# **Phytosterols as Functional Food Ingredients**

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Phytosterols are defined as plant sterols and plant stanols. Phytosterols lower total and LDL blood cholesterol by preventing cholesterol absorption from the intestine, so they have been known as blood cholesterol-lowering agents for over the last half century. Phytosterols are naturally found in vegetable products, principally oils. Dietary phytosterol intakes are normally range from 170 to 360 mg/day with variations depending on food culture and major food sources. Recent studies have shown that maximum cholesterol-lowering benefits are achieved at doses of 2-3 g per day. Therefore today's use implies the need for enriched functional food products, which give enough phytosterols intake.

Keywords: phytosterol, plant sterol, plant stanol, diet

#### Fonksiyonel Gıda Bileşenleri, Fitosteroller

Bitki sterolleri ve bitki stanolleri olarak bilinen fitosteroller, kolesterolün bağırsaktaki emilimini engelleyerek, kandaki toplam ve LDL kolesterol seviyelerini düşürücü etkiye sahiptirler. Bu yüzden, fitosteroller yaklaşık yarım asırdan beri kolesterol düşürücü ajanlar olarak bilinmektedirler. Fitosteroller bitkisel ürünlerde özellikle bitkisel yağlarda doğal halde bulunurlar. Normal diyetle alınan günlük fitosterol miktarı, tüketim alışkanlıkları ve gıda çeşidine bağlı olarak 170 mg'dan 360 mg'a kadar değişmektedir. Son yıllarda yapılan çalışmalar, kolesterol düşürücü etkiyi en üst seviyede oluşturan miktarın günlük 2–3 g olması gerektiğini göstermektedir. Bu seviyedeki fitosterol tüketimi, fitosterollerle zenginleştirilmiş gıda alımını gerektirmektedir.

Anahtar Kelimeler: Fitosterol, bitkisel sterol, bitkisel stanol, diyet

#### Introduction

Phytosterols (including plant sterols and stanols) cannot be synthesized by humans, and all plant sterols and stanols in the human body therefore originate from the diet (Jong et al., 2003). They are known to have several bioactive qualities with possible implications for human health (Normen et al., 2002). Their properties for reducing blood cholesterol levels, as well as their other beneficial health effects, have been known for many years (Quilez et al., 2003). It was recognized in the 1950s that plant sterols lower serum concentrations of cholesterol (Pollak, 1953). Plant sterols might also protect against certain types of cancer such as colon, breast and prostate (Rao and Koratkar, 1997; Awad and Fink, 2000). Scholarly reviews have all confirmed the health benefits and safety of phytosterols. Well-documented

reviews concerning phytosterols in human nutrition are to be found in the literature (Moghadasian, 2000; Ostlund, 2002; Moreau et al., 2002; Katan et al., 2003). The safety of plant sterols, plant stanols, and their esters has been affirmed by government agencies such as the US Food and Drug Administration and the European Union Scientific Committee (Kritchevsky and Chen, 2005). In addition, these plant fractions have been sanctioned for use in food.

People with high blood cholesterol levels are typically advised by health professionals to exercise and consume a diet high in fiber and low in saturated fats and cholesterol. Although these measures can reduce blood cholesterol, sometimes they don't go far enough. Other cholesterol-lowering interventions may be needed, including cholesterol-lowering medicines or adding phytosterol esters to the diet (Anon., 2003a). The enrichment of foods such as margarines with phytosterols is one of the recent developments in functional foods to enhance the cholesterol-lowering ability of traditional food products (Anon., 2005).

The objective of this article is to present briefly describes of the phytosterols, dietary sources and intakes, the enrichment of food products with phytosterols and legislation and labeling.

### **Chemical Structures of Phytosterols**

Sterols are ubiquitous in eukaryotic cells but absent from prokaryotes (Clifton, 2002). Sterols are an essential component of cell membranes, and both animals and plants produce them. They play a key role in cell membrane function (Law, 2000).

The sterol ring is common to all sterols; the differences are in the side chain (Law, 2000). Plant sterols include a wide variety of molecules that are structurally similar to cholesterol; the principal examples are 4desmethyl sterols (Quilez et al., 2003). Plant sterols are C-28 or C-29 sterols, differing from cholesterol (C-27) by the presence of an extra methyl (campesterol) or ethyl (sitosterol) group on the cholesterol side chain (Nguyen, 1999). While over 40 plant sterols from seven different plant classes have been identified (Bean, 1973), campesterol (C-28), stigmasterol (C-29), and especially  $\beta$ -sitosterol (C-29) are the most abundant (Law, 2000). The terms plant sterol and phytosterol are sometimes used as generic

terms to include both unsaturated sterols and saturated stanols, but they are used here to refer specifically to the unsaturated compounds (Clifton, 2002). Saturated plant sterols, referred to as plant stanols have no double bound in the ring structure. Plant stanols are produced by the hydrogenation of sterols and are not abundant in nature (Law, 2000). Sitostanol and campestanol are saturated plant sterols, which are found in nature in much smaller amounts than plant sterols (Jong et al., 2003). The structures of sitosterol, sitostanol, campesterol and campestanol are shown in Fig. 1. The structure of cholesterol, respectively, is shown for comparison. In foods, cholesterol occurs either as the free alcoholic sterol or as cholesteryl esters (Fenton, 1992), whereas plant sterols occur as free plant sterols, esterified plant sterols, plant steryl glycosides, and acylated plant steryl glycosides (Akihisa et al., 1991). It is obvious that chemical, physical and nutritional properties of these phytosterol classes may be very different (Piironen et al., 2000). The different fractions are assumed to exist in different parts of the plant cell. Free plant sterols are part of the cell wall with a structural property (Normen et al., 1999). Plant steryl esters are generally believed to be storage products. They can be found in the cytosol of plant cells, in droplets or vesicles (Lorenz, 1989). The largest amount of plant steryl glycosides has been found in the microsomal fraction of the plant cell and acylated steryl glycosides are believed to exist in mitochondria (Anon., 1989).



Fig 1. Chemical structures of cholesterol and some phytosterols (Gilbert et al., 2005).

| Food             | Serving                          | Total phytosterols (mg) |
|------------------|----------------------------------|-------------------------|
| Wheat germ       | $\frac{1}{2} \exp(57 \text{ g})$ | 197                     |
| Corn oil         | 1 tablespoon (14 g)              | 102                     |
| Canola oil       | 1 tablespoon (14 g)              | 91                      |
| Peanuts          | 1 ounce (28 g)                   | 62                      |
| Wheat bran       | ½ cup (29 g)                     | 58                      |
| Almonds          | 1 ounce (28 g)                   | 34                      |
| Brussels sprouts | ½ cup (78 g)                     | 34                      |
| Rye bread        | 2 slices (64 g)                  | 33                      |
| Macadamia nuts   | 1 ounce (28 g)                   | 33                      |
| Olive oil        | 1 tablespoon (14 g)              | 22                      |

Table 1. Total phytosterol contents of selected foods (Normen et al., 2002; Phillips et al., 2002).

#### **Dietary Sources and Intakes**

Phytosterols can be found at widely varying concentrations in the fat-soluble fractions of seed, root stems, branches, leaves and blossoms. They are constituents of both edible and ornamental plants, including herbs, shrubs (Clifton, 2002). As and trees natural constituents of the human diet, phytosterols are naturally found in all food items of plant origin, principally oils, but also pulses and dried fruits (Piironen et al., 2000). Their content is highest in edible oils, seeds and nuts (Weihrauch and Gardner, 1978). The total contents are very variable and range from nearly 8g/kg in corn oil to 0.5g/kg in palm oil, with intermediate levels being found in commonly used oils (Philips et al., 2002). Tall oil contains a higher proportion of plant stanols than do vegetable oils (Anon., 2005). Phytosterols are of products based on vegetable oils, such as margarines (Clifton, 2002). The refining process cited leads to a significant reduction in phytosterols in vegetable oils (Ferrari et al., 1997), and it would therefore be very interesting to develop industrial methods which minimize these losses (Ouilez et al., 2003). Table 1 shows the total phytosterol contents of selected foods.

The dietary intake of phytosterols among and within different human population varies greatly, depending on the type and amount of plant foods eaten. Although cooking oils, margarine and peanut butter are the main sources of phytosterols in the diet, phytosterols are also consumed in seed, nuts, cereals and legumes (Clifton, 2002). The consumption of phytosterols can range between 170mg/day in populations eating a Western diet and 360mg/day in diets rich in vegetable products (Vries et al., 1997). The dietary intake of plant stanols is usually only about 50mg/day unless the diet is supplement with tall oil, which is derived from conifers and is rich in sitostanol (Gilbert et al., 2005). The normal dietary intake of plant stanols is much less than that of plant sterols. Consequently, the normal dietary intake of plant stanols is negligible.

In order to achieve a cholesterol lowering benefit approximately 1 g/day of plant sterols or plant stanols need to consume (Anon., 2005). At the same time, recent studies of plant sterol and stanol esters in humans have shown, however, that maximum cholesterol-lowering benefit is achieved at doses of 2-3 g per day (Hallikainen et al., 2000; Jones et al., 2000; Maki et al., 2001). Although people consume phytosterols every day in food, the amounts are often not great enough to have significant cholesterol lowering effect (Anon., 2003a). Phytosterols can be incorporated into traditional food products. Also, finding and cultivating varieties with higher phytosterol contents will increase consumption in the population (Quilez et al., 2003). Genetic modification becomes a powerful tool related purposes for (Venkatramesh et al., 2003).

# The Enrichment of Food Products with Phytosterols

The use of foods containing phytosterols is a relatively recent development in human nutrition (Gilbert et al., 2005). Phytosterols, as functional ingredients in foods, appear to be a practical and safe option for improving cholesterol levels in the population (Quilez et al., 2003). These components are incorporated nowadays into a wide variety of food products. To produce functional foods containing elevated levels of plant sterols and stanols is the aim of many food companies. New techniques have allowed the incorporation of plant sterols and stanols into food forms without affecting the texture and taste (Nguyen, 1999). There has been increased interest in these natural compounds after canola oil-based margarine, enriched by sitostanol ester, was introduced commercially in Finland in 1995 and in the United States and European countries in 1999 (Miettinen et al., 1995; Salo and Wester, 2005). Margarine containing plant stanol esters is among the first examples of a functional food with proven LDL cholesterol-lowering efficacy (Plat and Mensink, 2005). Since fats are necessary to solubilize sterols, margarines are an ideal vehicle for them, although cream cheese, salad dressing, and yogurt are also used. Phytosterols can also be incorporated into baked products, fruit juice, ice cream, and other vehicles (Clifton, 2002). Commercially, phytosterols are currently contained in bars (Australia, England), vegetable oils (Japan, Israel), orange juice, mayonnaises (Australia), milk (England, Australia, and Argentina), yogurt (Australia, England), yogurt drinks, soy milk, meat and soups (Finland), and green teas (Korea) (Berger et al., 2004). One can foresee an endless list of food products into which phytosterols will be incorporated (Kritchevsky and Chen, 2005). Phytosterols are also being sold or developed mixed with other functional ingredients such as: fiber (France), healthy oils (England), non-absorbable diacylglycerol (Israel), almonds, soy protein and viscous fibers, and minerals (Berger et al., 2004).

In early studies of the cholesterol-lowering efficacy of phytosterols, plant sterols were in the form of a crystalline powder (unesterified form), and had to be used in relatively large quantities to achieve significant lowering of cholesterol (Lees et al., 1977). Unesterified plant sterols (or free) are not water soluble and their solubility in fat is limited, their use in food applications is restricted (Salo and Wester, 2005). Plant sterol and stanol esters are far more lipid soluble than plant sterols and stanols. The commercial esterification of plant sterols and stanols with fatty acids from vegetable oil has made it possible to produce

spreads and other food containing the desired esters (Clifton, 2002).

Plant stanols are prepared form the hydrogenation of naturally occurring, mixed phytosterols that are found in wood (tall oil) and various vegetable oils (Slesinski et al., 1999). Sitostanol and campestanol exist in quantifiable amounts in cereals, fruit and vegetables, but generally of less concentration than the unsaturated phytosterols (Dutta and Appelqvist, 1996). For use in the commercial product. stanol esters are prepared bv interesterification of stanols with the fatty acids of vegetable oils such as canola oil (Slesinski et al., 1999). Esterification of plant stanols with fatty acids derived from vegetable oils converts them from a crystalline powder with low lipid solubility into a fatty substance that can easily be incorporated into a variety of foods (Wester, 1999).

Initially, esterified plant stanols and sterols were commercially used in fat-based foods such as margarines, shortenings and mayonnaise, but recent clinical studies have shown that the cholesterol-lowering efficacy of esterified plant sterols and stanols is independent of the food matrix (Mensink et al., 2002; Clifton et al., 2004; Hyun et al., 2005). The use of a low fat vehicle for delivery of stanol esters was tested by Mensink et al. (2002), who randomized healthy individuals to receive low fat (<1%)vogurt with or without stanol ester. The results showed reductions in total and LDL cholesterol levels in individuals consuming stanol ester in vogurt that were comparable to those observed with similar amounts of stanol ester taken in the form of margarine (Plat and Mensink, 2000; Weststrate and Meijer, 1998). Salo and Wester (2005) also showed that cholesterol-lowering efficacy of plant stanol esters was independent of the food type (meat-based ready-made lowfat meals, pasta, and low-fat yogurt drinks) in which it is incorporated. Effect of stanol ester incorporated in low-fat (35% fat) margarine showed similar cholesterol-lowering efficacy when compared with regular margarine (80% fat) with added stanol ester (Miettinen et al., 1995; Hallikainen and Uusitupa, 1999). Gylling and Miettinen (1999) showed that plant stanol esterified with butter fatty acids (predominantly myristic acid) and dissolved in butter was just as effective in lowering LDL cholesterol as was plant stanol esterified with rapeseed oil and dissolve in margarine. Plant stanol ester contained in low-fat yogurt was effective in reducing cholesterol level in a habitual diet without restriction of fat and cholesterol intake (Hyun et al., 2005). With esterification of plant stanols, the food vehicle does not have to have a high fat content to be an effective means of delivery of plant stanol (Salo and Wester, 2005).

# Legislation and Labelling

In the USA, plant sterol, stanols and their esters were given Generally Recognized as Safe (GRAS) status. On the basis of this recognition, the US Food and Drug Administration (FDA) approved fat spreads containing up to 20% of either steryl or stanyl esters (Quilez et al., 2003). In Europe in 2000, the EU Scientific Committee on Food (SFC) approved the use of phytosterol esters in yellow fat spreads (maximum level of 8% of free phytosterols) (Anon., 2000). SCF and its successor, the European Food Safety Authority (EFSA) through its Scientific Panel on Dietetic Products, Nutrition and Allergies, have recommended that plant sterol containing foodstuffs should not be consumed in amounts resulting in total phytosterol intakes exceeding 3g/day (Anon., 2002).

In 2003, the FDA published the interim final rule (IFR) authorizing the use of a health claim for plant sterol/stanol esters and reduced risk for coronary heart disease on food labels. The IFR authorizes the use of a health claim relating between plant sterol/stanol esters and reduced risk of coronary heart disease on labeling of spreads and dressings for salad containing at least 0.65 grams of plant sterol esters per serving and spreads, dressings for salad, snack bars, dietary supplements in softgel form containing at least 1.7 grams plant stanol esters per serving (Anon., 2003b). In 2004 the published EU Commission Regulation 608/2004/EC concerning the labeling of foods and food ingredients with added phytosterols and/or phytosterol esters, requiring such

products to be labelled with additional information including the words "with added plant sterols/plant stanols". In England, the food with added phytosterols (labelling) Regulations 2004 were published to provide for the enforcement in England of Regulation 608/2004/EC (Anon., 2004).

## Conclusions

Over the past decade, the possibility of using phytosterols as ingredients in functional foods has led to numerous research studies in relation to their ability to reduce blood cholesterol. Phytosterols has also been shown that they can control certain illnesses if they are ingested in the same quantities required for cholesterol reduction (Quilez et al., 2003). Initially, phytosterol esters were commercially used in margarines, but recent clinical trials show that phytosterol esters effectively reduce blood cholesterol, even used in food vehicles with a low fat content.

The present sources of phytosterols are limited. It is estimated that 2500 tonnes of vegetable oil needs to be refined to produce 1 tonne of phytosterols. Another source of phytosterols is tall oil which is derived from the process of paper production from wood and approximately 2500 tonnes of pine is required to produce 1 tonne of phytosterols (Anon., 2005). In the longer term, the addition of plant sterols and stanols to foods could be an important public health policy if new technology and economies of scale can lower the cost and enable a greater demand to be met (Law, 2000).

Phytosterols are naturally found in all plant origin food products. People consume these components every day. Naturally available phytosterols have effects on cholesterol metabolism. However, cholesterol lowering effect attributed to phytosterols in food products would be of limited significance. Introducing phytosterols, as functional ingredients, into food products will increase consumption in the human population.

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