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CHOLESTEROL AND CHOLESTEROL OXIDATION PRODUCTS IN POLISH COMMERCIAL SAUSAGES

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ABSTRACT

A study was carried out to determine the cholesterol and cholesterol oxidation products (COPs, oxysterols) content of several Polish commercial sausages. Cholesterol content ranged from 244.42 µg/1 g of sample in salami to 847.87 µg/1 g of sample in pasztetowa no. 1. The content of the sum of cholesterol oxidation products ranged from 4.42 µg/1 g of the sample in parówkowa to 36.52 µg/1 g of the sample in metka łososiowa. The correlation between fat and cholesterol oxidation products showed that in case of the higher the fat content, the higher the sum of oxysterols content is observed. A correlation between fat content and cholesterol content was not found.

Key words: cholesterol oxidation products; cholesterol; Polish commercial sausages

INTRODUCTION

Cholesterol oxidation products (oxysterols; COPs) have received considerable attention in recent years because of their biological activities associated with human diseases. The implications of adverse biological effects such as atherogenesis, cytotoxicity, mutagenesis, and carcinogenesis from oxysterols have been reviewed [7, 9]. Animal studies suggested that COPs in the diet could be associated with heart and vascular diseases. Human studies also showed that the quantity of oxidized lipids in the diet was directly related to the level of oxidized lipids in serum postprandial chylomicrons [21], which provides a mechanism by which dietary oxidized lipids

can affect the oxidative states of endogenous lipoproteins. Staprans, Pan, and Rapp [20] showed that oxidized cholesterol in the diet could be directly absorbed into the circulation, and that COPs accelerated the development of atherosclerosis in rabbits. It is also convincing that oxysterols associated with lipid oxidation in meat arise from heating [8, 11], during storage [8], at various stages of processing, and type of meat product [6]. Moreover, cholesterol readily undergoes oxidation in the presence of oxygen, light, metal ions, radiation and other compounds which could generate reactive components [10, 12, 16, 23]. During food processing and storage, polyunsaturated fatty acids tend to be oxidized. Cholesterol can be oxidized by the same mechanism as fatty acids. Therefore, as Smith [19] suggested, hydroperoxides of polyunsaturated fatty acids formed during lipid oxidation can accelerate the formation of oxysterols from cholesterol.

Although the Polish people have commonly consumed various processed meat products for many years, little information is available on the occurrence of cholesterol and cholesterol oxidation products in commercially manufactured sausages.

In this study we investigated the content of cholesterol and its oxidation products in typical Polish sausages.

MATERIALS AND METHODS

Materials

All the sausages were produced according to Good Manufacturing Practice, on a commercial scale in a large plant located in Poznań. Freshly prepared, processed sausages were purchased from local markets. The analysis of the sausages took place two days after their production date and immediately upon acquisition.

Reagents

Methanol, ethanol, chloroform and 2,6-di-tert-butyl-4-methyl phenol (BHT) were purchased from POCH (Gliwice, Poland), hexane, acetonitrile, isopropanol, methyl tert-butyl ether (MTBE) and a 30% methanolic solution of sodium methylate and pyridine were purchased from Aldrich. A derivatization reagent, BSTFA (Bis(trimethylsilyl)trifluoro-acetamide), was obtained from Supelco, Inc. (Bellefonte, PA).

Standards

7β-hydroksycholesterol (7β-OHC), 20α -hydroksycholesterol (20α-OHC), 25-hydroksycholesterol (25-OHC), 27-hydroksycholesterol (27-OHC), cholesterol α -epoksy (α-epoksy-C), 7-ketocholesterol (7-keto-C), cholestane-3β,5α ,6β-triol (triol-C) and the internal standard 19-hydroksycholesterol (19-OHC) were obtained from Sigma Chemical Co.(St. Louis, MO), 7α-hydroksycholesterol (7α -OHC) and cholesterol β-epoksy (β -epoksy-C) was obtained from Steraloids Inc. (Wilton, NH).

Equipment

A Hewlett-Packard 6890 gas chromatograph with split/splitless injector and a FID detector was used for the analyses. Compounds were separated using DB-5 column (J&W, 30 m x 0.25 mm x 0.25 µm). The identity of oxysterols was confirmed on a Hewlett-Packard HP 5890 II gas chromatograph coupled to an quadrupole mass spectrometer.

Determination of cholesterol oxidation products

The determination of cholesterol oxidation products was made according to Przygoński, Jeleń and Wąsowicz [18] with the following modification:

Lipid was extracted using the Folch [5] method: A homogenized sample (1.000 g) was placed in an Erlenmeyer flask and an internal standard (250 µg 19-OHC) was added. Subsequently, 50 ml of chloroform/ methanol mixture (2:1) containing 0.006% BHT was added and the sample was homogenized for 3 min (8.000 rot/min.) using Ultra–Turrax T 25. The sample was then shaken in a shaker for 6 min. Afterwards the sample was filtered and transferred into a separatory funnel into which 15 ml of water was added. The lower, chloroform layer was filtered over anhydrous sodium sulfate (5 g) and the funnel was washed with chloroform (5 ml). The chloroform fraction was collected in a 100 ml flask, 1 ml of anhydrous ethanol was added and the sample was evaporated to dryness at 30°C under nitrogen.

Statistical analysis

The experimental design was intended to determine the cholesterol and its oxidation products content in Polish commercially sausages. The correlations between fat contents and cholesterol, cholesterol oxidation products contents was also calculated. Data was analyzed using a simple regression analysis. Significance was defined at $p = 0.05$. The experiment was carried out in three replications.

RESULTS AND DISCUSSION

The fat content, cholesterol and cholesterol oxidation products content of the different Polish commercial sausages are shown in Table 1.

Table 1. Fat content (%), cholesterol and sum of cholesterol oxidation products contents (µg/1 g of sample) of the analyzed sausages

No	Assortment	Fat	Cholesterol	Sum of COPs
1	**Piwna**	20.70	673.66	11.89
2	**Wiejska**	23.78	687.28	12.41
3	**Jałowcowa**	38.66	821.58	11.65
4	**Śląska**	19.38	271.84	22.02
5	**Kabanosy**	22.84	425.81	25.68
6	**Polska 1**	19.05	484.73	9.79
7	**Polska 2**	20.60	549.20	8.40
8	**Polska 3**	17.50	613.68	9.78
9	**Salami**	34.89	244.42	25.44
10	**Pasztetowa 1**	36.46	847.87	22.61
11	**Pasztetowa 2**	32.66	253.00	25.83
12	**Mortadela**	21.24	346.73	8.04
13	**Metka łososiowa**	51.54	353.16	36.52
14	**Parówkowa**	27.35	366.05	4.42
15	**Mielonka**	12.88	367.51	11.60
16	**Metka wędzona**	57.62	829.75	23.71

Fat, cholesterol and cholesterol oxidation products contents varied widely from one sausage to another. Metka wędzona was the fattiest (57.62 % of fat) and mielonka (12.88 % of fat) the leanest.

The cholesterol content ranged from 244.42 µg/1 g of sample in salami to 847.87 µg/1 g of sample in pasztetowa 1. The highest amount of total COPs was in metka łososiowa (36.52 µg/1g of sample), the smallest in parówkowa (4.42 µg/1 g of sample).

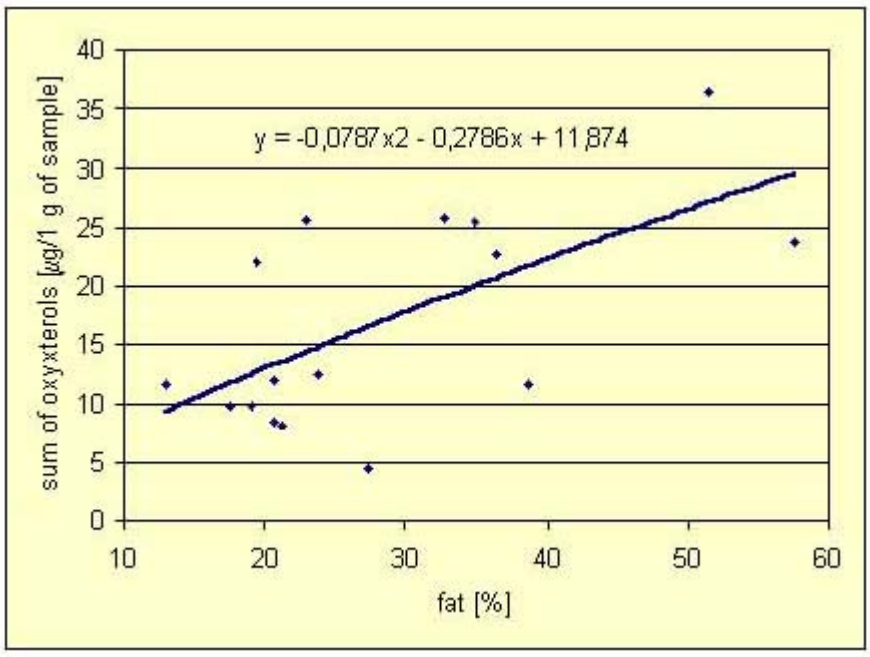
A Statistical analysis of the given results showed correlations between fat and total COPs content in 16 sausages ($R^2 = 0.394$). In spite of a low determination coefficient the result of a big dispersion in the given results, it is statistically significant at $p = 0.05$ (Table 2).

Table 2. Content of COPs [µg/1 g of sample] in selected Polish commercial sausages

No	Asortment	7α -OHC	7β -OHC	β - epoksy - C	α - epoksy -C	20 α - OHC	triol-C	25 -OHC	7 keto-C	27 -OHC
1	Piwna	0.17	0.08	0.20	0.99	4.23	1.16	0.28	0.10	4.71
2	Wiejska	0.08	0.07	0.36	0.75	4.00	2.59	0.44	0.08	4.04
3	Jałowcowa	2.21	0.69	5.54	0.40	0.17	0.38	1.00	1.06	0.20
4	Śląska	3.19	1.44	3.74	1.48	0.45	1.64	1.53	4.56	3.98
5	Kabanosy	6.47	1.77	9.03	0.90	0.37	1.02	1.40	2.77	1.96
6	Polska 1	2.38	0.93	0.37	1.08	0.05	0.75	0.21	2.31	1.71
7	Polska 2	0.31	0.81	0.55	1.25	0.48	1.33	0.50	1.16	2.01
8	Polska 3	1.75	1.24	0.19	0.81	0.45	2.08	0.66	1.20	1.40
9	Salami	4.57	2.21	4.89	2.42	0.95	1.01	1.95	5.56	1.89
10	Pasztetowa 1	4.29	1.41	10.75	0.69	1.52	1.01	0.66	1.72	0.56
11	Pasztetowa 2	2.81	1.16	3.21	1.74	1.16	2.98	4.17	6.34	2.26
12	Mortadela	2.81	1.10	1.70	0.13	0.42	1.01	0.11	0.61	0.15
13	Metka łososiowa	4.87	7.65	6.52	2.06	1.26	2.52	2.14	5.20	4.32
14	Parówkowa	1.01	0.15	0.19	0.17	0.17	0.47	0.31	0.77	1.19
15	Mielonka	1.87	1.29	2.19	1.00	0.20	0.58	0.60	2.29	1.58
16	Metka wędzona	0.63	1.63	9.49	1.06	1.63	4.65	1.35	1.12	2.15

As a rule, but not in all sausages, the greater the content of fat, the greater the total COPs content (Fig. 1).

Fig. 1. Correlation between fat content and total COPs content in 16 Polish commercially sausages



In the analyzed sausages we also determined the content of the 9 most popular animal origin food oxysterols: 7β-hydroksycholesterol (7β-OHC), 20α-hydroksycholesterol (20α-OHC), 25-hydroksycholesterol (25-OHC), 27-hydroksycholesterol (27-OHC), cholesterol α-epoksy (α -epoksy-C), 7-ketocholesterol (7-keto-C), cholestane-3β,5α,6β-triol (triol-C), 7α-hydroksycholesterol (7α-OHC) and cholesterol β-epoksy (β-epoksy-C). The given results are showed in Table 2.

The given results were calculated and a correlation between fat contents and cholesterol, total sum of oxysterols (COPs) and every single oxysterols content was also analyzed. The results of the correlation are shown in Table 3.

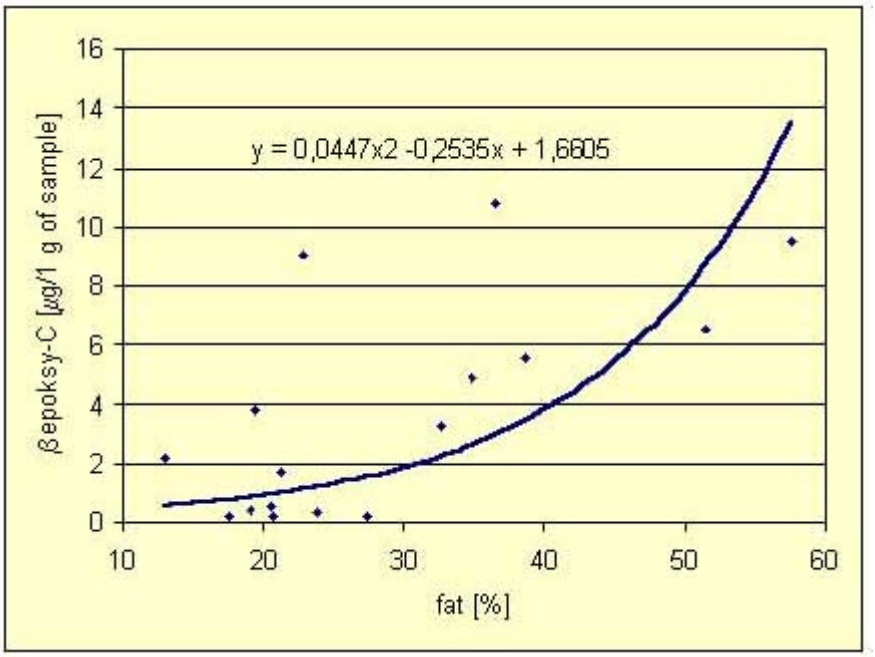
Table 3. Determination of coefficient (R2) between fat content and oxysterols, cholesterol and total sum of oxysterols content in Polish commercial sausages

Discriminant	Fat
β epoksy-C	**0.453***
20 α –OHC	0.012
27-OHC	0.001
7α -OHC	0.031
7 keto-C	0.059
α epoksy-C	0.081
25-OHC	0.180
7β -OHC	**0.289***
Triol-C	**0.327***
Sum of oxsysterols	**0.394***
Cholesterol	0.085

*** Statistically significant results at p =0.05**

We observed statistically significant results only between the fat content and β-epoksycholesterol, 7 β - hydroksycholesterol, cholestanetriol and, as mentioned above, the total sum of oxysterols. We did not observe any correlation between the fat content and the remainder of the oxysterols and cholesterol content. The highest β-epoksycholesterol was determined in pasztetowa 1 sausage (10.75 µg/1 g of sample), the smallest in parówkowa sausage (0.19 µg/1 g of sample). The higher the fat content, the higher the β-epoksycholesterol content (Fig. 2).

Fig 2. Correlation between fat content and β -epoksycholesterol content in 16 Polish commercially sausages



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7 β-hydroksycholesterol content was greatest in wiejska sausage (0.07 µg/1 g of sample), the smallest in metka łososiowa (7.65 µg/1 g of sample). In all the sausages we also observed an amount of toxic cholestanetriol. The content of the oxysterols was the greatest in metka wędzona (4.65 µg/1 g of sample), and the smallest in jałowcowa (0.38 µg/1 g of sample). As 7 β -hydroksycholesterol as cholestanetriol showed the same behavior as β-epoksycholesterol.

Previous studies showed that fresh raw meat contained none or only a trace amount of cholesterol oxidation products [1, 17]. However some studies show that some COPs such as 7α-hydroksycholesterol, 7β -hydroksycholesterol and 7-ketocholesterol existed in even fresh raw meat samples before storage Maerker [10].

Secondary COPs, which can be derived from primary COPs such as α-epoxides, β-epoksides, cholestanetriol and 20-hydroksycholesterol were also detected. Cholestanetriol and 25-hydroksycholesterol were reported to be the most atherogenic among oxysterols studied [22]. Peng et al. [15] reported that a remarkably acute injury to the endothelium of rabbits resulted from 25-hydroksycholesterol and cholestanetriol. Ahn et al. [2] reported that the cooking and processing of raw beef and pork increased the content of COPs. In world literature little information is available about COPs content in commercially manufactured sausages and given results are very often different. In selected Polish fresh sausages the content of COPs was rather low and never exceeded 11µg/1 g of fat. This data is in agreement with findings from other authors [13], who reported low levels of COPs in such pork derivatives as dry sausages, cooked sausages and bacon. A high correlation between cholesterol and fat content was found by Dorado [4]. In this study correlations between fat and β-epoksycholesterol, 7 β -hydroksycholesterol, cholestanetriol and total sum of oxysterols were found.

CONCLUSIONS

1. A considerable amount of COPs was found in fresh sausages.
2. The amounts and compositions of COPs in sausages are varied.
3. Correlations between fat and β-epoksycholesterol, 7 β- hydroksycholesterol, cholestanetriol and the total sum of oxysterols were found.

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