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PLANT STEROLS IN FOOD TECHNOLOGY

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Abstract. Phytosterols are one class of food constituent with serum cholesterol-lowering properties by inhibiting cholesterol absorption. These reasons led to an increasing availability of phytosterol-enriched foods. The content of phytosterols in rapeseeds ranged from 4.7 mg/g to 6.3 mg/g of seeds depends on variety. After pressed in industrial conditions their content in the oil was 8.7 mg/g. In extracted oil the level of phytosterols was 25% higher then in pressed oil. During refined process, especially neutralization, the decrease of phytosterols content was 20%. Using the rapeseed oil to multiple deep frying of French fries generated next 64% losses. The most important reasons for phytosterols content decrease are their autoxidation processes. Autoxidation of sterols is theorized to be a free radical process. Some processes, such as refining plant oils and deep frying or storage, intentionally induce oxidation to produce phytosterol oxidation products (oxyphytosterols). They were determined in rapeseeds on the level 10-15 μg/g of seeds. During industrial production of rapeseed oil the content of oxyphytosterols systematically increased and after refining it was 100-110 μg/g. During multiple deep-frying of French fries in rapeseed oil oxyphytosterols content increased and after 14th frying their content was 200 μg/g in used oil and 150 μg/g in French fries. Oxyphytosterols are absorbed by humans and their subsequent metabolic conversions may be of toxicological significance.

Key words: phytosterols, oxyphytosterols, plant oils, production, frying, food

INTRODUCTION

Plant sterols, named also as phytosterols, are components of the non-glyceride fraction of natural plant oils, nuts and seeds, cereals and beans. They are important components of plant membranes and serve to stabilize phospholipids bilayers in plant cell membranes [Piironen et al. 2000]. Four classes of sterols are present in food products: free alcoholic sterols, sterol esters, and steryl glycosides (free and acylated). The most common plant sterols are β-sitosterol, campesterol and stigmasterol, which are classified as 4-desmethylsterols of the cholestane series. Typical sterol for rapeseed is brassicasterol. The structure of these plant sterols is similar to that of cholesterol with an extra methyl or ethyl group and a double bond in the side chain. Saturated plant sterols, re-

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ferred to as stanols, have no double bond in the ring structure. The effect of plant sterols and stanols on health has been the subject of various studies [Hicks and Moreau 2001, Normén et al. 2000, Piironen et al. 2000, Plat and Mensink 2001]. Until recently, it was commonly thought that they are superior in lowering serum cholesterol levels and can play an important role in a reduction in the risk of heart disease. And, from the other side, it was demonstrated no toxicity in animal and human organisms. As already noted, the quantity of plant sterols in the normal diet is negatively correlated with cholesterol absorption and serum total and LDL cholesterol. They inhibit not only absorption of dietary but also that of biliary cholesterol. New findings indicate antipolymerizing activity of these compounds in the process of food frying [Boskou, 1998; Blekas and Boskou, 1999]. Phytosterols composition is unique for specific oil. During the last 10 years has been noted unprecedented escalation of interest in phytosterols. It has been proposed that some plant sterols act as antioxidants under frying conditions [Gordon and Magos 1983, White and Armstrong 1986]. New findings indicate antipolymerizing activity of these compounds in the process of food frying [Boskou 1998, Blekas and Boskou 1999]. Phytosterols during production processes, storage and deep-frying of plant oils undergo various physical and chemical processes, such as absorption on bentonite, or hydrogenation or oxidation. The reaction of phytosterols oxidation and formation of oxyphytosterols resembles similar processes observed for cholesterol. The literature data on oxyphytosterols are limited compared to cholesterol oxidation products (COP). Literature data on the presence of oxyphytosterols in various kinds of food is scarce. However, evidence on their potential diverse biological effects has focused scientists' attention on these compounds nowadays. Meyer and Spiteller [1997] showed the toxicity of epoxy derivatives of phytosterols and especially triols.

In this publication results of our experiments with appeared phytosterols in food products, and changes of their content during production, storage, heating and frying will be presented

PHYTOSTEROLS AS COMPOUNDS OF FOOD

Rapeseed oil is still the main source of commercial and household frying fats in Poland and some other countries. The production of refined rapeseed oil in Poland in 2002 year was about 350 thousands tons, and margarines about 370 thousands tons [Polish... 2003]. The hydrogenated frying fats based on the rapeseed oil have modified fatty acids composition but the heat stability of oils and fats depends not only on their fatty acids composition but also on the presence of non-glyceridic constituents such as phytosterols.

Plant sterols were detected in lipids extracted from different varieties of winter rape seeds at the level from 4.7 mg/g of seeds to 6.3 mg/g of seeds. Exemplary data showed in Table 1. The percentage composition of sterol fractions was similar and typical for rapeseed. The dominated sterol was β-sitosterol which was 43%-55%, campesterol – 30%-35% and brassicasterol – 11% in all varieties. During production of rapeseed oil the content of phytosterols decreased but the percentage composition was not changed. In pressed oil received from three Polish factories the content of plant sterols ranged from 8.41 mg/g to 8.72 mg/g and in extracted oil was 25% higher then in pressed oils.

Table 1. The amount of phytosterols in some food products
Tabela 1. Zawartość fitosteroli w wybranych produktach spożywczych

Food products Wybrane produkty spożywcze	Phytosterols content – Zawartość fitosteroli					
	Brassica-sterol Brassika-sterol	Campe-sterol Kampe-sterol	Stigma-sterol Stigma-sterol	Sitoste-rol Sitoste-rol	Avena-sterol Awena-sterol	Total sterols Całkowita zawartość fitosteroli
1	2	3	4	5	6	7
Rape seeds variety Bor, mg/g of seeds Nasiona rzepaku odmiany Bor, mg/g nasion	0.56	1.64	0.04	2.29	0.16	4.74
Rape seeds variety Kronos, mg/g of seeds Nasiona rzepaku odmiany Kronos, mg/g nasion	0.67	2.21	0.06	2.88	0.36	6.29
Rapeseed oil during industrial production, mg/g of oil* Olej rzepakowy z poszczególnych etapów produkcji, mg/g oleju*						
Pressed oil Olej tłoczony	1.13	2.92	0.04	4.27	0.32	8.72
Extracted oil Olej ekstrakcyjny	1.36	3.57	0.03	5.27	0.49	10.78
Degumming oil Olej odśluzowany	1.40	3.66	0.04	5.60	0.44	11.18
Neutralized oil Olej neutralizowany	1.07	2.95	0.03	4.20	0.54	8.85
Bleached oil Olej bielony	1.24	3.04	0.04	4.46	0.41	9.25
Refined oil Olej rafinowany	1.24	3.06	0.02	4.43	0.44	9.23
Cold pressed rapeseed oil, mg/g of oil Olej rzepakowy tłoczony na zimno, mg/g oleju	1.08	2.58	0.06	3.94	0.24	7.90
Rapeseed oil after 6 months stored, mg/g of oil Olej rzepakowy po 6 miesiącach przechowywania, mg/g oleju	0.86	2.25	Nd	3.01	0.36	6.48
Rapeseed oil after 14th frying of French fries, mg/g of oil Olej rzepakowy po 14 cyklach smażenia frytek, mg/g oleju	0.27	0.65	0.02	0.99	0.08	2.01
French fries after 14th frying, mg/g of extracted lipids Frytki po 14 cyklach zmażenia, mg/g wyekstrahowanego tłuszczu	0.12	0.44	Nd	0.48	0.05	1.09

1	2	3	4	5	6	7
Margarines, mg/g Margaryny, mg/g	0.66	2.18	0.19	3.51	0.14	6.76
Refined corn oil, mg/g of oil Olej kukurydziany rafinowany, mg/g oleju	Nd	2.03	0.66	6.33	0.86	9.88
Cold pressed corn oil, mg/g of oil Olej kukurydziany tłoczony na zimno, mg/g oleju	Nd	1.64	0.57	4.70	0.75	7.65
Refined corn oil after 6 months stored, mg/g of oil Olej kukurydziany rafinowany po 6 miesiącach przechowywania, mg/g oleju	Nd	1.33	0.56	4.50	0.75	7.14
Peanuts Orzeszki ziemne	Nd	0.84	0.39	2.81	0.63	4.67
Peanuts after 6 days incubation at 60°C Orzeszki ziemne po 6 dniach inkubacji w 60°C	Nd	0.53	0.37	2.10	0.67	3.69

* The average from three different industrial productions in the same factory.
* Średnia z trzech procesów produkcyjnych prowadzonych w jednym z zakładów przemysłu tłuszczowego.

During refined process, especially neutralization, the decrease of phytosterols content was about 20%. The alternative could be used cold pressed oils. Some cold pressed oils were analyzed and obtained data presented that the content of phytosterols in them is always lower then in adequate refined oils [Rudzińska et al. 2001]. For example, the cold pressed corn oil content 7.7 mg/g and the refined corn oil contents 9.9 mg/g these compounds. It is probably because, that extraction of oil in industrial production plays an important role in the recovering phytosterols.

Not only high temperature is source of phytosterols decrease. Even than plant oils were stored in room temperature the decrease of the content of phytosterols was detected at the level from 7% in corn oil, 11% in sunflower oil and 14% in rapeseed oil. In percentage composition of sterols fraction changes were low except for stigmasterol which showed the highest decrease.

Plant oils heating in model laboratory conditions at 120°C as well as 180°C by 4 hours resulted in decrease of phytosterols levels compared to unheated oils [Rudzińska et al. 2002]. In corn and soybean oils heated at 120°C the diminishment in phytosterols was 15%, whereas heating at 180°C effected in losses of 24% and 21% respectively. In contrast the reduction of phytosterols in rapeseed oil heated at 120°C and 180°C was the same and equalled 8%. Similarly, the decrease in sunflower oil was 12% after heating at both temperatures. Changes in concentration of particular sterols were proportional for particular compounds – no variations in percentage of evaluated sterols were observed.

During multiple deep frying of French fries in laboratory conditions, the total sterol content in rapeseed oil, used for frying, decreased systematically. The total sterols content at the beginning of frying in good quality rapeseed oil was 5.4 mg/g, and after 14th frying was 2.0 mg/g (Table 1). It is interesting that in lipids extracted from French fries

the amounts of sterols were always lower then in rapeseed oil used for frying. French fries prepared in the first stage of frying contained 2.9 mg of sterols in 1 g of extracted lipids, and after 14th frying had only 1.1 mg/g. The low amount of phytosterols in French fries is unfavourable from the nutritional point of view [Piironen et al. 2000]. In this situation, when consuming of phytosterols had great interest, the level of these compounds in food ought to be as high as it is possible. Presented data showed that the content of phytosterols in French fries depends on the time of using oil for deep frying and these differences are very significantly.

A source of phytosterols in human diet can be margarines, nuts and other products content plant fat. Especially recommended are products with elevated amount of phytosterols and phytostanols esters, which are regarded as functional food because they anticholesterolemic properties. It seems to be important monitoring the content of phytosterols during different processes of food production. This decrease of phytosterols content could be caused by some different factors, like polymerization, degradation or oxidation. The autoxidation is a source of cytotoxic substances, named as oxyphytosterols [Adcox et al. 2001].

FOOD TECHNOLOGY PROCESSES AS SOURCE OF PHYTOSTEROL OXIDATION PRODUCTS

The reaction of phytosterols oxidation and formation of oxyphytosterols resembles similar processes observed for cholesterol. However, the literature data on oxyphytosterols are limited compared to cholesterol oxidation products (COP). Research on COP carried out throughout the last few decades has proved their mutagenic, carcenogenic, cytotoxic and atherogenic properties. Plant sterols form oxidized forms analogous to cholesterol (i.e. 7-hydroxy epimers, epoxides, triols, 7-keto-derivatives). Their similarity to cholesterol structures may indicate similar mode of action and the negative influence on human health [Grandgirard 1999].

Phytosterol oxidation products are compounds formed by heating and radiation, by non-emzymatic processes involving reactive oxygen and free radical species, or enzymatically by specific monooxygenase. Because of that, their presence in good quality fresh rape seeds was not amazement. It was at the level about 10-15 μg/g of seeds and probably they were formed in enzymic reaction during metabolic changes and maturation of seeds. Derivatives 7β-hydroxy and β-epoxy-phytosterols were dominated and made 38-52% and 22-32% of oxyphytosterols fraction respectively. Triols were also detected and their level average 8% of oxyphytosterols fraction.

The content of oxyphytosterols in rapeseed oil systematically increased during its industrial production. In pressed oil the amount of these compounds was 42-48 μg/g, and in extracted oil – 52-59 μg/g. First stage of refining rapeseed oil – degumming – contributed to the rise of oxyphytosterols content to 66-72 μg/g. During neutralization their content increased next 7-14%, during bleaching 6-17% in comparison with neutralized oil. In analyzed refined oils the amount of oxyphytosterols was 100-110 μg/g and was 130% higher then in pressed oil. In all analyzed oils dominated 7β-hydroxy derivatives, like in rape seeds, and α-epoxy, which approximate respectively up to 30-50% and 40-60% of oxyphytosterols fraction.

Table 2. The content of oxyphytosterols in some food products
Tabela 2. Zawartość oksysteroli w wybranych produktach spożywczych

Food products – Wybrane produkty spożywcze	Total oxyphytosterols Całkowita zawartość oksysteroli
Rape seeds, μg/g of seeds Nasiona rzepaku, μg/g nasion	12-15
Rapeseed oil during industrial production, μg/g of oil* Olej rzepakowy z poszczególnych etapów produkcji, mg/g oleju*	
Pressed oil Olej tłoczony	42-48
Extracted oil Olej ekstrakcyjny	52-59
Degumming oil Olej odśluzowany	66-72
Neutralized oil Olej neutralizowany	75-78
Bleached oil Olej bielony	90-99
Refined oil Olej rafinowany	100-110
Cold pressed rapeseed oil, μg/g of oil Olej rzepakowy tłoczony na zimno, μg/g oleju	8
Rapeseed oil after 6 months stored, μg/g of oil Olej rzepakowy po 6 miesiąch pzrechowywania, μg/g oleju	127
Rapeseed oil after 14th frying of French fries, μg/g of oil Olej rzepakowy po 14 cyklach smażenia frytek, μg/g oleju	197
French fries after 14th frying, μg/g of extracted lipids Frytki po 14 cyklach zmażenia, μg/g wyekstrahowanego tłuszczu	148
Margarines, μg/g Margaryny, μg/g	74
Refined corn oil, μg/g of oil Olej kukurydziany rafinowany, μg/g oleju	36
Cold pressed corn oil, μg/g of oil Olej kukurydziany tłoczony na zimno, μg/g oleju	9
Refined corn oil after 6 months stored, μg/g of oil Olej kukurydziany rafinowany po 6 miesiącach przechowywania, μg/g oleju	89
Peanuts Orzeszki ziemne	49
Peanuts after 6 days incubation at 60°C Orzeszki ziemne po 6 dniach inkubacji w 60°C	93
Peanuts + rapeseed oil + black currant seeds extract after 6 days incubation at 60°C, μg/g Orzeszki ziemne + olej rzepakowy + ekstrakt z pestek czarnej porzeczki po 6 dniach inkubacji w 60°C, μg/g	40

* The data from three different industrial productions in the same factory.
* Dane z trzech procesów produkcyjnych prowadzonych w jednym z zakładów przemysłu tłuszczowego.

Heating process resulted in an increase of oxyphytosterols amount compared to unheated oil [Rudzińska et al. 2002]. In rapeseed oil the increase of oxyphytosterols was the lowest (130%) in oil heated 4 hours at 120°C and 150% in oil heated 4 hours at 180°C, whereas in soybean oil the rise in oxyphytosterols was the greatest and equalled 435% and 485% respectively. In sunflower oil it was 175% and 205%, whereas in corn oil 322% and 333%. When the oxyphytosterols fraction of refined oils was analysed the percentages of particular campesterol, stigmasterol and β-sitosterol oxidized derivatives was different than their parent sterols. It was observed that in the process of oil heating the formation of oxyphytosterols goes on differently for examined oils. In unheated corn oil the contents of 7-hydroxy, epoxy and triols was on a similar level of 10-12 μg/g of oil, whereas in heated oil the dominant were epimers of 7-hydroxy-phytosterol, which concentration was 60 μg/g of oil. In both unheated and heated rapeseed oil epoxy derivatives prevailed, specifically α-epoxysitosterol and α-epoxy campesterol. Their content in unheated oil was 60 μg/g, whereas in heated 80-85 μg/g, in sunflower oil unheated and subjected to heating dominant were 7-hydroxy and epoxy derivatives. Their content in unheated oil was 14 μg/g and 8 μg/g, while in a result of heating increased to 20 μg/g.

During storage of plant oils (corn, rapeseed, sunflower) at room temperature with light access the content of oxyphytosterols increased. In rapeseed oil amount of these compounds arise only 1.3-times, the lowest from all analyzed oils. In this oil dominated 7β-hydroxy and α-epoxy derivatives. Corn oil before storage content 36 μg/g, and after 6 months their amount increased 2.5-times, up to 90 μg/g. This oil characterized the highest level of 7β-hydroxy and α-epoxy derivatives.

As expected the content of total oxyphytosterols increased during frying in both rapeseed oil and lipids extracted from French fries about 8 and 9-times respectively. Despite, that the content of oxyphytosterols in rapeseed oil and lipids extracted from French fries were similar in μg/g, their content in relation to phytosterol fraction was 100% higher in lipids from French fries then rapeseed oil. In rapeseed oil dominated epoxy and 7hydroxy derivatives with α-configuration. In lipids extracted from French fries dominated epoxy and 7-hydroxy derivatives with β-configuration. Domination of α-epimers in rapeseed oil and β-epimers in lipids extracted from French fries was not explained.

Consumption of plant oils so preferred and recommended in human diet, can be a source of phytosterol oxidation products. Alternative could be cold pressed oil, which always content lower amount of these compounds then refined oils. For example cold pressed rapeseed oil showed 8 μg/g and corn oil 9 μg/g, while refined oils had these compounds several times more [Rudzińska et al. 2001].

The content of oxyphytosterols in margarines was also analyzed. Their total amount was 74-76 μg/g of margarines and α-epoxy derivatives dominated. In hard margarines very high level of triol was detected, while in soft margarines it was 10-times lower.

Oxidation process of phytosterols refers not only to pure plant oils but also to products that contain oils as one of constituents, such as infant formulas and fried products – French fries and chips.

Investigation on oxyphytosterols formation in fried products was carried out by Lee et al. [1984, 1985]. They determined content of these compounds by HPLC in fat from French fries and chips. Dutta and Appelqvist [1997] investigated phytosterols oxidation compounds in sunflower and palm oils mixed with hydrogenated canola oil, and in

French fries and chips fried in these oils. The total content of oxyphytosterols in oils ranged from 39.9 ppm to 46.7 ppm, and in French fries 32.0-53.7 ppm. In fast food products fried in a mixture of plant and animal fat, that were monitored for the sterol oxidation products contents, the following derivatives of cholesterol, sitosterol and campesterol were detected: α and β-epoxysitosterol, 7-hydroxysitosterol and 7-hydroxycampesterol, 7-ketositosterol and 7-ketocampesterol and sitosterol and campesterol triols [Dutta and Appelqvist 1997].

Peanuts, which can be a good source of phytosterols also content their oxidative derivatives at the level about 49 μg/g of product and it increased after 6 days of incubation in 60°C to 93 μg/g [Małecka et al. 2003].

The primary products of phytosterols oxidation are unstable peroxy radicals, which are finally reduced to hydroxyl groups. The epoxides and their common hydration products, triols, are formed by sterol oxidation. Numerous studies have indicated that epoxides are linked with atherosclerosis and mutagenicity [Guardiola et al. 1996, Morin et al. 2000, Grandgirard 2002]. It was reported by Aringer and Eneroth [1974] that α-epimers of oxyphytosterols could be preferentially transformed to triols. They could be better metabolized *in vivo* than the other phytosterol oxidation products and they could be transformed to the triols in the acidic conditions of the stomach or to the action of an epoxide hydralase in the intestinal cells [Grandgirard 2002, Aringer and Eneroth 1974, Hwang and Kelsey 1978, Maerker et al. 1988]. In the next step of autoxidation, epoxides are transformed to the triol derivatives, which are expected to be the most cytotoxic [Meyer et al. 1998]. Because of that, monitoring of epoxy-phytosterols increase is very important.

INHIBITION OF PHYTOSTEROLS OXIDATION BY ANTIOXIDANTS

Stability of plant sterols is important especially when consumption of processing food grows up. Inhibition of oxysterols formation by synthetic antioxidants was a subject of some researches [Rudzińska et al. 2003, 2004]. Addition of antioxidants BHT, α-tocopherol, ethanolic extracts of rosemary or green tea to solution of stigmasterol in TAG from sunflower oil contributed to statistically significant inhibition of stigmasterol degradation. Tocopherol showed the highest effectiveness in inhibition of formation of stigmasterol oxidation products. The content of oxystigmasterols after incubation TAG with that antioxidant at 3, 6 and 9 days, were ca. 15%, 21%, and 24% lower, respectively, than in control samples. But the content of these compounds in TAG in all incubated samples with added BHT was ca. 27%, 17% and 11% higher, respectively, than in control samples. Ethanolic extracts of rosemary and green tea showed the same effectiveness after 3 and 6 days of incubation. But after 9 days, ethanolic extract from rosemary showed higher effectiveness then green tea extract. Addition of rosemary extract inhibited ca. 22% of the formation of stigmasterol oxidation products after 9 days of incubation, but green tea extract showed only ca. 6% inhibition.

Other natural antioxidants were used in experiment referred by Małecka et al. [2003]. Treatment of peanuts with rapeseed oil enriched with antioxidant extracts of raspberry, black currant and tomato seeds obtained from the waste of food processing. The content of oxyphytosterols ranged from 40 μg/g in peanuts treated with rapeseed oil enriched with raspberry seeds extract to 95 μg/g in peanuts treated rapeseed oil without

any additives. The dominating oxyphytosterols in all samples were 7β-hydroxy- and epoxy-campesterol and -β-sitosterol derivatives. All extracts exhibited the protective effect towards sterols oxidation in peanut samples although the black currant seeds extract was the most effective one. The amount of oxyphytosterols in treated peanuts was 40 to 60% lower in comparison to the control peanuts sample stored without additives.

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STEROLE ROŚLINNE W TECHNOLOGII ŻYWNOŚCI

Streszczenie. Sterole roślinne (fitosterole) należą do grupy związków obniżających poziom cholesterolu we krwi poprzez hamowanie jego absorpcji. Stąd wzrosło zainteresowanie wykorzystaniem ich do wzbogacania żywności funkcjonalnej. Zawartość fitosteroli w nasionach rzepaku wynosiła 4,7-6,3 mg/g nasion w zależności od odmiany. W oleju tłoczonym w warunkach przemysłowych ich zawartość wynosiła około 8,7 mg/g. W oleju ekstrakcyjnym poziom fitosteroli był o 25% wyższy niż w oleju tłoczonym. Podczas rafinacji oleju, zwłaszcza procesu neutralizacji, ubytek fitosteroli wynosił 20%. Zastosowanie oleju rzepakowego do wielokrotnego smażenia frytek spowodowało 64-procentowy ubytek tych związków. Ubytek fitosteroli podczas procesów technologicznych związany jest głównie z ich wolnorodnikowym utlenianiem. Niektóre procesy takie, jak rafinacja olejów roślinnych, smażenie lub przechowywanie powodują tworzenie się pochodnych utlenionych fitosteroli (oksyfitosteroli). Związki te były oznaczane w nasionach rzepaku na poziomie 10-15 μg/g nasion. Podczas procesu produkcji oleju rzepakowego zawartość oksyfitosteroli wzrastała systematycznie do 100-110 μg/g. Wielokrotne smażenie frytek w oleju rzepakowym spowodowało wzrost zawartości tych związków do 200 μg/g w oleju i 150 μg/g we frytkach. Oksyfitosterole są absorbowane przez organizm człowieka i wykazują działanie toksyczne. Dlatego monitorowanie tworzenia się tych związków podczas procesów technologicznych jest konieczne.

Słowa kluczowe: fitosterole, oksyfitosterole, oleje roślinne, produkcja, smażenie, produkty spożywcze

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