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THE EFFECT OF RASPBERRY, BLACK CURRANT AND TOMATO SEED EXTRACTS ON OXYPHYTOSTEROLS FORMATION IN PEANUTS

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Fats, oils and other food products of plant origin contain phytosterols which may undergo oxidation during processing and storage. The aim of the study was to identify and quantify phytosterols and their oxidation products in peanuts using GC/MS. Treatment of peanuts with rapeseed oil enriched with antioxidant seed extracts was used to prevent phytosterols oxidation. Ethanolic extracts of raspberry, black currant and tomato seeds obtained from the waste of food processing were used as potential antioxidants. All extracts exhibited the protective effect towards fatty acids as well sterols oxidation in peanut samples although the black currant seed extract was the most effective one.

INTRODUCTION

Oxidation of lipids has been gaining a great concern for several years because of its influence on a decrease in the quality and nutritive value of fat and fat-containing food as well as of the diverse biological activity of the products formed [Wu *et al*., 1999; Frankel, 1998].

Plant sterols (phytosterols) exist in all food items of plant origin: vegetable oils, beans, peanuts, and avocado. They are known to have hypocholesterolemic, anticarcinogenic and other health benefits. The main plant sterol - β-sitosterol, may offer protection from colon, prostate and breast cancer [Moreau *et al*., 1999; Hicks & Moreau, 2001]. As health benefits of a diet become of prime consideration of consumer recently, many food producers have started adding plant sterols to food such as margarines, dressings, and bread. The growing demand on phytosterol-enriched products has increased the interest in phytosterols stability during food processing and storage.

The chemical structure of plant sterols is very similar to that of cholesterol and their reactivity is related [Dutta *et al*., 1997]. Cholesterol oxides are described to have harmful effects on human health, but little is known about the formation, presence and biological effects of oxidation products of plant sterols (oxyphytosterols). Literature data on the presence of oxyphytosterols in various foods is scarce, however evidences on their potential diverse biological effects have focused attention of scientists on these compounds nowadays. The presence of oxyphytosterols in rapeseed and rapeseed oil in relation to the plant metabolism, technological processes and storage was studied by Rudzińska *et al*. [2001, 2002]. There is no data concerning the effect of antioxidants on the oxyphytosterols formation.

Several researchers have suggested that prevention of cholesterol oxidation in processed foods should be similar to procedures undertaken to prevent fatty acids oxidation. Morgan and Armstrong [1987] displayed an inhibitory effect of butylated hydroxyanisole and butylated hydroxytoluene. The application of antioxidants to extend shelf-life of meat products has been reviewed by Gray and Pearson [1984]. Also the use of antioxidants incorporated into animal diets has evoked an effect on meat quality.

Antioxidants are widely used as food additives to prevent oxidation of fats and protect other susceptible food constituents against oxidative damage. Because of the concern over the safety of synthetic antioxidants in recent years, there has been noticed the interest in natural ones. Many compounds of plant origin have been found to posses antioxidant activity in food and model systems [Schwarz *et al*., 2001; Kaur & Kapoor, 2001; Kähkönen *et al*., 1999; Heinonen *et al*., 1998; Amarowicz *et al*., 1997, 1996].

The aim of this study was to analyze the phytosterol and oxyphytosterol composition in peanut samples and to evaluate the effect of rapeseed oil enriched with antioxidant extracts of black currant, raspberry and tomato seeds on sterol resistance against oxidative damage.

MATERIAL AND METHODS

Roasted peanuts were obtained from local chocolate producer "Terravita". Peanuts were then treated in a laboratory. Peanuts (200 g) were placed in a glass bottle and followed by 2 g of salt and 0.3 g of refined rapeseed oil enriched with 0.3% (w/w) seed extracts or without any additives. Samples were then mixed vigorously for 10 min.

Refined rapeseed oil, low erucic acid, was obtained from Z.T. Kruszwica S.A.

Black currant, raspberry and tomato seeds were separated from the waste of processing obtained from the

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food industry, dried, comminuted, defatted with hexane and extracted threefold using 80% aqueous ethanol. The combined extracts were evaporated under vacuum at 45°C and the dry residue was dissolved in ethanol (96%). The ethanolic extracts were characterized for their total phenolics content measured by Folin Ciocalteau colorimetric method [Singleton & Rossi, 1965].

In the experiment performed, the effect was studied of rapeseed oil enriched with seed extracts on the oxyphytosterols formation in peanut samples subjected to accelerated storage condition at 60°C. Rapeseed oil was added to the peanut samples in the amount of 0.12% (w/w). The concentration of seed extracts in rapeseed oil was 0.3%.

The oxidation of lipid fraction of peanut samples was followed by measurement of the peroxide value according to standard method [PN-ISO 3960:1996], and hexanal content determined by headspace gas chromatography (GC).

The GC analyses were performed using a gas chromatograph Varian 3800 equipped with FID detector, capillary column CP Sil 8CB (30 m x 0.53 mm x 1.5 m) and an autosampler headspace Tekmar 7000. Conditions of headspace analysis: platen temperature – 50°C, sample equilibration – 40 min, pressurization time – 0.2 min, loop fill – 0.35 min, injection time – 0.5 min, sample valve temperature – 110°C, transfer line temperature – 120°C. Conditions of GC analysis: the oven temperature was held at 40°C for 1 min after injection. Then it was raised to 120°C at a rate of 8°C/min and to 200°C at a rate of 20°C/min and held for 5 min. The detector temperature was 220°C. Helium was the carrier gas. Identification of peaks was based on retention time of standard sample. Quantitative calculations of hexanal content were carried out on the basis of standard curve.

The content of sterols in the analysed samples was determined by gas chromatography (Hewlett-Packard 5090) according to the method described by Rudzińska *et al*. [2001]. 5-Alpha-cholestan in the concentration of 500 μg/200 μL oil was used as an internal standard. Phytosterols were identified based on the comparison of their retention times with that of authentic standards and by gas chromatography with mass spectrometry (GC/MS).

Oxidized derivatives of plant sterols were determined as described earlier by Rudzińska *et al*. [2001]. As an internal standard 10 μg of 19-hydroxy cholesterol was added to each analysed sample. Oxyphytosterols were separated and identified using GC – Trace 200 and MS – Finnigan Polaris Q.

RESULTS AND DISCUSSION

Peanuts are a whole food naturally containing phytosterols and make an important contribution to the diet in many countries. Processing of peanuts, especially at high temperatures, may cause oxidation of fatty acids as well as phytosterols. The effect of oxidized phytosterol derivatives on health is not known.

Autooxidation of unsaturated fatty acids and unsaturated minor compounds undergoes the same free radical mechanism and oxidation products formed may influence each other [Kim & Nawar, 1991]. Oxidation could be stopped or inhibited by adding antioxidants which can inhibit oxidation and also protect desirable sensory characteristics of food.

Raspberry, black currant and tomato seed extracts contain phenolic compounds whose antioxidant activity is well known. The total content of phenolics in 100 g of dry matter ranged from 70 mg in tomato seeds, 340 mg in black currant to 2 370 mg in raspberry seeds. Antioxidant activity of black currant seed extracts in rapeseed oil and in β-carotene/linoleic acid system was described by Pachołek and Małecka [2000]. The influence of raspberry and black currant seed extract on the lipolytic enzymes activity was followed by Iskra *et al*. [2003].

Treatment of peanut samples with rapeseed oil enriched with seed extracts enabled elucidation of the influence of these natural antioxidants on fatty acids and phytosterol oxidation.

Figure 1 shows the extent of oxidation changes (in %) in the peanut samples after 6 days of incubation at 60°C. The degree of oxidation of the control samples (without any additives) is regarded as 100%.

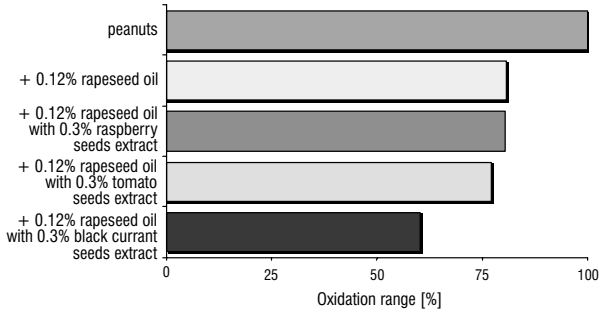

FIGURE 1. The extent of oxidation in peanut samples after 6 days of incubation at 60°C, based on PV determination.

The results of hexanal content are shown in Table 1. The ethanolic extracts of black currant, tomato and raspberry seeds were protective against fatty acids oxidation in peanut samples under the conditions applied. Black currant seed extract was the most effective in retarding the fatty acids oxidation.

TABLE 1. Hexanal content in the peanut samples before and after 6 days of incubation at 60°C.

Sample	Hexanal content [mg/kg]	
	Before	After
Peanuts	0.9 ± 0.0	24.4 ± 0.3
+ 0.12% rapeseed oil	0.9 ± 0.0	22.0 ± 0.1
+ 0.12% rapeseed oil with 0.3% of raspberry seeds extract	0.9 ± 0.0	15.3 ± 0.2
+ 0.12% rapeseed oil with 0.3% of tomato seeds extract	0.9 ± 0.0	18.8 ± 0.1
+ 0.12% rapeseed oil with 0.3% of black currant seeds extract	0.9 ± 0.0	14.2 ± 0.1

The content of phytosterols

Campesterol, stigmasterol, β-sitosterol and avenasterol were identified and quantified in the peanut samples. The total content of phytosterols in peanuts ranged from 3.7 mg/g of sample B and D (control peanuts and peanuts treated with rapeseed oil after incubation) to 6.5 mg/g of sample E (peanuts treated with rapeseed oil enriched with raspberry seed extract after incubation) (Table 2). After incubation of the samples A and C (peanuts and peanuts treated with rapeseed oil), the content of phytosterols

TABLE 2. The content of phytosterols in peanut samples [mg/g].

Sample	Campesterol	Stigmasterol	Sitosterol	Avenasterol	Sum
A	0.84 ± 0.06	0.39 ± 0.02	2.81 ± 0.03	0.63 ± 0.01	4.67
B	0.53 ± 0.10	0.37 ± 0.00	2.10 ± 0.34	0.69 ± 0.34	3.69
C	0.88 ± 0.06	0.62 ± 0.01	2.80 ± 0.02	0.77 ± 0.19	5.07
D	0.68 ± 0.03	0.43 ± 0.05	2.20 ± 0.19	0.47 ± 0.01	3.78
E	1.25 ± 0.15	0.53 ± 0.07	3.75 ± 0.09	0.96 ± 0.08	6.49
F	0.88 ± 0.10	0.76 ± 0.03	3.06 ± 0.08	0.95 ± 0.46	5.65
G	1.10 ± 0.10	0.80 ± 0.08	3.10 ± 0.02	0.92 ± 0.01	5.92

A – peanuts; B – peanuts after 6-day incubation at 60°C; C – peanuts + rapeseed oil; D – peanuts + rapeseed oil after 6day incubation at 60°C; E – peanuts + rapeseed oil + raspberry seed extract after 6-day incubation at 60°C; F – peanuts + rapeseed oil + tomato seed extract after 6-day incubation at 60°C; G – peanuts + rapeseed oil + black currant seed extract after 6-day incubation at 60°C.

decreased by more than 20%. In thesamples of peanuts treated with rapeseed oil enriched with seeds extracts the content of phytosterols was 20–30% higher then in control samples.

In all analysed samples the most abundant sterol was β-sitosterol which covered over 50% of their total content.

The content of oxyphytosterols

Phytosterol oxidation products identified and quantified in peanut samples are listed in Table 3. Twelve oxidized derivatives of phytosterols were identified: 7-α and 7β-hydroxy, β- and α-epoxy-, triol-, 7 keto-campesterol and –sitosterol. The amount of oxidized phytosterols in the analyzed samples ranged from 40 μg/g in peanuts treated with rapeseed oil enriched with black currant extract to 95 μg/g in sample D (peanuts treated with rapeseed oil after incubation at 60°C) (Table 2). The content of oxyphytosterols after incubation of control samples A (peanuts, control) and C (peanuts treated with rapeseed oil) increased by *ca.* 90% in both samples (B and D). The addition of raspberry, tomato and black currant seed extracts resulted in a decrease in oxyphytosterols amount compared to control samples (B and D). In peanuts with black currant extract, the decrease was the highest (60%) but in the other two samples it was also significant and equalled 50% in sample F and 40% in sample E.

When the oxyphytosterol fraction of peanuts was analyzed, the percentage of particular campesterol and sitosterol oxidized derivatives was different than their parent sterols. The most significant was the domination of β-sitosterol oxidized derivatives in the whole oxyphytosteros fraction, which ranged from 72% in sample A to 88% in sample C (Figure 2). The composition of oxyphytosterols fraction underwent changes depending on the additives. Control samples of peanuts (A and B, not treated) contained about 28%, peanuts treated with rapeseed oil (C and D) – about 14%, peanuts with raspberry and tomato seeds extracts – 22%, and peanuts with black currant extract – 14% of campesterol oxidation products.

The abundant oxyphytosterols in all samples were 7β-hydroxy- and α-epoxy-campesterol and -sitosterol derivatives (Figure 3). 7β-hydroxy-campesterol and -sitosterol were present at the concentration of 6–30 μg/g of products, what covered 14–32% of total oxyphytosterols. The percentage concentration of α-epoxy-phytosterol was similar in all samples and ranged from 35% to 48% (18–33 μg/g of products).

The experiment performed shows a significant effect of ethanolic extracts of raspberry, tomato seeds and black currant on the amount of oxyphytosterols in the analyzed peanut samples. The addition of ethanolic seed extracts to peanuts resulted in a decrease in oxyphytosterol content compared to peanuts stored without these. The total content of oxyphytosterols in control sample was 95 μg/g. After treatment with rapeseed oil enriched with seed extracts, the total content of oxidized phytosterols was 59 μg/g in the presence of raspberry extract, 50 μg/g with tomato extract and 40 μg/g with black currant extract.

The amount of phytosterol oxidation products formed was not equal to the sterols lost in peanut samples during

TABLE 3. The content of phytosterol oxidation products in peanut samples [μg/g].

Oxyphytosterols	A	B	C	D	E	F	G
7α-OH-Campesterol	0.55 ± 0.01	3.06 ± 0.06	0.54 ±0.01	4.25 ± 0.09	nd	nd	nd
7α-OH-Sitosterol	2.15 ± 0.36	5.09 ± 1.61	3.21 ± 0.14	5.72 ± 0.72	7.13 ± 0.12	3.58 ± 0.17	2.01 ± 0.16
7β-OH-Campesterol	6.11 ± 0.01	13.66 ± 1.83	4.05 ± 0.13	4.94 ± 0.00	6.91 ± 0.82	5.09 ± 0.19	2.58 ± 0.21
7β-OH-Sitosterol	6.16 ± 0.66	16.80 ± 0.27	5.691 ± 0.06	15.99 ± 0.31	7.12 ± 0.17	6.25 ± 0.13	3.05 ± 0.16
β-epoxy-Campesterol	1.02 ± 0.23	0.80 ± 0.01	nd	nd	nd	nd	nd
β-epoxy-Sitosterol	8.59 ± 1.47	16.59 ± 0.27	13.65 ±0.61	19.84 ± 0.85	10.55 ± 0.65	9.14 ± 0.25	10.81 ± 0.15
α-epoxy-Campesterol	4.37 ± 0.46	6.32 ± 0.21	nd	nd	5.71 ± 0.67	5.85 ± 0.16	2.75 ± 0.12
α-epoxy-Sitosterol	17.31 ± 1.20	26.44 ± 0.04	18.25 ± 0.07	33.65 ± 0.14	18.72 ±0.42	18.21 ± 2.35	16.37 ± 2.31
Triol-Campesterol	1.00 ± 0.00	nd	nd	nd	nd	nd	nd
Triol-Sitosterol	0.75± 0.28	1.95 ± 0.00	1.12 ± 0.00	3.48 ± 0.00	2.04 ± 0.00	nd	2.18 ± 0.12
7-keto-Campesterol	0.33 ± 0.01	1.00 ± 0.00	1.56 ± 0.35	4.96 ± 0.55	0.48 ± 0.01	0.74 ± 0.21	0.21 ± 0.12
7keto-Sitosterol	0.34 ± 0.01	1.52 ± 0.01	2.45 ± 0.00	2.55 ±0.33	0.84 ± 0.01	0.95 ± 0.09	0.32 ± 0.32
Sum	48.68	93.23	50.52	95.38	59.50	49.81	40.28

A – peanuts; B – peanuts after 6-day incubation at 60°C; C – peanuts + rapeseed oil; D – peanuts + rapeseed oil after 6-day incubation at 60°C; E – peanuts + rapeseed oil + raspberry seed extract after 6-day incubation at 60°C; F – peanuts + rapeseed oil + tomato seed extract after 6-day incubation at 60°C; G – peanuts + rapeseed oil + black currant seed extract after 6-day incubation at 60°C.

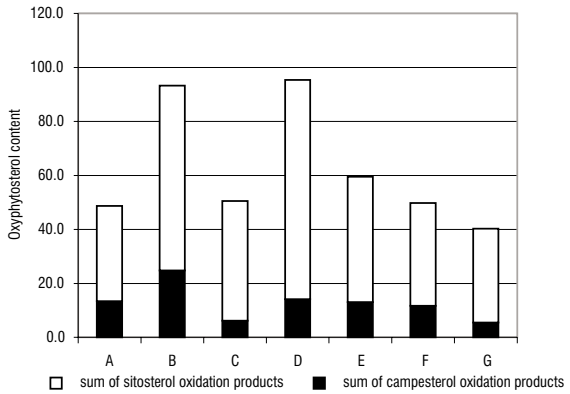

FIGURE 2. Oxidation products of sitosterol and campesterol in peanut samples [g/g].
A – peanuts; B – peanuts after 6-day incubation at 60°C; C – peanuts + rapeseed oil; D – peanuts + rapeseed oil after 6-day incubation at 60°C; E – peanuts + rapeseed oil + raspberry seed extract after 6-day incubation at 60°C; F – peanuts + rapeseed oil + tomato seed extract after 6-day incubation at 60°C; G – peanuts + rapeseed oil +black currant seed extract after 6-day incubation at 60°C.

incubation at 60°C. No oxidation products of avenasterol as well stigmasterol were identified in this experiment. The volatile oxidation products of phytosterol fraction could also originate.

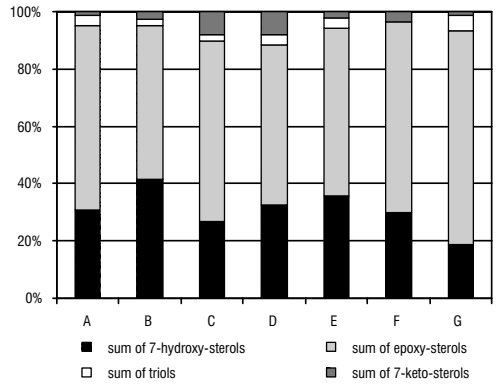

FIGURE 3. Percentage composition of oxyphytosterol derivatives in peanut samples.
A – peanuts; B – peanuts after 6-day incubation at 60°C; C – peanuts + rapeseed oil; D – peanuts + rapeseed oil after 6-day incubation at 60°C; E – peanuts + rapeseed oil + raspberry seed extract after 6-day incubation at 60°C; F – peanuts + rapeseed oil + tomato seed extract after 6-day incubation at 60°C; G – peanuts + rapeseed oil + black currant seed extract after 6-day incubation at 60°C.

CONCLUSION

The results obtained indicate the protective effect of the ethanolic extracts of black currant, raspberry and tomato seed against fatty acids and phytosterol oxidation in peanuts. According to the peroxide value and hexanal content, the most effective as an antioxidant was the ethanolic extract of black currant seeds added in the amount of 0.3%.

In peanut samples treated with rapeseed oil enriched with ethanolic seed extracts, 12 oxidized derivatives of phytosterols were identified (7-α and 7β-hydroxy, β- and α-epoxy, triol and 7-keto-kampesterol and β-sitosterol), and their amount ranged from 40 μg/g in peanuts treated with rapessed oil enriched with raspberry seeds extract to 95 μg/g in peanuts treated with rapeseed oil without any additives. The dominating oxyphytosterols in all samples were 7β-hydroxy- and epoxy-campesterol and β-bsitosterol derivatives.

The addition of rapeseed oil enriched with ethanolic seed extracts to peanuts resulted in a significant retarding of oxyphytosterols formation during incubation at 60°C. Their amount in treated peanuts was lower by 40 to 60% in comparison to the control peanut sample stored without additives. Black currant seed extract was the most effective in retarding oxidation of fatty acids and phytosterols in peanuts.

The total content of phenolic compounds in the analysed extracts does not correlate with their antioxidant activity. The study on the profile of phenolic compounds in raspberry, black currant and tomato seeds is in progress.

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