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EFFECT OF STORAGE ON OXIDATION OF CHOLESTEROL AND LIPIDS IN LIVER PATE TYPE SAUSAGE

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ABSTRACT

The effect of storage on the contents of cholesterol, its oxidation products (oxysterols), lipid oxidation and hydrolytic changes in liver pate type sausage was studied. Lipid oxidation, hydrolysis and oxysterols level were examined after 1, 3, 6, 8 and 10 days of storage at 4°C. 7β-hydroxycholesterol and 7 – ketocholesterol were the major cholesterol oxidation products formed in samples during storage and their amounts increased throughout storage time. Storage time had no significant effect on acid number and lipid oxidation products (TBARS) in sausage. Significant increase ($p = 0.05$) of peroxide value, 7β-hydroxycholesterol, 7–ketocholesterol levels and total of oxysterols was noted. Storage time had a significant effect on the decrease of cholesterol. The contents of particular oxysterols and their sum were closely related to the peroxide value. The results indicate that both cholesterol and lipids undergo the same oxidation process.
Key words: liver pate, lipid oxidation, cholesterol, cholesterol oxidation products (oxysterols)

INTRODUCTION

Cholesterol oxidation products (oxysterols) 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 of oxysterols has been reviewed [8, 10]. It has been proven that oxysterols associated with lipid oxidation in meat arise from heating [9, 12], storage [9], various stages of processing, and type of meat product [7]. Moreover, cholesterol rapidly undergoes oxidation in the presence of oxygen, light, metal ions, radiation and other compounds, which could generate free radicals [13, 11, 16, 20]. During food processing and storage, polyunsaturated fatty acids tend to oxidize. Cholesterol can be oxidized as a result of the same mechanism as fatty acids. Therefore, lipid radicals formed during processing and storage of food, and also meat products, can accelerate formation of oxysterols [2, 15].

Despite the widespread existence of oxysterols in foods and their adverse effect on health, little work has been done on meat products. Particularly in liver pate type sausages the presence of oxysterols has not been investigated.

Therefore the objective of this study was to determine the effect of storage time on cholesterol level and process of lipid oxidation in liver pate type sausage.

MATERIALS AND METHODS

The liver pate type sausage was produced according to Good Manufacturing Practice, on the commercial scale in a large plant located in Poznań. The sausage was stored during 10 days, at 4°C, in darkness. The proximate analysis was made according to Polish Standars. Cholesterol contents (µg/1g of sample) and oxysterols contents (% of total oxides) were determinated following the GC and GC/MS procedure described by Przygoński et al. [19]. The following main products of cholesterol oxidation were determined: 7–ketocholesterol (7 keto–C and 7β–hydroxycholesterol (7 β-OH-C). Additionally the sum of oxysterols was measured as the total amount of following compounds: 7 α-OH-C, 7 β-OH-C, α epoksy-C, β epoksy-C, 20 α-OH-C, triol-C, 25 OH-C, 7 keto-C and 27 OH-C. The rate of oxidative and hydrolitic changes of fat in sausages was assessed by determination of peroxide value, acid number and TBARS. The peroxide value (PV) was measured using the method described by Charzyński [3] and expressed in [ml 0.002 n $Na_2S_2O_3$/ 1g of fat]. Acid number (AN) [mg 0.1 n KOH/ 1 g of fat] was measured according to Polish Standard (PN-74/R-66165) and TBA value (TBARS) asdescribed by Pikul et al. [17] and expressed in [mg malonaldehyde /kg of sample]. Analyses were performed after 1, 3, 6, 8 and 10 days of storage.

RESULTS AND DISCUSSION

The results of investigations have been presented in Table 1. Chemical composition of analysed sausage was in accordance with Polish Standard: fat – 36.46%, protein – 11.81% and dry matter – 61.35%.

Table 1. The effect of storage (at 4°C) on some indicators of lipid oxidative and hydrolytic changes in liver pate type sausage.

Time storage	PV [ml 0,002 n $Na_2S_2O_3$/1 g of fat]	AN [mg 0,1n KOH/1 g of fat]	TBARS [mg malonaldehyde/ kg of sample]	Cholesterol [µg/ 1 g of sample]	7 β -OHC [% sum of oxysterols]	7 keto–C [% sum of oxysterols]	Sum of oxysterols [µ g/ 1 g of sample]
1	2.03	8.9	1.04	847.87	6.24	7.61	11.97

3	3.38	8.0	1.12	834.54	7.54	10.38	13.03
6	4.01	6.02	1.43	831.12	10.57	11.56	13.06
8	4.56	8.41	1.38	832.09	11.89	11.17	17.25
10	7.97	8.68	0.95	809.99	13.37	15.12	22.61

The oxidative parameters measured were peroxide value (PV) and TBA number (TBARS). PV is the most common method to measure hydroperoxides, which are the primary products of lipid oxidation. TBA number is a measure of malonaldehyde (MDA) a secondary product of lipids oxidation. The hydrolysis changes of sample were measured by determination of acid number. As previously shown different researchers oxidative changes of lipids are closely associated with cholesterol oxidation [6, 4] and therefore in the experiment we determined the degree of cholesterol oxidation and two main cholesterol oxidation products formation.

Statistical treatment of analytical data showed significant influence of time storage liver pate type sausage on peroxide value, cholesterol level and total sum of oxysterols. Particulary, high and significant influence of time storage on 7 β -OHC i 7 keto-C was found. According to data reported by different researchers [4, 21, 5] 7 β-OHC and 7 keto-C are main cholesterol oxidation products rising during meat products storage. On storage in the refrigeration (4° C) amounts of both cholesterol oxidation products increased progressively over the teen days period as shown in Table 1 respectively from 6.24 % of total oxides to 13.37 % of total oxides for 7β-OHC (Fig. 1) and from 7.61 % of total oxides to 15.12 % of total oxides for 7 keto-C (Fig. 2). Similar increases in oxysterol contents during refrigerated storage were reported by Kesava Rao [9]. The acceleration of cholesterol oxidation in meat as a result of storage has been previously documented [15].

Fig. 1. Correlation of storage time with 7β–OHC for sausage "pasztetowa" type stored 1,3,6,8, and 10 days at 4°C.

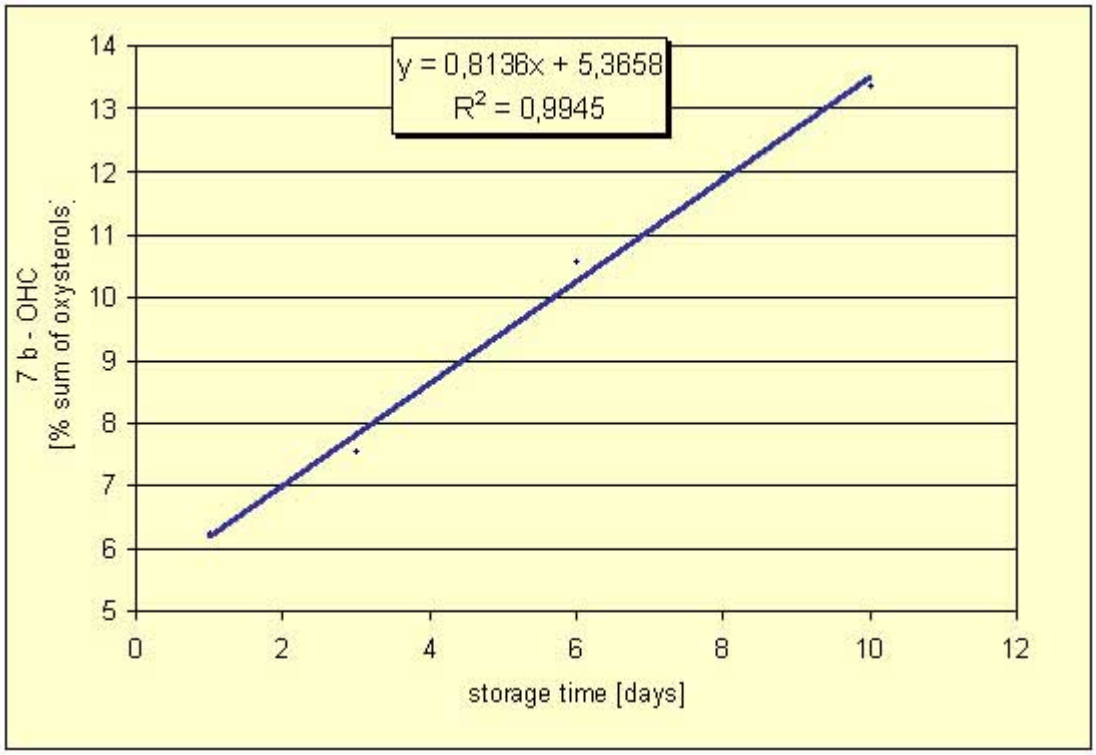
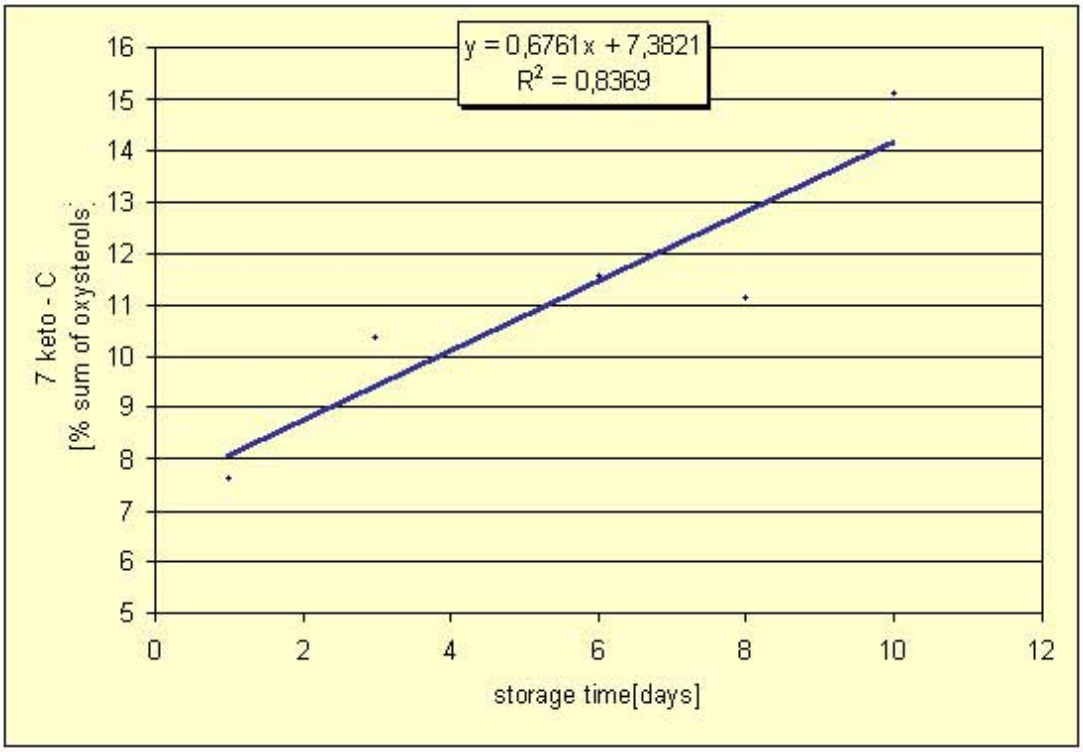


Fig. 2. Correlation of storage time with 7 keto-C for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.



Total sum of oxysterols showed the same behavior as the individual ones. The sum of oxysterols doubled the orginal value after storage. It arrised from 11,97 [µg/1g of sample] to 22.61 I[µ g/1g of sample] in last day (Fig. 3). As total sum of oxysterols increased, cholesterol level decrease was observed. Total cholesterol contents in liver pate type sausage (Table 1) ranged from a maximum of about 847.87 [µg/1g of sample] to a minimum of about 809.99 [µg/1g of sample] (Fig. 4). As could be expected time storage had significant influence on peroxide values for analyzed sample. PV increased progressively from 2.03 [ml 0.002 n $Na_2S_20_3$/1 g of fat] whitin 10 days of storage (Fig. 5). However no significant influence of storage time on TBA value and acid number was observed. A decline in TBA value might have occurred by generation of different decomposition products. MDA is only one of the compounds that originate from the peroxids breakdown. It can also be degraded via oxidation and the lowering TBA value could be due to its degradation or further reactions between aldehydes themeselves [1].

Data collected in Table 1 were used to calculated correlation coefficient between all mentioned paraeters. Obtained correlation coefficient have been presented in Table 2.

Fig. 3. Correlation of storage time with sum of oxysterols for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

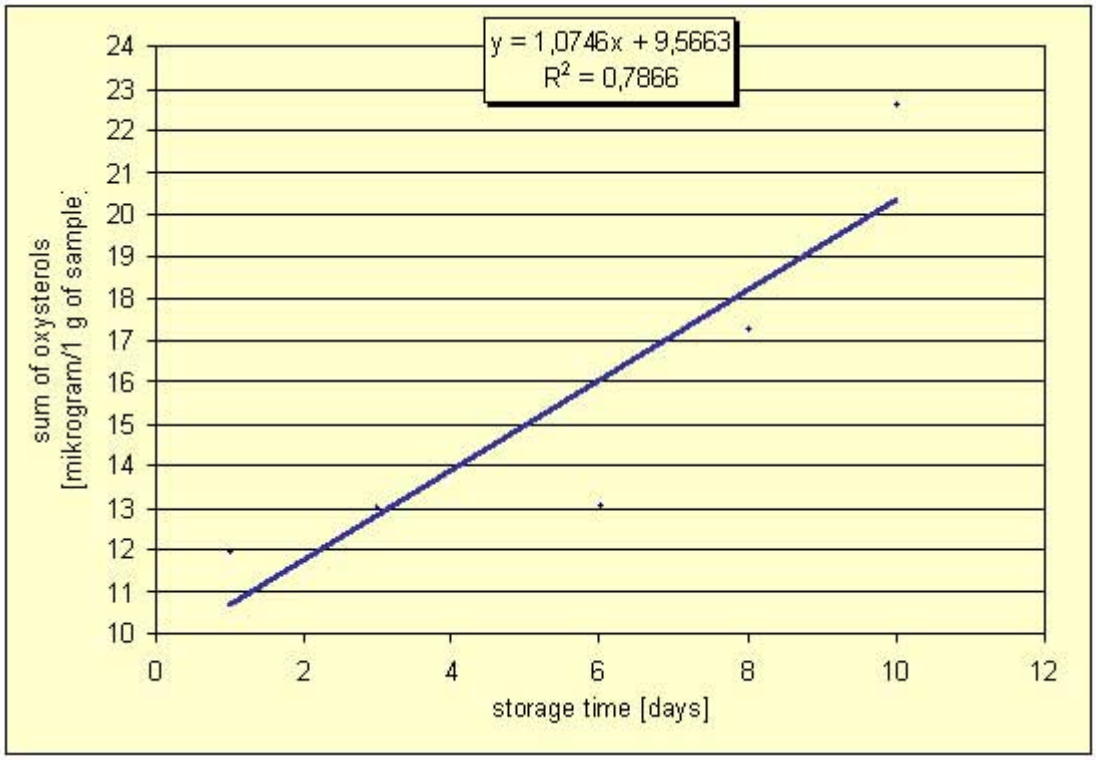


Fig. 4. Correlation of storage time with cholesterol level for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

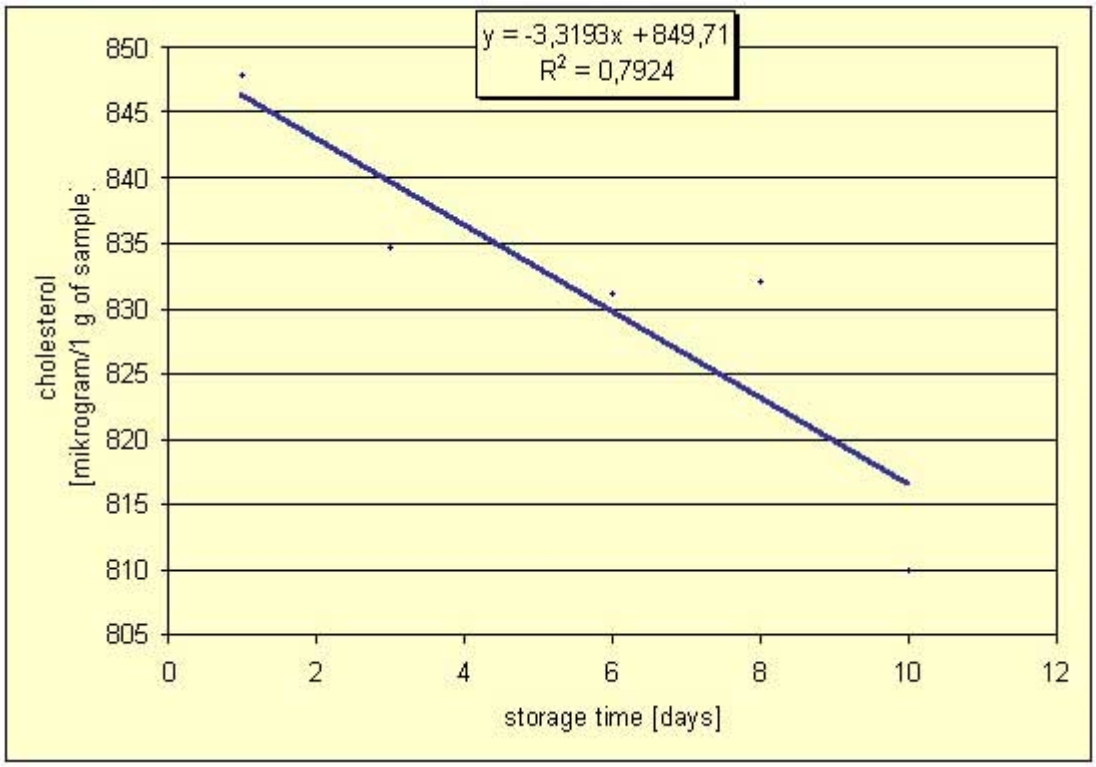


Fig. 5. Correlation of time storage with peroxide value for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

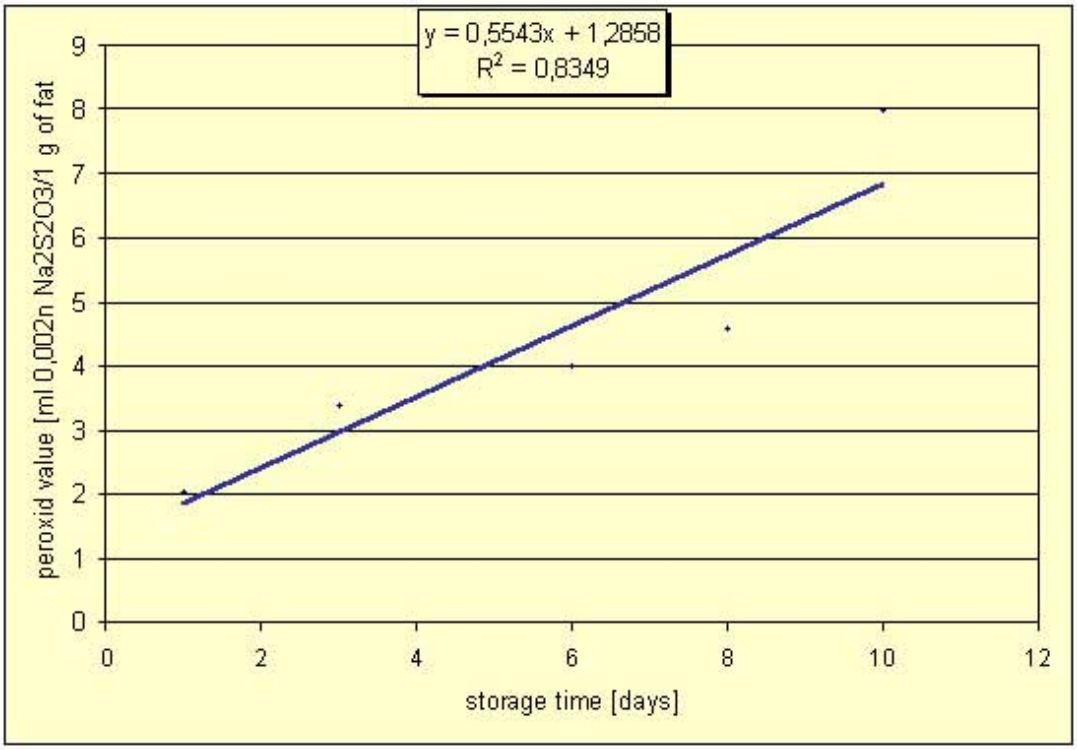


Table 2. Correlation coefficients for analyzed parameters in liver pate type sausage.

	Time storage	PV	AN	TBARS	Cholesterol	7β - OHC	7 keto–C	Sum of oxysterols
Time storage	x	**0.835**		0.014	**0.792**	**0.994**	**0.837**	**0.787**
PV		x	0.077	0.069	**0.969**	**0.798**	**0.946**	**0.920**
AN			x	0.02	0.0003	0.010	0.012	0.120
TBARS				x	0.047	0.028	0.013	0.113
Cholesterol					x	**0.756**	**0.989**	**0.808**
7β -OHC						x	**0.810**	0.741
7 keto–C							x	**0.765**
Sum of oxysterols								x

Presented results indicate high correlation between peroxide value and level of cholesterol, 7 β-OHC, 7 keto-C and total sum of oxysterols (Fig. 6, 7, 8, 9). Increase of peroxide value paralelled the increase of both oxysterols and total sum of oxysterols levels. At the same time cholesterol level decreased. Due to proceed by generation of oxidation products. Correlations between cholesterol level and total sum of oxysterols (Fig. 10), 7 β-OHC (Fig. 11), 7 keto-C (Fig. 12) and correlation between this two oxysterols (Fig. 13). Were also notheworthy. In time when cholesterol level decreased, the level of sum of oxysterols and of 7β-OH and 7 keto-C increased.

Fig. 6. Correlation of peroxide value with cholesterol level for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

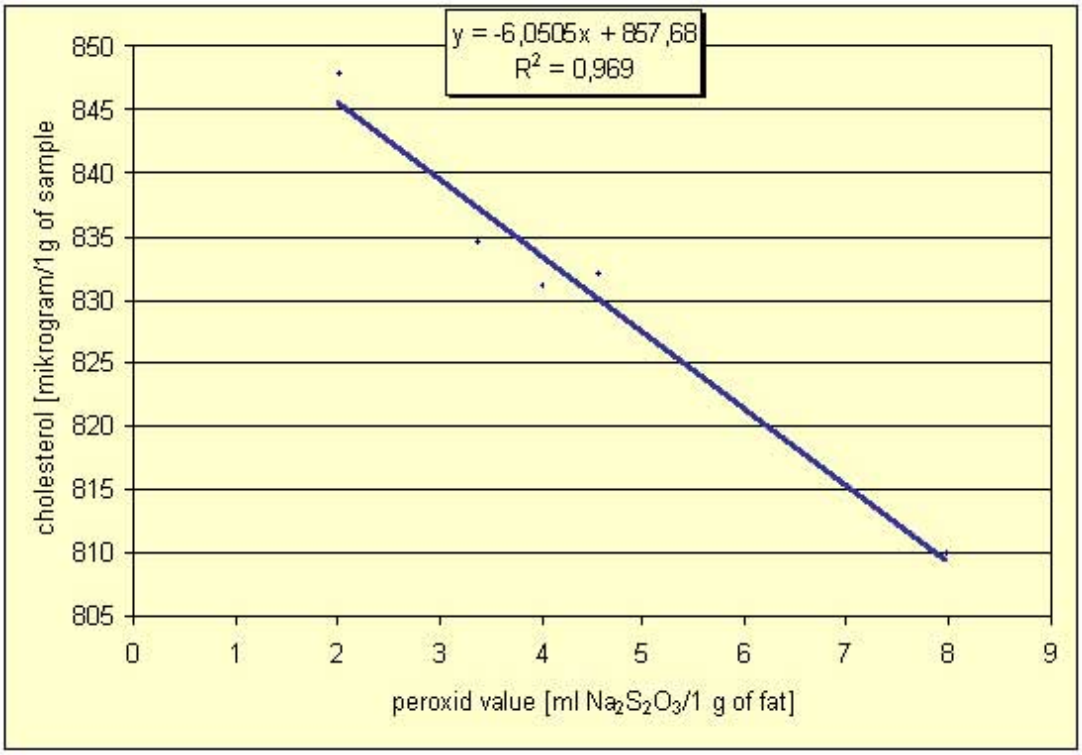


Fig. 7. Correlation of peroxide value with 7 β-OHC for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

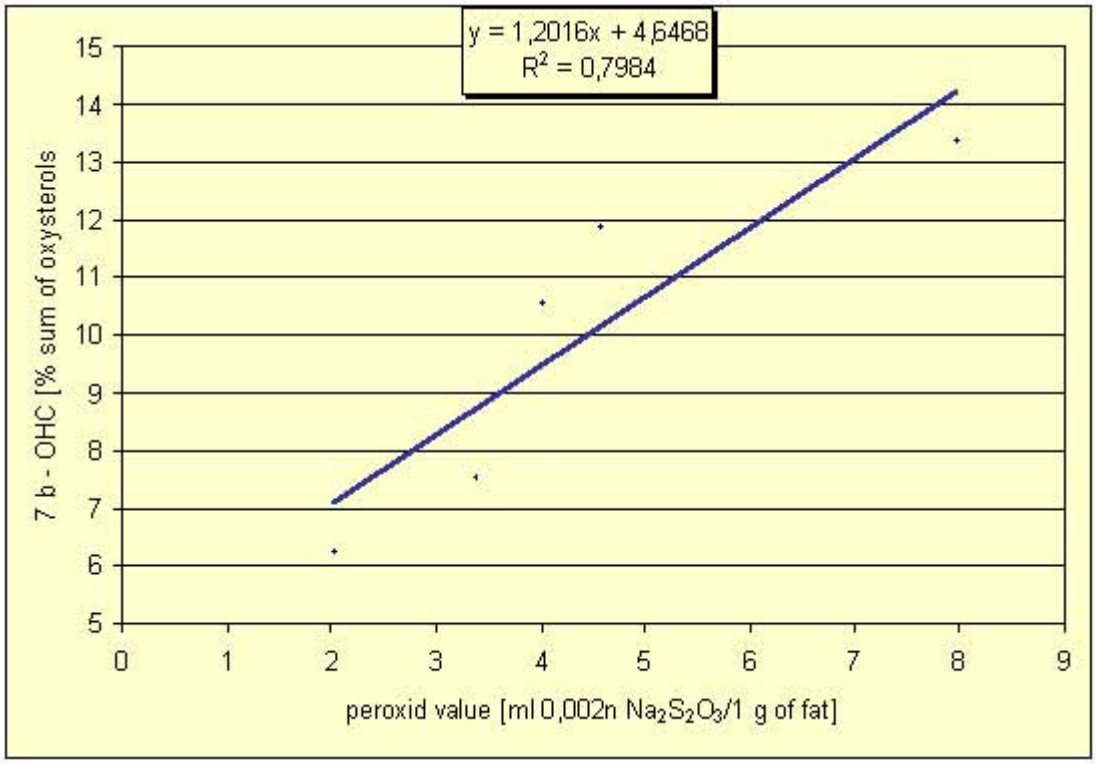


Fig. 8 Correlation of peroxide value with 7-keto-C for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

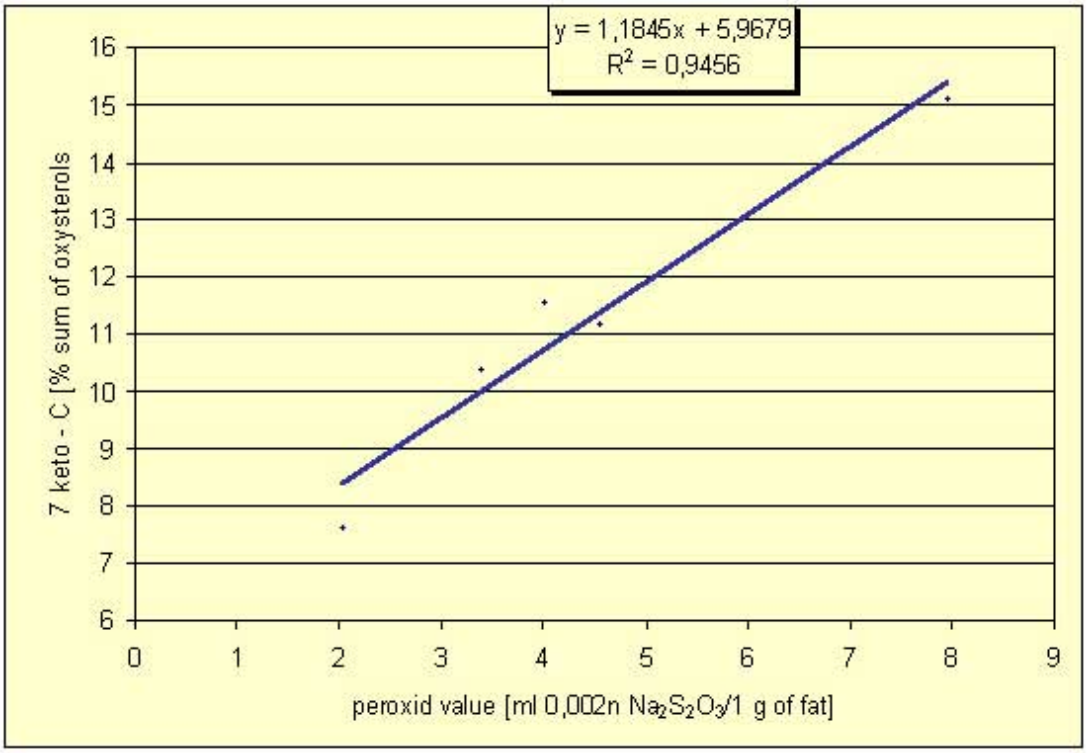


Fig. 9. Correlation of peroxide value with sum of oxysterols for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

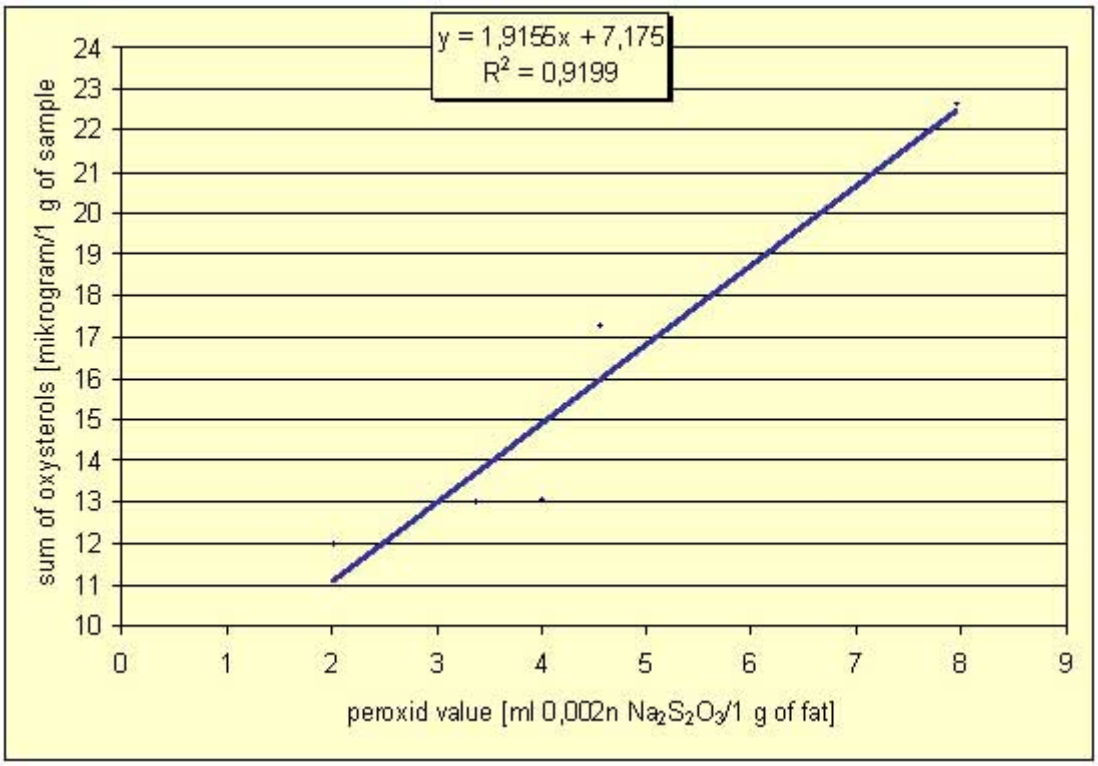


Fig. 10. Correlation of cholesterol level with sum of oxysterols for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

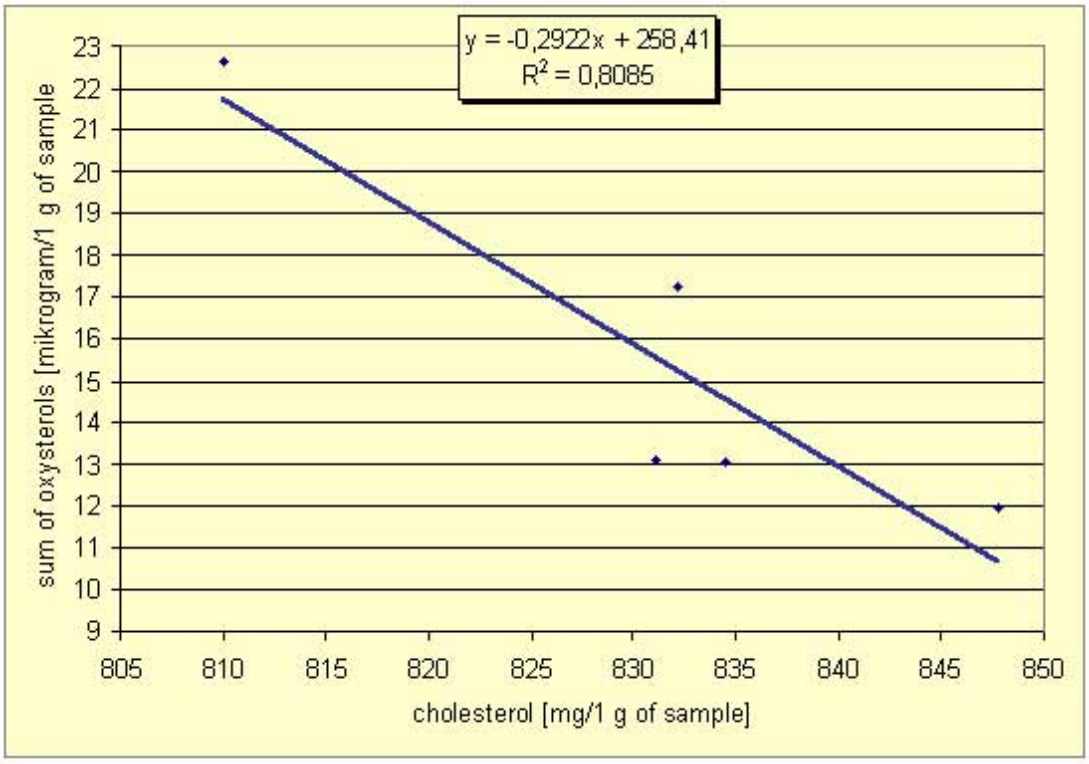


Fig. 11. Correlation of cholesterol level with 7 β-OHC for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

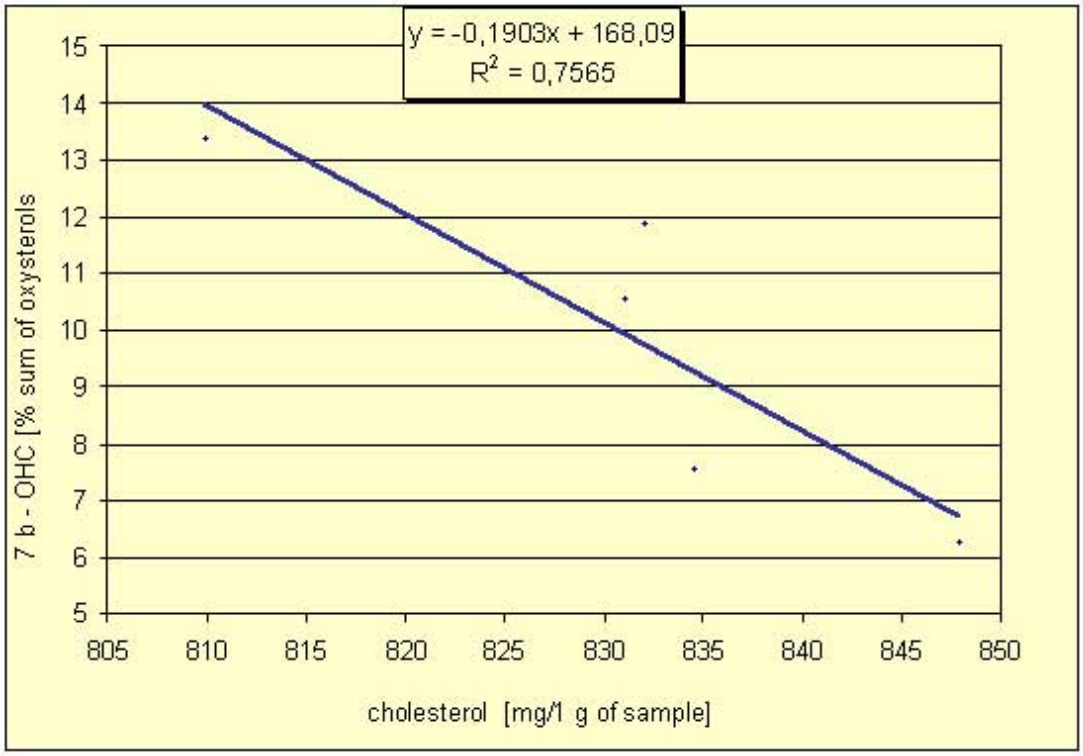


Fig. 12. Correlation of cholesterol level with 7 keto-C for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.

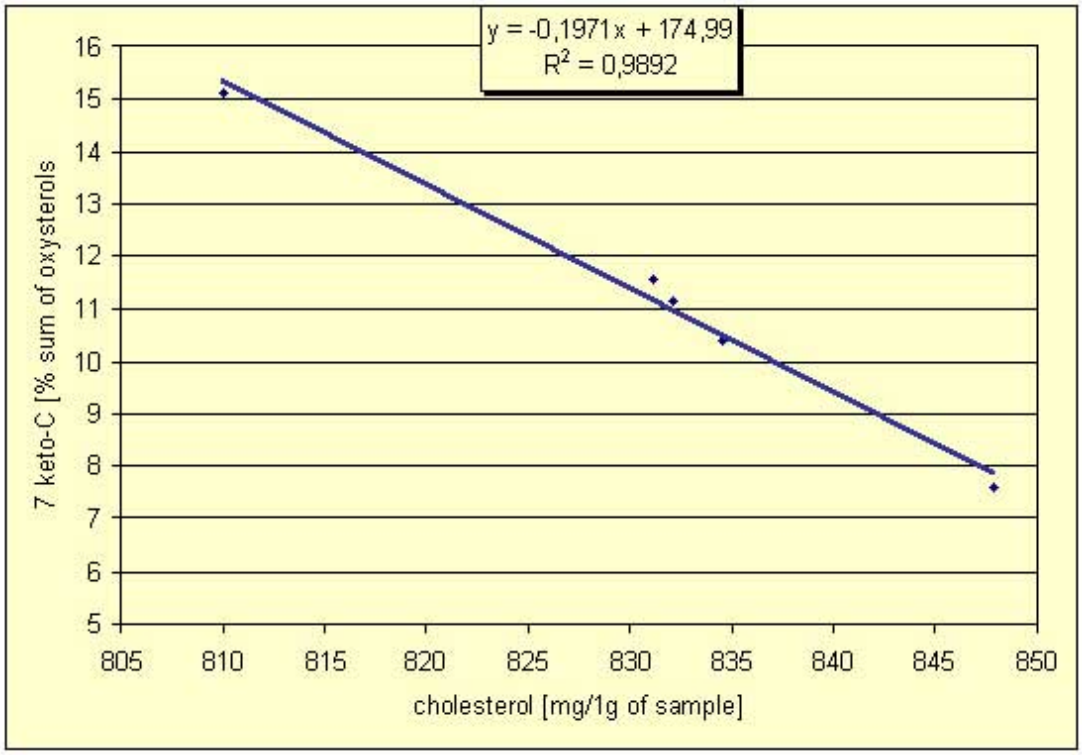
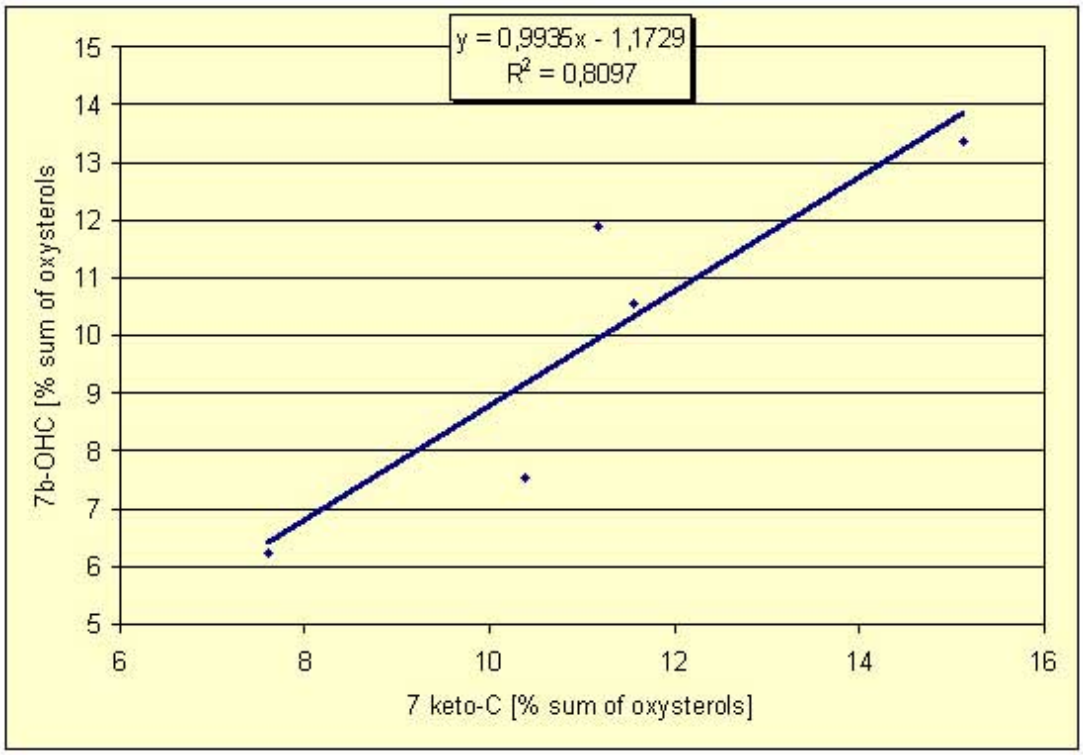


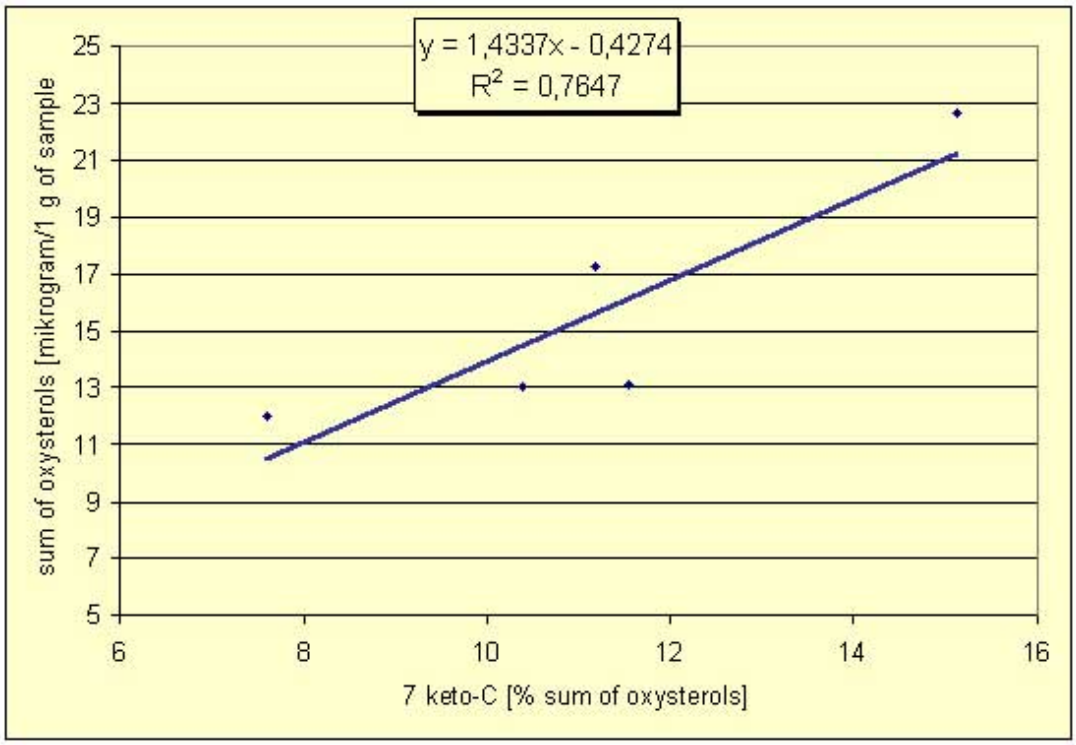
Fig. 13. Correlation of 7 β -OHC with 7 keto-C for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.



However, membrane-bound cholesterol is closely associated with the polyunsaturated fatty acids of membranal phospholipids and oxidises under similar conditions. Hence, cholesterol oxidation is accelerated by those factors which promote lipid oxidation, including processing, heating, and the presence of pro- and antioxidants. The cholesterol oxidation is positively associated with the degree of unsaturation of neighbouring fatty acids and is, therefore, likely to involve attack by fatty acyl radicals formed in close proximity to membrane-bound cholesterol molecules [14].

The correlation between sum of oxysterols and 7 keto-C was also obserwed (Fig. 14). Both oxysterols increased in the same time, but 7 keto-C arised faster than 7 β-OHC. No correlation between acid number, TBA value and remaining parameters has been found. The lack of correlation between acid number and sterols can indicate that fatty acid hydrolysis and cholesterol oxidation followed a different pattern during processing and further storage at 4°C for 10 days.

Fig. 14. Correlation of sum of oxysterols with 7 keto-C for sausage "pasztetowa" type, stored 1,3,6,8 and 10 days at 4°C.



CONCLUSIONS

1. Storage time influenced level of sum of total oxysterols, cholesterol, 7 β-OHC, 7 keto-C and peroxide value.
2. Lipids peroxidation was significantly correlated with cholesterol, 7 β-OHC, 7 keto-C and total oxysterols.
3. Acid number and TBA value was not correlated with cholesterol and oxysterols contents.

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