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OXYSTEROL CONTENT IN SELECTED MEATS AND MEAT PRODUCTS

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Background. High consumption of oxysterols contributes to the development of arteriosclerosis. Thus it is necessary to monitor changes of their concentration in foodstuffs.
The aim of this study was to determine the content of oxysterols in selected meats and meat products before and after heat treatment.
Material and methods. Meats and meat products were pan fried in rapeseed oil for 10 minutes. Oxysterols methodology applied for the study of fat extraction, saponification, derivatization and determination by gas chromatography coupled with mass spectrometer.
Results. The content of cholesterol oxidation products in meats and meat products after heat treatment (17.5 to 34.9 μg/g of fat) was statistically higher than before frying (2.2 to 10.7 μg/g of fat). Raw meats and processed meat products contained mainly cholesterol oxidation products which equalled from 1.0 to 8.3% of cholesterol content. In fried meats and meat products has been found phytosterol oxidation products (0.1-1.7 μg/g of fat) but only in small amounts.
Conclusions. The increase in the content of phytosterol oxidation products in analysed meat samples after frying was probably the result of intensive phytosterol oxidation included in the rapeseed oil, also induced by haeme dyes within meat. From the results of the samples analyzed, it seems that multiple parameters are associated with the formation of oxysterols. Further studies should be performed to identify the factors e.g. water content, pro-oxidants, exposure to light, storage time and conditions, that may affect oxysterol formation during home frying.

Key words: cholesterol, phytosterol, sterol oxidation products, thermal processing

INTRODUCTION

Sterols included in fats undergo the oxidation processes and their oxidation takes place mainly as the result of interaction with: oxygen, light, higher temperature, ultra-

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violet and gamma radiation, and the presence of unsaturated fatty acids, free radicals and peroxides, enzymes, metal ions (especially of iron and copper), natural dyes e.g. chlorophyll [Johnsson et al. 2003, Baggio and Bragagnolo 2006]. During the processing and storage of animal foodstuffs, variables that are crucial in formation of oxysterols are: composition of the matrix, polyunsaturated fatty acids content, sterols level, processing method, processing time and temperature, pH, pro- and antioxidants and water activity [Morrissey and Kiely 2006].

Sterol oxidation products can reach human organism along with food or they can form as the result of enzymatic or no enzymatic transformations taking place inside human organism. It is estimated that an average western diet consists of roughly 1% oxidized cholesterol [Brown and Jessup 1999]. Products of sterol oxidation can be built into human body cells changing their permeability and stability or they can be expelled through the synthesis of bile acids [Meynier et al. 2005].

Cholesterol oxidation products (COPs) contribute to the development of arteriosclerosis and are characterized by the following activities: mutagenic, cancerogenic, antitoxic, cytotoxic, immunosuppressive. Moreover, they slow down the synthesis of DNA and cholesterol biosynthesis and they are also the calmodulin inhibitors disturbing activity of cell membranes [Guardiola et al. 1996, Chang et al. 1997, Johnsson 2004].

It was the subject of many conducted tests to determine the influence of phytosterol oxidation products (POPs) on a human organism. Ryan and coworkers [2005] compared the cytotoxic and apoptic effects of phytosterol and cholesterol oxidation products on model human cells. Research shows that phytosterol oxidation products are characterised by the similar effect on both cells and cholesterol oxidation products. However, the much higher dosage of these compounds must be used to achieve the same toxicity effect. Adcox and partners [2001] conducted similar experimental research which showed that cholesterol, sitosterol and campesterol oxidation products produce similar cytotoxic effect on macrophage cells. However, oxidized cholesterol compounds (7-ketocholesterol) were characterised by the most harmful effects. Because of the harmful effects of sterol oxidation products on human organism there is a strong need to monitor the content of these compounds in food products. For instance content of oxyphytosterols in Polish rapeseeds was analysed. Rudzińska and partners published the result of experiments where they confirmed that content of phytosterol oxidation products in rapeseeds was between 10 to 15 μg/g [Wąsowicz et al. 2004]. On the other hand plant cooking oils are known as a source of antioxidants (tocopherols and tocotrienols), which can prevent the lipid oxidation and also cholesterol oxidation. Xu and partners [2005] claimed that the antioxidants from cooking oils may contribute to the inhibition of cholesterol loss and formation of COPs during heating. To minimize the formation of the oxysterols in foods, it is crucial that the generation of oxysterols be assessed during different stages of production and handling. In this study we examined the influence of thermal processing of meats and meats products with usage of rapeseed oil.

MATERIAL AND METHODS

The materials investigated were pork and beef minced meat, and frozen cordon-blue and turkey chops. From every assortment three samples were taken to the study before and after thermal processing. Minced meat and meat products were pan fried in shallow layer (3 ±0.5 mm) of rapeseed oil. Thermal processing lasted about 10 minutes.

Sterol oxidation products methodology applied for the study of fat extraction, saponification, derivatization and determination by gas chromatography coupled with mass spectrometer.

In the begging the extraction of fat was performed. 1-2 g of pates or ground sausage sample was mixed with 16 ml of chloroform – methanol (2:1, v/v) and 3 ml of saturated solution of sodium chloride in water and put in the flask and shaken at 8000 rpm for the time period of 10 minutes. After centrifugation chloroform layer was collected and filtered through filter paper with 0.5 g of anhydrous sodium sulfate to remove residual moisture. The filtrate was evaporated to dryness under nitrogen and dissolved in 2 ml of hexane and 100 µl of internal standards 19-hydroxycholesterol (1 ppm) and 5α-cholestane (4 ppm) were added. The mixture was saponificated by adding 0.5 ml of sodium hydroxide solution in methanol (2 N, room temperature for 1-2 hours). 200 µl of hexane layer was transported into 1.5 ml vial insert and after evaporation to dryness under nitrogen, the residue was dissolved in 100 µl of pyridine and 100 µl BSTFA with 1% TCMS and left to remain in the dark for 24 hours to complete derivatization. Then, 1 mL of hexane was added and 1 µL of mixture was collected for GC-MS analysis. A DB5ms capillary column was used to separate oxysterols. Helium was used as a carrier gas with a flow rate of 0.9 mL/min. The injector temperature was 230°C, and the column temperature was programmed as follows: 50°C in the beginning for 2 min, subsequent increase to 230°C at the rate of 15°C/min, to 310°C at the rate of 3°C/min maintained for 10 min. The interface temperature for GC-MS was 240°C. Temperature of ion source was 220°C, ionization energy was 70V. Qualitative analysis of cholesterol and phytosterol oxidation products was done by comparing the retention times of reference materials and to mass spectrum libraries. Quantitative analysis of sterols and oxysterols was performed by addition of internal standards 5α-cholestane and 19-hydrosycholesterol, respectively.

The obtained results were statistically worked out using Statgraphics Plus 4.1 programme. To appraise the significance of the differences between the means COPs content in particular samples of meat or meat products, Tuckey's test was used, at significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The analysed products were characterised by a diverse content of cholesterol typical for products of animal origin and also by a low content of phytosterols. The content of fat in the analysed products increased from 17% to 28% during heat treatment in comparison to its initial content which was the result of rapeseed oil used for frying. The phytosterol content in tested products was rising either due to the frying method used (rapeseed oil) or it was the resultant of prescription composition of tested products (Table 1). The differences between sterol content in minced meats and meat products before and after heat treatment probably resulted from the fact that the minced meat did not have any breadcrumb coating which highly absorbs rapeseed oil used during heat treatment. The content of cholesterol in all products decreased during heat treatment: by 40% in pork, 50% in beef, 46% in cordon blue, 20% in turkey chop. The aggregate phytosterol content in minced meats and readymade chops increased almost two times during heating, which was the result of rapeseed oil used for frying.

Table 1. Fat (g/100 g of product) and sterol (mg/100 g of fat) in meats and meat products before and after thermal processing

		Pork chops without breadcrumbs A	Beef chops without breadcrumbs B	Turkey chops coated with breadcrumbs C	Cordon blue chops coated with breadcrumbs D
Fat content	before	21.7 ±1.4[a]	23.0 ±0.8[a]	20.5 ±1.2[a]	23.4 ±1.6[a]
	after	29.3 ±0.5[b]	32.0 ±2.0[b]	25.4 ±1.0[a]	28.4 ±2.4[a]
Sum of sterols	before	340.3 ±30.1[a]	205.2 ±11.8[a]	170.2 ±16.4[a]	1194.3 ±74.5[a]
	after	240.4 ±20.0[b]	139.8 ±13.0[b]	207.4 ±18.7[b]	1324.2 ±91.2[a]
Cholesterol	before	319.5 ±28.5[a]	182.3 ±8.5[a]	129.0 ±13.3[a]	212.2 ±13.7[a]
	after	190.6 ±16.7[b]	92.9 ±7.4[b]	103.1 ±7.7[a]	113.7 ±3.9[b]
Brassicasterol	before	0.7 ±0.1[a]	2.4 ±0.2[a]	2.5 ±0.3[a]	13.4 ±3.1[a]
	after	2.1 ±0.5[b]	4.6 ±0.7[b]	13.8 ±2.0[b]	7.2 ±1.3[b]
Campesterol	before	7.7 ±1.0[a]	6.3 ±1.1[a]	8.8 ±0.7[a]	8.0 ±0.1[a]
	after	18.3 ±0.6[a]	16.3 ±0.5[b]	35.0 ±4.0[b]	24.5 ±1.6[b]
Stigmasterol	before	0.7 ±0.1[a]	3.6 ±0.4[a]	3.3 ±0.3[a]	1.4 ±0.4[a]
	after	2.7 ±0.3[b]	5.2 ±0.8[b]	1.9 ±0.3[b]	nd
Sitosterol	before	11.8 ±0.5[a]	10.6 ±1.6[a]	26.6 ±1.8[a]	959.3 ±57.2[a]
	after	26.7 ±1.9[a]	20.8 ±3.6[b]	53.5 ±4.7[b]	1178.7 ±84.4[b]

[a,b]Values within a row with different letters are significantly different ($p < 0.05$) in products before and after thermal processing.
nd – not detected.
Before – before thermal processing.
After – after thermal proceessing.

Conducted tests have confirmed that raw minced meats and frozen meat products contained cholesterol oxidation products in the amount from 2.2 to 10.7 μg/g of fat, and the presence of phytosterol oxidation products was detected only in the turkey chop (0.1 μg/g of fat; Table 2, 3). The results of conducted analyses find their confirmation in literature. Osada et al. [2000] observed similar content of cholesterol oxidation products in raw beef hamburgers i.e. 2.3 μg/g of fat. Baggio and partners, however, have found that the content of these compounds in turkey raw hamburgers was significantly lower – from 0.07 to 0.35 μg/g of fat.

Conducted heat treatment of meats and meat products lead to the statistical increase of cholesterol oxidation products content. Cordon blue chops had lower level of COPs before frying compared with meats and turkey chops. The increase of COPs during thermal processing was more pronounced in the fried cordon blue chops: the level of COPs increased from 2.2 μg/g of fat to 34.9 μg/g after frying. The level of COPs in remaining samples was 8.7, 6.4 and 10.7 μg/g of fat in pork, beef and turkey chops, respectively. After frying total COPs content elevated and was 19.3, 25.9 and 17.5 in pork, beef and turkey chops, respectively. The distribution of particular COPs in mentioned above chops was comparable to those in cordon blue chops, where 7-keto-

Table 2. Cholesterol oxidation products content (μg/g of fat) in meats and meat products before and after thermal processing

		Pork chops without breadcrumbs A	Beef chops without bread-crumbs B	Turkey chops coated with bread-crumbs C	Cordon blue chops coated with bread-crumbs D
Sum of COPs	before	8.7 ±1.4[a]	6.4 ±0.3[a]	10.7 ±0.9[a]	2.2 ±0.25[a]
	after	19.3 ±1.5[b]	25.9 ±0.4[b]	17.5 ±1.1[b]	34.9 ±1.4[b]
7β-hydroxy-cholesterol	before	0.1 ±0.1[a]	0.1 ±0.1[a]	7.0 ±0.4[a]	0.1 ±0.05[a]
	after	3.9 ±0.3[b]	0.8 ±0.1[b]	9.0 ±0.3[b]	9.0 ±0.3[b]
Triol	before	nd	nd	nd	nd
	after	nd	nd	nd	2.9 ±0.2[b]
25-hydroxy-cholesterol	before	0.2 ±0.1a	0.4 ±0.1[a]	0.2 ±0.1[a]	0.9 ±0.1[a]
	after	5.8 ±0.8[b]	0.2 ±0.1[b]	2.9 ±0.2[b]	5.6 ±0.6[b]
7-ketochole-sterol	before	8.4 ±1.2[a]	5.9 ±0.1[a]	3.5 ±0.4[a]	1.2 ±0.1[a]
	after	9.6 ±0.4[b]	24.9 ±0.3[b]	5.6 ±0.6[b]	17.4 ±0.3[b]

[a,b]Values within a row with different letters are significantly different ($p < 0.05$) in products before and after thermal processing.
nd – not detected.
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cholesterol, 7β-hydroxycholesterol and 25-hydroxycholesterol dominated. Only small amounts of triol were found after thermal processing of cordon blue chops. The given observation probably derives from the fact that cordon blue chops belong to the group of highly processed products. The protein structure begins to denaturate during technological process of chops which leads (among others) to a decreased activity of antioxidant enzymes, disintegration of cell membranes and releasing of polyunsaturated fatty acids causing oxidation processes [Grau et al. 2001]. Larkenson and partners [2000] conducted studies which confirmed the presence of six cholesterol oxidation products in fresh meat, tentatively fried meatballs along with beef and pork hamburgers. The aggregate content of cholesterol oxidation products in raw meatballs and hamburgers amounted from 3.3 to 8.4 μg/g of fat. It has been observed that an average content of oxysterols in the analysed samples after heat treatment of about 150-160°C (without any additional frying fats) increased to the amount from 6.7 to 29.4 μg/g of fat [Larkenson et al. 2000]. In our study we demostrated that the total content of COPs in fried meats and meats products was very similar, it shows that addition of rapeseed oil during thermal processing did not effected on COPs formation, which was also noticed by Echarte and coworkers [2001]. They could not find relationship between content of unsaturated faty acids in frying matter and formation of COPs. Worth mentioning is the fact that total content of COPs in thermal processed meats and meat products was between 1.0 to 8.3% of cholesterol content. It shows that loss of cholesterol content is not equal with the formation of COPs, and remaining loss of cholesterol was caused by thermal degradation of the sterol.

Table 3. Phytosterol oxidation products content (μg/g of fat) in meats and meat products before and after thermal processing

		Pork chops without breadcrumbs A	Beef chops without breadcrumbs B	Turkey chops coated with breadcrumbs C	Cordon blue chops coated with breadcrumbs D
Sum of POPs	before	nd	nd	0.1 ±0.05	nd
	after	0.2 ±0.1	1.7 ±0.3	< LOQ	< LOQ
5α,6α-epoxy-sitosterol	before	nd	nd	nd	nd
	after	nd	nd	< LOQ	nd
5β,6β- epoxy-campesterol	before	nd	nd	nd	nd
	after	nd	nd	< LOQ	nd
5α,6α-epoxy-campesterol	before	nd	nd	nd	nd
	after	nd	nd	< LOQ	nd
7-ketocampe-sterol	before	nd	nd	nd	nd
	after	0.1 ±0.05	0.1 ±0.5	< LOQ	< LOQ
7-ketositoste-rol	before	nd	nd	0.1 ±0.05[a]	nd
	after	0.1 ±0.05	1.6 ±0.2	< LOQ	< LOQ

[a,b]Values within a row with different letters are significantly different ($p < 0.05$) in products before and after thermal processing.
nd – not detected.
< LOQ – below limit of quantification of the method.
Before – before thermal processing.
After – after thermal processing.

It is worth noting that high temperature of heat treatment is a very important factor in the intensity of sterol oxidation process. The slight increase in the content of cholesterol oxidation products has been noted during heat treatment of minced pork meat at 120°C for about 60 minutes [Obiedziński et al. 1999]. Initially the meat contained 4.37 μg of sum of COPs expressed in 1 g of product, and after 60 minute heat treatment this value reached 6.64 μg/g.

In fried minced meats and chops the phytosterol oxidation products have been found (5α,6α-epoxysitosterol, 5β,6β-epoxysitosterol, 5α,6α-epoxycampesterol, 7-ketocampesterol and 7-ketositosterol). Only trace amounts of phytosterol oxidation products have been found in fried chops. In beef and pork meat, however, the aggregate content of phytosterol oxidation products equaled to 0.2 and 1.7 μg/g of fat respectively (Table 3). The presence of these products in analyzed meat samples is probably the result of an intensive phytosterol oxidation contained in rapeseed oil, which is also induced by haeme dyes in meat. It is important to note that the rapeseed oil did not contain phytosterol oxidation products in numbers possible to be determined. Results of these observations find approval in related literature. Soupas and partners [2007] proved that the appropriate heat treatment plays an important role in formation of phytosterol oxidation products. Model based testing has shown that 5-10 min pan frying

results in phytosterol oxidation close to oil oxidation in an oven at the temperature of 180°C for the period from 0.5 to 2 hours. The author notes that using a shallow layer of fat during frying results in the acceleration of the process of sterol thermal oxidation due to the high availability of oxygen [Soupas et al. 2007]. Also Rudzińska and partner [2002] have proved that heating of corn, rapeseed, sunflower and soybean oil led to increase of concentration of phytosterol oxidation products about 130-485%. Johnsson and Dutta [2006] demonstrated that the content of phytosterol oxidation products may increase during oil heating (taking into consideration such oils as olive oil, corn oil, peanut oil) at the temperature of 180°C for two hours. In case of olive oil the aggregate content of phytosterol oxidation products increased from 7.7 to 17.6 μg/g, in case of corn oil from 4.3 to 12.2 μg/g, and in peanut oil it did not change [Johnsson and Dutta 2006].

CONCLUSIONS

The present study is the first to record the total and particular content of sterol oxidation products in commercial and thermaly processed meats and meats products consumed in Poland. The content of cholesterol oxidation products in meats and meat products after heat treatment (17.5 to 34.9 μg/g of fat) was statistically higher than before frying (2.2 to 10.7 μg/g of fat). Raw meats and processed meat products contained mainly cholesterol oxidation products which equaled from 1.0 to 8.3% of cholesterol content. Loss of cholesterol was caused not only by oxidation but also by thermal degradation. In fried meats and meat products has been found phytosterol oxidation products (0.1-1.7 μg/g of fat) but only in small amounts. The increase in the content of phytosterol oxidation products in analysed meat samples after frying was probably the result of intensive phytosterol oxidation included in the rapeseed oil, also induced by haeme dyes within meat. From the results of the samples analysed, it seems that multiple parameters are associated with the formation of sterol oxidation products. Further studies should be performed to identify the factors e.g. water content, pro-oxidants, exposure to light, storage time and conditions, that may affect oxysterol formation during home frying.

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ZAWARTOŚĆ OKSYSTEROLI W WYBRANYCH MIĘSACH ORAZ PRODUKTACH MIĘSNYCH

Wstęp. Duże spożycie oksysteroli przyczynia się do rozwoju arteriosklerozy. Dlatego jest konieczne monitorowanie ich zawartości w produktach spożywczych. Celem pracy było oznaczenie zawartości oksysteroli w wybranych mięsach mielonych oraz produktach mięsnych przed i po procesie obróbki termicznej.

Materiał i metodyka. Mięsa oraz produkty mięsne były smażone z użyciem oleju rzepakowego przez 10 minut. Zastosowana metodyka oznaczania oksysteroli obejmowała ekstrakcję tłuszczu, zmydlanie, derywatyzację oraz rozdział z użyciem chromatografu gazowego sprzężonego ze spektrometrem mas.
Wyniki. Zawartość produktów utleniania cholesterolu w mięsach i produktach mięsnych po smażeniu (17,5-34,9 μg/g tłuszczu) była statystycznie wyższa niż przed smażeniem (2,2-10,7 μg/g tłuszczu). Surowe mięsa i przetwarzane produkty mięsne zawierały głównie produkty utleniania cholesterolu, które stanowiły od 1,0 do 8,3% zawartości cholesterolu. W smażonych mięsach i produktach mięsnych stwierdzono obecność produktów utleniania fitosteroli (0,1-1,7 μg/g tłuszczu), lecz w małych ilościach.
Wnioski. Wzrost zawartości produktów utleniania fitosteroli w analizowanych próbkach po przeprowadzeniu obróbki termicznej wynikał prawdopodobnie z intensywnej oksydacji fitosteroli występujących w oleju rzepakowym oraz obecności barwników hemowych w mięsie. Wyniki pracy wskazują, że wiele czynników ma wpływ na proces tworzenia się oksysteroli. Istnieje potrzeba prowadzenia dalszych badań mających na celu określenie pozostałych czynników, które mają wpływ na tworzenie się oksysteroli podczas smażenia.

Słowa kluczowe: cholesterol, fitosterole, produkty utleniania steroli, obróbka termiczna

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