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# Stabilisation of phytosterols by natural and synthetic antioxidants in high temperature conditions

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ABSTRACT

The aim of the study was to assess the potential applicability of natural antioxidants in the stabilisation of phytosterols. A mixture of β-sitosterol and campesterol was incorporated into triacylglycerols (TAGs). The following antioxidants were added to the prepared matrix: green tea extract, rosemary extract, a mix of tocopherols from rapeseed oil, a mix of synthetic tocopherols, phenolic compounds extracted from rapeseed meal, sinapic acid and butylated hydroxytoluene (BHT). Samples were heated at a temperature of 180 °C for 4 h. After the completion of heating, the losses of phytosterols were analysed, as well as the contents of β-sitosterol and campesterol oxidation products. The total content of phytosterol oxidation products in samples ranged from 96.69 to 268.35 μg/g of oil. The effectiveness of antioxidants decreased in the following order: phenolic compounds from rapeseed meal > rosemary extract > mix of tocopherols from rapeseed oil > mix of synthetic tocopherols > green tea extract > sinapic acid > BHT.



## 1. Introduction

The capacity of phytosterols to reduce blood cholesterol levels, observed as early as the 1950s (Pollak, 1953), is at present the primary property with which these compounds are associated. Numerous findings have confirmed the positive effect of the daily consumption of 2–3 g phytosterols and phytostanols (saturated forms of sterols) on the reduction of the total contents of cholesterol and its LDL fraction in human blood by several to around a dozen percentage points. Previous investigations resulted in the launch of the first margarine with an addition of plant sterols, Benecol®, in 1995 by Raiso, Finland. At present, there are numerous products available on the food market (milk, cheeses, yoghurts, mayonnaises, chocolate products) enriched with plant sterols and stanols and aimed at a reduction of blood cholesterol levels.

However, phytosterols undergo oxidation. The presence of double bonds in phytosterol molecules (Fig. 1) makes them sensitive to the effect of light, metal ions, pigments, enzymes and elevated temperature. Under the influence of these factors, phytosterol oxidation occurs, most frequently as a result of autoxidation reactions, leading to the formation of phytosterol oxidation products (POPs), such as 7-hydroxy, 7-keto, epoxy, 25-hydroxy and triols of sterols (Soupas, 2006). The reaction scheme of the formation of POPs during autooxidation is shown in Fig. 2. Since vegetable oils are the primary natural source of phytosterols in the human diet and they contain 70–1100 mg sterols per 100 g oil, they may also be a source of POPs formed during oxidation which have an adverse effect on the human organism. The content of phytosterol oxidation products in oils may depend, e.g., on the manner of their production, storage method or the type of oil (Dutta, 2004; Rudzińska, Kazuś, & Wąsowicz, 2001). A much higher increase in the levels of derivatives of oxidised phytosterols is observed during the storage of oil at elevated temperature (Cercaci, Rodriguez-Estrada, Lercker, & Decker, 2007), as well as during the heating of vegetable oils (Johnsson & Dutta, 2006; Kmiecik et al., 2011).

The rate of thermo-oxidative changes may be reduced due to the application of substances with antioxidant properties. These

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substances may be synthetic (butylated hydroxytoluene – BHT, butylated hydroxyanisole – BHA, *tert*-Butylhydroquinone –TBHQ) or come from natural sources. Natural antioxidants, which are at present used by many researchers to stabilise fats, are a much bigger group of compounds and originate mainly from two sources: vegetable oils and tissues of other plants, vegetables and herbs. Native antioxidants found in oils include tocopherols, tocotrienols, sesamol, sesaminol and their isomers, oryzanol, squalene and certain phytosterols (Warner, 2005). Another group of natural antioxidants comprises extracts produced from tea leaves, rosemary, sage, as well as other plants such as thyme, oregano, marjoram, oat or peanut husks. The antioxidant properties of these extracts result from the presence of the flavonoids, phenolic acids and selected sterols they contain (Amarowicz et al., 2008; Kobus et al., 2009).

The potential applicability of natural antioxidants in the stabilisation of oil and its components subjected to heating has been confirmed, e.g., by Khan and Shahidi (2001) and Besmira, Jiang, Nsabimana, and Jian (2007). Applied natural antioxidants and their mixtures frequently exhibit a similar or higher antioxidant activity than the applied synthetic antioxidants.

The aim of this study was to assess the potential applicability of antioxidants obtained from natural sources (rapeseed meal, rosemary, green tea and crude oil) in the stabilisation of phytosterols during the heating of the triacylglycerols of oil in model systems.

## 2. Materials and methods

### 2.1. Materials

Triacylglycerols (TAGs) were collected from rapeseed oil purchased in a local market using column chromatography according to Chimi, Cillard, and Cillard (1994). Rapeseed oil was dissolved in hexanes (1:3, v/v) and run through a glass column packed with activated carbon, aluminium oxide (activated at 300 °C), and anhydrous sodium sulphate. During the refining process, the column and receiver system were shielded from external light. Next, the recovered hexanes were removed under a vacuum at <40 °C, after which the triacylglycerols free of endogenous antioxidants were sealed in an amber vial under a nitrogen headspace. TAGs were stripped from the oil directly before conducting the planned experiments.

Rosemary extract was prepared from rosemary leaves (*Rosmarinus officinalis* L.) via ethanolic extraction according to Gramza-Michałowska, Abramowski, and Jovel (2008). Green tea extract was prepared from China Lung Ching leaves (*Camellia sinensis* L.) via ethanolic extraction according to Gramza-Michałowska, Hęś, and Korczak (2008). Natural tocopherols were obtained from crude rapeseed oil via a three-step crystallisation process according to Szulczewska-Remi, Kałucka-Nogala, Kwiatkowski, Lampart-Szczapa, and Rudzińska (2005). Phenolic compounds from rapeseed meal were obtained according to Wanasundara, Amarowicz, and Shahidi (1994). Briefly, rapeseed was ground and defeated with petroleum for 12 h using a Soxlet apparatus and dried. Phenolic compounds were obtained via double ethanolic extraction with 95% ethanol for 20 min at 80 °C. After extraction, alcohol was evaporated under a vacuum at 40 °C.

The mix of phytosterols (β-sitosterol – 75%, campesterol – 14.5%, β-sitostanol – 10.5%) and sinapic acid were purchased from Sigma-Aldrich (Sigma–Aldrich St. Louis, MO, USA). α-tocopherol (synthetic, 95% grade) was purchased from Sigma (Germany). β-, δ-, γ-tocopherols (synthetic) were purchased from Eisai (Japan). Butylated hydroxytoluene (BHT) was purchased from Merck (Germany). Standards for the identification of phytosterols (β-sitosterol, campesterol, stigmasterol, and brassicasterol) and oxyphytosterols (5α-cholestane, cholestane-3β,5α,6β-triol, 5α, 6α-epoxy-cholestane-3β-ol, 5-cholestane-3β,19-diol, 5-cholestane-3β,7β-diol, 5β,6β-epoxy-cholestane-3β-ol, 5-cholestane-3β-ol-7-one) were purchased from both Sigma–Aldrich (Sigma–Aldrich St. Louis, MO, USA) and Steraloids (Steraloids, Newport, RI, USA).

### 2.2. Sample preparation

In the first step, a mix of phytosterols (2%) was dissolved in chloroform, added to triacylglycerols and then the solvent was removed under a vacuum at <40 °C. In the second step, the following were added to a mix of TAGs and phytosterols (5 g): ethanol extract of rosemary (0.02%), ethanol extract of green tea (0.1%), a mix of tocopherols extracted from oil (natural tocopherols) (0.02%), a mix of synthetic tocopherols (0.02%), phenolic compounds extracted from rapeseed meal (0.02%), sinapic acid (0.02%) and BHT (0.02%). Then, the solvent was evaporated. The mix of synthetic tocopherols consisted of α-, β-, δ- and γ-tocopoherol. The concentrations of these components were 24, 0.1, 45 and

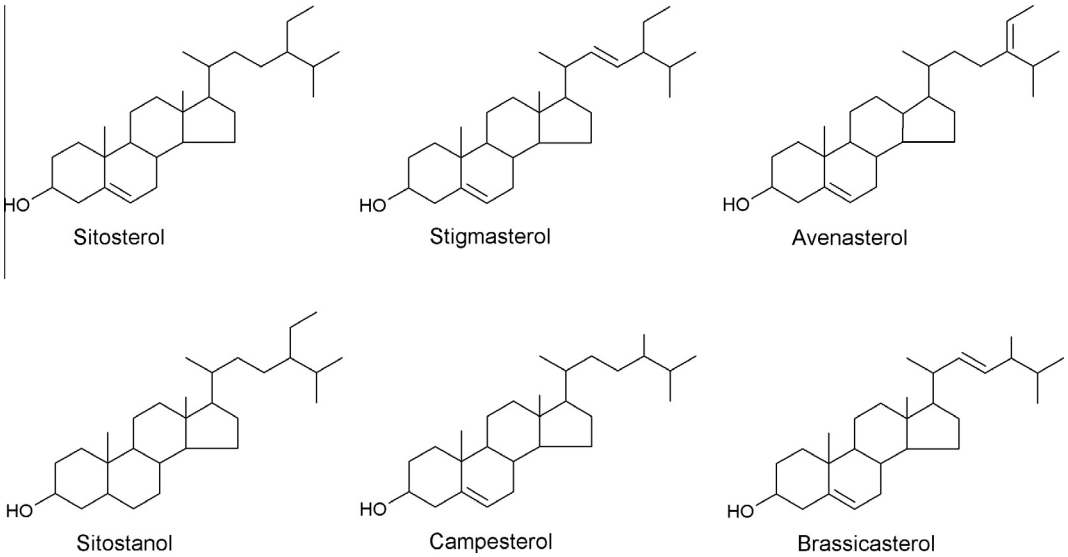


**Fig. 1.** Chemical structures of phytosterols and phytostanols.