

## THE EFFECT OF SOY HYDROLYSATES ON CHANGES IN CHOLESTEROL CONTENT AND ITS OXIDATION PRODUCTS IN FINE – GROUND MODEL SAUSAGES

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**Background.** Meat products belong to products particularly at risk of fat oxidation processes. One of the methods to prevent disadvantageous oxidative changes of lipids in food is the application of antioxidants.

**Material and methods.** The experimental material consisted of fine – ground model sausages. Produced processed meats differed in terms of the presence and amount of acid and enzymatic soy hydrolysates (0.3% and 0.7%). The reference sample comprised processed meat product with no hydrolysate added. Model processed meat products were stored at 4°C for 29 days. The analyses included changes in peroxide value, changes in cholesterol and its oxidation products.

**Results.** It was found that changes of peroxide value, 7 $\alpha$ -OHC, 7 $\beta$ -OHC,  $\alpha$ -epoxy-C,  $\beta$ -epoxy-C, 20 $\alpha$ -OHC, 25-OHC and total oxisterols were statistically significantly affected, apart from storage time, also by the type and level of applied hydrolysates. The addition of enzymatic and acid hydrolysates to batter of experimental sausages effectively inhibited the process of fat oxidation.

**Conclusions.** In samples with enzymatic hydrolysate an approx. 20% loss of initial cholesterol content was recorded. In contrast, in the other samples this loss amounted to approx. 10%.

The process of cholesterol metabolism in tested processed meat products was affected by their storage time and the type of added hydrolysate. It was observed that the highest dynamics of cholesterol metabolism occurred in a sample with no hydrolysate added. The level of total oxisterols in the sample with no addition of hydrolysate was over two times higher than in samples with an addition of hydrolysate.

**Key words:** protein hydrolysates, cholesterol, cholesterol oxidation products, oxisterols, peroxide value

## INTRODUCTION

Meat products belong to products particularly at risk of fat oxidation processes. Their consequence is a reduction of their shelf life, resulting from a deterioration of their sensory attributes, reduced nutritive value and deteriorated health safety of these foodstuffs. These changes pertain not only to fatty acids, but also components of the non-glyceride fraction, such as vitamins or sterols [Peña-Ramos and Xiong 2003, Wąsowicz et al. 2004, Gramza-Michałowska et al. 2008].

Lipids and cholesterol itself easily undergo free radical oxidation, which occurs especially at the interface. Products of cholesterol oxidation, also referred to as oxisterols, similarly as lipid oxidation products (hydroperoxides, ketone compounds, aldehydes as well as free radicals) reduce the nutritive value of products and some, e.g. oxisterols, constitute a health hazard [Guardiola et al. 1996, Addis 1986, Valenzuela et al. 2003]. Sterol oxidation products formed most frequently in food include 7 $\alpha$ - and 7 $\beta$ -hydroxysterols, 5,6 $\alpha$ - and 5,6 $\beta$ -epoxysterols, 7-ketosterols and triols [Derewiaka and Obiedziński 2007, Ziarno 2008]. One of the methods to prevent disadvantageous oxidative changes of lipids in food is the application of antioxidants [Peña-Ramos and Xiong 2003, Samotyja and Urbanowicz 2005, Sikora et al. 2008].

Extensive applications have been found for protein hydrolysates in food industry. These hydrolysates are produced as a result of hydrolysis of plant or animal origin raw materials rich in proteins. They are applied in the amount of 0.5-2.0% and sometimes even 3.0% in relation to the weight of the final product. They not only give foodstuffs a specific flavour, but also enhance and improve their taste [Flaczyk 1997 a, 2005, Komorowska and Stecka 1998]. Moreover, they are capable of reducing water activity and they exhibit antioxidant properties [Peña-Ramos and Xiong 2003, Wu et al. 2003]. Antioxidant properties may be explained by the capacity to regenerate primary antioxidants, reaction with free radicals of fats, formation of complexes with pro-oxidative metal ions, reaction with free fatty acids and blocking oxidisable methylene groups [Szukalska 1999, Flaczyk 1997 b, 2005].

## AIM OF STUDY

The aim of the study was to assess the effect of two soy hydrolysates: acid and enzymatic, on changes in contents of cholesterol and its oxidation products in a fine – ground model sausages, cold stored for 29 days.

## MATERIAL AND METHODS

The experimental material consisted of fine – ground model sausages in a Betan Naturin polyamide casing with the following formulation: 44% beef grade II, 28% pork grade II and 28% yowl. Produced processed meats differed in terms of the presence and amount of acid and enzymatic hydrolysates (0.3% and 0.7%). The reference sample comprised processed meat product with no hydrolysate added.

Model processed meat products were stored at 4°C for 29 days. During storage of model processed meat products the analyses included changes in peroxide value [PN-ISO 3960 1996], changes in cholesterol and its oxidation products. Cholesterol content in experimental processed meat products was determined by gas chromatography, based on a method described by Fenton and Sim. Analysis of silyl esters of oxisterols was performed using high performance gas chromatography using a flame ionization detector (FID) [Przygoński et al. 2000]. Analyses were conducted at day 1, 8, 15, 22 and 29 after production.

All results were subjected to basic statistical analysis using the STATISTICA 6.0 and Microsoft Excel 2007 software. Results were interpreted at a significance level  $\alpha = 0.05$ .

## DISCUSSION AND RESULTS

At the beginning of the experiment the basic composition of the analysed sausages was determined. It was found that contents of fat, protein and water met the requirements of standard PN-A-82007/A1 for finely comminuted sausages.

Recorded results were subjected to a three-way analysis of variance, where the sources of variation were the type of hydrolysate (A), the level of hydrolysate (B) and storage time (C). Table 1 presents significance coefficients for the analysed dependencies.

Table 1. A list of significance coefficients F ( $\alpha \leq 0.05$ )

Analysed parameter	Source of variation					
	type of hydrolysate		level of hydrolysate		storage time	
	F <sub>obl</sub>	F <sub>tab.</sub>	F <sub>obl</sub>	F <sub>tab.</sub>	F <sub>obl</sub>	F <sub>tab.</sub>
Peroxide value	27.127	4.171	254.358	3.316	4 642.016	2.690
Cholesterol	5.369		3.134		89.367	
7 $\alpha$ -OHC	9.192		1 047.742		1 528.935	
7 $\beta$ -OHC	2 834.793		6 609.076		19 824.004	
$\alpha$ -epoxy-C	335.935		10 415.569		4 227.497	
$\beta$ -epoxy-C	12.564		658.824		616.297	
20 $\alpha$ -OHC	815.541	4.001	2 505.808	3.150	4 642.086	2.525
25-OHC	7.804		471.410		3 562.467	
Triol	1.479		2 915.960		1 393.269	
7-keto-C	0.069		2.001		64.793	
Total oxisterols	1 110.353		18 104.644		21 536.700	

Conducted analysis of variance showed a highly significant effect of the type and amount of added hydrolysate and storage time on changes in peroxide value, 7 $\alpha$ -OHC, 7 $\beta$ -OHC,  $\alpha$ -epoxy-C,  $\beta$ -epoxy-C, 20 $\alpha$ -OHC, 25-OHC and total oxisterols. A statistically

significant effect on changes in triol content was found for the amount of hydrolysate and storage time, while changes of 7-keto-C were statistically significantly affected only by storage time. It was observed that the type of hydrolysate and storage time had a statistically significant effect on changes in cholesterol.

Peroxide value is an index of primary oxidation products (peroxides). It characterizes the degree of peroxide fat spoilage and it is connected mainly with the formation of epihydrine aldehyde. Results of analyses showed that with an extension of storage time for experimental processed meat products (up to day 15 after production) the value of peroxide number increased gradually, but the dynamics of this growth varied (Fig. 1). Further storage of these processed meat products resulted in a statistically significant decrease in the value of this attribute. Moreover, it was observed that an addition of enzymatic and acid hydrolysates to batter of experimental sausages effectively inhibited the process of fat oxidation. The smallest changes in peroxide value were recorded in sample A (0.3% HE) and in sample D (0.7% HKw).

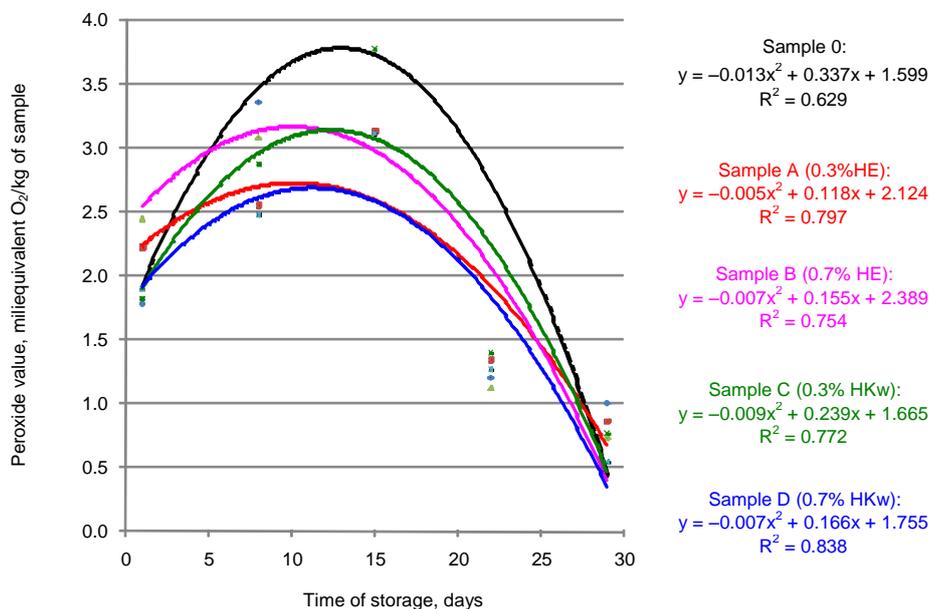


Fig. 1. The effect of storage time on changes in value of peroxide number in experimental sausages

Moreover, it was found that during storage the peroxide value decreased the fastest in samples with a 0.7% addition of hydrolysate. No statistically significant differences were observed between samples with an addition of enzymatic and acid hydrolysates. In contrast, statistically significant differences were found between the zero sample and samples with an addition of hydrolysates.

In all examined experimental sausages a trend was observed for cholesterol content to decrease during storage (Fig. 2). In sample 0 (with no hydrolysate added) and in samples with a 0.3% and 0.7% addition of acid hydrolysate the amount of cholesterol

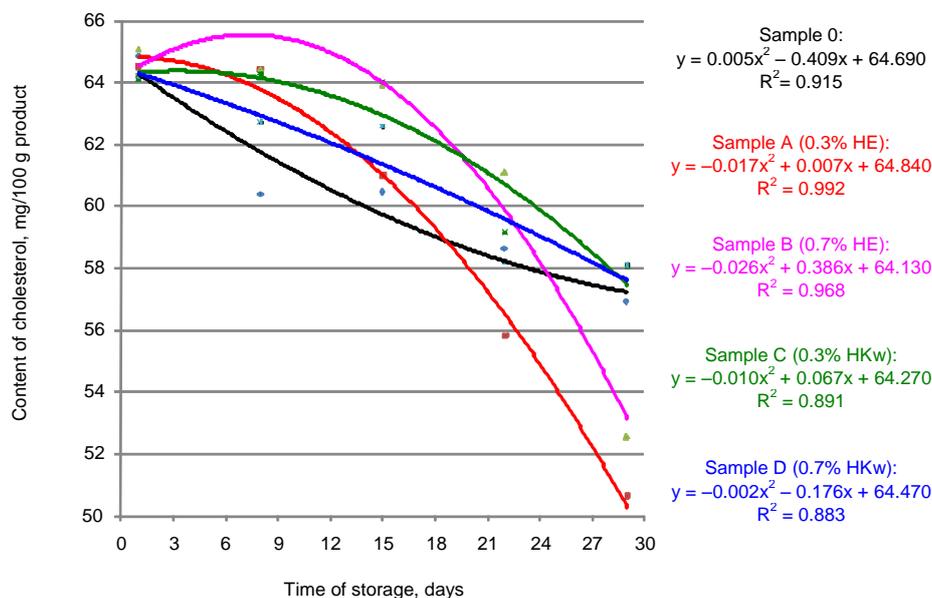


Fig. 2. The effect of storage time on changes in cholesterol content in experimental sausages

decreased on average from 64.2 to 57.5 mg/100 g product, which constituted approx. 10% loss of initial cholesterol content. In turn, in samples with an addition of enzymatic hydrolysate, cold stored for 29 days, the content of cholesterol decreased from 64.6 to 51.6 mg/100 g product, which amounts to approx. 20% loss of initial cholesterol content.

Applied technological processes (e.g. temperature of the technological process, oxidation time, the presence of water, pH value, etc.) as well as the method of storage in animal origin products result in degradation and oxidation of cholesterol, forming cholesterol oxidation products, i.e. oxisterols [Adcox et al. 2001]. In analyzed experimental meat products the following cholesterol oxidation products were found: 7 $\alpha$ -OHC, 7 $\beta$ -OHC,  $\alpha$ -epoxy-C,  $\beta$ -epoxy-C, 20 $\alpha$ -OHC, 25-OHC, triol and 7-keto-C. During 29-day storage total content of cholesterol oxidation products increased systematically in analyzed products (Fig. 3).

The highest increase was recorded in sample 0 (with no hydrolysates added) from 2.93  $\mu$ g/g product to 184.17  $\mu$ g/g product, while the smallest in a sample with an addition of acid hydrolysate amounting to 0.7% (from 1.64 to 65.91  $\mu$ g/g product). In samples with an addition of enzymatic hydrolysate, irrespective of the level of the applied addition, total content of cholesterol oxidation products increased on average from 2.45 to 78.44  $\mu$ g/g product. In turn, in samples with an addition of acid hydrolysate a statistically significant effect of the level of applied addition on total oxisterols was found. Sample C, with a 0.3% addition of acid hydrolysate, total content of cholesterol oxidation products was observed to be over 80% higher than in sample D (with a 0.7% addition of acid hydrolysate).

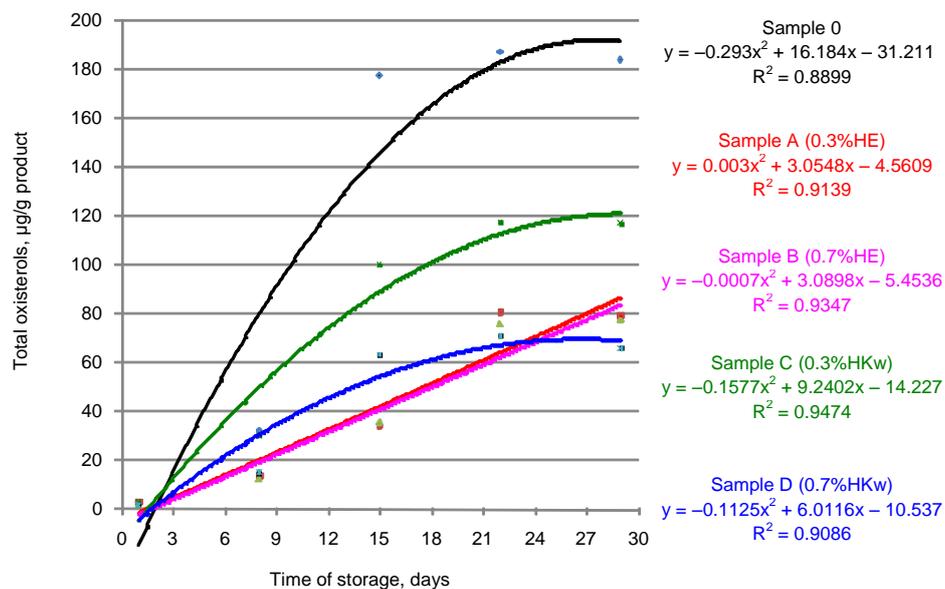


Fig. 3. The effect of storage time on changes in total oxisterols in experimental sausages

## CONCLUSIONS

1. An addition of enzymatic and acid hydrolysates to batter of experimental sausages effectively inhibited the process of fat oxidation.
2. Apart from storage time, the type and level of applied hydrolysates had a statistically significant effect on changes in peroxide value.
3. Samples with an addition of acid hydrolysate were characterized by a 10% loss of initial cholesterol content. In turn, during storage in samples with an addition of enzymatic hydrolysate this loss was approx. 20%.
4. The process of cholesterol metabolism in analysed processed meat products was influenced by storage time and the type of added hydrolysate. It was observed that the biggest dynamics of cholesterol metabolism was found for a sample with an addition of enzymatic hydrolysate.
5. Total cholesterol oxidation products in a sample with no hydrolysate added was over two-fold higher than in samples with an addition of hydrolysate.

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## WPLYW HYDROLIZATÓW SOJOWYCH NA ZMIANY ZAWARTOŚCI CHOLESTEROLU I PRODUKTÓW JEGO UTLENIANIA W KIEŁBASIE MODELOWEJ TYPU PARÓWKOWA

**Wprowadzenie.** Wyroby mięsne należą do produktów szczególnie narażonych na procesy utleniania tłuszczu. Jednym ze sposobów zapobiegania niekorzystnym zmianom oksydacyjnym lipidów żywności jest stosowanie przeciwutleniaczy.

**Materiał i metody.** Materiałem doświadczalnym była kiełbasa modelowa typu parówkowa. Wyprodukowane wędliny różniły się ilością hydrolizatów sojowych: kwasowego i enzymatycznego (0,3% i 0,7%). Próbą odniesienia była wędlina bez dodatku hydrolizatu. Wędliny modelowe przechowywano w temperaturze 4°C przez 29 dni. Badania wykonano w 1, 8, 15, 22 i 29 dniu po produkcji. Oznaczano zmiany: liczby nadtlenkowej, cholesterolu i produktów jego utleniania.

**Wyniki.** Stwierdzono, że na zmiany liczby nadtlenkowej, 7 $\alpha$ -OHC, 7 $\beta$ -OHC,  $\alpha$ -epoxy-C,  $\beta$ -epoxy-C, 20 $\alpha$ -OHC, 25-OHC oraz na sumę oksysteroli, oprócz czasu przechowywania, statystycznie istotny wpływ miał rodzaj i poziom zastosowanych hydrolizatów. Dodatek hydrolizatów: enzymatycznego i kwasowego do farszu kiełbas doświadczalnych skutecznie hamował proces utleniania tłuszczu.

**Wnioski.** W próbach z hydrolizatem enzymatycznym stwierdzono około 20-procentowy ubytek początkowej zawartości cholesterolu. Natomiast w pozostałych próbach ubytek wyniósł około 10%. Na proces przemian cholesterolu w badanych wędlinach miał wpływ czas przechowywania oraz rodzaj dodanego hydrolizatu. Zauważono, że największą dynamiką przemian cholesterolu charakteryzowała się próba bez dodatku hydrolizatu. Suma oksysteroli w próbce bez dodatku hydrolizatu była ponad dwa razy większa niż w próbach z dodatkiem hydrolizatu.

**Słowa kluczowe:** hydrolizaty białkowe, cholesterol, produkty utleniania cholesterolu, oksysterole, liczba nadtlenkowa

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