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Determination of phytosterol oxides in some food products by using an optimized transesterification method

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Abstract

An increased intake of phytosterols (PS), as well as new products launched on the market enriched with PS and phytostanols, increases the demands for improved analytical techniques. In the present study, a method for the determination of phytosterol oxidation products (POP) was evaluated and optimized. The method included transesterification, enrichment with amino SPE cartridges and quantification by GC. The methodology was evaluated with authentic samples of sito-, campe- and stigma-sterol oxidation products and the linearity and recovery for some POP were >0.95 and 80–120%, respectively. The lowest spiking level determined was 0.5 μg and lower levels could be quantified. In addition, the formation of POP during thermal oxidation of vegetable oils at 180 °C for 0–2 h was studied and increased levels were observed. A commercially available PS ester-enriched margarine was also included in the investigation and the level of POP was 12 μg/g of spread.

Keywords: Analysis; Campesterol; Enriched spread; Phytosterol oxidation products; Stigmasterol; Sitosterol; Transesterification; Vegetable oils

1. Introduction

Phytosterols (PS) and phytosterol oxidation products (POP) have lately attracted more interest due to the increased consumption of vegetable food products and the introduction of products enriched with PS or phytostanols. Validated analytical methods for the unoxidized PS in different matrices have previously been described (Abidi, 2001; AOAC, 2000). However, for the analysis of POP, the method characteristics are limited and, so far, no well-documented method has been described in the literature. The development of reliable analytical methods and possibilities of calculating the daily intake of POP are of great importance for consumers.

The most commonly used method in the analysis of PS and POP is extraction of the lipids from the sample, followed by saponification with an ethanolic or methanolic solution of KOH. Enrichment, using the solid phase extraction (SPE) technique, is usually performed prior to quantification by gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC) or high performance liquid chromatography–mass spectrometry (HPLC–MS) (Dutta, Przybylski, & Appelqvist, 1996). This methodology was used to study the formation of POP in different vegetable oils and products prepared in these oils (Dutta, 1997; Dutta & Appelqvist, 1997). A twofold SPE enrichment step prior to quantification increased the efficiency of the technique and it was stated by the authors that the elimination of unoxidized PS prior to determination of POP was crucial in order to achieve better sensitivity of the method. It was also reported, in those studies, that the levels of POP increased, both in the oils and in the food products during frying.

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In another study, the formation of POP in soy, olive, maize and peanut oil was reported during heating at 180 °C for various times (Lebovics et al., 1999). Heating at 180 °C during 240 min converted 3.9 ≅ 6.7% of the PS originally present in the oils to oxidized products. Increased levels of POP were also reported when rapeseed oil was heated at 180 °C for up to 24 h (Lampi, Juntunen, Toivo, & Piironen, 2002). Samples were taken every 6th h and the major POP formed were the epimeric 7-hydroxysterols, epoxides and 7-ketosterols. In addition, some method characteristics were determined for the analytical procedure, using cholesterol oxidation products as standards (COP), and a relative recovery of more than 90% was reported. The major sample losses were supposed to be in the saponification step (Lampi et al., 2002). Recently, a cold saponification technique, in combination with GC-FID, was used in the investigation of POP in a fortified spread (Grandgirard, Martine, Joffre, Juaneda, & Berdeaux, 2004). The separation of the POP was performed on two capillary GC columns and numerous POP were identified in the spread. A rough estimation of the POP content was calculated to be 68 μg/g spread.

Besides saponification, transesterification can be used when bulk lipids are separated from the POP. The methodology involves the cleavage of ester bondings, followed by formation of methyl esters of fatty acids (FAME) (Liu, 1994; Stransky & Jursik, 1996). However, data in this area are limited.

Biedermann, Konrad, and Mariani (1993) investigated the content of unoxidized sterols in different edible oils and fats and compared a method based on saponification with a method based on transesterification. The transesterification of the triacylglycerols and of the sterol esters was completed after 5 and 15 min, respectively. Data from the transesterification analyses showed good repeatability when numerous analyses on sunflower oil were performed, and the results obtained were comparable with previous reported data using saponification.

In another study, based on transesterification, SPE and GC were used for the determination of COP in sausages and cheeses (Schmarr, Gross, & Shibamoto, 1996). Satisfactory recovery data were indicated for both polar and moderate polar oxidation products and even for the most polar product, cholestane-3β,5α,6β-triol, a recovery of over 85% was obtained. The authors also reported sufficient separation of unoxidized cholesterol and COP when enrichment by SPE was used.

In order to calculate the daily intake of POP it is of great importance that the analytical methods are well validated. A properly performed method validation is therefore crucial for the results to be considered reliable and accurate. The method characteristics should include evaluations and determination of specificity, precision, accuracy, sensitivity, as well as limitations of the method (IUPAC, 2002; McCluskey & Devery, 1993). So far, the analytical methods for POP have been developed with COP as model substances (Lampi et al., 2002) because of structural similarities between the molecules and also because POP are not commercially available. However, even though the chemical structures of cholesterol and phytosterols are similar, the methods should be evaluated individually.

The aim of this study was to optimize an analytical method for the determination of POP in food products by using transesterification of the lipid extract. The method characteristics were determined by using authentic oxidation products of stigmasterol, sitosterol and campesterol. In addition, the optimized method was tested and evaluated during the determination of the levels of POP in heated vegetable oils over 2 h as well as in a commercial available PS ester-enriched spread.

2. Materials and methods

2.1. Chemicals and materials

The unoxidized standard sample of stigmasterol was purchased from Sigma–Aldrich (Stockholm, Sweden) and the unoxidized standard sample of a mixture of sitosterol and campesterol was purchased from Research Plus Inc. (Bayonne, NJ, USA). All other solvents or chemicals were of analytical grade and purchased from VWR (Stockholm, Sweden). The amino SPE columns (0.5 g, 6 ml) were purchased from International Sorbent Technology Ltd. (Mid Glamorgan, UK).

2.2. Thermal oxidation, separation and identification of POP

The analytical procedure, including autoxidation, purification, separation and identification of the different POP, has previously been described in detail (Johnsson, Andersson, & Dutta, 2003; Johnsson & Dutta, 2003).

2.3. Transesterification

The transesterification procedure was a modification of a previously described method by Schmarr et al. (1996). 0.5 g of unoxidized maize oil was weighed into a 15 ml glass tube with a stopper. The sample was spiked with known amounts of POP and 5 ml of 10% sodium methylate in dry methanol, diluted with MTBE (4:6, v:v), were added. The sample was mixed by vortex for 30 s and, after 20 min, the sample was mixed by vortex for another 30 s. After an additional 20 min, 1.5 ml of water and 4 ml of dichloromethane were added and the sample was mixed by vortex again. The tube was centrifuged at 3000 rpm for 3 min and the upper aqueous layer was discarded. The remaining organic phase

was neutralized with 2 ml of 1% citric acid, mixed by vortex, centrifuged at 3000 rpm for 3 min and again the upper layer was discarded. Finally, the organic phase was evaporated under nitrogen and some anhydrous sodium sulphate was added with 5 ml of hexane. Prior to SPE purification, the sample was centrifuged at 3000 rpm for 3 min.

2.4. Enrichment of POP from vegetable oil by SPE

After the transesterification, single and a twofold SPE enrichment steps were evaluated for the enrichment of POP from the sample. A 0.5 g amino SPE cartridge was pre-treated with 5 ml hexane and the sample was applied in 5 ml hexane. The unwanted esters were eluted with 2×5 ml of hexane:MTBE (2:1, v:v) and finally the POP were eluted with 5 ml of acetone. The acetone was evaporated to dryness under a stream of nitrogen and the sterol products were reconstituted in 5 ml hexane and, for the twofold enrichment step, the same procedure as previously described was performed an additional time. Prior to GC separation the internal standard, 5α-cholestane, was added and the mixture was derivatized to trimethylsilyl-ethers, as described elsewhere (Johnsson et al., 2003).

2.5. Method optimization

The method was optimized by using authentic samples of POP, purified as described in Section 2.2. The completeness of the transesterification and the following SPE-purification were investigated and evaluated visually by thin-layer chromatography (TLC). Further, 0.5 g of unoxidized maize oil was spiked with POP in the range 0.5–10 μg in order to investigate the linearity, repeatability, recovery and limit of detection, using five sample rounds at each level.

2.6. GC separation

The GC-FID analyses were performed on a combination of two capillary columns of the same dimensions (25 m × 0.2 mm, 0.33 μm) combined with a press-fit connector (NTK kemi, Uppsala, Sweden). The moderate polar capillary column, DB35-MS (International Sorbent Technology Ltd., Mid Glamorgan, UK) was connected to the injector and the non-polar, DBS MS (International Sorbent Technology Ltd., Mid Glamorgan, UK) was connected to the detector. All parameters for the GC analysis have been described elsewhere (Johnsson & Dutta, 2005).

2.7. Oxidation of vegetable oils

Approximately 400 ml of commercially available vegetable oil were oxidized at 180 ± 5 °C for 0–2 h in an 800 ml glass bottle with additional stirring. Samples were taken once per hour and, stored at room temperature in darkness for subsequent analysis. The fatty acid composition and sterol composition of the oils were determined as described elsewhere (Dutta, Helmersson, Kebedu, Alemax, & Appelqvist, 1994) and the POP content as described in previous sections.

2.8. Determination of POP in a spread enriched with PS esters

A margarine sample enriched with PS esters was purchased at a local store. The margarine contained a mixture of stigmasterol, sitosterol and campesterol esters. The spread was extracted with a mixture of hexane and isopropanol (3:2, viv), as previously described by Hara and Radin (1978). The sterol composition and fatty acid composition were determined as previously reported (Dutta et al., 1994) and the POP composition was determined as described in the previous section. Mean values from duplicate analyses were reported from both the investigations of vegetable oils. The relative response factor and recovery calculations were not included when POP levels were calculated.

3. Results and discussion

3.1. Method optimization

Analytical TLC was used to investigate the completeness of the transesterification reaction visually. It was observed that all fatty acids, including esterified sterols (unoxidized and oxidized esterified phytosterols), were esterified during the process. Due to the moderate analytical conditions, no artifact formation was observed on TLC.

By using a single SPE clean-up step, insufficient separation of FAME from POP was noted in TLC analysis. Therefore, a double SPE-step was introduced and analytical TLC indicated that the FAME was quantitative. The final procedure in the SPE-enrichment step was the elution of POP with 5 ml of acetone. This step was also investigated with analytical TLC and reported to be quantitative. However, due to strong interactions with the cartridge material and co-elution of peaks from the unoxidized maize oil, 4-campesten-6α-ol-3-one, 4-campesten-6β-ol-3-one, 4-sitosten-6α-ol-3-one, 4-sitosten-6β-ol-3-one, 24-hydroxycampesterol and 24-hydroxysitosterol were excluded from further optimization.

In the present study, the recovery of stigmasterol oxidation products was investigated in 0.5 g of unoxidized maize oil at three levels (1, 5 and 10 μg) with five replicates at each level. The recoveries were calculated using 5α-cholestane as an internal standard and, also, the background noise was taken into consideration. At the

lowest spiking level (1 μg), the recoveries ranged from 93% to 112% for 7-ketostigmasterol and 24-hydroxystigmasterol, respectively (Table 1). The recoveries reported for the two higher spiking levels (5 and 10 μg) ranged from 95% to 105% for 7α-hydroxystigmasterol (5 μg) and 25-hydroxystigmasterol (10 μg), respectively. Data for the stigmasterol oxidation products clearly indicated that the methodology was comparable with other existing methods with acceptable recoveries for both medium polar and polar oxidation products.

For sito- and campe-sterols, the recoveries had a wider range, probably because the commercially available sterols were in a mixture when the oxidation products were prepared (Johnsson & Dutta, 2003). At the lowest spiking level, the recoveries ranged from 84% to 121% for campesterol-5α,6α-epoxide and 7β-hydroxysitosterol, respectively (Table 1). For the epoxides, recoveries were below 90% except for 0.97 μg campesterol-5β,6β-epoxide (114%). Recoveries over 100% were achieved for 7β-hydroxysterols, from both sitosterol and campesterol. For further knowledge of the method characteristics, the use of extremely pure authentic samples of POP is necessary.

The recovery results from the present study were in line with previously reported data where the transesterification procedure has been used for the analysis of COP and unoxidized sterols (Biedermann et al., 1993; Schmarr et al., 1996). Schmarr et al. (1996) reported recoveries of COP between 86% and 107%, and the lowest recoveries were reported for cholestane-3β,5α,6β-triol and 19-hydroxycholesterol. Data recently published, reported recoveries of 16–89% for numerous COP when transesterification methodology was used in the analysis of lard (Ubhayasekera, Verleyen, & Dutta, 2004). However, the authors postulated that the SPE cartridges might have been overloaded. Recoveries below 100% are commonly reported and might be caused by interactions with the SPE-cartridge or other losses during the analytical procedure. Due to matrix effects, or other analytical difficulties, recoveries in excess of 100% are also commonly reported (Lampi et al., 2002; Schmarr et al., 1996). In general, the measurement uncertainty is often affected by factors such as volumes, weights, temperature, storage and treatment and concentration of the sample. All these parameters are important to note when analytical methods are developed and optimized (IUPAC, 2002).

The precision of the method was determined as the repeatability (McCluskey & Devery, 1993) with five analyses of each oxidation product, at three different levels (Table 1). The, coefficient of variance (CV) for most of the products, for all levels investigated, was less than or just above 15%. The results were in line with a recently published evaluation of different methods for the analysis of COP in lard (Ubhayasekera et al., 2004). In that study, three spiking levels (5, 10 and 20 μg) were investigated and CV values in excess of 10% were reported for many of the investigated COP at these levels. Good precision was reported when a method for the determination of POP was evaluated with COP as standard substances (Lampi et al., 2002). However, a CV-value of 12% was reported for the

Table 1
The repeatability and the recovery, investigated using authentic POP spiked at different levels. The reported values are average, of five replicates

Phytosterol	POP	Level[a]	Recovery[b]	CV[b]	Level[a]	Recovery[b]	CV[b]	Level[a]	Recovery[b]	CV[b]
Stigmasterol	7α-Hydroxy	1.08	93.3	9.9	5.04	94.8	8.7	10	103	5.9
	7β-Hydroxy	1.21	105	10.4	4.84	95.5	11.8	9.67	98.6	7.2
	5β,6β-Epoxide	1.05	97.1	9.4	–[c]	–	–	–	–	–
	5α,6α-Epoxide	0.93	95.5	7.0	–	–	–	–	–	–
	Triol	0.99	106	3	8.9	–	–	–	–	–
	25-Hydroxy	0.97	96.1	5.5	5.03	97.2	7.7	10.1	105	5.9
	24-Hydroxy	1.00	112	11.5	–	–	–	–	–	
	7-Keto	1.01	92.7	13.7	4.84	100	5.8	9.68	104	4.1
Campesterol	7α-Hydroxy	0.63	105	12.8	1.94	78.1	6.3	4.95	99.5	11.5
	7β-Hydroxy	0.6.8	106	6.0	2.89	99.3	7.2	5.54	103	6.8
	5β,6β-Epoxide	0.97	114	16.1	1.95	85.8	7.9	–	–	–
	5α,6α-Epoxide	0.60	84.0	8.3	5.45	86.1	13.8	–	–	–
	Triol	0.58	98.8	11.0	–	–	–	–	–	–
	7α-Keto	0.69	86.3	13.5	3.21	95.3	6.1	6.25	92.7	5.7
Sitosterol	7α-Hydroxy	1.52	109	8.9	4.89	80.4	3.8	11.8	96.8	11.1
	7β-Hydroxy	1.70	121	6.7	7.08	110	6.2	12.6	105	7.7
	5β,6β-Epoxide	0.92	87.7	7.9	3.43	83.8	8.6	–	–	–
	5α,6α-Epoxide	1.29	85.9	8.1	5.05	83.7	11.4	–	–	–
	Triol	1.48	109	11.7	–	–	–	–	–	–
	7-Keto	1.47	112	15.1	5.72	93.3	8.0	9.68	78.9	3.0

[a] Spiked levels in μg.
[b] Recoveries and CV measured in %.
[c] Not detected.

Table 2
The linearity, determined over the range 0.5–10 μg for some POP

Oxidation product	Sitosterol	Campesterol	Stigmasterol
7α-Hydroxy	0.998	0.996	0.999
7β-Hydroxy	0.971	0.981	0.988
25-Hydroxy	–	–	0.999
7-Keto	0.956	0.982	0.998

All products evaluated had correlation coefficients above 0.95.

determination of cholestanetriol. In another report, Horwitz summarized over 150 independent AOCS inter-laboratory studies covering numerous topics (Horwitz, 1982; Horwitz, 1998). From the investigation, a generalized formula was reported for the appearance of uncertainty at different levels in food chemical analysis. The formula was independent of the nature of the analyte or analytical technique used for the determination. The established empirical formula established that, for analytical methods working in the ppm-region, CV-values for the repeatability up to 16% were acceptable.

The linearities for 7α-hydroxy-, 7β-hydroxy- and 7-ketoproducts of the three PS, as well as for 25-hydroxystigmasterol, were determined over the range 0.5–10 μg. The correlation coefficient (r^2) was studied, where $r^2 = 1$ represents a perfect fit to a set of data points. Correlation coefficients were higher for the oxidation products of stigmasterol (0.999.0., 8,0.999 and 0.998 for 7α-hydroxy-, 7β-hydroxy-, 25-hydroxy- and 7-ketostigmasterol, respectively), than for the corresponding sito- and campe-sterol products (Table 2).

The natural levels of POP in unoxidized maize oil were determined to be low and therefore maize oil was used as a standard oil in this study for the calculation of limit of detection (LOD). These results were in line with previously reported data (Lebovics et al., 1999). The lowest spiking level used in the present study was 0.5 μg/0.5 g of unoxidized maize oil. However, the detector response of the relevant peaks indicated that levels of POP below the spiking levels could be quantified. It was estimated that the lowest amount of detectable POP was less than 0.1 μg/g oil, using the method procedure described in previous sections. However, it has to be carefully considered that every matrix should be evaluated individually since the background noise differs in different matrices.

The relative response factor for the POP was not evaluated in this study since it has been reported elsewhere that it may be disregarded in the analysis of POP (Lampi et al., 2002; Nourooz-Zadeh & Appelqvist, 1992). The risk with this assumption was a small over- or underestimation of the amount of POP.

3.2. POP in heated vegetable oils

The analytical procedure optimized in this study was tested in the investigation of the formation of POP during heating of some commercial available vegetable oils. The oxidation of the oils was conducted during 2 h in order to evaluate the method and to study the formation of POP. The fatty acid (FA) profile of the oils was reported to be similar to previously reported data (Leissner et al., 1989). In addition, the PS composition of the oils was determined (Table 3). Maize oil contained the most unoxidized sterols, although considerably lower amounts than previously published values, followed by peanut oil and olive oil (Lebovics et al., 1999; Piironen & Lampi, 2004). The dominant PS in all three oils was sitosterol (Table 3).

Table 3
Sterol composition of the food products included in the present study

Vegetable oil	Campesterol[a]	Stigmasterol[a]	Sitosterol[a]	Total[a]
Maize oil	42	37	179	258
Peanut oil	36	31	143	210
Olive oil	18	6	78	103

[a] mg/100 g food product.

In the literature, many different oxidation methods have been reported for the investigation of POP formation in vegetable oils (Dutta, 1997; Lampi et al., 2002; Oehrl, Hansen, Rohrer, & Fenner, 2001). In the present study, approximately 400 ml of oil were oxidized in an 800 ml glass bottle with continuously stirring. During oxidation of the vegetable oils the major POP formed were epimeric 7-hydroxysterols, epoxides, triols and 7-ketosterols. During heating of olive oil for 0–2 h the total levels of POP increased from 7.7 μg/g oil to 17.6 μg/g oil. The same trend was reported from maize oil and this was in line with previously published results (Dutta, 1997) (Table 4 and Fig. 1). During heating of peanut oil, the levels of POP were almost unchanged (7.1–6.8 μg/g oil). Slight decrease in the levels of oxidation products has previously been reported (Lampi et al., 2002; Osada, Kodama, Yamada, & Sugano, 1993). The lowering effect for some POP might be caused by sample preparation or other simultaneous reactions in the oil, such as isomerization and decomposition.

3.3. POP in a spread enriched with PS esters

Recently, enriched products containing esters of phytosterols and phytostanols have been launched on the market and additional products will soon be available for consumers. However, the investigations of the levels of POP in these products are so far limited and, as far as we know, only one investigation regarding POP in fortified spread has been published (Grandgirard et al., 2004). In that study, POP were separated using two different capillary GC-columns and numerous oxidation products were identified in the spread. When using the enriched spread as recommended, the daily intake of

Table 4
POP composition of vegetable oils during heating at 180 ± 5 °C for 0–2 h

Oxidation product	Olive oil[a]			Peanut oil[a]			Maize oil[a]		
	0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
7α-Hydroxycampesterol	0.2	0.2	0.6	0.3	0.3	0.3	0.1	0.6	0.7
7α-Hydroxystigmasterol	0.6	0.6	1.2	0.3	0.3	0.3	0.2	0.3	0.5
7α-Hydroxysitosteroi	0.3	1.7	4.7	0.4	0.5	0.8	0.3	1.8	1.3
7β-Hydroxycampesterol	–[b]	–	–	0.2	–	–	–	–	0.9
7β-Hydroxystigmasterol	0.3	0.3	0.8	0.4	0.2	0.2	–	–	0.7
7β-Hydroxysitosterol	0.2	0.5	3.2	0.6	0.2	0.9	0.3	2.7	2.8
24-Hydroxystigmasterol	0.4	0.6	–	–	–	–	–	0.2	0.9
24-Hydroxycampesterol	–	–	–	0.4	0.4	0.7	–	–	–
24-Hydroxysitosterol	–	–	–	0.2	0.2	–	–	–	–
Campesterol-5β,6β-epoxide	0.1	–	0.3	–	–	0.2	0.2	0.3	0.3
Campesterol-5α,6α-epoxide	0.1	0.6	0.3	0.3	0.2	0.2	0.2	0.3	0.2
Stigmasterol-5β,6β-epoxide	–	0.2	–	–	–	–	–	–	–
Stigmasterol-5α,6α-epoxide	–	–	–	–	0.2	–	–	–	–
Sitosterol-5β,6β-epoxide	–	–	–	–	–	0.2	0.6	0.7	0.5
Sitosterol-5α,6α-epoxide	–	–	–	0.3	0.6	0.3	0.5	0.9	0.2
Campestanetriol	–	–	–	0.8	0.2	–	0.3	0.5	0.2
Stigmastanetriol	4.3	3.8	4.4	–	0.2	0.6	0.2	0.3	0.3
Sitostanetriol	0.4	0.4	0.5	0.9	0.2	–	0.3	1.6	0.8
7-Ketocampesterol	–	–	–	–	0.2	0.3	–	–	0.4
7-Ketostigmasterol	0.1	–	–	0.7	0.6	0.5	0.1	0.3	–
7-Ketositosterol	0.8	1.3	1.6	1.3	1.1	1.3	1.0	1.8	1.5
Total amount of POP[a]	7.7	10.2	17.6	7.1	5.4	6.8	4.3	12.4	12.2

The POP were identified using relative retention times from previously characterized standards.
[a] μg/g oil.
[b] Not detected.

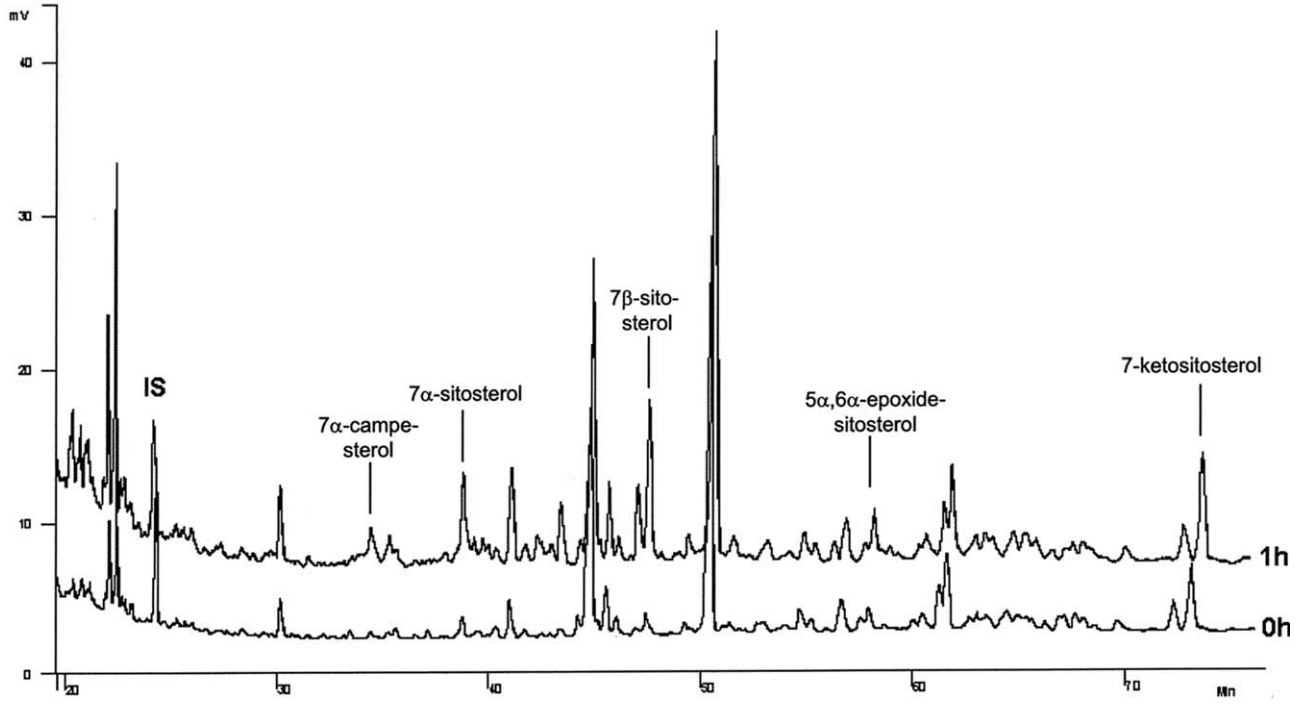

Fig. 1. GC-chromatograms of POP from unoxidized maize oil (lower chromatogram) and maize oil oxidized at 180 °C during 1 h (upper chromatogram). Increased formation during heating is illustrated for some common POP.

POP was estimated to 1.3–1.7 mg and the levels of POP in the spread were roughly estimated to be 68 μg/g of spread. Since POP have been observed in human plasma, as well as in enriched spreads, the authors concluded that further studies in this area should be conducted (Grandgirard et al., 2004).

Table 5
POP composition of a PS esters-enriched spread

Oxidation product	Level[a]
7α-Hydroxycampesterol	0.8
7α-Hydroxystigmasterol	1.1
7α-Hydroxysitosterol	1.6
7β-Hydroxycampesterol	1.1
7β-Hydroxystlgmasterol	1.4
7β-Hydroxysitosterol	2.5
Sitosterol-5β,6β-epoxide	1.3
Stigmasterol-5β,6β-epoxide	1.3
Campesterol-5α,6α-epoxide	1.6
Sitostanetriol	1.7
Campestanetriol	1.4
Stigmastanetriol	1.5
24-Hydroxystigmasterol	2.0
24-Hydroxysitosterol	2.3
24-Hydroxycampesterol	1.8
7-Ketocampesterol	3.3
7-Ketostigmasterol	2.5
7-Ketositosterol	5.5
Total amount of POP	34.7

The POP were identified using relative retention times from previously characterized standards.
[a] μg/g lipid.

In the present study, a PS ester-enriched spread, purchased at a local store, was characterized using various methods. The dominating fatty acids were the unsaturated oleic acid (18:1, 28%) and linoleic acid (18:2, 44%) but 6% linolenic acid (18:3) was also determined. Major phytosterols in the spread were sitosterol (44%), followed by campesterol (29%) and stigmasterol (27%). An ordinary non-enriched spread was also characterized and the sterol composition was sitosterol (75%), campesterol (18%) and stigmasterol (7%). Many POP were identified in the enriched spread, using relative retention times of known peaks in the GC-system described in the previous section. The total amount of identified POP was calculated to be almost 35 μg/g lipid in the spread (12 μg/g spread) and major oxidation products identified were epimeric 7-hydroxysterol, 24-hydroxysterol, epoxides, triols and 7-ketosterol (Table 5). However, more efficient separation of the POP and the use of mass spectrometry are crucial for further identification and characterization of POP in enriched food products.

4. Conclusions

The number of food products enriched with PS and phytostanol esters on the commercial market will increase in the future and it is an urgent need that efficient and accurate analytical methods for the determination of PS and POP in food products are developed and optimized. In this study, the use of transesterification, in combination with SPE, appears to be a rapid and efficient technique for determining the enrichment of POP in different food products.

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