HEART HEALTH BENEFITS AND REGULATORY STATUS OF PLANT STEROLS

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Contents

Summary

Consumption of functional foods enriched with plant sterols may provide a risk reduction for coronary heart disease. Sterols are structurally related to cholesterol and are found in oil seeds, vegetables, coniferous trees, and other plants. They represent one of the most thoroughly researched phytochemicals that provide both complementary and alternative treatment to reliably lower total- and LDL-cholesterols, thus providing value for safeguarding cardiovascular heart health. Based on findings derived from numerous clinical studies, the US National Cholesterol Education Program has advocated plant sterol's consumption in the range of 2g/day as part of the recommended life style changes considered important to reduce the risk of heart disease. Clinical studies conducted using plant sterols, have reported a reduction in LDL-cholesterol in the range of 10-15% when daily doses of 1-3 g of stanols or sterols are used. Other studies have characterized the stability of plant sterols and confirmed the safety of consuming established dosages of sterols that are required for efficacy.

Moreover, there is current research to also show that plant sterols are effective regardless of whether they are consumed as free or esterified formats. Plant sterol-containing foods, such as vegetable fat spreads, juices, dairy and cereal products have regulatory approval for use in the US, Europe, Australia, Japan and other Asian countries as well as Canada. Most of the regulatory jurisdictions recommend a daily consumption of 2g of sterols and allow for specific health claims on food product labels.

1. Introduction

Coronary Heart Disease (CHD) is leading cause of death in the World today. It has been estimated that about 20 million people worldwide will die from cardiovascular disease by 2015. There are many known risk factors associated with CHD, including genetics, obesity, diabetes, sedentary life style, high blood pressure and elevated blood cholesterol. A diet rich in fat and/or cholesterol can initiate the onset of the disease; the result being that high blood cholesterol is a recognized major risk factor for CHD for more than 40 years. Serum cholesterol has a major detrimental role in the formation of endothelial atherosclerotic plagues and numerous epidemiological studies have shown that lowering both total-cholesterol and low-density lipoprotein (LDL) cholesterol will reduce the risk of CHD. According to the West of Scotland Coronary Prevention Study, lowering LDL cholesterol by approximately 10 to 19% resulted in a reduced risk of CHD by about 41%. A 10% decrease of blood cholesterol in mildly-hypercholesterolemic subjects (e.g. ranging from 5.3 mmol/L to 6.0 mmol/L) may also prevent an estimated 100,000 deaths annually.

Cholesterol-lowering drugs, such as statins, known to be effective inhibitors of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase, a rate limiting enzyme for cholesterol synthesis and thus synthesis of biliary cholesterol, will control for high blood cholesterol in hypercholesterolemic patients. Some patients however, fail to respond to the statin treatment and moreover, some indeed exhibit a variety of adverse side effects. While statin drugs help to protect against high blood cholesterol levels, there is a significant portion of the population that requires non-pharmaceutical intervention to assist them with the prevention of elevated blood cholesterol levels.

One of the most effective means in controlling for high circulating cholesterol levels is through the use of dietary components, such as plant sterols. This group of nutraceuticals has been studied for the last 60 years and during the last decade in particular, recognized as an effective adjunctive lipid-lowering agent. With nearly 100 million Americans and another 100 million Europeans experiencing elevated blood cholesterol concentrations, plant sterol containing functional foods offer feasible, alternative, preventive solutions to effectively manage hypercholesterolemia, without posing significant adverse side effects.

Plant sterols are complex lipid-like compounds that are related both structurally and biosynthetically to animal cholesterol, but found in vegetable oil, seeds, nuts and coniferous trees where they have essential roles in stabilizing cell membranes and other diverse cellular functions. An important feature of plant sterols as they relate to human health is the affinity to actively reduce cholesterol absorption in the intestine, which in turn will result in lowering total serum cholesterol and LDL-cholesterol levels. The affinity of plant sterols to this end is governed by a competition between plant sterols and cholesterol for the inclusion into a mixed micelle; the affinity to compete with dietary cholesterol is based on the similarities in chemical structure that exists between both sterol compounds. The hypocholesterolemic character of plant sterols is a key element of numerous functional foods and nutraceuticals that are available today on the global market.

2. Chemistry, Properties and Source of Sterols

Whereas cholesterol intake is derived mainly from animal food products, such as dairy, eggs and red meat sources, including seafood, plant sterols are derived from plants and consumed from a variety of primary sources, such as oils, seeds, as well as products that are fortified with plant sterols mixtures, namely spreads and milk. They are a major constituent of the nonsaponifiable matter of oils with principle plant sterols being both 28- and 29- carbon steroid alcohols; campesterol ($C_{28}H_{46}O$; m.p. 158^{o}C), β-sitosterol ($C_{29}H_{50}O$; m.p. 140^{o}C) and stigmasterol ($C_{29}H_{48}O$; m.p. 170^{o}C). Brassicasterol, the Δ^5 and Δ^7 – avenasterols and Δ^7 – stigmasterol are also present in lesser quantities depending on the plant source. Plant stanols are also less abundant type of sterols that are present in oil seeds and coniferous trees. Therefore commercial plant stanols used for enrichment of foods are prepared by hydrogenation of plant sterols. Cholesterol and plant sterols have similar chemical structures, both chemically characterized as being steroids with 4 ring structures and which contain a hydroxyl group on carbon 3 of the A-ring (Figure 1). Plant sterol structure, specifically, is based on a tetracyclic cyclopenta[*a*]phenanthrene structure that contains a side chain on carbon 17, and two methyl groups on carbons C-10 and C-13, respectively, and along with the hydroxyl group located on C-3 (thus defined is 5α-cholestan-3β-ol). Plant stanols on the other hand are saturated counterparts of sterols, without double bonds at the C-5 position. Plant sterols, like cholesterol, are vulnerable to oxidation reactions, largely due to the presence of unsaturated bonds located at C-5 and C-6 within the steroid nucleus. However, relatively greater resistance to oxidation potential exists for plant sterols as compared to cholesterol due to subtle differences associated with the C-24 side chain. Whereas oxidation of cholesterol occurs both with endogenous enzymatic reactions as well as from external oxidation, the latter attributed to heat and light, plant sterols principally undergo oxidation catalyzed by heat. The unique differences in sterol structure explains the distinct patterns of a number of oxidation products that arise between animal and plant sterol sources exposed to oxidative and thermolytic reactions (Table 1).

The most important major plant sterols consumed in the diet are β-sitosterol, campesterol, and stigmasterol. These plant sterols are often present in an esterified form that is derivatized to C12 - C18 fatty acids. Unrefined vegetable oils, such as rice bran, corn, sesame, soybean and olive contain 100 - 500 mg of plant sterols per 100 g of vegetable oil (Moreau *et al*., 2002). Certain vegetable oils that are rich sources of sterols, like rice bran, wheat germ, and oats may contain up to 4% sterols. Other foods such as nuts, cereals, beans, fruits and vegetable contain a significantly lesser amount of plant sterols, but they also represent a significant dietary source of sterols because of the higher consumption by consumers.

Recent estimates of dietary intakes of plant sterols range from 150-463 mg/day from UK and Germany and 320 mg/day from Spain. The average daily consumption of plant sterols in the Western diet approaches 250 mg, almost equivalent to that of cholesterol, which is 300 mg/day. In contrast, vegetarian and Japanese diets produce a daily consumption of sterols that reach as high as 450 mg/day. Some reduced fat and low fat spreads currently available in the market place may also contain natural plant sterol levels at trace amounts of 0.3% to 0.4%. Collectively these levels of dietary intake from

natural sources represent insufficient amounts to establish meaningful reductions in serum cholesterol. An excellent review of the plant sterol occurrence, nomenclature, chemical structure and detection methods was published by Moreau et al. (2002).

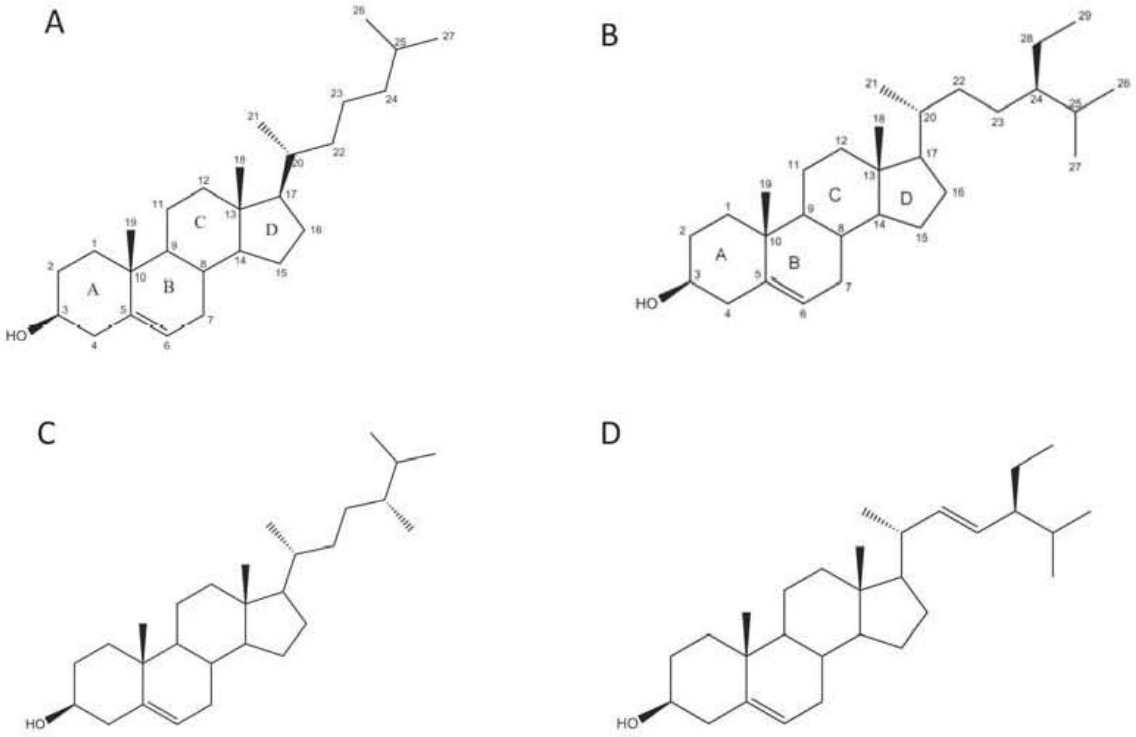

Figure 1. Structures of cholesterol (A) and plant sterols: sitosterol (B), campesterol (C) and stigmasterol (D).

Cholesterol derived: (COPs)	Plant sterol derived (POPs)
7α-hydroxyl cholesterol	7β-hydroxysitosterol
7β-hydroxyl cholesterol	7α-hydroxysitosterol
7-keto cholesterol	7-ketositosterol
5α, 6α-epoxycholesterol	5.6α,β-epoxysitosterol
5β, 6β-epoxycholesterol	25-hydroxysitostanol
3β, 5α, 6β-cholestanetriol	25-hydroxycampesterol
3β, 5α, 6β-cholestanetriol	6-β hydroxycampesterol
24-hydroxy cholesterol	6-β hydroxysitosterol

[1]Yuan *et al*., 1998: Dzeletovic *et al*., 1995; Grandigirard *et al*, 2004; Soupas *et al.* 2005; Menendez-Carreno *et al*., 2008.

Table 1. Known oxidation products derived from animal and plant sterols.[1]

2.1. Solubility

The lipophilic nature of plant sterols implies that they are insoluble in aqueous solutions; however, solubility characteristics of sterols in fat vary depending on many factors, such as the source of the sterol mixture, the type of fat or oil used for its dispersion, and furthermore, the temperature that is used to solubilize the sterol mixture. In general, sterols and mixtures of sterols with stanols have higher solubility in oil compared to stanols alone. This was shown with wood sterol/stanols mixtures, which in one study had relatively greater solubility than sterol mixtures derived from soya. Plant sterol solubility is also decreased two-fold in formulations where water is the continuous phase. Low solubility of sterols in oil/water emulsions has been attributed to the formation of monohydrate molecules. Limited solubility (e.g. 2-6%) of sterols in vegetable oil will also occur at low temperatures below 30^{o}C. However, raising temperatures to a 50-80^{o}C range will improve the solubility by 4 to 5 fold; albeit, recrystallization of the sterol mixture usually occurs when the heated mixture is cooled. The melting point of sterols, which ranges between 138–145°C, will also affect solubility character. Esterifying sterol/stanol mixtures with mid-chain C8-C12 carbon fatty acids lowers the melting point by nearly 40^{o}C. Similar results have been reported with esterifying β-sitosterol, using C6-C14 fatty acids that lowered melting points within a range of 74-87^{o}C. This characteristic follows the inverse relationship known to hold true between log solubility of many aromatic compounds and the melting point. Thus esterification of plant sterols with long-chain polyunsaturated fatty acids will increase solubility by tenfold in fats, thus enabling better use of plant sterols in vegetable fat spreads. Both the hydrocarbon chain length and the degree of unsaturation of the fatty acid used to esterify sterols will influence the relative improvement of sterol solubility within the lipid phase.

2.2. Oxidation Reactions

Cholesterol can be oxidized to a number of products (COPs), both by endogenous P450 enzymes as well as by non-enzymatic, external reactions that involve exposure to heat, air or ultra-violet light that will induce photoxidation. In the case of plant sterols, non-enzymatic, thermolytic reactions result in a large number of potential oxidation products (Figure 2a, b). One interesting exception is the finding that POPs are derived from some cosmetic products applied to the skin and reacted by UV radiation. Plant sterols are however, relatively resistant to thermal processing, compared to cholesterol; albeit, there are some factors that predispose them to specific rates of oxidation and subsequent degradation. These factors include the specific sources of plant sterols, conditions of heat treatment, such as temperature and time interactions, and also specific difference in chemical make up of the oil matrices to which they are heated in. For example, 47, 41 and 40 ppm of POPs were detected in heat processed refined oils such as high-oleic sunflower, palm-rapeseed oil blend and sunflower oil, respectively; and in part reflected the fatty acid composition of the different oils, notwithstanding also the presence of natural antioxidants. As is the case with the oxidation of cholesterol, plant sterol oxidation results in a similar pattern of ketones, alcohols, epoxides, dienes and trienes composed products that characterize both the initiation and propagation phase of the oxidation reaction (Figure 2a, b). In general, sterol oxides generated from plant sterols resemble those of cholesterol oxides; this being largely attributed to the commonality in

various reaction products that occur between the two sterol sources once exposed to oxygen or thermal oxidation conditions (Figure 2a). Subsequent autoxidation reactions occur with primary oxidation products, or hydroperoxides (Figure 2b). Reactions between sterols and oxygen to produce peroxyl radicals lead to a large number of oxidation products, most notably hydroperoxide derivatives, which are typical of the initiation of plant sterol oxidation. The generation of these products contributes to further autoxi autoxidation of sterols at rates that are relatively less, compared to fatty acid oxidation. Notwithstanding this, however, is the accumulation of greater quantities of primary oxidation products that eventually lead to the generation of secondary oxidation products. The rate of sterol peroxide formation and subsequent accumulation are factors that influence the overall rate of sterol autoxi autoxidation during the initial stages of the oxidation reaction. These reactions also collectively explain the contrasting rates of sterol oxidation when in the presence of fatty acids that vary in unsaturation index.

Oxidation of sterols is initiated by oxygen species attacking the plant sterol, resulting in numerous primary and secondary oxidation products that are similar to cholesterol. Plant sterol oxidation produces a number of reaction products identified in Table 1 and possible schemes presented in Figure 2a and 2b. POPs, namely 7β-hydroxy, 7α-hydroxy and 7-keto, epoxy, and triol derivatives are generated from β-sitosterol and campesterol, in variable quantities in different oil blends heated at very high temperatures of 250^{o}C. Generation of 7-keto-β-sitosterol, a non-toxic sterol derived from β-sitosterol oxidation and first reported is regarded as a chemical marker for early phytosterol oxidation. Subsequently, 7-keto derivatives, typically generated from β-sitosterol and campesterol and stigmasterol are commonly recovered and identified from individual elution patterns of reference standards and mass spectrometric information for characterizing sterol oxidation in different foods. Six major oxidation products including, 7α-hydroxy, 7β-hydroxy, 5α,6α-epoxy, 5β,6β-epoxy, 7-keto and 25-hydroxy were detected for β-sitosterol, stigmasterol and campesterol at varying concentrations in rapeseed oil within the first hour of high temperature (e.g. 180^{o}C) processing. Subtle differences in chemical structure of plant sterols can also influence thermal stability characteristics. For example, β-sitostanol is more resistant to high temperature processing (e.g. 180^{o}C) than ergosterol, when heated in rapeseed oil for 24 hr. Similarly, lower losses of β-sitostanol, compared to campesterol, stigmasterol, and sitosterol, respectively, have been reported with deep-fat frying using corn oil. Esterification of sterols with specific fatty acids for the purpose of enhancing solubility can also influence phytosterol oxidation rates. Sterols naturally present and not esterified, had actually lower oxidation rates at 100^{o}C compared to those that were esterified and used for food fortification purposes. There is no appreciable difference in the susceptibility to thermal oxidation between esterified or natural sterols/stanols at higher temperatures (e.g. 180^{o}C), however.

There are numerous studies that have shown that storage duration of foods, rich in animal sterols will influence the generation of COPs; however, storage conditions do not have the same impact to generate POP formation. Heat treatment of animal sterols under similar conditions also produces relatively higher concentrations of COPs compared to POPs. For example, POPs identified from heating β-sitosterol for 1 hour at 150^{o}C could not be detected when the same mixture was heated at lower temperatures

of 120°C and 100°C, respectively. Heating plant sterol mixtures at much higher temperatures of 180°C in the presence of oxygen also resulted in loss of detectable POPs; a finding which was related to the degradation of oxidized products.

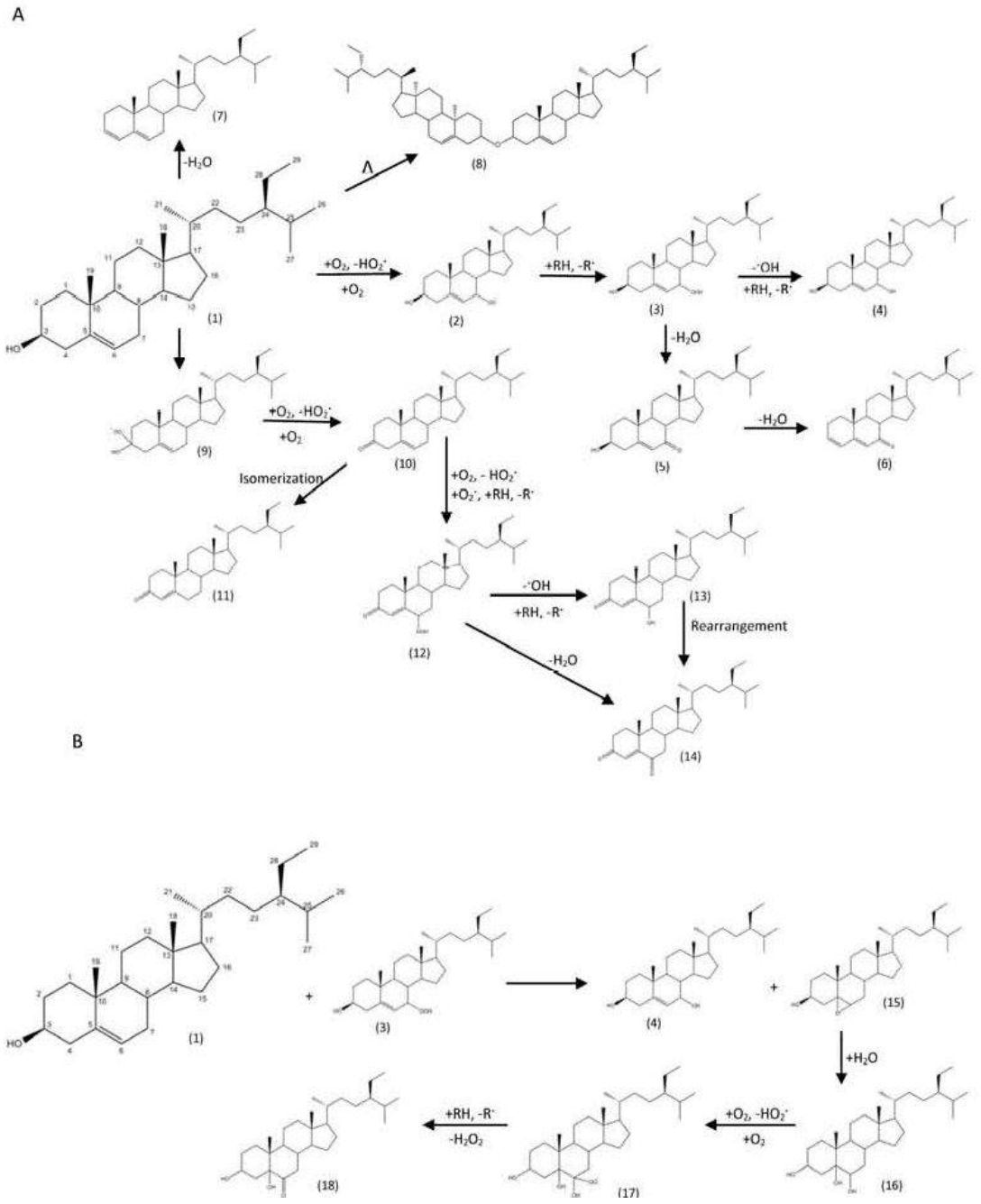

Figure 2. a) Summary of oxidation reactions characterizing the products of β-sitosterol specific to A and B rings. (1)- β-sitosterol, (2,9)-7 peroxyl; 3 peroxyl radicals, (3)-sitosterol hydroperoxide, (4)-sitosterol 7β hydroxide,(5)- 7-keto-3β-sitosterol, (6)-7-keto-sitosta 3,5,diene, (7)-3,5-sitostadiene, (8)-di-β-sitosteryl ether, (10,11)-3-keto-sitosta 3,5, diene isomers, (12)-3-keto-hydroperoxide derivative, (13)-3 keto, hydroxyl derivative, (14)-3,6, keto derivative. b) Autooxidation scheme for β-sitosterol oxidation. (1)- β-sitosterol, (3)-sitosterol hydroperoxide, (4)- 7α hydroxyl sitosterol, (15)- 5α,6α epoxide,(16)- sitostan-3β,5α,6β triol (17) peroxyl radical (18)- 6 keto, 3β ,5α, sitosterol

A similar finding has been reported for POPs degradation in sterol enriched milk, where main factors such as level of heat treatment and time were shown to be relevant in reducing POPs that were microwaved for two minutes, compared to 1.5 minutes. Finally, higher concentrations of POPs in crude compared to heat processed, refined oils have been reported, thus supporting the theory that oxidation products derived from plant sterols are heat liable and will degrade at high temperatures.

The chemical composition of the food matrix which contains plant sterols is another important factor in the generation of POPs. It has been observed that a mixture of β-sitosterol, stigmasterol and campesterol was effective at inhibiting methyl linoleate oxidation during a heating process. Wood sterols in free form were more effective at protecting against thermal oxidation of canola oil when compared to sterol esters. For example, heat processing a mixture of 5% wood sterols in different oils at 180^{o}C for 6 hours produced losses in sterol concentrations that ranged between 5 to 20%; a finding that was also dependent on the source of the vegetable oil used in the thermal process and the susceptibility to degradation. Moreover, reduced polymerization of oxidized oils, attributed mostly to thermal degradation of oils, arising from heat induced oxidation during frying, has also been reported for non-esterified phytosterol mixtures. Many workers have postulated that the relative thermal instability observed for plant sterols is dependent on the interaction between temperature of thermal processing and the degree of unsaturation of the cooking oil. For example, losses of sterols in sterol enriched rapeseed oil have been reported to be relatively higher in hydrogenated oil compared to regular deodorized oil. POPs generated in palm-rapeseed oil blends far exceeded those generated using high-oleic acid sunflower oil when heated at 250^{o}C for 15 min. Furthermore, the presence of sterols in olive oil inhibited thermal oxidation of the oil when heated at 180^{o}C. Thus, sterols are more susceptible to thermal oxidation when the lipid matrix is predominately saturated (Lampi *et al*., 2002). The specific temperature of heating however is important in recognizing the relative importance of lipid matrix composition on sterol oxidation rates. Comparatively lower heating temperatures below 120^{o}C result in higher POPs when the food matrix is predominantly unsaturated lipid. In contrast POPs are generated more so when the matrix is saturated fat when exposed to higher temperatures (e.g. >150^{o}C) (Soupas *et al*., 2004). This observation suggests that sterol oxidation rates, arising from exposure to high temperature processing are influenced by the presence of unsaturated fatty acids, which are more prone to oxidation. As a result, fatty acid(s) present in the matrix are preferentially oxidized when in combination and thereby provide some degree of protection to the plant sterols (Soupas *et al*., 2005). The extent of sterol oxidation is also greater when present in emulsions, compared to bulk oils, due to the surface activity of the sterol/stanol, which in turn enhances the probability of chemical interactions associated with prooxidant reactions. The localization of sterols into the lipid phase of an oil-to-water emulsion increases the susceptibility to oxidize. Taken together, these results indicate that the induction of free lipid radicals when accelerated by thermal oxidation will indeed react preferentially with plant sterols at allylic carbon atom sites, thus forming stable isomers that in turn retard further oxidation of fatty acids, by interrupting the process of autoxidation. A scheme for this is shown in Figure 2a. This mechanism is supported by data that showed avenasterol, but not β-sitosterol, was affective at preventing deterioration of soybean oil at 180^{o}C. β-sitosterol lacks a double bond on its side chain, thus providing a lesser capacity to sequester lipid radicals. This

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