitation from the solvent. More commonly the tall oil soap is acidified to produce an oily phase which is a mixture of free rosin and fatty acids and neutral components, most importantly consisting of sterols, fatty alcohols, squalene, waxes and other esters. This mixture is referred to as crude tall oil.

Crude tall oil is refined into different fractions (e.g. rosin acids, fatty acids) by distillation, where the phytosterols are concentrated, mostly as phytosterol esters, in the residue. This is known as tall oil pitch and serves as the raw material for the production of tall oil phytosterols. The concentration of phytosterols in tall oil pitch is in the range of 5-15%.

Pure phytosterols are obtained from the tall oil pitch, mainly containing high boiling fatty acid esters, resin acids and the phytosterols. The tall oil pitch is saponified 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. The residual pitch is distilled in a number of steps to recover the phytosterol fraction. This fraction is finally purified via solvent re-crystallization using food-grade solvents.

3.3 Production of phytostanols from phytosterols

Starting with the unsaturated phytosterols from any of the processes described above, pure saturated phytostanols can be obtained by hydrogenation. In this process the double-bond in the sterol molecule (see figure 1) 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).

Phytostanols thus produced mainly consist of sitostanol and campestanol. Phytostanols produced from tall oil sterols typically contain ~ 90% sitostanol and ~ 10% campestanol, whereas a blend of stanols obtained from vegetable oils, typically from soybean oil, contains 68 – 75% sitostanol and 25 - 32% campestanol.

It should be noted that stanols are also naturally-occurring. Especially in tall oil phytosterols, the level of phytostanols can be as high as 15%.

3.4 Production of phytosterol and phytostanol esters

Phytostanol and phytosterol esters are produced via esterification of plant stanols or sterols with fatty acids from common vegetable oils. Thus, the fatty acid composition of the esters is similar to the parent vegetable oil used as a source of the fatty acids.

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. The proportion of the phytosterol backbone is approximately 60 % by weight of the ester and that of the fatty acid tail approximately 40 % by weight.

The phytosterols and phytostanols can be esterified with fatty acids from vegetable oils by two different routes:

- Direct esterification using free fatty acids;
- Trans-esterification using fatty acid methylesters.

3.4.1 Free fatty acid route

This process consists of two consecutive steps:

i) preparation of free fatty acids and;
ii) esterification of free fatty acids and phytosterols/phytostanols.

The first step comprises hydrolysis of edible vegetable oil, e.g. sunflower oil triacylglycerides to form free fatty acids. After separation of the glycerol formed, the free fatty acids are purified from the unsaponifiable fraction and residual partial glycerides by distillation. In the second step the free fatty acids and phytosterols/phytostanols are esterified to form phytosterol or phytostanol esters in a process similar to the conventional manufacture of mono-acylglycerides. This reaction is carried out at elevated temperature (>180°C) using a food grade catalyst. The reaction is carefully controlled with respect to reaction temperature and time. After the esterification reaction the excess of free fatty acids is removed by distillation.

3.4.2 Methylester route

In this process two similar steps are involved:
i) preparation of fatty acid methyl esters and;
ii) inter-esterification of the fatty acid methyl esters and the phytosterols/phytostanols.

The first step comprises the methanolysis of edible vegetable oils to fatty acid methylesters and glycerol. In the second step these fatty methylesters are interesterified with the phytosterols and/or phytostanols by a similar process as used for the conventional chemical interesterification of fat blends. Also here the final purification involves deodorization to remove the excess methylesters and produce bland tasting and stable esters.

3.5 Commercial suppliers

Depending on the manufacturer, the commercial product may be a mixture of the extracted sterols, a mixture of free sterols and stanols, sterol and stanol esters or stanol esters. A list of commercial suppliers is given in Table 1.

Manufacturer	**Brand name**	**Source[1]**
Raisio Plc.	Benecol ®	TO & VO stanol esters
Cognis	Vegapure ®	TO & VO sterol esters
Archer Daniels Midland Co.	CardioAid TM	VO sterols & esters
Cargill Inc.	Corowise TM	VO sterols
Triple Crown	Prolocol TM	TO sterols
Pharmaconsult Oy Ltd	Multibene ®	TO & VO sterols/esters
Teriaka Ltd.	Diminicol TM	TO & VO sterols
Forbes Medi-Tech Inc.	Reducol TM	TO sterols & stanols
Arboris	AS-2 TM	TO sterols
PrimaPharm B.V.	Beta sitosterol	TO sterols
Fenchem Enterprises Ltd.	Cholevel TM	VO sterols
DRT	Phytopin ®	TO sterols
DDO Processing LLC	Nutraphyl TM	TO sterols
Degussa Food Ingr. GmbH	Cholestatin TM	VO sterols
Phyto-Source LP	Phyto-S-Sterol TM	TO sterols
Lipofoods	Lipophytol TM	VO sterols
Enzymotech Ltd.	CardiaBeat TM	TO & VO sterols

Table 1. Commercial suppliers of phytosterols, phytostanols and/or their esters; [1)] TO: tall oil; VO: vegetable oil.

4. Chemical Characterization

4.1 Composition and properties

The physical characteristics and composition of different commercial phytosterols, phytostanols and their esters are summarized in Table 2.

Phytostanol and phytosterol esters are chemically stable, fat-type materials, having comparable chemical and physical properties to edible fats and oils. The product is insoluble in water, but 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 esters is comparable to or even better than that of the parent vegetable oil or oil blend from which the fatty acids were derived. During shelf-life studies (long term storage), as pure material or in a product, phytostanol- and 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 phytosterol and phytostanol moieties are very stable at ambient temperatures. At higher temperatures some oxidation may occur. For this topic see section 7.

	Tall oil sterols / stanols	**Vegetable oil Sterols**	**Sterol- and stanol esters**
Colour	White to off-white		Whitish solid, pale yellow liquid
Appearance	Crystalline waxy powder or prills		Solid or liquid
Solubility	<0.01 g in 100 g water; 2.5% in fat at ambient temperature; soluble in acetone, ethyl acetate, isopropanol		Insoluble in water; soluble in oils and fats; soluble in non-polar solvents
Melting point	138 - 158 ºC		-25.7 - 38.8 ºC
Sitosterol (%)	36 - 80	43 - 52	
Sitostanol (%)	6 - 34	0 - 3	90[1] / 68 – 75[2]
Campesterol (%)	4 - 25	24-28	
Campestanol (%)	0 – 14	0 - 2	10[1] / 25 - 32[2]
Stigmasterol (%)	< 1	14 - 24	
Brassicasterol (%)	< 1	0 - 9	
Minor sterols (%)	0 - 2	< 3	<5[3]
Solvents	MeOH <0.2%	MeOH 40 mg/kg; Ethanol 1.0 g/kg; Hexane 15 mg/kg	
Water (%)		<1	<0.1
Ash, residue on ignition (%)	<0.1		
Mercury (mg/kg)		<0.1	
Lead (mg/kg)	<0.1	<0.1	<0.1
Cadmium (mg/kg)		<0.1	
Arsenic (mg/kg)		<0.1	<0.1
Total heavy metals (mg/kg)		<20	
PAHs (ppb)	< 2		
Dioxins and Dioxin-like PCB’s (ng-TEQ/kg)	< 1.5		
Pesticides (ppb)	absent		

Table 2: Physical characteristics and composition of different commercial phytosterols, phytostanols and their esters; [1)] from TO sterols; [2)] from VO sterols; [3)] mainly sitosterol and campesterol.

4.2 Quality of phytosterols, phytostanols and their esters

Phytosterols, phytostanols and their fatty acid esters should not contain contaminants or other impurities in concentrations that may prevent or limit their use in food products. Major contaminants to analyze or monitor in the final ingredients are:

- heavy metals (Cd, Pb, Hg, As)
- pesticides
- dioxins/furans/PCBs
- polycyclic aromatic hydrocarbons (PAHs)

Analysis of total phytostanol/phytosterol and ester content is generally measured on both the pure ingredients as well as the final food products. If required the level of free phytosterols and phytostanols can be separately analyzed. The product specification of the esters may set a minimum value for esterification and limit the free stanol/sterol content.

The conventional fats and oils analyses of water content, free fatty acids and peroxide value are generally performed to ensure good stability of the phytosterol, phytostanols and their esters during storage in pure form as well as in the supplemented foods.

4.3 Analytical methods

Quantitative determination of phytosterols and phytostanols as their trimethylsilyl (TMS) derivatives in the presence of an internal standard is carried out by capillary gas chromatography (GC) equipped with a flame ionization detector (FID). In addition to quantitative amounts of stanols/sterols in the sample, the GC analysis provides information also on the distribution of individual sterol components and possible sterol degradation products. So far, there are no official reference methods developed particularly for the analysis of phytosterols or phytostanols present in sterol-enriched foodstuffs or ingredients. Some international reference methods exist for the analysis of sterols as natural minor food components (sterol content 1 % or less), whereas the sterol/stanol concentrations in phytostanol ester ingredients or enriched functional food products may be as high as 8%.

A number of methods are available for the analysis of naturally occurring or added phytosterols/ phytostanols in foods. Most methods are based on hot saponification (ISO 12228 or similar standard methods). In principle, the sample is saponified in the presence of an internal standard with potassium hydroxide in ethanol to break the ester bonds. The unsaponifiable material containing phytosterols/phytostanols is extracted with an organic solvent and evaporated to dryness under a stream of nitrogen gas. Sterols/stanols are silylated and analyzed by GC-FID. A GC/FID method commonly used is based on the AOAC Official Method 994.10 for "Cholesterol in Foods". This method has been developed and validated for determining sitosterol, campesterol and stigmasterol in saw palmetto and dietary supplements, and it may be suitable for analyzing the main sterols/stanols in different types of food. However, different sample preparation procedures may be required, and saponification of the sample is required if esterified plant sterols are present. Alternatively, a GC/FID method for the analysis of phytosterols in margarine may be used.

For mixtures of phytosterols, phytostanols and their esters the separation of esterified and unesterified sterols and stanols is necessary. This may be achieved by using thin layer chromatography (TLC) or solid phase extraction. The fraction containing free phytosterols and phytostanols can be quantified by separating them from the esters and other fat and oil components by TLC.

The purity of sterol and stanol raw materials may be monitored by GC-FID. The sample is silylated in the presence of an internal standard and analyzed by GC. No saponification step is necessary, as the sterols and stanols are unesterified. TLC or LC may be used as a preparative step, if necessary. Quantification relies on relating peak area to the internal standard concentration. Identification of the main sterols is possible through the use of standards and information in the literature. GS-MS provides data that can be used for sterol structure elucidation and to confirm peak identification. However, identification of trace level sterols is more difficult. Only some of the peaks can be identified as sterols, whereas other peaks remain unidentified. Because many compounds are not commercially available at sufficient purity, a single response factor is often used. Other properties of fats and oils, e.g. free fatty acids, peroxide value and moisture content, are determined using standard methods, e.g. AOCS methods.

5. Regulatory status

Phytosterols, phytostanols and their esters have been evaluated globally by various authorities. Following thorough assessment, positive approval for phytosterols has been obtained in the European Union (EU), Australia, Switzerland, Norway, Iceland, Brazil, South Africa, Japan, Turkey and Israel. Furthermore in the USA a 'self-GRAS' (GRAS = Generally Recognized As Safe) procedure has been followed for both phytosterols and phytostanols, to which the US FDA raised no objections. The first GRAS approval was obtained in 1999 for phytosterol esters for use as an ingredient in vegetable oil–based spreads. In addition to receiving approval by the FDA, phytosterol esters have also been evaluated by major health organizations in the United States.

In the European Union (comprising 27 countries), the use of phytosterols, phytostanols and their esters in foods is regulated under Regulation (EC) No. 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Before a novel food or food ingredient can be placed on the market, it must go through an applicant specific authorisation procedure which involves a rigorous safety assessment. The first approval was granted in 2000 for the use of phytosterol esters as a novel food ingredient in yellow fat spreads. Up to September 2008, several approvals have been granted for different types of foods (yellow fat spreads, salad dressing, milk type products, fermented milk type products, yoghurt type products, milk based fruit drinks, milk based beverages, soy drinks, rice drinks, cheese type product, spicy sauces, cereal based products and rye bread). It should be noted that the plant stanol ester products are excluded from having to comply with the EC Regulation as they were on the market before the law came into force.

6. Functional use

6.1 Function in products

Phytosterols and phytostanols, in free or esterified form, are added to foods for their properties to reduce absorption of cholesterol in the gut and thereby lower blood cholesterol levels. It is now generally accepted that sterols and stanols have the same cholesterol lowering efficacy.

The daily doses, considered optimal for the purpose of lowering blood cholesterol levels, are 2-3 g of phytostanols and/or phytosterols, which translates to 3.4-5.2 g in esterified form. This recommended daily dose is typically divided in 1-3 portions of food providing 1.7-5.2 g ester, which equals 1-3 g phytostanol and/or phytosterol equivalents.

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. Moreover, phytostanol esters may be used to modify the fatty acid composition of a fat blend and replace part of the hard fat in margarines and spreads. Furthermore these esters can provide a crispy texture (prevents sogginess) to cereal products by coating the product surface. Both phytosterol and phytostanol esters give an enhanced creamy texture to low fat dairy products (yoghurt/ drinking yoghurt). They may also improve the taste of food products by masking bitterness and hence reduce the amount of sugar or other sweetener required to obtain a pleasant taste and mouth feel (e.g. in soy drinks).

6.2 Food categories and use levels

Phytosterols, phytostanols and their esters are incorporated into a variety of foods and beverages and supplements, produced by a growing number of food- and beverage manufacturers.

6.2.1 Phytosterol(ester) products

The main product formats (phytosterol ester level given between brackets) are:

- margarines and low fat spreads (3.4g per 30g)
- yogurts (1.25g per 125ml)
- yogurt drinks (3.4g per 100ml)
- milk (5g per litre)

As the EU approvals stipulate that products have to be easily divided into portions that contain either a maximum of 3g (in case of one portion per day) or a maximum of 1g (in case of three portions per day) of added phytosterols, products on the EU market are primarily marketed as a range or as a single portion product.

Main foods- and/or beverage manufacturers (non-exhaustive list) are:

- Danone (Danacol brand)
- GFA Foods (Smart balance brand)
- Meadow Lea (Logicol brand)
- Nestlé (Nesvita brand)
- Unilever (pro.activ brand)

An increasing number of retailers are carrying similar type products under their own brands (so-called Distributer Owned Brands (DOBs)), for example:

- Albert Hein (NL)
- Aldi (EU)
- Asda (UK)
- Carrefour (EU)
- Kesko (Finland)
- Migros (CH)
- Sainsbury (UK)
- Tesco (UK)

The Unilever phytosterol enriched spread was first launched in 1999 in the USA (Promise activ®, formerly Take Control®,) and in 2000 in the EU (pro.activ®), following approvals referred to in section 5. Currently pro.activ products (spreads, yoghurts, milk and yoghurt drinks) are on the market in the following countries:
Australia, Austria, Bahrain, Belgium, Brazil, Chile, Cyprus, Czech Rep., Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Latvia, Lithuania, Luxemburg, Malta, Netherlands, Netherlands Antilles, New Zealand, Norway, Poland, Portugal, Singapore, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, UK, USA.

6.2.2 Phytostanol ester products

Phytostanol esters are marketed under the Benecol® brand. In terms of consumer products these ingredients are branded also sub-branded by major foods and beverage manufacturers.

- Raisio (Benecol brand)
- McNeil (Benecol brand)
- Minerva (Minerva Benecol brand)
- Emmi Dairy (Emmi Benecol brand)
- Kaiku Dairy (Kaiku Benecol brand)
- Ûlker (Kalbim Benecol brand)

The first phytostanol ester enriched product, a spread, was launched in Finland in late 1995. Since then, phytostanol ester enriched margarines with fat contents from 60 to 65 % have been launched in countries in the EU and in the USA. Phytostanol ester low fat spreads, containing 30-55 % fat, are sold in Argentina and several EU countries. In Portugal, an olive oil with added phytostanol ester is commercially available. Since the first launch of phytostanol ester enriched yoghurt in 1999 (on the UK and Irish market) a number of different types of dairy applications of phytostanol ester enriched foods have been launched in several EU countries. Also, cheese type products, frankfurters, sausages, ready-to-eat meals, salad dressings, snack bars and candies have been introduced on the market.

The first cereal based Benecol® food was dry pasta launched in Finland in the beginning of 2003 and later instant oat meal was marketed. Food products with added phytostanol ester, which are or have been on the market in the following countries:
Argentina, Austria, Belgium, Chile, Ecuador, Estonia, Finland, France, Germany, Greece, Iceland, Indonesia, Ireland, Italy, Malta, The Netherlands, Luxembourg, Poland, Portugal South Africa, Spain, Sweden, Switzerland, Turkey, United Arab Emirates, UK, USA.

Food products with added phytostanol esters and portion sizes are given in Table 3. Possible future food categories of phytostanol ester enriched products are not limited to the current food categories.

Table 3. Phytostanol concentrations in food products on the market, including portion sizes

Country	Food product	Phytostanol ester content/daily portion (equals 2g phytostanols)
Argentina	Spread	3.4g / 30 g
Austria	Yoghurt drink	3.4 g / 65 ml
Belgium	Spread	3.4 g / 30 g
	Cream cheese	3.4 g / 50 g
	Yoghurt	3.4 g / 125 g
	Yoghurt drink	3.4 g/ 70 g
	Soy yoghurt drink	3.4 g / 65 ml
Chile	Milk drink	3.4 g / 200 ml
Ecuador	Cream cheese	3.4 g / 50 g
	Milk drink	3.4 g / 250 ml
	Yoghurt drink	3.4 g / 120 ml
Estonia	Spread	3.4 g / 30 g
Finland	Spread	3.4 g / 25 g
	Cream cheese	3.4 g / 40 g
	Liquid Rapeseed oil product	3.4 g / 40 g
	Cheese type product	3.4 g / 50 g
	Frankfurters	3.4 g / 300 g
	Turkey Liver Sausage	3.4 g / 60 g
	Turkey Sausage	3.4 g / 125 g
	Mayonnaise based Salad	3.4 g / 200 g
	Broiler casserole	3.4 g / 300 g
	Chicken balls	3.4 g / 234 g
	Pasta	3.4 g / 140 g
	Yoghurt	3.4 g / 150 g
	Buttermilk	3.4 g / 300 ml
	Yoghurt drink	3.4 g / 100 ml
	Milk drink	3.4 g / 500 ml
	Instant Oat Meal	3.4 g / 35 g
	Capsules	3.4 g / 4 capsules
France	Spread	3.4 g / 30 g
	Yoghurt	3.4 g / 250 g
Germany	Yoghurt drink	3.4 g / 65 ml
Greece	Cream cheese	3.4 g / 30 g

Country	Food product	Phytostanol ester content/daily portion (equals 2g phytostanols)
	Rye bread Spread	3.4 g / 4 slices 3.4 g / 30 g
Iceland	Yoghurt drink	3.4 g / 65 ml
Indonesia	Acid milk	3.4 g / 100 ml
Ireland	Spread Cream cheese Milk drink Yoghurt Yoghurt drink Soy yoghurt drink Snack Bar	3.4 g / 30 g 3.4 g / 50 g 3.4 g / 250 ml 3.5 g / 125 g 3.4 g / 70 g 3.4 g / 65 ml 3.4 g / 50 g
Italy	Yoghurt drink	3.4 g / 65 ml
Malta	Spread	3.4 g / 30 g
The Netherlands	Yoghurt Yoghurt drink	3.4 g / 500 g 3.4 g / 500 g
Luxembourg	Spread Cream cheese Yoghurt Yoghurt drink	3.4 g / 30 g 3.4 g / 50 g 3.4 g/ 125 g 3.4 g/ 70 g
Poland	Spread Yoghurt drink	3.4 g / 25 g 3.4 g / 100 ml
Portugal	Yoghurt drink Milk drink Olive oil	3.4 g / 65 ml 3.4 g / 333 ml 3.4 g / 45 ml
South Africa	Yoghurt drink	3.4 g/ 100 ml
Spain	Yoghurt drink Milk drink Yoghurt Spread	3.4 g / 65 ml 3.4 g / 333 ml 3.4 g / 125 g 3.4 g / 30 g
Sweden	Spread	3.4 g / 25 g
Switzerland	Yoghurt drink Yoghurt Spread	3.4 g / 65 ml 3.4 g / 150 g 3.4 g / 20 g
Turkey	Yoghurt drink Milk drink Yoghurt Spread	3.4 g / 100 ml 3.4 g / 250 ml 3.4 g / 115 g 3.4 g / 25 g
United Arab Emirates	Milk drink Yoghurt	3.4 g / 500 ml 3.4 g / 125 g
UK	Spread Cream cheese Milk drink Yoghurt Yoghurt drink Orange juice Soy yoghurt drink Snack Bar	3.4 g / 30 g 3.4 g / 50 g 3.4 g / 250 ml 3.4 g / 125 g 3.4 g / 70 g 3.4 g / 500 ml 3.4 g / 65 ml 3.4 g / 50 g
USA	Spread Dressing Snack Bars Candy Chews Capsules	3.4 g / 56 g 3.4 g / 30 ml 3.4 g / 62 g 3.4 g / 2 candies 3.4 g / 6 capsules

7. Reactions and fate in foods

7.1 Stability at high temperatures

Phytosterols and their fatty acid esters are quite stable compounds and undergo only limited degradation during oil processing. Only under harsh conditions, such as high temperatures (>100°C) in the presence of oxygen, oxidation of the phytosterol moiety may occur, in the same way as for cholesterol. As mentioned in section 3, phytosterols are mono-unsaturated compounds (double bond in the B-ring), which 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, such as during shallow frying, sterol oxidation products form only slowly. Under conditions of use for shallow frying by consumers (temperatures 160-200 °C, 5-10 minutes of frying) the level of oxidation of sitosterol esters remains below 1.3% when the matrix consists of liquid oil of liquid margarine. Using free sterols these levels are somewhat higher at 2.5% and 5.1%, respectively.

Factors affecting phytosterol oxidation include, as would be expected, temperature and heating time, but also the composition of the lipid matrix. Phytosterol esters were found to be more susceptible to oxidation at elevated temperatures than free phytosterols.

Phytostanols are generally heat stable and phytostanol esters also show an oxidative stability.

It should be noted that most spreads on the market are actually low fat spreads (< 40% fat). These are intended for spreading, not for shallow frying. Higher fat spreads (>60% fat) spreads however are suitable for shallow frying but are certainly not intended for deep fat frying.

7.2 Stability during product manufacturing and storage

Phytosterols and phytostanols are microbiologically largely inert as shown by the absence of an effect during the fermentation process used to produce yoghurt. Furthermore, the ester added to various food products show excellent stability at different pH values during long term storage (up to at least a year). Phytostanol and phytosterol esters are also stable in milk and fermented milk and products with viable bacteria like yoghurts and yoghurt drinks.

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