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CONTENTS OF PHYTOSTEROLS AND OXYPHYTOSTEROLS IN SOYBEAN OIL WITH DIFFERENT ADDITION OF SEA BUCKTHORN SEED OIL AFTER THERMAL PROCESSING

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Key words: phytosterols, phytosterols oxidation products, plant oils

The aim of the study was to determine contents of phytosterol and oxyphytosterols in soybean oil and soybean oil with the addition of sea buckthorn seed oil after thermal processing, and to assay the oxidative stability of the oils.

The analysis verified that the thermal processing of soybean oil at 120°C induced a decrease in total phytosterols (phytosterols concentration was reduced by 5.2 to 21.0%) and formation of the following phytosterol oxidation products: 7-ketositosterol, 5α,6α-epoxysitosterol, 7-ketocampesterol, 5α,6α-epoxycampesterol, 7-ketostigmasterol, and 5α,6α-epoxystigmasterol. The content of phytosterol oxidation products (POPs) increased with the higher sea buckthorn seed oil addition to soybean oil (from 1.2 mg/100 g in soybean oil with 1% addition of sea buckthorn oil to 11.4 mg/100 g in soybean oil with 5% addition of sea buckthorn oil) during Rancimat test.

INTRODUCTION

Thermal processing is one of the most popular methods of food preparation. During the process, fat undergoes chemical and physical alterations, namely fat components are oxidized and flavour properties are changed due to the formation of volatile, non-volatile and polar compounds [Rudzińska *et al*., 2004].

There are many methods of monitoring fat changes. One of these is Rancimat test, which consists in accelerated fat oxidation and determination of the oxidative stability. Another indicator of oxidation are concentrations of primary and secondary oxidation products of fatty acids and phytosterols. Phytosterols are present in foodstuffs of plant origin and the most abundant are β-sitosterol, stigmasterol, campesterol and brassicasterol. In the highest amount phytosterols occur in oils, nuts, fats, and cereal products [Thanh *et al*., 2006]. Phytosterols have chemical structure that resembles that of cholesterol, but plant sterols contain an additional ethyl and methyl group in the side of the chain [Krygier, 2004]. Those additional groups result in relatively poor intestine absorption of phytosterols in comparison with cholesterol. Cholesterol intestine absorption ranges from 20% to 80%, whereas phytosterol absorption is about 5% [Ostund, 2002]. The chemical similarity of phytosterols and cholesterol results in lowering serum cholesterol level in humans because phytosterols are absorbed in the gastrointestinal tract instead of cholesterol. A number of studies have shown that regular consumption of phytosterols leads to a decrease in serum level of low-density lipoprotein (LDL) cholesterol, without any influence on the high-density (HDL) cholesterol [Wolfs *et al.*, 2006]. The intake of food enriched with phytosterols can contribute to decreasing the risk of coronary heart diseases [Krygier, 2004; Thanh *et al.,* 2006].

Phytosterols are susceptible to oxidation and form phytosterol oxidation products during the industrial processing, storage and thermal processing. POPs have been identified in some foods, for instance in flour, coffee, fried potato products, vegetable oil, and phytosterol-enriched spread fats [Dutta, 2004; Soupas *et al*., 2004]. A number of investigations have proven atherogenic, cytotoxic, mutagenic or carcinogenic effects of POPs [Rudzińska, 2003]. However some authors claim that phytosterol oxidation products have less adverse effects on human health compared to cholesterol oxidation products [Dutta, 2004].

Formation of phytosterol and cholesterol oxidation products may be suppressed by preventing plant and animal origin foodstuffs from light, high temperature, oxygen, or by the addition of antioxidants [Rudzińska *et al*., 2004]. Many studies have shown that the addition of plant extracts, *e.g.* herbs and spices extracts, can also prevent fat oxidation [Anwar *et al*., 2007].

Sea buckthorn (*Hippophaë rhamnoides*) is a plant that is recognised as a source of bioactive compounds. Sea buckthorn fruits contain high amounts of antioxidants, vitamins (tocopherols, tocotrienols), phytosterols (especially β-sitosterol) and carotenoids (especially β-carotene), while seed berries contain 6-11% of oil [Zadernowski *et al*., 1997]. The fatty acids composition of sea buckthorn seed oil is characterised by a high contribution of poly- and monounsaturated fatty acids, *e.g.* linoleic acid (18:2 *n*-6) – 30-40%, linolenic acid (18:3 *n*-3) – 20-35%, and oleic acid (18:1 *n*-9) – 13-30% [Yang *et al*.,

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2003; Yang & Kallio, 2002]. Sea buckthorn seed oil consumption enhances antioxidative effects in human body, as well as stimulates immune and cardiovascular systems, mucosa and skin functions [Yang & Kallio, 2002].

The aim of the study was to determine contents of phytosterols and oxyphytosterols in soybean oil and soybean oil with the addition of sea buckthorn seed oil after thermal processing, and to assay the oxidative stability of the oils.

MATERIALS AND METHODS

Oils sampling

Soybean oil and sea buckthorn seed oils were purchased in November 2006 on the Warsaw market.

Chemicals

Internal standards: 5α-cholestane was purchased from Sigma–Aldrich, and 19-hydroxycholesterol was purchased from Steraloids. The following organic solvents were used: hexane, isooctane, methanol, 2-propanol, all solvents were of analytical grade.

Determination of oxidative stability of oils

The 679 Rancimat apparatus (Metrohm Ltd., Herisau, Switzerland) was used for the Rancimat method test. A fat sample (2.5 g) was put into a glass tube. Dry air was set at 20 L/h and the temperature of the heating block was set at 120°C. The outlet vapours were collected in distilled water in which the conductivity was monitored continuously at room temperature until the induction was researched. All the tests were conducted in triplicate.

Phytosterols and phytosterol oxidation products determination

About 0.1 g of oil was weighed and dissolved in 2 mL of hexane, then internal standards: 5α-cholestane (16 μg) and 19-hydroxycholesterol (10 μg) were added. The samples were saponificated with the addition of 0.5 mL of 2 mol/L KOH in methanol for 1 h and were periodically shaken. Afterwards, 200 μL of hexane layer were collected and evaporated under a nitrogen stream, whereas trimethylsilyl esters derivatives were made by the addition of 200 μL of a silylating reagent (BSTFA with 1% TCMS) and 200 μL of pyridine, at a room temperature for 18 h. Then, the sample was evaporated under a nitrogen stream and 1 mL of hexane was added [Grandgirard *et al*., 2004; Johnsson & Dutta, 2006; Yang *et al*., 2003].

The analyses were carried out using a gas chromatograph coupled with a mass spectrometer (Shimadzu GC-2010 with Shimadzu GC-MSQ2010S) equipped with a DB5ms capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness). The carrier gas was helium at a flow rate of 0.94 mL/min. The temperature programme was: initial 50°C for 2 min, 50°C-230°C with 15°C/min increase, then 230°C-310°C with 3°C increase, final temperature was held for 10 min. The injector temperature was 230°C. Temperature of ion source was 220°C, ionization energy was 70V. The total ion monitoring mode (100-600 m/z) was used to detect phytosterols and oxyphytosterols.

The quantitative and qualitative analyses of phytosterols were performed by the comparison of retention times of their standards. β-Sitosterol, campesterol and stigmasterol oxidation products were identified based on mass spectra from recent literature [Grandgirard *et al*., 2004; Rudzińska, 2003; Yang *et al*., 2003] and on the basis of mass spectra from NIST 147 and Wiley 175 libraries. The quantitative analyses were made using the internal standards. Three replicates per each sample were analysed.

The results were processed statistically with the use of Statistica 6.0 program. The results were subjected to the statistical one-way analysis of variance. The simple correlation coefficient was calculated. A significance level of $p=0.05$ was adopted in calculations.

RESULTS AND DISCUSSION

Results of the determination of oxidative stability of soybean oil and soybean oil with the addition of 1, 3, and 5% of sea buckthorn seed oil did not show any statistically significant differences in the induction periods of the analysed oils oxidation. The addition of 1, 3 and 5% of sea buckthorn seed oil to soybean oil caused extension of oxidation induction periods by 0.1 h on average (Figure 1). The induction period of sea buckthorn seed oil oxidation (7.72 h) was twofold longer than that of soybean oil (3.11 h). Statistical treatment of analytical data showed a significant correlation between the induction times of oxidation of soybean oil, soybean oils with the addition of sea buckthorn seed oil and sea buckthorn seed oil. The multinomial model was applied in the study. The multinomial coefficient was 0.86, which means that the greater addition of sea buckthorn seed oil affected the extension of the induction time of soybean oil oxidation (Figure 1).

The induction period of soybean oil oxidation reported by Anwar *et al*. [2003] was 2.61 h and was shorter than that determined in our experiment (3.11 h). This difference was, probably, due to Rancimat test methodology. However, Wroniak *et al*. [2006] achieved similar results of soybean oil Rancimat tests – 3.8 h. In another study Anwar *et al*. [2007] showed that each addition of 20% of the *Moringa oleifera* oil to soybean and sunflower oil was lengthening the induction period by 1.47 h and 1.58 h, respectively. Moreover the addition of 80% *Moringa oleifera* oil to soybean and sunflower oil

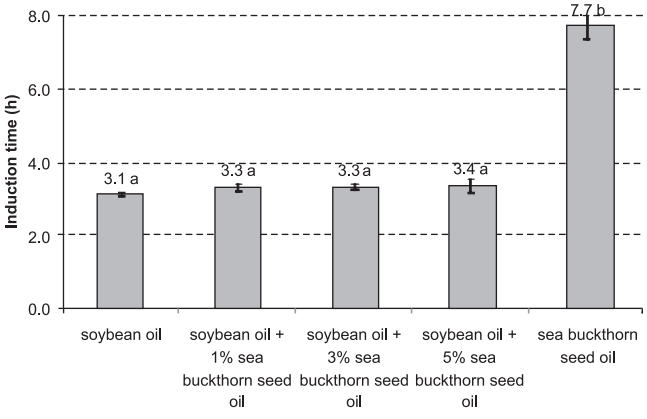

FIGURE 1. Induction times of soybean oil oxidation with various sea buckthorn seed oil addition (a,b -values with different letters are significantly different ($p<0.05$) in oils).

prolonged the induction time from 1.47 h to 6.22 h and from 1.12 h to 5.99 h, respectively [Anwar *et al*., 2007].

Various levels of the addition of sea buckthorn seed oil to soybean oil resulted in changes of phytosterols content. β-Sitosterol, campesterol and stigmasterol were determined in all analysed samples (Table 1). Thermally-processed samples of soybean oil with the addition of sea buckthorn seed oil contained a lower concentration of phytosterols compared with oil samples that were not processed. A loss of particular phytosterols in soybean oil with the addition of sea buckthorn seed oils was found to depend on the level of this addition. The highest losses of the total phytosterols after the Rancimat test were observed in sea buckthorn seed oil. Their concentration decreased from 121.0 to 82.3 mg/100 g, which means their losses reached about 32%. Similar losses of phytosterols were observed during thermal processing of soybean oil and soybean oils with the addition of 1 and 3% of sea buckthorn seed oil, *i.e.* from 12.0 to 14.1%. The total concentration of phytosterols in soybean oil with 5% of sea buckthorn seed oil addition after the Rancimat test decreased from 307.5 to 275.1 mg/100 g. The loss of total phytosterols reached 10.6% and was lower than in soybean oil. This observation may suggest that the addition of 5% of sea buckthorn seed oil to soybean oil could prevent phytosterol oxidation during thermal processing. This is likely to result from the high content of tocopherols in sea buckthorn oil, *e.g.* the total content of tocopherols in berries ranges from 101.4 to 128.3 mg/100 g [Zadernowski *et al.,* 2003].

Other experiments were aimed at investigating losses of phytosterols during refining. Verleyen *et al*. [2002] stated that the physical and chemical method of refining soybean oil led to a reduction in phytosterols content. Increasing the temperature of refining from 220 to 260°C led to ever greater losses of the total phytosterols in the oils analysed. After physical refining, a decrease of the total phytosterols content ranged from 325 mg/100 g to 220 mg/100 g in soybean oil sample refined at 260°C for 60 min. In turn, the chemical method of refining led to the total phytosterols content reduction from 331 mg/100 g to 208 mg/100 g in soybean oil sample refined at the same conditions [Verleyen *et al*., 2002]. Oehrl *et al*. [2001] investigated concentrations of phytosterols and phytosterol oxidation products after thermal processing of soybean oil at 100°C for 20 h. Losses of β-sitosterol accounted for 37%, and these of campesterol – for about 44%. Rudzińska *et al.* [2003] heated soybean, sunflower and rapeseed oils at 120°C for 4 h and reported 4.3, 3.7 and 2.6% losses of the total phytosterols. The differences between results of the present study and other researchers' data might be due to various material examined (soybean origin, or genetic modification) or to conditions of the heat treatment [Vlahakis & Hazebroek, 2000; Yamaya *et al.,* 2007].

The non-heated oils did not contain phytosterol oxidation products. After thermal processing the POPs were formed in oil samples, and the following phytosterol oxidation products were determined: 5α,6α-epoxysitosterol, 7-ketositosterol, 5α,6α-epoxycampesterol, 7-ketocampesterol, 5α,6α-epoxystigmasterol, and 7-ketostigmasterol. Rudzińska *et al*. [2005] published similar results of heating rapeseed oil and oil used during thermal processing of French fries at 180°C.

TABLE 1. Contents of phytsterols and phytosterol oxidation products (mg/100 g) in soybean oil with various addition of sea buckthorn seed oil before and after Rancimat test (RT).

Samples and treatement		Phytosterols (mg/100 g)				Phytosterol oxidation products (mg/100 g)						Phytosterols losses after Rancimat test(%)
		β-sitosterol	Campesterol	Stigmasterol	Total phytosterols content	7-ketositosterol	5α,6α-epoxysitosterol	7-ketocampesterol	5α,6α-epoxycampesterol	7-ketostigma sterol	5α,6α-epoxystigmasterol	Total oxysterols content	
Soybean oil	Before RT	224.5±14.7^{a}	46,3±5,9^{a}	45,6±4,2^{a}	320.7±4.7^{a}	n.d.	nd.	nd.	nd.	nd.	nd.	nd.	12.0
	After RT	205.9±12.2^{a}	39.4±2,8^{a}	36.2±3,5^{a}	281.5±11.4^{b}	0.6±0.02	nd.	nd.	nd.	nd.	nd.	0.6±0.02	
Soybean oil + 1% sea buckthorn seed oil	Before RT	222.1±4.7^{a}	50.5±1.4^{a}	46.0±2.1^{a}	318.6±8.6^{a}	n.d.	nd.	nd.	nd.	nd.	nd.	nd.	13.4
	After RT	191.7±8.3^{a}	43.8±3.8^{a}	40.4±1.8^{a}	275.9±2.9^{b}	0.2±0.08	0.5±0.1	0.3±0.1	0.2±0.1	0.1±0.05	nd	1.3±0.43	
Soybean oil + 3% sea buckthorn seed oil	Before RT	219.0±6.3^{a}	47.2±3.6^{a}	45.9±3.7^{a}	312.7±6.2^{a}	n.d.	nd.	nd.	nd.	nd.	nd.	nd.	14.1
	After RT	190.8±9.3^{a}	40.7±1.5^{a}	36.7±2.1^{a}	268.1±5.7^{b}	0.2±0.05	0.7±0.4	0.4±0.1	0.4±0.1	0.2±0.1	0.1±0.05	2.0±0.8	
Soybean oil + 5% sea buckthorn seed oil	Before RT	217.1±6.6^{a}	46.6±2.1^{a}	43.8±1.8^{a}	307.5±6.3^{b}	n.d.	nd.	nd.	nd.	nd.	nd.	nd.	10.6
	After RT	190.1±7.6^{a}	42.9±1.1^{a}	42.1±0.6^{a}	275.1±7.1^{b}	0.3±0.05	1.3±0.4	0.5±0.1	0.9±0.2	0.3±0.1	0.2±0.05	3.5±0.9	
Sea buckthorn seed oil	Before RT	109.8±6.5^{a}	5.0±1.1^{a}	6.2±0.6^{a}	121.0±4.9^{a}	n.d.	n.d.	n.d.	n.d.	nd.	nd.	n.d.	32.0
	After RT	81.0±4.1^{b}	1.3±0.3^{b}	n.d.^{b}	82.3±4.4^{b}	2.7±0.4	1.4±0.2	n.d.	0.1±0.03	nd.	nd.	4.2±0.63	

a,b -Values within a row with different letters are significantly different ($p<0.05$) in oils before and after Rancimat test; nd – not detected.

During thermal processing, they noticed a decrease in the total phytosterols and formation of epoxysterol, 7-ketosterols, 7-hydroxysterols and triols.

Oxyphytosterols were determined only in oils after Rancimat test. The total concentration of POPs in heated soybean oil and soybean with 1, 3 and 5% addition of sea buckthorn seed oil increased with the greater addition of sea buckthorn seed oil (Table 1). The thermal heating of soybean oil affected the formation of 0.6 mg of POPs in 100 g of oil. In heated soybean oils with the addition of 1, 3 and 5% of sea buckthorn seed oil to soybean oil the total POPs concentration was 1.3, 2.0 and 3.5 mg/100 g, respectively. Sea buckthorn seed oil after Rancimat test contained 4.2 mg of POPs. The statistical analysis of correlation was conducted between sea buckthorn seed oil addition to soybean oil and concentration of total oxyphytosterols applying a linear model. The linear coefficient was 0.96, and there was a strong positive correlation between increasing addition of sea buckthorn seed oil and the content of phytosterol oxidation products. It is worth mentioning that the concentration of POPs during the Rancimat test is not equal to phytosterol concentrations losses due to potential phytosterol degradation. It indicates that the formation of phytosterol oxidation products is only an intermediate stage of phytosterol changes during heating. The losses of phytosterols may be a consequences of not only oxidation but also of polymerisation and degradation of fat [Adcox *et al*., 2001].

Similar results were observed by Oerhl *et al*. [2001] after thermal processing of soybean oil at 100°C for 20 h and with the addition of β-sitosterol (90 mg/100 g), *i.e.* levels of 7-ketositosterol, 5α,6α-epoxysitosterol and 5α,6α-epoxycampesterol accounted for 0.45 mg/100 g, 1.07 mg/100 g and 0.55 mg/100 g oil, respectively.

The addition of sea buckthorn seed oil to the soybean oil resulted in increased formation of POPs during the Rancimat test. It may be a consequence of the higher concentration of polyunsaturated fatty acids in the investigated oil, which can contribute to phytosterol oxidation products formation. Sea buckthorn seed oil contains the highest percentage of linoleic acid (18:2 *n*-6): about 30-40%, linolenic acid(18:3 *n*-3): about 20-35% and oleic acid (18:3 *n*-3): about 20-35% [Yang & Kallio, 2002]. On the other hand, the addition of 5% of sea buckthorn seed oil to soybean oil prevented a decrease in phytosterols content. In many studies it was affirmed that the addition of the plant antioxidant extracts (in the appropriate dosage) to oils prevented oxidation during thermal processing and storage [Anwar *et al*., 2007]. It is likely that the same effect would be exerted by sea buckthorn seed extract, however this needs further investigations.

CONCLUSIONS

The addition of 1, 3 and 5% of sea buckthorn seed oil to the soybean oil did not significantly extend the induction period of Rancimat test. Phytosterols content was significantly decreased in all analysed oils after the Rancimat test, while the phytosterol oxidation products content was increased at the same time. The relatively small losses of the total phytosterols were observed in soybean oil and soybean oil with the addition of sea buckthorn seed oil after the Rancimat test (10.6–14.1%), and the highest losses were noticed in sea buckthorn seed oil (32.0%). It has been observed that POPs content in thermally-processed soybean oil and soybean oil with the addition of sea buckthorn seed oil was higher the higher addition of sea buckthorn oil was. Additionally it has been observed that phytosterol oxidation was only an intermediate stage of phytosterol changes during heating.

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