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Review - Natural Food Extracts and Additives Section

PHYTOSTEROLS – BIOLOGICAL ACTIVE COMPOUNDS IN FOOD

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Abstract

The purpose of this review is to be a comprehensive treatise on biological, chemical and nutraceutical aspects of phytosterols, as well as extraction methods and new trends in food industry. The term "phytosterols" covers plant sterols and their hydrogenation products, the stanols. The phytosterols are effective in lowering plasma total and low density lipoprotein (LDL) cholesterol, by inhibiting the absorption of cholesterol from the small intestine. The phytosterols isolation method is using nonpolar solvents (such as hexane, methylene chloride, ethanol and isopropanol) near their supercritical region where they have high extraction properties. The enrichment of foods such as margarine with plant sterol/stanol ester is one of the recent developments in functional foods to enhance the cholesterol lowering ability of traditional food products.
Keywords*: phytosterol, functional food, bio-active compound, extraction, supercritical fluid*

Introduction

A balance diet is defined as one which contained a variety of food in such quantities and proportion that the need for energy, amino acids, vitamins, minerals, fats, carbohydrates and other nutrients are adequately met for maintaining health vitality and general well being.

In 1996 Health Canada did define functional foods as foods that contain bioactive component / components that provide health benefits beyond basic nutritional value. (Therefore, food enriched with nutrient constituents such as vitamins and minerals should not be considered functional foods, while foods with chemical compounds known to lower blood cholesterol fit the definition) (Zawistowski, 2002).

Phytonutrient, within the context of natural health and nutrition, has come to refer to bio-active plant chemicals that humans eat and have or may well have significant positive effects on human metabolism. Phytonutrients are not essential for life, but they appear to be essential for optimal health and longevity. Phytochemicals that are concentrated or prepared in such a dosage as to have likely therapeutic effects are generally becoming referred to as nutraceuticals (Maher, 2003). Nutraceuticals may be considered as health bio-actives that may be used in functional foods and one of the most researched groups of nutraceuticals in the area of cardiovascular diseases is phytosterols (Zawistowski, 2002).

New trends in food industry

During the past decade, consumers have become more aware to the importance of nutraceuticals and functional foods. Although many countries are still in the process of developing regulations for this new type of foods, Japan, the U.S., and, to some extent, Europe, have in place of regulatory system to deal with this issue. In Canada, functional food regulation is still under development (Zawistowski, 2002).

Phytosterols represent a diverse group of natural products, and knowledge about their occurrence in various plants and their chemical composition has gradually accumulated during the last 100 years. Much early research and utilization of phytosterols focused on their value as precursors in the synthetic synthesis of several steroid hormones (Moreau 2002). During the last 10 years, there has been an unprecedented escalation of interest in phytosterols. Most of this interest has focused on the cholesterol-lowering properties (both dietary and endogenously-produced) of 4-desmethyl phytosterols and phytostanols, this result in a decrease in serum total and low-density lipoprotein cholesterol (LDL-C) (Ling 1995, cited by Moreau). Evidence of this phenomenon include at least 25 clinical studies, 20 patents and at lest 10 major commercial phytosterol products currently being marketed in many parts of the world.

Phytosterols are natural components of human diets, largely derived from vegetable oils, cereals, fruits and vegetables. Phytostanols are much less abundant in nature than phytosterols and common dietary sources of phytostanols are corn, wheat, rye and rice. For many years, the existence and dietary effects of these minor sterols were largely ignored and poorly understood. Sterol chemists and biochemists focused their efforts on cholesterol because elevated serum cholesterol levels were shown to be a prominent risk factor for cardiovascular disease. Recent strategies for lowering serum cholesterol utilize dietary restrictions to limit cholesterol intake and / or require the use of drugs such as the popular statins which inhibit cholesterol biosynthesis in humans. The prospect of lowering cholesterol levels by consuming functional foods fortified with natural phytonutrients would seem more attractive to many than use of drugs or dietary restrictions. Since the mid 1990s, there has been considerable interest and commercial marketing of phytosterol products for this purpose.

Phytosterols – types, isolation

Numerous phytosterols have been isolated from the unsaponifiable part of vegetable fats, for example: brassicasterol (C_{28}) from rape oil (*Brassica rapa*), campesterol (C_{28}) from *Brassica campestris,* spinasterol, from spinach and other plants, sitosterol (C_{28}), one of most common, as a mixture of three isomers α-, β-, γ- and a dehidro-β-sitosterol, stigmasterol (C_{29}), from soy oil and the bean *Physostigma venenosum* (Moreau, 2002) (Figure 1)

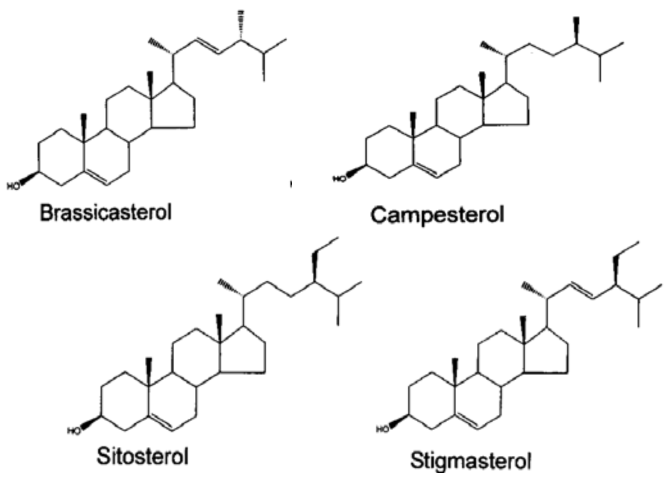

Fig.1. Types of phytosterols

In all plant tissues, phytosterols occur in five common forms (Figure2): as the free alcohol (FS), as fatty-acid esters (SE), as steryl glycosides (SG), as acylated steryl glycosides (ASG) and as phytosteryl hydroxycinnamic-acid esters (HSE).

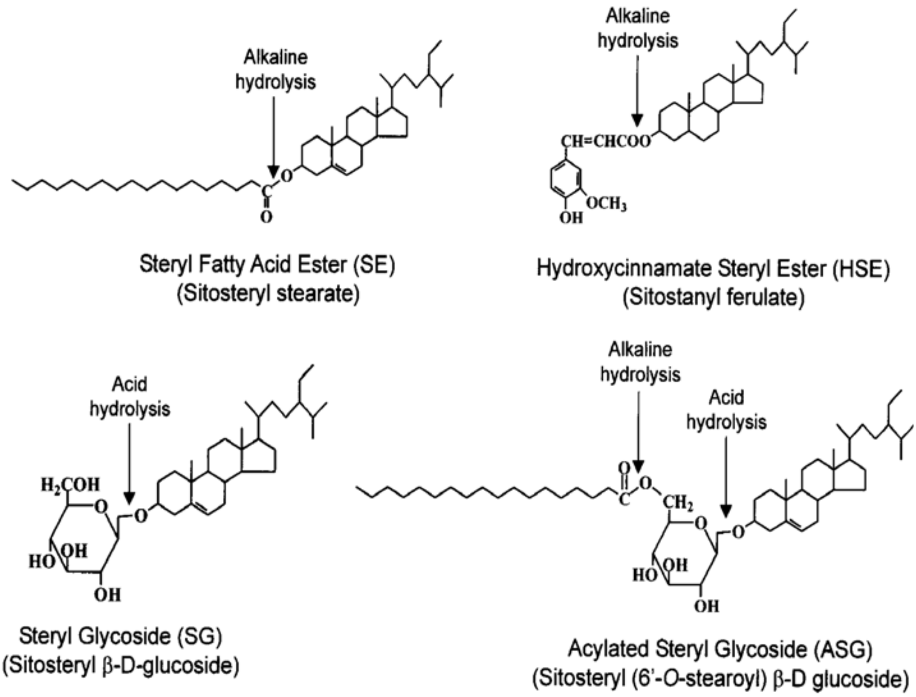

Fig. 2. Structures of phytosterol conjugate. The sites of cleavage via alkaline hydrolysis (saponification) and acid hydrolysis are indicated with arrows.

The fourth three forms (SE, SG, ASG and HSE) are generically called “phytosterol conjugates”. In free phytosterols (FS), the 3β-OH group on the A-ring of the sterol nucleus is underivatized, whereas in the conjugates the OH is covalently bound with another constituent. The OH group is ester-linked with a fatty acid in SE and linked by a 1-*O*-β-glycosidic bond with a hexose (most commonly glucose) in SG (Power 1913, cited by Moreau). The third group of phytosterol conjugates, ASG, differ from SG by the addition of a fatty acid esterified to the 6-OH of the hexose moiety (Lepage 1964, cited by Moreau). Seeds of corn and rice and other grains contain a fourth type of phytosterol conjugate, HSE, in which the sterol 3β-OH group is esterified to ferulic or *p*-coumaric acid.

Most common methods for the extraction of lipids also extract phytosterols. Nonpolar solvents such as hexane (commonly used to extract most types of vegetable oils), quantitatively extract free phytosterols and phytosteryl fatty-acid esters (Piironen 2000). The extraction of free phytosterols, phytosteryl fatty-acid esters and ferulate phytosteryl esters from corn fibre was compared with four different solvents (hexane, methylene chloride, ethanol and isopropanol) and each solvent extracted >95% of these three sterol lipid classes (Moreau 1996). Steryl glycosides and fatty acylated steryl glycosides are only partially extracted with hexane and increasing the polarity of the solvent gave a higher percentage of extraction (Goad 1991, cited by Moreau). It is routinely used the Bligh and Dyer chloroform-methanol extraction method to extract all sterol lipid classes (Bligh 1959, cited by Moreau).

Precipitation of cholesterol with digitonin (a type of steroidal saponin) was a technique commonly used to remove other lipids and obtain a cholesterol-enriched fraction. Digitonin precipitation also has been used in studies (Whitaker 1987, cited by Moreau) to isolate the free phytosterols fraction (binding of digitonin to a sterol and consequent precipitation of the complex, requires a free 3β-OH group). However, there is evidence that certain types of phytosterols are not quantitatively precipitated by digitonin, and this technique is now infrequently used to isolate free phytosterols.

Pressurized fluid extraction (PFE) is similar to Soxhlet extraction, except that the solvents are used near their supercritical region where they have high extraction properties. In that physical region the high temperature enables high solubility and high diffusion rate of lipid solutes in the solvent, while the high pressure, in keeping the solvent below its boiling point, enables a high penetration of the solvent in the sample. Thus, PFE permits high extraction efficiency with a low solvent volume (15-40 ml) and a short extraction time (15-20 min). That procedure is also known as "Accelerated Solvent Extraction" (ASE) (Richter 1996).

The PFE device includes an extraction cell (1 up to 100 ml) maintained at a temperature between 80 and 200°C into which a solvent is pumped and maintained at 10-20 MPa for some minutes.

Then, the extract is pushed into a collection vial by a second volume of solvent and finally the whole solvent is pushed with an inert gas flow (Figure 3).

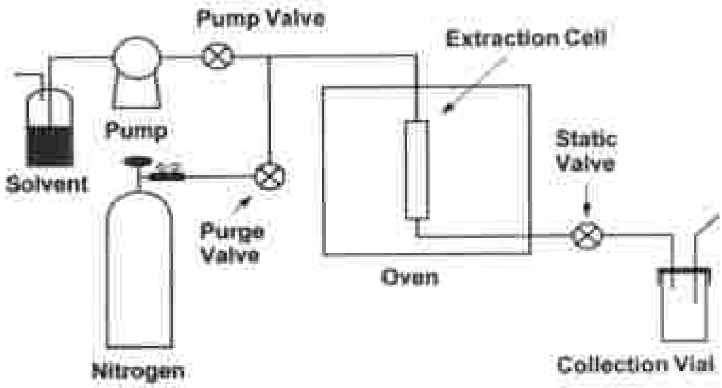

Fig 3. Scheme of Pressurized Fluid Extraction (PFE)

It was reported (Taylor 2002) that corn bran contains ferulate-phytosterol esters (FPE), with sitostanolferulate being the most abundant molecular species. A preparative-scale procedure has been developed for the supercritical fluid extraction/supercritical fluid chromatography (SFE / SFC) of oil from corn bran. The oil is removed from the corn bran by utilizing supercritical carbon dioxide (SC-CO_2), and then fractionated by on-line SFC to obtain a fraction enriched in FPE.

SFE was conducted on corn bran (175 g) with SC-CO_2 at 34.5 MPa and 40°C using 600 L CO_2 at a flow rate of 5 L/min for 120 min. The extract-laden stream was directed through the pressure-reducing regulator prior to its deposition on to the head of the chromatographic column. This allowed the pressure to be reduced to 6.2 from 34.5 MPa, allowing the extract to be concentrated at the top of the column without initially eluting any of the extract from the column. In addition, this gradual letdown of the pressure avoided freezing of the regulator from the Joule-Thompson expansion effect. After the set volume of CO_2 was used, the extraction was terminated and the extraction cell was bypassed. The CO_2 stream was then directed in to the column for fractionation of the corn bran extract. SFC was performed in three steps, followed by sorbent bed reconditioning between runs. Fractions were collected during each SFC step and analyzed *via* HPLC. Round-bottomed flasks were used for collection during SFC. SFC steps one and four employed 250-mL flasks, and the second and third steps used 500-mL flasks. During the SFC steps employing ethanol-modified CO_2, multiple collection flasks were required owing to the large volume of ethanol accumulated. Fractions were collected at equal volume intervals of CO_2 (500 L). The fractions collected during the second and third SFC steps were combined, respectively, and the EtOH was removed under reduced pressure at 60°C. Each fraction was then reconstituted in hexane at 5 mg/mL for HPLC analysis (Figure 4) (Taylor 2002).

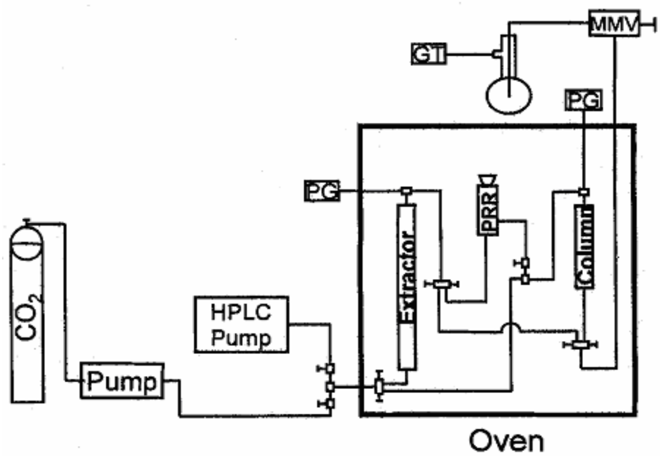

Fig. 4. Schematic of supercritical fluid extraction /supercritical fluid Chromatography (SFE / SFC) processing system.
Pressure gauge (PG); gas totalizer (GT); micrometering valve (MMV); pressure-reducing regulator (PRR).

Phytosterol / phytostanol products: past, present and in development

Stanyl ester products entered the market in Finland in the mild 1990s, followed by both stanyl and steryl ester products in other European countries. Stanyl and steryl ester products entered the US market in 1999 after considerable regulatory discussions and delay. Based upon conclusions of a panel of independent experts in the USA, plant steryl and stanyl esters were approved for use as ingredient in vegetable oil-based spreads in amount up to 20% (Moreau 2002).

More than 20 patents on phytosterol and phytostanol products or processes have issued in US in the last 10 years. These patents fall into several categories. The earliest and larges group are patents on phytostanyl esters filed by Raisio and McNeil. Lipton (Unilever) has several patents on phytosterol / phytostanol technology. Other major patents include various one from Forbes Medi-Tech, a lecitin-phytosterol preparation owned by Washington University, another phytosterol-protein complex owned by Monsanato, a phytosterol-emulsifier complex owned by Kraft (Moreau 2002).

Another patent represent a nanoscale process to produce emulsions containing microscopic particles of phytosterols (Kropf 2001). Several patents involve multi-component phytosterols products include: patent on the extraction, composition of mater and cholesterol-lowering applications of corn fibre oil (which contains natural steryl esters, free sterols, hydroxycinnamate steryl and stanyl esters, tocopherols and carotenoids) (Moreau 1998); a multi-component formulation that combines tocopherols and tocotrienols, free sterols, steryl esters, cycloartenols and saturated fats (Berry 2001) and a formulation that contains phytosterols (1.2-20 wt.%) dissolved in diacylglycerol (Goto 2001).

In 1999 Lipton and its parent company, Unilever, launched their product, “Take Control”, which contained vegetable oil-derived steryl esters. Because Becouse Take Control’s active ingredients, steryl esters, were simpler and less expensive to prepare (no hydrogenation required), Lipton’s product could be sold at a low price (Moreau 2002)

In October 2000, Forbes Medi Tech Inc. announced the first consumer product test market of its phytosterol-based cholesterol lowering ingredient, Phytrol (unesterified tall oil phytosterols) in Australia and the USA. Forbes claims that Phytrol can be used in a wide variety of food products besides fatty foods (Moreau 2002).

Corn fibre oil has both phytosterol and phytostanyl esters, naturally esterified with either fatty acids or phenolic acids, such as ferulic acid, a potent antioxidant. In fact, corn fibre oil appears to be the richest source of natural stanols and stanyl esters ever reported the levels of total phytosterols range from about 15 to 50%, depending on extraction and fibre pre-treatment conditions. The Agricultural Research Service scientists have worked with several major US corporations to move the corn fibre oil towards commercial product, which could include spreads, chocolates, dairy products, cooking oils, and other foodstuffs with health-promoting properties (Moreau 1996).

During the years 2000 and 2001, Procter & Gamble test marketed a new line of phytosterol-containing cooking oils under the brand name "Cook Smart". These oils contained soy phytosteryl esters. P & G was the first company to market phytosterol-containing cooking oil which conceivably could add cholesterol-lowering phytosterols to fried foods such as French fries (Moreau 2002).

Forbes Medi-Tech Inc. announced in December 2000 its development of "designer oil" which reduces LDL-cholesterol and increases energy expenditure, and hence may prevent people from gaining weight. The oil contains Forbes' phytosterol based ingredient, Phytrol, which is incorporated into the oil by a proprietary process that preserves clarity of the oil (Moreau 2002).

Some consumer resistance to current commercial products stems from the high fat content of the spreads and margarines in which they are formulated. Recent research shows that phytosterols delivered in a low fat spread are as effective as those in higher fat formulations. Low fat spreads containing phytosterols are now commercially available (Moreau 2002).

There is a demand for phytosterol formulations that could be included in beverages, dairy drinks and non-fat foods and it was developed a patent-pending phytosterol formulation that allows introduction of sterols in a dispersible form in an aqueous application (Moreau 2002).

Conclusions

The developments of functional foods represent a unique way towards improvement of the food quality and consumer health. The use of phytosterols in functional food formats to lower blood cholesterol levels began in the 1990s, when it was discovered that sterol and stanol fatty acyl ester derivatives could be efficiently incorporated into fatty foods, such as fat spreads and salad dressings. The reported SFE /SFC system represent a two-step process to enrich and fractionate high-value nutraceutical components.

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