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Oxidation of sitosterol and campesterol in foods upon cooking with liquid margarines without and with added plant sterol esters

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Chemical compounds studied in this article:
Sitosterol (PubChem CID: 222284)
7-Hydroxysitosterol (PubChem CID: 161816)
7-Ketositosterol (PubChem CID: 160608)
5,6-Epoxysitosterol (PubChem CID: 11258970)
Sitostanetriol (PubChem CID: 3036251)
Campesterol (PubChem CID: 173183)
7-Hydroxycampesterol (PubChem CID: 101112100)
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5,6-Epoxycampesterol (PubChem CID: 101112101)
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ABSTRACT

Plant sterol (PS) oxidation products (POP) derived from sitosterol and campesterol were measured in 15 foods cooked with liquid margarine without (control) and with added 7.5% PS. POP were analyzed using a GC–MS method. PS liquid vs. control margarine resulted in a higher median POP content per food portion (1.35 mg, range 0.08–13.20 mg versus 0.23 mg, 0.06–0.90 mg), a lower PS oxidation rate (0.63 vs. 1.29%) and lower oxidation susceptibility of sitosterol vs. campesterol. POP formation was highest in shallow-fried potatoes with PS liquid margarine (64.44 mg per portion food plus residual fat). Mean relative abundances of epoxy-, 7-keto-, 7-hydroxy- and triol-PS derived from sitosterol and campesterol were 40.0, 34.4, 21.5 and 4.0% with control vs. 44.1, 23.8, 29.6 and 2.4% with PS liquid margarine. In conclusion, PS liquid margarine increased POP content in foods with a POP profile characterized by a higher ratio of epoxy- to 7-keto-derivatives.

1. Introduction

Plant sterols (PS) are naturally occurring in all plant-based foods and are especially found in vegetable oils, nuts, seeds and grains. Because of their established plasma cholesterol lowering activity, (Gylling et al., 2014; Ras, Geleijnse, & Trautwein, 2014) PS mostly in the form of PS esters are added to foods, most commonly to fat-based products like margarine and spreads (Garcia-Llatas & Rodriguez-Estrada, 2011; Willems, Blommaert, & Trautwein, 2013). PS in foods are susceptible to auto-oxidation (also called non-enzymatic oxidation) to form PS oxidation products (POP). Adding PS to fat-based products may increase POP contents in foods, especially when these fat products are exposed to heat treatment such as during household cooking (Lin, Knol, & Trautwein, 2016). It has been reviewed that POP may have both beneficial (anti-diabetic and anti-carcinogenic properties) and undesirable effects (associated with atherogenicity, inflammation, cytotoxicity, and oxidative stress) based on in vitro and animal studies (Alemany, Barbera, Alegria, & Laparra, 2014; Garcia-Llatas & Rodriguez-Estrada, 2011; O'Callaghan, McCarthy, & O'Brien, 2014; Vanmierlo, Husche, Schott, Pettersson, & Lutjohann, 2013). Data about physiological effects of POP in humans are scarce.

Typical POP formed in foods via auto-oxidation are 7-hydroxy- (7-OH-), 7-keto-, 5,6-epoxy-(epoxy-) and 3,5,6-triol-(triol) derivatives of PS (Alemany et al., 2014; Lin, Knol, & Trautwein, 2016). These individual POP can also be found in human plasma (Baumgartner, Mensink, Husche, Lutjohann, & Plat, 2013; Grandgirard et al., 2004; Husche et al., 2011). In humans, consuming ca. 0.6 mg/d POP with a PS-added margarine (3 g PS/d) for 4 weeks resulted in a small

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increase of plasma total POP by 0.0015 μmol/L (Baumgartner et al., 2013; Husche et al., 2011).

Available data show that individual POP seem to be different in their biological properties. For example, Grandgirard et al. (Grandgirard, Sergiel, Nour, Demaison-Meloche, & Ginies, 1999) described that epoxy derivatives of PS had a higher absorption rate than 7-keto derivatives in rats (4.7% versus 1.5%). Tomoyori et al. (Tomoyori et al., 2004) reported that in rats oxidation products of sitosterol (POP-sito) had a lower absorption rate than corresponding oxidation products of campesterol (POP-camp) (9.1% versus 15.9%). Further, in vitro studies suggest that individual POP have different physiological and pathological properties from both a qualitative and quantitative point of view (Maguire, Konoplyannikov, Ford, Maguire, & O'Brien, 2003; Ryan, Chopra, McCarthy, Maguire, & O'Brien, 2005). For example, 7β-OH-sitosterol and 7-keto-sitosterol at 60 μM increased apoptosis in cultured U937 cells (a human lymphoblast cell line), while epoxy- and triol-derivatives of sitosterol did not at the same concentration (Ryan, Chopra, McCarthy, Maguire, & O'Brien, 2005). Roussi et al. (Roussi et al., 2005) demonstrated that 7β-OH-sitosterol exerted less severe cytotoxic effects than 7β-OH-cholesterol in Caco-2 cells, in spite of their structural similarities. 7-keto-stigmasterol showed no cytotoxicity and even reduced the toxicity of 7-keto-cholesterol in Caco-2 cells (Alemany, Laparra, Barbera, & Alegria, 2012). Clearly, not only POP contents, but also the POP profiles, i.e. the relative abundance of individual POP, are important parameters that should be considered. Previous studies including our own (Lin, Knol, Menendez-Carreno, et al., 2016; Lin, Knol, & Trautwein, 2016; Scholz, Guth, Engel, & Steinberg, 2015) report total POP contents in various foods prepared with different cooking and baking methods. However, the POP profiles, i.e. the relative abundance of 7-OH-PS, 7-keto-PS, epoxy-PS and triol-PS derived from individual PS, in various foods cooked with PS-added fat products such as margarine are scarcely described. Although Soupas et al. (Soupas, Huikko, Lampi, & Piironen, 2007) and our own study (Lin et al., 2017) assessed individual POP formation using various fat products as model for pan-frying in the absence of foods, these studies do not properly reflect individual POP formation in foods prepared under household cooking conditions. Sitosterol and campesterol are the two major individual PS present in foods and in the PS ester formulation that is typically added to foods for PS enrichment. Whether sitosterol and campesterol are equally susceptible to oxidation under different food cooking methods is unknown. Furthermore, profiles of POP derived from sitosterol (POP-sito) and campesterol (POP-camp) in foods upon cooking have so far not been reported in detail.

Therefore, the aim of this study was to investigate formation of POP and especially the profiles of POP-sito and POP-camp in foods prepared by typical household cooking methods including shallow- and stir-frying, stewing, roasting and microwave cooking using liquid margarines without and with added PS.

2. Materials and methods

2.1. Margarines and food ingredients used for cooking

Liquid margarines made from mixtures of vegetable oils without (control) and with added 12.5% PS esters (equivalent to 7.5% free PS, referred as PS liquid margarine) were manufactured at Unilever R & D, Vlaardingen, NL. The PS esters were obtained from BASF, Germany. PS were esterified to sunflower oil. The added PS esters mainly replaced water content of the margarine. No antioxidants e.g. beta-carotene were added to these margarines. The composition of the two margarines are shown in Table 1. They were similar in total fatty acid content (66–68 g/100 g), while compared to control, PS liquid margarine contained more monounsaturated fatty acids and less polyunsaturated fatty acids, which is not fully attributed to the profile of fatty acids esterified with PS. Sitosterol was the most abundant PS, followed by campesterol and stigmasterol. Measured amounts of total POP in the two margarines were low (< 1 mg/100 g product) and consisted mainly of 7-keto-PS in control and epoxy-PS in PS liquid margarine (Table 1).

Table 1
Composition of the control and PS liquid margarine per 100 g product.

	Control liquid margarine	PS liquid margarine
Energy, kJ (kcal)	2430 (586)	2515 (602)
Total fatty acids (FA), g	65.6	67.9
Saturated FA, g	6.3	5.4
Monounsaturated FA, g	29.2	41.1
Polyunsaturated FA, g	29.6	20.8
Added plant sterols (PS)[$], g	–	7.5
Measured PS[^], mg	347.6 ± 13.0	7619.4 ± 359.1
Sitosterol, mg	207.9 ± 7.0	6278.1 ± 296.9
Campesterol, mg	96.6 ± 2.8	1052.8 ± 77.3
Stigmasterol, mg	9.5 ± 1.2	38.6 ± 14.5
Brassicasterol, mg	33.6 ± 2.6	249.9 ± 31.4
Measured POP[#], mg	0.40 ± 0.10	0.75 ± 0.07
7-OH, mg	0.06 ± 0.01	0.12 ± 0.03
7-keto, mg	0.31 ± 0.10	0.23 ± 0.05
5,6 epoxy, mg	0.01 ± 0.00	0.33 ± 0.06
Triols, mg	0.01 ± 0.01	0.07 ± 0.04
Water, g	32.7	22.9
Others[*], g	1.7	1.7

[$]PS were added as 12.5 g PS esters, representing 7.5 g PS and 5 g fatty acids.
[^]Only the listed four major plant sterols were included in the sum, while stanols and minor plant sterols were not accounted. Data are mean ± SD of 8 measurements.
[#]Individual POP derived from sitosterol, campesterol and stigmasterol were summed up. Data are mean ± SD of 8–9 measurements.
[*]Includes small amounts of protein, carbohydrates, vitamins and minerals.

Fresh vegetables (green beans, cabbage and onions), fish (cod, salmon and frozen fish fingers), meats (pork, beef and chicken) and eggs used for the cooking experiments were purchased at whole sale in the Netherlands. Pre-cooked small potatoes and minced meat (50/50 beef/pork mixture) were purchased in a local supermarket. Portion sizes of each food ingredient varied from 100 to 250 g (before cooking) representing a typical portion customarily consumed per eating occasion by 1–2 individuals (Supplementary Table I). Typically, 20 g of control or PS liquid margarine was used for cooking one portion of food; only for roasting beef, 70 g of control or PS liquid margarine and ca. 1 kg of beef were used (Supplementary Table I) and the portion size was defined as 200 g roasted (cooked) beef.

2.2. Cooking methods

The applied cooking methods (shallow- and stir-frying, stewing, roasting and microwaving) were described in detail previously (Lin, Knol, Menendez-Carreno, et al., 2016). A digital infrared thermometer was used to measure the temperature on the surface of the pan or wok during cooking. An overview of cooking methods and amounts of food ingredients used, cooking temperatures and times and weights of foods and residual fat after cooking is presented in Supplementary Table I. In total 15 different foods were prepared. Each food preparation using either the control or PS liquid margarine was repeated five times.

All used cooking utilities and equipment, like a stir-frying wok (cast iron bottom ø 16 cm and upper side ø 36 cm), a frying pan (Teflon coated cast iron bottom ø 22 cm), electromagnetic (induction) stove, microwave oven and electric oven were domestic appliances.

2.3. Food sampling, lipid extraction and chemical analysis of POP

Weights before (raw materials) and after cooking were measured for each food. For cooked foods (except roasted beef), the total amount of prepared food and the total amount of residual fat remaining in the pan or wok were separately collected. For roasted beef, one quarter of the total roasted meat was sampled by cutting the piece of meat lengthwise and crosswise. All food samples were put into 500 mL pre-labelled dark glass containers, and the residual fat into 20 mL glass containers, all