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Model studies on the formation of volatile compounds generated by a thermal treatment of steryl esters with different fatty acid moieties

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ABSTRACT

The consumption of plant sterols is reported to have a beneficial effects on human health, i.e. phytosterols are known for their cholesterol-lowering properties. Whereas, they are prone to oxidation and currently there is ongoing worldwide research aimed at the biological effect of phytosterol oxides. In this study volatile compounds formed during thermal degradation of stigmasteryl esters were identified. The research was conducted using standards of stigmasterol, fatty acids and stigmasteryl esters as well as fat enriched with stigmasteryl esters which were thermally treated at 60 °C and 180 °C for 12 h. Volatile compounds were characterised by SPME-GC-MS. Among the volatiles formed during heating of stigmasteryl esters aldehydes, ketones, alcohols and hydrocarbons were found. The mechanism of the formation of volatile compounds from sterol esters was related to oxidation of steryl and fatty acid moieties. In particular, 2-methyl-3-pentanone and 5-ethyl-6-methyl-3-hepten-2-one were identified as unique degradation products formed from degradation of the steryl moiety specifically, and a mechanism of their formation was suggested. Both volatiles could be a good indicator of thermo-oxidative degradation of functional food products enriched in phytosterols and their esters.

1. Introduction

The consumption of plant sterols, also referred to as phytosterols, is reported to have beneficial effects on human blood lipids. These compounds lower the total and LDL fractions of plasma cholesterol and by this function reduce the risk of coronary heart disease (CHD), which nowadays is the major risk factor causing death in the world. Because of these properties, an increasing number of commercial food products is enriched in phytosterols. However, solubility of free plant sterols in food products is limited and to improve solubility in lipids they are transferred into fatty acid esters (Moreau, Whitaker, & Hicks, 2002). Phytosteryl esters after ingestion are hydrolysed to free sterols which limit absorption of cholesterol from the intestine (Thompson & Grundy, 2005). Currently, many products are fortified with steryl esters, including: fat spreads, pasta, milk based fruit drinks, cheese and egg products, sauces, salad dressings, bread, sausages, soups and puddings (FDA, 2005; , 2006a; , 2006b). Cooking oils enriched with phytosteryl esters are also marketed to restaurants and catering companies in the US and Japan (Watkins, 2005).

Phytosteryl esters, endogenous vegetable oil components are present at different amounts and variety in vegetable oils. In canola, rapeseed, corn, peanut, avocado, evening primrose and sunflower oils esterified sterols are predominate components of unsaponifiable matter, where free sterols contributing 32–44% of total sterols content (Phillips, Ruggio, Toivo, Swank, & Simpkins, 2002). Both moieties of phytosteryl esters are susceptible to oxidative degradation which usually leads to nutritional losses, formation of undesirable flavour, colour and toxic compounds, which make food unacceptable to human consumption. Oxidation of phytosterols is leading to the formation of oxidation products (oxyphytosterols, POPs), followed by their degradation to volatile compounds (Rudzińska, Przybylski, & Wąsowicz, 2009). The stability of phytosterols during heating and storage has been studied and its degradation is affected by chemical structure, temperature and time of heating (Lampi, Juntunen, Toivo, & Piironen, 2002; Rudzińska, Przybylski, & Wąsowicz, 2014; Sosińska, Przybylski, Hazendonk, Zhao, & Curtis, 2013). Numerous volatile compounds have been observed during thermo-oxidative degradation of phytosterols, and most of them are typical compounds which forming off-flavour and rancidity in lipids (Rudzińska et al., 2009).

When cholesterol was stored at ambient temperature, 14 volatile compounds were identified as decomposition products of cholesterol hydroperoxides (Van Lier, Da Costa, & Smith, 1975). Since phytosterols

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share a similar chemical structure, formation of volatile compounds follow the same mechanism. However, the presence of double bond(s) in the side chain may further stimulate degradation process. Rudzińska et al. (2009) proposed possible degradation mechanism of sterols forming fragmented sterol molecules and volatile compounds.

The volatiles produced from oxidation of fatty acids are influenced by the type of double bonds cleavage, leading to the formation of off-flavour and detrimental compounds in stored and thermally treated oils (Kim & Min, 2008). Additionally, the formation of volatiles and other components is a source of free radical formation, directly stimulating/initiating oxidative degradation of other endogenous oil/food constituents. Volatile compounds formed during oxidation of fatty acids are applied as quality indicators for fats and oils. During phytosterols oxidative degradation off-flavour compounds also are formed (Rudzińska et al., 2009).

Up to our knowledge, research describing the effect of fatty acid unsaturation on the stability and degradation of phytosteryl esters is lacking and rather describe the effect of fatty acids used as heating medium. Xu, Sun, Liang, Yang, and Chen (2011) studied the cholesterol and β-sitosterol degradation during heating at 180 °C for 120 min using different fatty acids as matrix. It was concluded that the effect of a fatty acid on oxidation of cholesterol and β-sitosterol was time-dependent and most unlikely related to the amount of double bonds. Functional additives added to food products consist of fatty acids and sterols connected in one molecule of esters. Margarines enriched with phytosteryl esters are labelled as applicable for cooking and baking, following its thermal instability this is an overstatement.

The goal of this study was to establish thermal degradation mechanism of volatile compounds formation from phytosteryl esters, the later often used as functional ingredients in food products designed for thermal processing. Thus, for a comprehensive study of volatiles formed during thermal oxidation of phytosteryl esters four different types of experiments were performed: (i) determination of volatiles originating from fatty acids after heating; (ii) determination of volatiles originating from free stigmasterol after heating, (iii) determination of volatiles from heated stigmasteryl esters, and (iv) identification of stigmasterol volatile derivatives in fat enriched in stigmasteryl esters.

2. Materials and methods

2.1. Materials

Stigmasterol (~95%), stearic acid (≥98.5%), oleic acid (≥99%), linoleic acid (≥99%), linolenic acid (≥99%), dicyclohexyl carbodiimide (DCC), 4-dimethylamino pyridine (DMAP), dichloromethane (≥99.8%), hexane (≥97%), ethyl acetate (≥99.7%), silica gel (70–230 mesh, high purity), cholesteryl esters: stearate (96%), oleate (98%), linoleate (98%), linolenate (97%) were purchased from Sigma-Aldrich (St. Luis, MO, USA). The divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fibre was purchased from Supelco (Bellefonte, USA). Palm stearin was obtained from ZT "Kruszwica" SA (Poland). Its fatty acid composition was as follows: C14:0–1%, C16:0–55%, C18:0–5%, C18:1–32% and C18:2–7%. The total content of phytosterols was 0.2 mg/g, while that of stigmasterol was 0.02 mg/g.

2.2. Esterification of stigmasterol

Chemical esterification (Neises & Steglich, 1978) was used to obtain esters of stearic, oleic, linoleic and linolenic acids and stigmasterol. Briefly, 500 mg of stigmasterol was dissolved in 30 mL of dichloromethane and placed in a three-necked flask. The air was replaced by argon and catalysts – DCC (500 mg) and DMAP (15 mg), and 600 mg of fatty acid were added. Esterification was run at room temperature for 24 h in the dark. Then reaction mixture was placed in a separatory funnel, 10 mL of distilled water was added and the entire mixture was shaken. The lower layer was collected into the flask. The removing of water fraction was repeated three times. The solvent from the collected fractions was evaporated in vacuum at 30 °C and the residue was dissolved in 20 mL of hexane. The mixture after esterification was further cleaned using a silica gel column (45 cm × 2.5 cm), eluting the clean fraction with 450 mL of hexane: ethyl acetate (9:1, v/v). Purity of the fraction was verified by TLC comparing it with the cholesteryl oleate standard. Quality of esterified esters was determined by NMR and GC-MS.

Four esters, stigmasteryl stearate (StS), stigmasteryl oleate (StO), stigmasteryl linoleate (StL) and stigmasteryl linolenate (StLn), were synthesised.

2.3. Preparation of fat enriched in stigmasteryl esters

Fat was enriched in stigmasteryl esters using their mixture. It contained StS, StO, StL and StLn (1:1:1:1, w/w/w/w). A sample of 1 g fat was supplemented with 80 mg ester mixture.

2.4. Sample heating

Samples of 1.5 g enriched fat or 0.5 g of each stigmasteryl ester as well as free stigmasterol and fatty acids were singly placed into 200 mL glass ampoules and before sealing filled with stoichiometric amounts of oxygen to prevent oxygen starvation. Samples were heated at 60 °C and 180 °C for 12 h. All heat treatments were performed in duplicate. Volatiles were extracted three times from individual ampoules. The non-heated samples of esters were used as the control.

2.5. Analysis of volatile compounds by SPME-GC-MS

Headspace solid-phase micro-extraction (SPME) was used to isolate volatile compounds from non-heated and heated samples. Volatiles were adsorbed on the three-phase fibre (DVB/CAR/PDMS) as previously described (Rudzińska et al., 2009). The fibre was firstly conditioned in the GC injection port at 270 °C for 4 h. The conditioned fibre was exposed to a headspace of the sample for 5 min at room temperature, after extraction the fibre was retracted into a needle and transferred to an injection port of a gas chromatograph. Desorption of volatiles was run in the GC injection port for 5 min at 260 °C in the splitless mode. Volatiles were analysed on a 7890A GC system (Agilent Technologies) coupled to a 5975C VL Triple-Axis mass detector (Agilent Technologies). Separation was run on a DB-5MS capillary column (25 m × 0.2 mm; 0.33 μm film thickness; J&W, Folsom, CA) with helium as a carrier gas at a flow rate of 0.6 mL/min. The injector and transfer line were held at 260 °C and 280 °C, respectively. The column temperature was programmed as following: the initial temperature at 40 °C was held for 3 min, then increased at 4 °C/min to 160 °C and further increased at 10 °C/min to 280 °C, with the final temperature held for 3 min. Mass spectra were recorded in an electron impact mode (70 eV) and masses were scanned from 33 to 333 Da. Relative peak areas were used for sample comparison. Retention indices were calculated for each compound using a homologous series of C7–C24 n-alkanes (van den Dool & Kratz, 1963). Volatiles were identified based on the comparison of the mass spectrum of a compound with the NIST 05 library match and comparison of retention indices (RI) with those available in literature (Adams, 2007).

3. Results and discussion

Because the standards of phytosteryl esters are not commercially available, we esterified stigmasterol using fatty acids with different unsaturation levels and heated them at 60 °C and 180 °C, respectively, to simulate storage (as in the accelerated Schaal oven test) and frying conditions. Stigmasterol was used in these experiments as a plant sterol representative, because the highest purity standard was commercially available. Volatile compounds formed after heating were identified by SPME-GC-MS.