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Pan-frying may induce phytosterol oxidation

Laura Soupas *, Laura Huikko, Anna-maija Lampi, Vieno Piironen

Department of Applied Chemistry and Microbiology, University of Helsinki, Latokartanonkaari 11, P.O. Box 27, FIN-00014, Helsinki, Finland

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Abstract

The oxidative stability of different phytosterol compounds during pan-frying was evaluated. Rapeseed oil, rapeseed oil-based liquid margarine and butter oil were used as lipid matrices. Gas chromatographic–mass spectrometric analyses indicated that pan-frying may induce phytosterol oxidation but has no marked effect on phytostanol oxidation; up to 5.1% of original sitosterol and 0.1% of original sitostanol were found as oxides. High-performance size-exclusion chromatographic determinations also revealed a moderate degree of polymerization of lipid matrices. The extents of these deteriorative reactions were determined by frying temperature and time, but also by unsaturation degree and water content of the lipid matrix as well as by structure of the phytosterol compound added. The highest phytosterol oxide content was measured in saturated butter oil enriched with free phytosterols, whereas the highest matrix dimer and polymer contents were found in free phytosterol-enriched liquid margarine, which contained 20% of water.

Keywords: Phytosterol; Phytosteryl ester; Phytostanyl ester; Oxidative stability; Pan-frying

1. Introduction

Fried foods, both deep-fried and pan-fried, are enjoyed by people worldwide. Both frying methods are popular because they are fast and produce palatable foods with unique sensory properties, namely colour, flavor and texture. The formation of these positive features is, however, accompanied by undesirable phenomena; during frying, the oil, as a frying medium, undergoes a series of physical and chemical reactions, including hydrolysis, oxidation and thermal decomposition (Dobarganes, Màrquez-Ruiz, & Velasco, 2000; Gupta, 2004; Orthoefer & Cooper, 1996). As these reactions proceed, the functional, sensory and nutritional qualities of the frying medium change, eventually reaching a point where it is no longer possible to prepare a high-quality fried food (Warner, 2004). The quality of the frying medium is important since, through absorption, it contributes to the quality of the final product (Orthoefer, Gurkin, & Liu, 1996).

An interesting up-to-date aspect of frying is the possibility to use spreads enriched with phytosterol compounds for frying purposes. At the moment, phytostanol-enriched spreads, at least, are advertised as suitable for cooking and frying. The possibility of developing phytosterol-enriched cooking oils has also been considered (Hicks & Moreau, 2001). Not much, however, is known about the effects of the frying process on the oxidative stability of phytosterol/-stanol-enriched frying media. Only a few studies have been conducted on phytosterol oxidation during deep-frying (Dutta & Appelqvist, 1996; Dutta, 1997), and none, to our knowledge, on pan-frying.

Based on studies assessing the oxidative stability of different vegetable oils during pan-frying, the combination of high temperature and a large surface-to-volume ratio of the frying medium induces oxidation, making pan-frying a deteriorative process (Soheili, Artz, & Tippayawat, 2002; Takaoka & Kobayashi, 1986; Usuki, Fukui, Kamata, &

* Corresponding author. Tel.: +358 9 191 58 228; fax: +358 9 191 58 475.
E-mail address: laura.soupas@helsinki.fi (L. Soupas).

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Kaneda, 1980). When, for instance, a thin film of low-linolenic acid soybean oil was fried in a Teflon pan at 180 °C, the polymer content exceeded 20% already after 10 min of frying (Soheili et al., 2002).

In phytosterol-enriched frying media, in addition to changes occurring in the oil matrix, the formation of phytosterol oxides during frying is of interest because of potential adverse effects of these oxides on human health (Adcox, Boyd, Oehrl, Allen, & Fenner, 2001; Guardiola, 2004; Maguire, Konoplyannikov, Ford, Maguire, & O'Brien, 2003). Already in the 1980s, the partition of phytosterol oxides, formed in plant-derived oils during frying, into foods was discussed (Finocchiaro & Richardson, 1983). Later, Dutta and Appelqvist (1996) determined sterols and sterol oxides in fried potato products and found considerably greater amounts of sterol oxides in French fries prepared in rapeseed oil/palm oil blend than in fries prepared in sunflower or high-oleic sunflower oil. They suggested that differences in sterol oxide content in fried products originate from different sterol contents in these oils, i.e., the more phytosterols in the frying medium, the more oxidized phytosterols were in the fried food item. Similarly, due to different phytosterol content of frying media, potato chips fried in palm oil contain significantly lower levels of phytosterol oxides than those fried in sunflower oil or high-oleic sunflower oil (Dutta & Appelqvist, 1997).

The necessity of using good-quality frying media is obvious, and studies in which the oxidative stability of frying media is examined in detail should be conducted. Evidence gained from previous research indicates that the oxidative stability of phytosterol/-stanol compounds is significantly affected by molecular structure, as well as by lipid matrix composition, and, for instance, heating temperature – and interactions between these variables (Soupas, Juntunen, Lampi, & Piironen, 2004; Soupas, Huikko, Lampi, & Piironen, 2005). Presumably, these factors also contribute to phytosterol oxidation during frying.

Our aim here was to investigate the oxidative stability of different phytosterol compounds (including phytostanol) in pan-frying, using various frying temperatures and times. We also had two more specific objectives. In the first part of the study, we examined differences in oxidation susceptibility of indigenous and added phytosterols in a rapeseed oil matrix. In the second part of the study, we examined the effect of different lipid matrices on phytosterol oxidation. Temperatures and times were selected to cover frying conditions normally used in cooking. The selection of phytosterol compounds was based on structures widely used in functional foods, whereas lipid matrices were chosen to cover different fatty acid compositions. To evaluate oxidative stability of the samples during frying, the formation and distribution of the main phytosterol oxides were studied, as well as the loss of original phytosterols. In addition, the polymerization of lipid matrices was measured.

2. Materials and methods

2.1. Materials and reagents

24β-Ethylcholest-5-en-3β-ol (β-sitosterol) was purchased from Fluka Chemie (Buchs, Switzerland). 5-Cholesten-3β,19-ol (19-hydroxycholesterol) and 3β-hydroxy-5α-cholestane (dihydrocholesterol; 95%), which were used as internal standards (ISTD), were from Steraloids (Newport, RI, USA) and Sigma (St. Louis, MO, USA), respectively. Phytosteryl and phytostanyl fatty acid esters, rapeseed oil and rapeseed oil-based liquid margarine were kindly donated by Raisio plc. (Raisio, Finland), and butter oil by Valio Ltd. (Helsinki, Finland). The materials and reagents used for saponification, extraction, solid-phase extraction and derivatization of the samples are described in detail in our previous publications (Lampi, Juntunen, Toivo, & Piironen, 2002; Soupas, Juntunen, Lampi, et al., 2004; Soupas, Juntunen, Säynäjoki, Lampi, & Piironen, 2004).

The phytosteryl and phytostanyl ester preparates were mixtures prepared from soybean oil-originated sterols and rapeseed oil fatty acids. Two batches of phytosteryl ester preparate were used. In the batch used in the first frying experiment, the main phytosterols were sitosterol (45%), campesterol (26%) and stigmasterol (15%), whereas the batch in the second frying experiment consisted of sitosterol (45%), campesterol (25%) and stigmasterol (18%). In the phytostanyl ester preparation, the main phytostanols were sitostanol (65%) and campestanol (33%). The commercial β-sitosterol consisted primarily of sitosterol (75%) and sitostanol (12%). The main phytosterols in the rapeseed oil were sitosterol (51%) and campesterol (37%), the total phytosterol content being approximately 0.8%.

In rapeseed oil and liquid margarine (containing 80% of fat), the proportions of total saturated, monounsaturated and polyunsaturated fatty acids, as reported by the manufacturer, were 7/100, 59/100 and 34 g/100 g and 6/100, 48/100 and 26 g/100 g of the product, respectively. In butter oil, corresponding proportions were 71/100, 25/100 and 3 g/100 g (National Public Health Institute, Finland).

2.2. Pan-frying experiments

In the first pan-frying experiment, rapeseed oil (25 g) was pan-fried as such and as enriched with commercial β-sitosterol or with phytosteryl or phytostanyl esters, at a level equivalent to 8% sterol/stanol. An iron pan with an inner diameter of 21 cm was used for frying. A copper thermoelement (2 cm × 2.5 cm) coupled to a Honeywell digital recorder and PC software (version 5.3A) (Morristown, NJ, USA) were used to monitor the temperature at the centre of the iron pan as well as on the hot plate. Temperatures were recorded at 15 s intervals. Frying conditions were set as follows: at 160 °C (156 ± 6 °C, number of measurements = 440) for 5 and 10 min, at 180 °C (180 ± 6 °C,

number of measurements = 440) for 5 and 10 min, and at 200 °C (184 ± 5 °C, number of measurements = 160) for 5 min. To stabilize the temperature of the iron pan, it was preheated for 5 min before adding of the sample. After frying, the oil samples were cooled in a desiccator before undergoing further analyses.

In the second frying experiment, rapeseed oil, liquid margarine and melted butter oil (25 g) were enriched with commercial β-sitosterol or with phytosteryl or phytostanyl esters, at a level equivalent to 8% sterol/stanol. Samples were pan-fried at 160 °C (154 ± 4 °C, number of measurements = 990) and 180 °C (174 ± 3 °C, number of measurements = 990) for 5 and 10 min. Otherwise, this frying experiment was carried out as was the first one.

All heating experiments were carried out twice, and each sample was analysed in duplicate. To equalize possible prooxidative effects caused by interactions between the iron pan and the thin film of oil, all fryings were randomized. Between fryings, the iron pan was washed with hot water and then heated with water. To ensure that no phytosterols/-stanols were left on the pan after washing, a preliminary experiment was done.

During the aforementioned frying experiments the water in the liquid margarine evaporated. The amount of this water was determined by heating liquid margarine in an oven at 160 °C for 5 and 10 min and by weighing samples before and after heating. By using this procedure, the amount of water in the margarine that had evaporated during frying was established to be 18.3% ($n = 2$). The evaporation of water was taken into account in all calculations.

2.3. Determination of phytosterol/-stanol oxides

Samples for phytosterol/-stanol oxide determination were prepared according to the method published previously (Lampi et al., 2002). In brief, this method consisted of an overnight cold saponification of lipid samples in the dark at 25 °C, extraction of unsaponifiable material with diethyl ether, enrichment of oxides with SiOH solid-phase extraction (SPE) and preparation of trimethylsilyl ether (TMS ether) derivatives. As a modification of this method, the amount of 19-hydroxycholesterol (ISTD), added prior to cold saponification, was reduced to 19, 8 or 2 μg to adjust its amount to the predicted amounts of phytosterol/-stanol oxides.

Analyses of phytosterol/-stanol oxides, as TMS ether derivatives, were carried out by gas chromatography–mass spectrometry (GC–MS) in an electron-ionization (70 eV) mode. The GC–MS apparatus consisted of a Hewlett-Packard 6890 GC (Wilmington, PA, USA) coupled to an Agilent 5973 MS (Palo Alto, CA, USA). The injection technique used was on-column injection. To separate the phytosterol/-stanol oxides, an RTX-5MS w/Integra Guard (crossbond 5% diphenyl – 95% dimethyl polysiloxane) capillary column (60 m × 0.25 mm i.d., 0.10 μm film) (Restek, Bellefonte, PA, USA) was used. Other GC–MS conditions were the same as described in our earlier publications (Soupas, Juntunen, Lampi, et al., 2004; Soupas, Juntunen, Säynäjoki, et al., 2004).

Identification of phytosterol/-stanol oxide TMS ether derivatives was carried out in full scan mode (m/z 100–600), and quantification of the main oxides in selected ion monitoring (SIM) mode. In the absence of commercial phytosterol/-stanol oxide standards, the calibration curves for sitosterol, campesterol and sitostanol oxides were constructed indirectly via gas chromatography-flame ionization detection (GC-FID) in a manner similar to our previous studies (Soupas, Juntunen, Säynäjoki, et al., 2004; Soupas et al., 2005; Soupas, Huikko, Lampi, & Piironen, 2006). The quantification of the main sitosterol and campesterol oxide TMS ether derivatives formed during pan-frying was achieved by SIM acquisition of the following target and qualifier ions: m/z 353.3 and 366.4 (19-hydroxycholesterol, ISTD), m/z 470.4 and 471.4 (7α- and 7β-hydroxycampesterol), m/z 484.4 and 485.4 (7α- and 7β-hydroxysitosterol), m/z 488.4 and 398.4 (5α,6α- and 5β,6β-epoxycampesterol), m/z 502.5 and 412.4 (5α, 6α- and 5β,6β-epoxysitosterol), m/z 486.4 and 381.3 (7-ketocampesterol) and m/z 500.4 and 395.4 (7-ketositosterol). The limits of determination for sitosterol and campesterol oxides were set to the lowest level of each calibration curve, thus being 0.6–2.1 μg/g of the lipid matrix.

In phytostanyl ester-enriched samples, the formation of 7α- and 7β-hydroxysitostanols, 6α-hydroxysitostanol and 15α-hydroxysitostanol was used as a marker of sitostanol oxidation during pan-frying because of their rather good separation and abundance in GC analysis (Soupas, Juntunen, Säynäjoki, et al., 2004). The quantification of TMS ether derivatives of these oxides was achieved by SIM acquisition of the following target and qualifier ions: m/z 353.3 and 366.3 (19-hydroxycholesterol, ISTD), m/z 486.4, 487.4 and 471.3 (7α-hydroxysitostanol), m/z 486.4, 487.4 and 471.4 (7β-hydroxysitostanol), m/z 204.1, 205.1 and 191.1 (6α-hydroxysitostanol), and m/z 269.2, 270.2, and 486.4 (15α-hydroxysitostanol). The limits of determination for sitostanol oxides were set to the lowest level of each calibration curve, thus being 0.2–0.9 μg/g of the lipid matrix.

The GC performance was monitored daily by injecting a sterol standard mixture containing dihydrocholesterol, cholesterol and stigmasterol. Assessment of whether the separation was acceptable was based on the area ratio between cholesterol and dihydrocholesterol in the mixture. Ratios that were within two standard deviations of the mean value ($n = 35$) were considered acceptable. In addition, the mass spectrum of stigmasterol in this mixture was also monitored and evaluated daily.

2.4. Determination of non-oxidized phytosterols/-stanols

To determine the phytosterol/-stanol content in enriched lipid matrices, before and after frying experiments, a direct hot saponification method was used (Piironen, Toivo, & Lampi, 2002). After saponification, the

samples were subjected to extraction of the unsaponifiables with hexane-diethyl ether after the addition of water. Dihydrocholesterol was used as an ISTD and added to the sample prior to saponification (Lampi et al., 2002; Soupas et al., 2005). Phytosterols/-stanols were analysed as TMS ether derivatives by a GC-FID system consisting of a Hewlett-Packard 5890 Series II GC (Karlsruhe, Germany), an automated on-column injection system and an FID. Phytosterols/-stanols were separated on an RTX-5 w/Integra Guard (crossbond 5% diphenyl – 95% dimethyl polysiloxane) capillary column (60 m × 0.32 mm i.d., 0.10 μm film) (Restek, Bellefonte, PA, USA) (Lampi et al., 2002). Other GC-FID conditions were the same as those described previously (Lampi et al., 2002; Soupas, Juntunen, Säynäjoki, et al., 2004).

The limit of determination for phytosterols in GC-FID analysis was 2 mg/100 g of product in enriched lipid samples and 0.5 mg/100 g in native rapeseed oil (Piironen et al., 2002). To confirm day-to-day repeatability of the method, two in-house reference samples were used: native and sitosterol-enriched rapeseed oil. The coefficient of variation (CV) for the total phytosterol determinations in the native rapeseed oil was 2.1% ($n = 11$). In the enriched rapeseed oil, the CV for the sitosterol determination was 3% ($n = 4$). The GC performance was monitored daily by injecting a sterol standard mixture as described in Section 2.3.

2.5. Analysis of polymerized lipid matrix

To clarify the overall changes occurring in enriched lipid samples during frying, the deterioration of lipid matrices was also studied in terms of polymerization. Each fried sample was subjected to high-performance size-exclusion chromatographic (HPSEC) analysis, as described previously (Lampi & Kamal-Eldin, 1998). In brief, a Hewlett-Packard 1090 Series II high-performance liquid chromatograph (HPLC) and a Hewlett-Packard 1047A refractive index detector were used (Waldbronn, Germany). Separation was performed on one 100-Å and two 50-Å PLGel columns (5 μm, 300 mm × 7.5 mm i.d.) (Polymer Laboratories Inc., Amherst, MA, USA). Samples were taken from both frying experiments and analysed in duplicate ($n = 4$). The amounts of dimers and polymers were given as percentages, based on peak areas, assuming equal responses of each lipid class. The limits of detection and determination for these analyses were 0.1% and 0.2% of dimers and polymers in the lipid sample, respectively. Tripalmitin, oxidized for 3 h at 180 °C, was analysed in each sample batch as an in-house reference sample.

2.6. Analysis of α-tocopherol in lipid matrices

The amount of α-tocopherol was determined by normal-phase HPLC (NP-HPLC), as described recently by Nyström, Achrenius, Lampi, Moreau, and Piironen (submitted for publication). In brief, a Hewlett-Packard 1090 Series II HPLC and a Hewlett-Packard 1046A fluorescence detector were used (Waldbronn, Germany). Separation was performed on a diol column (LiChrosorb Diol 5 μm, 100 mm × 3.0 mm, VDS Optilab, Berlin, Germany). All matrices were analysed in duplicate, and two injections from each sample were made ($n = 4$). The determination limit was 7.5 ng/injection.

3. Results and discussion

3.1. General remarks about the pan-frying experiment

The pan-frying experiment was a simulation of the actual cooking process. Thus, rapeseed oil and rapeseed oil-based liquid margarine were not purified from antioxidants or pro-oxidants, and an iron pan was used, although its usage could lead to interactions between iron and the frying oil. Variables that could affect phytosterol oxidation were therefore not strictly controlled. Noteworthy also is that, in addition to enrichment with 8% of phytosterols, indigenous phytosterols (approximately 0.8%) were present in rapeseed oil and rapeseed oil-based liquid margarine. The amount of indigenous phytosterols was, however, so small, compared with the enrichment, that its effect on total oxidation of phytosterols during frying was considered negligible.

The pan-frying itself was challenging to perform. Adequate frying temperatures, selected according to the recommendation of the Information Centre of Margarine Industry in Finland, were rather difficult to reach and maintain. This was because of the air stream. The highest temperature attained was 184 ± 5 °C, as described in Section 2. Despite the effect of the air stream, the repeatability of the frying experiments was good. This can be concluded by comparing the oxidation percentages of free and esterified phytosterols in the rapeseed oil matrix fried at 160 and 180 °C during the first (Table 1) and the second (Tables 2 and 3) frying. The oxidation percentages were rather similar although over six months had elapsed between experiments.

3.2. Structures of phytosterol/-stanol oxides formed during pan-frying

Due to the phytosterol/-stanol composition of samples studied, the main phytosterol oxides analysed in pan-frying were sitosterol or sitostanol oxides. In phytosterol-enriched samples, campesterol oxides were also detected in quantifiable amounts.

In addition to the quantified main sitosterol and campesterol oxides – the epimers of 7-hydroxysterols, the epimers of 5,6-epoxysterols and 7-ketosterols – small amounts of several other oxides, including 6-ketositostanol, 6α- and 6β-hydroxysitosterol, 6α-hydroxysitostanol and 6α-hydroxycampestanol were detected (Table 4, Fig. 1). Moreover, traces of sitostanetriol ((24*R*)-ethylcholestan-3β,5α,6β-triol) were detected in some samples.

Table 1
Effect of pan-frying on oxidation of sitosterol/sitostanol and polymerization of lipid matrix in native and phytosterol/-stanol-enriched (8%) rapeseed oil (RSO)

Pan-frying conditions	Native RSO			Added free phytosterols (8%) in RSO			Added phytosteryl esters (8%) in RSO			Added phytostanyl esters (8%) in RSO		
	Oxidized sitosterol (%)[a]	Non-oxidized sitosterol (mg/100 g of matrix)	Matrix di- and polymers (%)	Oxidized sitosterol (%)[a]	Non-oxidized sitosterol (mg/100 g of matrix)	Matrix di- and polymers (%)	Oxidized sitosterol (%)[a]	Non-oxidized sitosterol (mg/100 g of matrix)	Matrix di- and polymers (%)	Oxidized sitostanol (%)[a]	Non-oxidized sitostanol (mg/100 g of matrix)	Matrix di- and polymers (%)
160 °C												
0 min	0.1 ± 0.0	422 ± 3	0.4[b]	0.0 ± 0.0	6336 ± 5	0.3[b]	0.1 ± 0.0	4205 ± 14	0.5[b]	0.1 ± 0.0	5852 ± 9	0.2[b]
5 min	0.2[b]	404[b]	0.8[b]	0.1 ± 0.0	6268 ± 42	0.3 ± 0.1	0.2 ± 0.0	4116 ± 29	0.5 ± 0.2	0.1 ± 0.0	5830 ± 66	0.3 ± 0.0
10 min	0.3[b]	428[b]	1.5[b]	0.4 ± 0.0	6165 ± 92	0.4 ± 0.1	0.5[b]	4067[b]	0.6[b]	0.1 ± 0.0	5863 ± 47	0.4 ± 0.1
180 °C												
0 min	0.1 ± 0.0	422 ± 3	0.4[b]	0.0 ± 0.0	6336 ± 5	0.3[b]	0.1 ± 0.0	4205 ± 14	0.5[b]	0.1 ± 0.0	5852 ± 9	0.2[b]
5 min	0.7 ± 0.1	425 ± 6	0.7 ± 0.2	0.5 ± 0.0	6026 ± 69	0.8 ± 0.2	0.5 ± 0.0	4000 ± 53	0.8 ± 0.1	0.1 ± 0.0	5826 ± 68	0.6 ± 0.1
10 min	1.7[b]	402[b]	2.6[b]	1.8 ± 0.0	6032 ± 73	2.3 ± 0.3	1.8 ± 0.1	3971 ± 61	2.8 ± 0.3	0.1[b]	5705[a]	2.5[b]
200 °C												
0 min	0.1 ± 0.0	422 ± 3	0.4[b]	0.0 ± 0.0	6336 ± 5	0.3[b]	0.1 ± 0.0	4205 ± 14	0.5[b]	0.1 ± 0.0	5852 ± 9	0.2[b]
5 min	0.9[b]	421[b]	1.5[b]	0.9 ± 0.0	6084 ± 76	1.3 ± 0.1	0.9 ± 0.0	4107 ± 23	1.6 ± 0.2	0.1 ± 0.0	5939 ± 19	1.4 ± 0.1

[a] Percentage of total oxides from the original non-oxidized sitosterol/-stanol.
[b] For these samples, standard deviations could not be calculated since the number of determinations was only two.

Table 2
Effect of pan-frying at 160 °C on oxidation of sitosterol/sitostanol and polymerization of lipid matrix in rapeseed oil, liquid margarine and butter oil enriched with free or esterified phytosterols or with phytostanyl esters at a level equivalent to 8% sterol/stanol

Lipid matrix and frying time	8% Free phytosterols			8% Phytosteryl esters			8% Phytostanyl esters		
	Oxidized sitosterol (%)[a]	Oxidized sitosterol (μg/g of matrix)	Matrix di- and polymers (%)	Oxidized sitosterol (%)[a]	Oxidized sitosterol (μg/g of matrix)	Matrix di- and polymers (%)	Oxidized sitostanol (%)[a]	Oxidized sitostanol (μg/g of matrix)	Matrix di- and polymers (%)
Rapeseed oil									
0 min	0.0[b]	23.2[b]	0.4[b]	0.1[b]	38.9[b]	–	0.1[b]	43.2[b]	–
5 min	0.2 ± 0.0	119 ± 2	0.5 ± 0.0	0.2 ± 0.0	93 ± 4	0.5 ± 0.1	0.1 ± 0.0	44.8 ± 3.9	0.6 ± 0.2
10 min	0.4 ± 0.0	239 ± 23	0.8 ± 0.3	0.3 ± 0.0	169 ± 48	0.9 ± 0.0	0.1 ± 0.0	45.0 ± 2.7	0.7 ± 0.2
Liquid margarine									
0 min	0.0[b]	22.8[b]	–	0.1[b]	39.6[b]	–	0.1[b]	36.3[b]	–
5 min	0.3 ± 0.0	246 ± 12	0.8 ± 0.4	0.3 ± 0.0	133 ± 2	0.7 ± 0.3	0.1 ± 0.0	48.0 ± 1.5	1.0 ± 0.0
10 min	1.0 ± 0.1	731 ± 12	1.7 ± 0.6	0.6 ± 0.0	318 ± 13	1.5 ± 0.0	0.1 ± 0.0	55.2 ± 2.3	1.7 ± 0.1
Butter oil									
0 min	0.0[b]	20.2[b]	–	0.1[b]	33.9[b]	–	0.0[b]	4.2[b]	–
5 min	0.6 ± 0.2	344 ± 138	–	0.4 ± 0.2	153 ± 68	–	0.0 ± 0.0	7.0 ± 2.4	–
10 min	2.1 ± 0.1	1214 ± 30	<0.2	1.0 ± 0.2	381 ± 58	0.3[b]	0.0 ± 0.0	11.9 ± 0.4	–

(–) not detected (detection limit 0.1% dimers- and polymers in lipid sample); (<) concentration remained below the determination limit (0.2% dimers- and polymers in lipid sample).
[a] Percentage of total oxides from the original non-oxidized sitosterol/-stanol.
[b] For these samples, standard deviations could not be calculated since the number of determinations was only two.

Table 3
Effect of pan-frying at 180 °C on oxidation of sitosterol/sitostanol and polymerization of lipid matrix in rapeseed oil, liquid margarine and butter oil enriched with free or esterified phytosterols or with phytostanyl esters at a level equivalent to 8% sterol/stanol

Lipid matrix and frying time	8% Free phytosterols			8% Phytosteryl esters			8% Phytostanyl esters		
	Oxidized sitosterol (%)[a]	Oxidized sitosterol (μg/g of matrix)	Matrix di- and polymers (%)	Oxidized sitosterol (%)[a]	Oxidized sitosterol (μg/g of matrix)	Matrix di- and polymers (%)	Oxidized sitostanol (%)[a]	Oxidized sitostanol (μg/g of matrix)	Matrix di- and polymers (%)
Rapeseed oil									
0 min	0.0[b]	23.2[b]	0.4[b]	0.1[b]	38.9[b]	–	0.1[b]	43.2[b]	–
5 min	0.6 ± 0.0	355 ± 16	1.2 ± 0.2	0.6 ± 0.1	242 ± 34	1.2 ± 0.4	0.1 ± 0.0	46.9 ± 3.8	1.1 ± 0.0
10 min	1.8 ± 0.2	1112 ± 108	3.2 ± 0.5	1.4 ± 0.0	585 ± 10	3.1 ± 0.3	0.1 ± 0.0	60.6 ± 1.8	2.6 ± 0.2
Liquid margarine									
0 min	0.0[b]	22.8[b]	–	0.1[b]	39.6[b]	–	0.1[b]	36.3[b]	–
5 min	1.0 ± 0.1	744 ± 75	1.5 ± 0.0	0.6 ± 0.0	291 ± 19	1.3 ± 0.1	0.1 ± 0.0	48.5 ± 0.6	1.1 ± 0.0
10 min	2.5 ± 0.3	1874 ± 226	4.8 ± 0.1	1.3 ± 0.1	668 ± 55	3.5 ± 0.0	0.1 ± 0.0	62.9 ± 1.4	3.0 ± 0.1
Butter oil									
0 min	0.0[b]	20.2[b]	–	0.1[b]	33.9[b]	–	0.0[b]	4.2[b]	–
5 min	2.0 ± 0.0	1183 ± 6	0.5 ± 0.1	1.4 ± 0.1	515 ± 20	0.4 ± 0.2	0.0 ± 0.0	20.4 ± 5.0	1.0[b]
10 min	5.1 ± 0.2	2961 ± 143	0.9 ± 0.2	3.2 ± 0.3	1231 ± 119	1.2 ± 0.1	0.1 ± 0.0	38.9 ± 0.3	1.1 ± 0.0

(–) not detected (detection limit 0.1% dimers- and polymers in lipid sample).
[a] Percentage of total oxides from the original non-oxidized sitosterol/-stanol.
[b] For these samples, standard deviations could not be calculated since the number of determinations was only two.

In phytosteryl ester-enriched samples, stigmasterol and brassicasterol oxides were also detected (Table 4, Fig. 1). Naturally, the spectrum and the amount of oxides formed were strongly dependent on phytosterol composition of the phytosterol preparation used in enrichment. All detected oxidation products were identified by their elution order and mass spectral properties (Grandgirard, Martine, Joffre, Juaneda, & Berdeaux, 2004; Lambelet et al., 2003; Lampi et al., 2002). According to a recent study, the aforementioned oxides were also present in a commercial phytosterol-enriched spread (Grandgirard et al., 2004).

Interestingly, 6-ketositostanol seemed to be characteristic of samples enriched with free phytosterols, and 6α-hydroxysitostanol of samples in which rapeseed oil was used as lipid matrix. The initial amounts of these two oxides were higher than those of the other oxides, but for some reason, their amounts did not increase as much during frying as did the others. As an example, before frying, the amount of 6-ketositostanol was four times higher than that of 7α-hydroxysitosterol but, after 5 min of frying at 160 °C, the amount of 7α-hydroxysitosterol increased 10-fold while no changes occurred in the amount of 6-ketositostanol.

The presence of 6-hydroxystanols in refined rapeseed oil has also been noted in previous studies (Grandgirard et al., 2004; Lambelet et al., 2003). In our samples, 6α-hydroxysitostanol may have been derived from the rapeseed oil matrix. The explanation for the presence of 6-ketositostanol mainly in free phytosterol-enriched samples remains unclear. Only two suggestions concerning the formation of 6-ketostanols can be found in the literature. According to the study of Yanislieva-Maslarova, Schiller, and Seher (1982), esterification of the 3β-hydroxyl group increases the C4–H bond sensitivity towards the oxygen attack, leading to the formation of 6-ketostanol. Alternatively, the rearrangement of 5,6-epoxysterols may lead to the formation of 6-ketostanol (Yanislieva-Maslarova et al., 1982).

In addition to quantified sitostanol oxides 7α-, 7β-, 6α- and 15α-hydroxysitostanols, also 7-ketositostanol (Soupas, Juntunen, Säynäjoki, et al., 2004) was detected in phytostanyl ester-enriched samples. As mentioned above, 6α-hydroxysitostanol may have been derived from the rapeseed oil matrix. As small amounts of 6α-hydroxysitostanol were also quantified in phytostanyl ester-enriched butter oil, 6α-hydroxysitostanol may also have originated from the rapeseed oil used in the esterification process of sitostanol. However, since the amount of this oxide was observed to further increase during frying, it was used as one marker of sitostanol oxidation.

Since butter oil contains approximately 0.2% indigenous cholesterol (National Public Health Institute, Finland), cholesterol oxides were also observed. In all butter oil samples, 7α- and 7β-hydroxycholesterol and 7-ketocholesterol were detected. However, quantification of these oxides was beyond the scope of this study.

Table 4
Nomenclature and gas chromatographic data of phytosterol oxides analysed in phytosterol-enriched pan-fried lipid samples

Systematic name	Trivial name	RRT[a]	Number in Fig. 1
(24*S*)-Methylcholest-5,22-diene-3β,7α-diol	7α-Hydroxybrassicasterol	0.94	1
(24*R*)-Methylcholest-5-ene-3β,7α-diol	7α-Hydroxycampesterol	1.01	4
(24*S*)-Ethylcholest-5,22-diene-3β,7α-diol	7α-Hydroxystigmasterol	1.03	5
(24*S*)-Methylcholest-5,22-diene-3β,7β-diol	7β-Hydroxybrassicasterol	1.08	6
(24*R*)-Methylcholest-4-ene-3β,6β-diol	6β-Hydroxycampesterol	1.11	7
(24*R*)-Ethylcholest-5-ene-3β,7α-diol	7α-Hydroxysitosterol	1.11	8
(24*S*)-Ethylcholest-4,22-diene-3β,6β-diol	6β-Hydroxystigmasterol	1.13	9
(24*S*)-5β,6β-Epoxy-24-methylcholest-22-en-3β-ol	5,6β-Epoxybrassicasterol	1.15	10
(24*R*)-Methylcholest-5-ene-3β,7β-diol	7β-Hydroxycampesterol	1.18	11
(24*S*)-Ethylcholest-5,22-diene-3β,7β-diol	7β-Hydroxystigmasterol	1.19	12
(24*R*)-Ethylcholest-4-ene-3β,6β-diol	6β-Hydroxysitosterol	1.22	13
(24*R*)-5β,6β-Epoxy-24-methylcholestan-3β-ol	5,6β-Epoxycampesterol	1.24	14
(24*R*)-Methylcholestane-3β,6α-diol	6α-Hydroxycampestanol	1.25	15
(24*R*)-5α,6α-Epoxy-24-methylcholestan-3β-ol	5,6α-Epoxycampesterol	1.27	16
(24*S*)-5β,6β-Epoxy-24-ethylcholest-22-en-3β-ol	5,6β-Epoxystigmasterol	1.29	17
(24*R*)-Ethylcholest-5-ene-3β,7β-diol	7β-Hydroxysitosterol	1.31	18
(24*S*)-5α,6α-Epoxy-24-ethylcholest-22-en-3β-ol	5,6α-Epoxystigmasterol	1.32	19
(24*R*)-Ethylcholest-4-ene-3β,6α-diol	6α-Hydroxysitosterol	1.34	20
(24*R*)-5β,6β-Epoxy-24-ethylcholestan-3β-ol	5,6β-Epoxysitosterol	1.39	21
(24*R*)-Ethylcholestane-3β,6α-diol	6α-Hydroxysitostanol	1.41	22
(24*R*)-5α,6α-Epoxy-24-ethylcholestan-3β-ol	5,6α-Epoxysitosterol	1.42	23
(24*R*)-Methylcholest-5-ene-3β,25-diol	25-Hydroxycampesterol	1.50	24
(24*R*)-Methylcholest-5-ene-3β-ol-7-one	7-Ketocampesterol	1.55	25
(24*S*)-Ethylcholest-5,22-diene-3β-ol-7-one	7-Ketostigmasterol	1.62	26
(24*R*)-Ethylcholest-5-ene-3β,25-diol	25-Hydroxysitosterol	1.68	27
(24*R*)-Ethylcholestan-3β-ol-6-one	6-Ketositostanol	1.72	28
(24*R*)-Ethylcholest-5-ene-3β-ol-7-one	7-Ketositosterol	1.76	29

[a] RRT, retention times of phytosterol oxides (TMS ethers) in relation to 19-OH-cholesterol (TMS ether, ISTD). For analytical conditions, see Section 2.3.

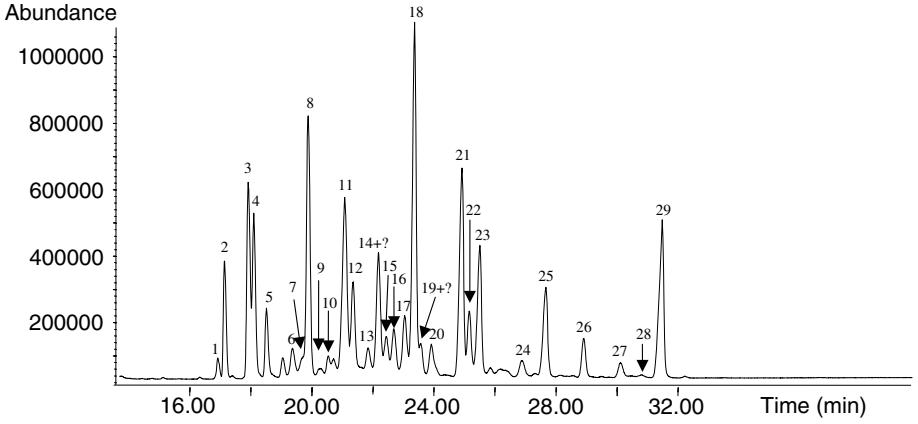

Fig. 1. Total ion current GC–MS chromatogram of phytosterol oxide TMS ether derivatives of pan-fried (180 °C/10 min) rapeseed oil enriched with phytosteryl esters (8%) on an Rtx-5 MS w/Integra Guard capillary column (60 m × 0.25 mm i.d., 0.1 μm film). For other analytical conditions, see Section 2.3. The peak numbers represent the compounds listed in Table 4. Peaks number 2 (dihydrocholesterol) and 3 (19-OH-cholesterol) were added as references and the latter was used as an internal standard in quantification. Peak numbers 14 and 19 were impure.

3.3. Oxidative stability of phytosteroll-stanol during pan-frying experiments

3.3.1. General

The amounts of sitosterol and sitostanol oxides formed during pan-frying are presented in Tables 1–3. To make the data easier to compare, most of the results are presented as percentages of phytosterol/-stanol oxides of the original non-oxidized phytosterol/-stanol.

Since the oxidative stability of campesterol was similar to that of sitosterol, the data are not shown. As an example, in native rapeseed oil fried at 160 °C, at the beginning and after 5 and 10 min of frying, 0.1%, 0.2% and 0.3% of both sitosterol (Table 1) and campesterol were oxidized,

respectively. In phytosteryl ester-enriched rapeseed oil, fried for 0, 5 and 10 min at 180 °C, 0.1%, 0.6% and 1.7% of campesterol and 0.1%, 0.5% and 1.8% of sitosterol (Table 1) were oxidized, respectively.

In general, pan-frying seemed to induce phytosterol oxidation but had no significant effect on phytostanol oxidation. During pan-frying, phytosterols oxidized significantly more than, for example, during heating in an oven. In our previous studies, we have measured the oxidation of added phytosterols and phytosteryl esters in rapeseed oil (purified from antioxidants) or in tripalmitin heated in an oven at 180 °C and have observed oxidation percentages similar to these in this study (1–6%) after 0.5–2 h of heating (Soupas, Juntunen, Lampi, et al., 2004; Soupas et al., 2005). Pan-frying may induce phytosterol oxidation because of its high temperature and large surface-to-volume ratio, which allows considerable oxygen adsorption per unit of frying oil (Usuki et al., 1980). Hence, when comparing the phytosterol oxidation during heating in an oven and during pan-frying, differences in surface-to-volume ratios should be kept in mind. Furthermore, in this study, interactions between the iron pan and the thin film of oil could have accelerated the overall deterioration of samples – a phenomenon also observed by Takaoka and Kobayashi (1986).

Moreover, we noted that the higher the temperature during frying, the more phytosterols were oxidized; after 5 min of frying at 200 °C, 0.9% of free sitosterol was oxidized, but at 160 °C only 0.1% (Table 1). Generally, frying time also affected phytosterol oxidation such that the longer the frying, the more were the phytosterols oxidized; after 5 min of frying at 180 °C, 0.5% of sitosteryl esters were oxidized in rapeseed oil, and after 10 min as much as 1.8% (Table 1).

During the first pan-frying experiment we also measured the loss of original phytosterols (Table 1). Despite the high frying temperature, the greatest sterol loss observed was 5.6%, being measured for the added sitosteryl esters in rapeseed oil fried for 10 min at 180 °C. At this same time point, the sitosterol oxidation percentage in the sample studied was 1.8%. The "gap" between sitosterol loss and formation of the main secondary oxidation products was thus 3.8%. For comparison, when we studied the oxidation of sitosterol in a tripalmitin matrix during 3 h of oven-heating at 180 °C, a "gap" of 27.5% was measured. These observations indicate that the formation of oxidation products other than the main secondary oxides is rather small during short-term pan-frying. For this reason, sterol loss was not measured during the second frying experiment.

3.3.2. Observations of phytosteroll-stanol oxidation during the first pan-frying experiment

During the first pan-frying experiment we could find out that phytosterol oxidation proceeded similarly in natural and enriched rapeseed oils. For instance, at 180 °C, after 0, 5 and 10 min of frying, 0.1%, 0.7% and 1.7% of indigenous sitosterol, 0.0%, 0.5% and 1.8% of added free sitosterol and 0.1%, 0.5% and 1.8% of added sitosteryl esters were oxidized, respectively (Table 1). This indicates that indigenous and added phytosterols can be similarly bound by the lipid matrix, and thus, similarly exposed to oxidation. Neither were any differences observed between the oxidation susceptibility of added free and esterified phytosterols. Although absolute phytosterol oxide amounts can be misleading, examples of the oxide levels may be illustrative to represent. The earlier mentioned oxidation percentages after 0, 5 and 10 min of frying at 180 °C corresponded to absolute total sitosterol oxide amounts of 4.3, 28 and 72 μg/g of lipid matrix in native rapeseed oil, 27, 304 and 1127 μg/g of lipid matrix in rapeseed oil enriched with free phytosterols and 50, 228 and 736 μg/g of lipid matrix in rapeseed oil enriched with phytosteryl esters, respectively (data not shown). When comparing these results, one should take into account that rapeseed oils were enriched with mixtures of phytosterols at a level equivalent to 8% sterol.

Our previous studies have demonstrated that the saturated phytostanol structure is less reactive than the unsaturated phytosterol structure in terms of oxidation (Soupas, Juntunen, Lampi, et al., 2004; Soupas et al., 2005). The greater stability of stanol compounds was also established in pan-frying; oxidation of sitostanol did not proceed markedly during frying at any temperature tested compared with the initial oxidation percentage 0.1% (Table 1), which corresponds to 38 μg of sitostanol oxides per gramme of lipid matrix. Noteworthy, however, is that different oxides have been used as markers of oxidation of phytosterols and stanols in our studies.

In addition to the total amounts of phytosterol/-stanol oxides, the distribution of these oxides was studied. Both frying temperature and time as well, as the structure of the phytosterol compound, seemed to affect distribution. However, comparisons between sterol structures were difficult since the product profiles were quite different in these samples already at the beginning; the main oxides in native, in free phytosterol and in phytosteryl ester-enriched rapeseed oils were 7-ketositosterol (66%), 5α,6α-epoxysitosterol (31%) and 7β-hydroxysitosterol (31%), respectively (data not shown).

The main observations concerning oxide distribution were the proportion of 7-ketositosterol decreasing during frying and the proportion of especially 7β-hydroxysitosterol increasing. The higher the frying temperature, the faster did these changes appear to happen. Interestingly, at the beginning of heating, the amount of 5α,6α-epoxysitosterol was always much higher than that of 5β,6β-epoxysitosterol. However, during frying, the proportion of 5β,6β-epoxysitosterol rapidly increased, while the proportion of 5α,6α-epoxysitosterol slightly decreased. In phytostanyl ester-enriched samples, the main product at the beginning of heating was 6α-hydroxysitostanol. At 160 °C, the amounts of other products started to increase within 10 min of frying and, at higher temperatures, already within 5 min of frying.

3.3.3. Observations of phytosteroll-stanol oxidation during the second pan-frying experiment

The second pan-frying experiment dealt with the effects of different lipid matrices on phytosterol/-stanol oxidation. During this experiment, up to 5.1% of original sitosterol was found as oxides (Table 3). This was significantly more than the highest sitosterol oxidation percentage, 1.8%, observed in the first experiment in the rapeseed oil matrix (Table 1). The highest oxide contents were measured in butter oil samples at both temperatures and frying times studied (Tables 2 and 3). This means that the percentage of sterol oxidation increased when a saturated lipid matrix was used. This observation is in accordance with our earlier study where we noted that, in an unreadily oxidizable saturated lipid matrix, the high temperature forces the more reactive lipid components, phytosterols, to react (Soupas, Juntunen, Lampi, et al., 2004). Or on the other hand, when the lipid matrix is unsaturated, it protects phytosterols from oxidation by oxidizing itself.

The existence of water in the lipid matrix also affected phytosterol oxidation. At both temperatures studied, phytosterols oxidized less in rapeseed oil than in rapeseed oil-based liquid margarine, which contained approximately 20% water. After 5 and 10 min of frying at 160 °C, 0.2% and 0.4% of free sitosterol was oxidized in rapeseed oil and 0.3% and 1.0% in liquid margarine, respectively (Table 2). Since the fatty acid composition of these two matrices were almost the same (see Section 2.1), we concluded that the reaction of water with frying oil resulted in the development of free fatty acids, which in turn accelerated the oxidation of phytosterols. We also determined the content of α-tocopherol in both of these matrices and, after taking water content in the liquid margarine into account, the amounts of α-tocopherol were 14.4/100 and 16.1 mg/100 g of product in liquid margarine and rapeseed oil, respectively. Using this information, we concluded that differences in phytosterol oxidation could not be due to different amounts of antioxidant in these matrices.

The presence of water in frying plays an important role. As mentioned above, water produces steam that causes hydrolysis of fat, resulting in the formation of free fatty acids. These fatty acids are rapidly oxidizable and promote thermal oxidation by solubilizing metal catalysts. The steam could also blanket the surface of frying oil, thus delaying the rate of lipid oxidation by reducing the availability of oxygen from the air (Frankel, 1998). In practice, frying oil deterioration could occur nearly two times quicker when oil is heated without food than during continuous frying with food. However, despite the important effects of moisture in frying, in the actual frying process oxidation and polymerization are more prevalent than hydrolytic reactions (Kochhar & Gertz, 2004).

During the second frying experiment we observed that phytosterols oxidized more in free form than as esters, especially in liquid margarine and butter oil. However, no large differences between the oxidation susceptibilities of these compounds were noted in the first part of the study, in which rapeseed oil was used as lipid matrix. This observation is interesting but requires further studies to elucidate the impact of fatty acid moiety and its unsaturation degree on sterol oxidation. As reported earlier, the fatty acid moiety in the phytosteryl ester molecule could increase or decrease the oxidation susceptibility of the phytosterol moiety, depending on, for example, the heating temperature (Soupas et al., 2005). In this study, in the saturated lipid matrix, the unsaturated fatty acid moiety seemed to protect the sterol moiety from oxidation. A similar phenomenon was observed during the oxidation of cholesterol in free form and as stearate, oleate and linoleate forms: free cholesterol oxidized significantly more than cholesterol as a linoleate when heated at 180 °C in a tripalmitin matrix (Soupas et al., 2006).

As can be seen in Tables 2 and 3, the oxidation of phytostanyl esters was rather low compared with oxidation of free and esterified phytosterols. For example, in enriched butter oil, at the beginning and after 5 and 10 min of frying at 180 °C, the respective amounts of oxides formed were 0.0%, 2.0% and 5.1% (free sitosterol), 0.1%, 1.4% and 3.2% (sitosteryl esters) and 0.0%, 0.0% and 0.1% (sitostanyl esters). Furthermore, in contrast with our observations of phytosterol oxidation in butter oil, less phytostanol oxidation was measured in butter oil than in rapeseed oil or liquid margarine (Tables 2 and 3). This was, however, mainly due to the earlier-mentioned problem with 6α-hydroxysitostanol; i.e., 6α-hydroxysitostanol could partially have been derived from rapeseed oil or rapeseed oil-based liquid margarine, which were used as lipid matrices. Already at the beginning of frying, the amount of 6α-hydroxysitostanol was approximately ten times higher in rapeseed oil and liquid margarine than in butter oil. The initial amounts of 6α-hydroxysitostanol in rapeseed oil, liquid margarine and butter oil were 43, 36 and 4 μg/g of lipid matrix, respectively. After 5 and 10 min of frying at 180 °C, corresponding amounts were 42, 44 and 10 μg/g of lipid matrix, and 48, 48 and 16 μg/g of lipid matrix (data not shown). When comparing only the amounts of 7α-, 7β- and 15α-hydroxysitostanols, the same trend as with phytosterols, more oxides in butter oil than in other matrices, was observed.

The trends in changes in oxide profiles during frying agreed with those seen in the first frying experiment, i.e., frying temperatures and times mainly affected the distribution. Fig. 2 presents the sitosterol oxide profiles in the enriched butter oil samples, in which the oxidative changes were the largest, and thus, the changes in oxide profiles the most obvious. The figure clearly shows the proportional decrease of 7-ketositosterol during frying and the simultaneous increase of 7β-hydroxysitosterol. α- and β-Epimers of 5,6-epoxysitosterols behaved as mentioned earlier, i.e., at the beginning of heating, the amount of 5α,6α-epoxysitosterol was higher than that of 5β,6β-epoxysitosterol but, during frying, the proportion of the former decreased while the proportion of the latter increased. In phytostanyl ester-enriched samples, the

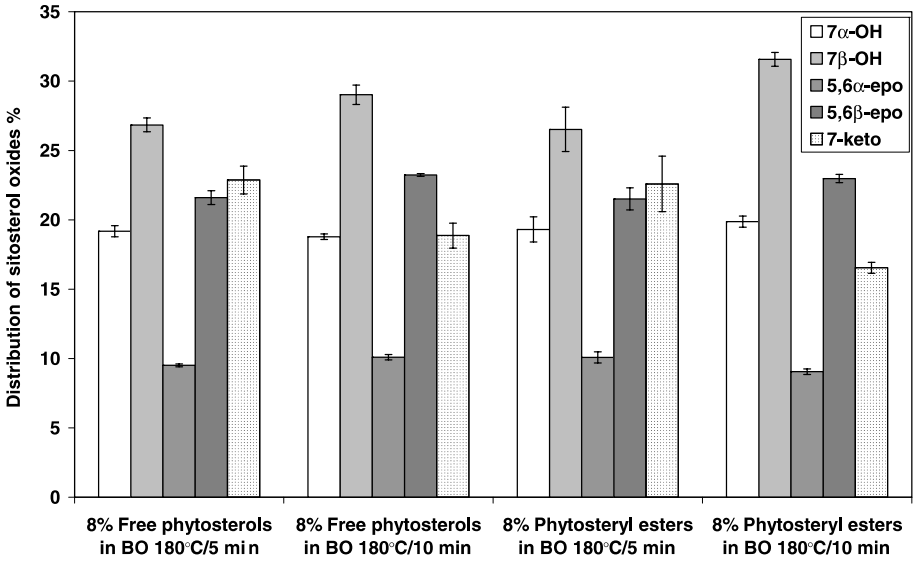

Fig. 2. Distribution (%) of sitosterol oxides in pan-fried (180 °C/5–10 min) butter oil (BO) enriched with free or esterified phytosterols at a level equivalent to 8% sterol.

proportions of sitostanol oxides changed differently in different lipid matrices. At the beginning of frying, the main product was always 6α-hydroxysitostanol (100%) but, within 5 min of frying, its proportion started to decrease and, after 10 min of frying at 180 °C, its proportions in rapeseed oil, liquid margarine and butter oil were 80%, 76% and 42%, respectively (data not shown). The lipid matrix also seemed to affect the distribution.

When summarizing all of the above-mentioned phenomena concerning phytosterol/-stanol oxidation in frying media, one should bear in mind that, in this study, only enriched oils were fried, and thus, neither the influence of food on frying oil stability nor the influence of frying oil on food quality was considered. Different foods can change the composition and stability of the frying medium during frying in many ways, and this frying medium can then be absorbed by foods in amounts varying from 5% to 40% (Frankel, 1998). To obtain more information on this topic, we have conducted a pan-frying study in which natural and phytosterol-enriched wheat bread slices were pan-fried in native or phytosterol-enriched rapeseed oil. This study revealed that a large amount of frying oil was absorbed by the bread slices. Interestingly, the quality of phytosterol-enriched breads decreased, mainly because of the absorbed phytosterol oxides formed in phytosterol-enriched frying oil – not because of the oxidation of phytosterols in phytosterol-enriched bread (unpublished data).

3.4. Formation of matrix dimers and polymers during pan-frying experiments

To clarify the overall changes that occur in phytosterol/-stanol-enriched lipid samples during frying, the deterioration of lipid matrices were also studied in terms of polymerization. The data obtained are presented in Tables 1–3. As can be seen from the data, the standard deviations were higher in dimer and polymer analyses than in phytosterol oxide analyses. This was due to rather low polymer amounts.

Polymerization degree of up to 2.3–2.8% of total lipids was measured during the first frying experiment (Table 1). During the second experiment, up to 4.8% of the lipid matrix was found as dimers and polymers (Table 3). The formation of dimers- and polymers in lipid matrices was mainly associated with the frying temperature and the frying time, i.e., the higher the temperature and the longer the frying time, the more lipid matrices were polymerized. The second frying experiment revealed that the unsaturation degree of the lipid matrix also had an important role in polymerization, as did the existence of water in the lipid matrix; the more unsaturated the matrix and the more water it contained, the more it polymerized. The highest dimer and polymer contents were measured in liquid margarine enriched with free phytosterols and fried for 10 min at 180 °C (Table 3). This is consistent with the knowledge that oils deteriorate because of high heat and moisture (Warner, 2004).

In liquid margarine, other interesting observations were also made: in contrast to the other matrices and temperatures studied, liquid margarine polymerized differently during frying at 180 °C, depending on the phytosterol compound used in enrichment (Table 3). The more the phytosterol compound oxidized, the more did the liquid margarine appear to polymerize (Table 3). Interestingly, when Marinova, Yanishlieva, and Toneva (2005) studied the influence of cholesterol on lipid autoxidation through the peroxide values and the content of conjugated dienes, they observed that added cholesterol (10%) accelerated the autoxidation process of triacylglycerols of sunflower oil at high temperatures. Noteworthy, however, is that, in

our study and in the cited study, sterols were added in relatively high concentrations.

In comparing phytosterol oxidation and lipid matrix polymerization at 180 °C, changes occurring during frying were more drastic in lipid matrices than in sterols in the case of rapeseed oil and liquid margarine. In butter oil, the situation was the opposite (Tables 2 and 3). In phytostanyl ester-enriched samples fried at 180 °C, all lipid matrices were much more reactive than the saturated stanol compound. During frying at 160 °C only slight increases in polymer contents were observed, and in many cases, dimer and polymer contents remained below the detection limit of 0.1% of the lipid sample (Table 2).

As already stated, phytosterols could enhance polymerization of liquid margarine. However, added phytosterols/-stanols might slightly inhibit polymerization of rapeseed oil during frying at 160 °C. In native rapeseed oil, the dimer and polymer contents were 0.4%, 0.8% and 1.5% after 0, 5 and 10 min of frying and, for example, in phytosteryl ester-enriched rapeseed oil, the respective contents were 0.5%, 0.5% and 0.6% (Table 1). No clear differences between different phytosterol structures or between phytosterols and phytostanyl ester in relation to matrix polymerization were noted. At higher temperatures, no polymerization-inhibiting effects of phytosterol/-stanol compounds were observed (Table 1). In the literature, the data have shown that sterol fractions containing higher levels of delta-5 and delta-7 sterols or sitosterol, or even brassicasterol, can significantly improve the stability of frying oils when evaluated by the OSET (oxidative stability at elevated temperatures) index (Kochhar & Gertz, 2004). The slight oxidation-promoting and -inhibiting effects of phytosterols observed here require confirmation by more detailed studies.

4. Conclusions

Our study showed that pan-frying at high temperatures may induce phytosterol oxidation but has no marked effect on phytostanol oxidation. In general, the higher the frying temperature and the longer the frying time, the more did phytosterols oxidize. Furthermore, a saturated lipid matrix and the existence of water in the lipid matrix can accelerate phytosterol oxidation. Pan-frying also causes a moderate degree of lipid matrix polymerization. The fact that the oxidation percentages (1–6%) observed during 5–10 min of pan-frying can only be reached after 0.5–2 h of heating in an oven at 180 °C indicates that pan-frying is a rather deteriorative process. Attention should therefore be paid to the quality of the frying medium during pan-frying.

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