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## INFLUENCE OF LIPID STABILIZATION ON THE RETENTION OF AVAILABLE LYSINE AND METHIONINE IN STORED RAW POLISH SAUSAGE

### Summary

The aim of the study was to determine the effect of the addition of antioxidants on changes in contents of available lysine and methionine in raw Polish sausage.

Experimental material consisted of four variants of experimental sausages. One variant consisted of a sample with no antioxidant added (control), while the following were added to the other variants: BHT (0.02%), rosemary ethanol extract (0.05%) and soy protein hydrolysate (2%). Oxidation degree of lipid using peroxide value (PV), anisidine value (AV), thiobarbituric acid reactive substances (TBARS) and the Totox index was controlled and content of available lysine and methionine were determined periodically.

In order to determine the effect of the addition of antioxidants on the stability of available lysine and methionine, values of the coefficient of the slope of curve of changes in time (coefficient  $a/24$  h) were analyzed along with the half-life period of the amino acids ( $T_{IC50}$ ).

Antioxidants exhibited an inhibitory effect on the advancement of lipid autoxidation reactions and reduced quantitative losses of available lysine and methionine in analyzed sausages. Natural antioxidants exhibited a lower capacity to reduce losses of nutritive value of protein than it was found for BHT.

**Key words:** raw sausage, lipid oxidation, antioxidants, lysine, methionine

### Introduction

Reactions of oxidative nature occurring in meat and processed meats are major causes deteriorating their quality. They are responsible for the degradation of colour, flavour and texture, as well as the loss of their nutritive value [10, 18]. The deterioration of nutritive value may result from the interactions of very reactive lipid oxidation

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products with proteins, which belong to the most valuable components of our diet and need to be especially protected in all processes connected with technological processing. Nutritive value of meat proteins is determined by the quantitative and qualitative composition of amino acids and the hydrolyzability of proteins by digestive enzymes [13]. Digestibility and availability of amino acids decrease as a result of the formation of cross-linking bonds in protein-lipid complexes and reactions of functional groups of amino acids with lipid oxidation products. This pertains especially to the amino, sulfhydryl and hydroxyl groups [19]. In nutrition losses of lysine and amino acids containing sulfur are crucial, as they are exogenous amino acids and at the same time they limit the nutritive value of protein in most products.

A reduction or inhibition of negative oxidative changes in lipids of meat and meat products may be the effect of added antioxidants [7]. The application of synthetic compounds, i.e. BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), in many countries is limited due to the results of toxicological analyses and the opinion of consumer organizations [5, 6]. As a consequence there is a need to search for alternative solutions concerning lipid stabilization. One of them may be the use of effective and cheap natural antioxidants. The most important group of substances with antioxidative properties is represented by polyphenol compounds, belonging to secondary metabolites widely found in plants. Rich sources of these substances include vegetables, fruit, some cereals, tea and many spices [3, 24]. Numerous studies show that good antioxidative properties are also found in protein hydrolysates. Their activity is connected with the presence of amino acids, peptides and Maillard reaction products [15, 23]. These natural compounds preventing oxidation may at the same time modify the nutritive value of food [11, 16]. This makes it possible to supply consumers with safe foodstuffs with prolonged shelf-life and enhanced nutritive value.

The aim of the study was to evaluate the antioxidant properties of natural substances in stored raw sausages, and their influence on the stability of lysine and methionine by retardation of lipid oxidation products-protein interactions.

### **Material and methods**

Experimental material consisted of Polish raw sausage with the addition of rosemary ethanol extract (0.05%), soy protein hydrolysate (2%) and BHT in the amount of 0.02% (% of meat weight). Soy protein acidithal hydrolysate, was made by the Kalisz Institute of Food Concentrates "Winiary", Poland. Dried rosemary leaves (*Rosmarinus off.* L.) was of Polish origin (Polish Herbs "Pharma" Poznań). Extract was prepared by mixing 100g of dried material with 1 L 96% ethanol and macerated overnight in ambient temperature. The suspension was filtered, the residue mixed with another portion of the same solvent, and the procedure was repeated four times. The filtrates were com-

bined, and the respective solvent was evaporated. BHT (butylated hydroxytoluene) was purchased from Merck (Germany).

Sausage links were left for 24 h to set. They were smoked at 22 – 25°C for 6 – 8 h. Afterwards sausages were placed in an aging room at 10°C and relative humidity of 75% ± 2% and stored for 30 days.

Oxidation degree of fat using peroxide value (PV), anisidine value (AV), thiobarbituric acid reactive substances (TBARS), Totox coefficient was controlled and content of available lysine and methionine were determined periodically [21].

In order to determine the effect of the addition of antioxidants on quantitative changes in available lysine and methionine, values of the coefficient of the slope of curve of changes in time (coefficient  $a/24$  h) were analyzed along with the half-life period of the amino acids ( $T_{IC50}$ ).

### Results and discussion

In order to determine the antioxidative activities of analyzed additives slopes of straight lines (coefficient  $a/24$  h) were determined, being angles of inclination of regression curves plotted from measurement points of a given attribute in time. Figure 1 presents results of statistical analysis of the effect of antioxidants on values of slopes of lines for analyzed sausages. Results clearly showed the inhibitory effect of antioxidants on the advancement of lipid autoxidation in the analyzed processed meats. All lipid oxidation indexes monitored during sample storage were increasing more slowly in products with their addition. The effectiveness of individual antioxidants varied markedly. The lowest increase of all lipid oxidation indexes per 24 h was observed in processed meat products produced with the addition of BHT (the lowest coefficient  $a/24$  h).

Natural antioxidants exhibited lower effectiveness; however, in comparison to control samples they also significantly inhibited lipid oxidation rate. Rosemary extract showed a better protective action than that of soy hydrolysate.

A similar effect of the addition of rosemary was observed on sausages made from pork [9], from turkey [4] as well as poultry and pork frankfurters [8, 22]. It was also shown that the 0.05% addition of rosemary extract effectively inhibits the increase of TBARS content in fermented sausages made from goat meat, stored at room temperature [17]. High antioxidative activity in fermented sausages was also observed when applying synthetic antioxidants, such as BHT and BHA [1, 2].

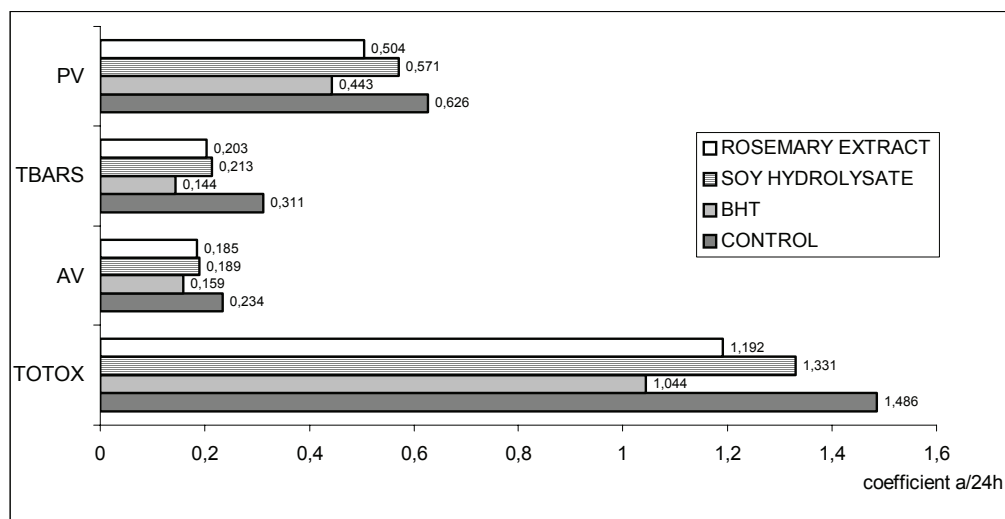


Fig. 1. Statistical analysis results of antioxidants influence on straight lines inclination in stored raw Polish sausage

Rys. 1. Ocena wpływu przeciwutleniaczy na wartość współczynników kierunkowych wskaźników utleniania lipidów w przechowywanej kielbasie typu „polska surowa”

Conducted studies showed that the addition of antioxidants significantly inhibited losses of analyzed amino acids. The most objective parameter when comparing the rate of changes was time, in which the initial amounts of lysine and methionine decreased by 50% ( $T_{IC50}$ ). Results of analyses determining the potential application of BHT, rosemary extract and soy hydrolysate as substances contributing to a reduction of losses of available lysine and methionine in stored sausages, are presented in Table 1.

Half-life of lysine and methionine in the samples with added BHT was 1.8 times longer than in the control samples. The application of rosemary extract and soy hydrolysate made it possible to extend this time 1.4 and 1.2 times, respectively.

The phenomenon of blocking active protein groups by lipid oxidation products was studied by Pokorny and Davídek [19], who showed that reactions of protein crosslinking, amino acid oxidation and the transformation of their amino groups into imino groups are initiated first of all by hydroperoxides. Hexanal, similarly as other aldehydes, may initiate protein cross-linking and blocking and transformation of functional groups [12, 20, 25]. Aldehydes, reacting with the sulfhydryl group of cysteine, form mercaptal, while bonding with the amino group of lysine they form Schiff bases [19]. Kinetics of aldehyde reactions is much lower in relation to hydroperoxides, which reactions with proteins may be violent even at room temperature [14].

Table 1

Influence of antioxidants addition on half-life period of lysine and methionine in stored raw Polish sausage  
 Wpływ dodatku przeciwutleniaczy na czas połowicznego rozpadu lizyny i metioniny w przechowywanej kiełbasie typu „polska surowa”

Kind of addition	Half-life period $T_{IC50}$ (days)	
	LYSINE	METHIONINE
Without addition	42.9	42.5
BHT	75.8	75.0
Rosemary extract	59.6	59.1
Soy hydrolysate	49.5	49.8

The destructive effect of lipid oxidation products on contents of available forms of lysine and methionine in frozen pork meatballs was shown also by Korczak et al. [16] and Heś et al. [11]. Results of their studies indicated as well that the addition of antioxidants significantly reduces quantitative losses of available lysine and methionine in stored processed meat products.

### Conclusions

1. Antioxidants exhibited an inhibitory effect on the advancement of lipid autoxidation reactions in analyzed sausages.
2. The addition of antioxidants significantly reduced quantitative losses of available lysine and methionine. Natural antioxidants exhibited a lower capacity to reduce losses of nutritive value of protein than it was found for BHT.
3. The addition of antioxidants may affect the maintenance of a higher nutritive value of protein in stored raw sausages.

### References

- [1] Ansorena D., Astiasarán I.: Effect of storage and packaging on fatty acid composition and oxidation in dry fermented sausages made with added olive oil and antioxidants. *Meat Sci.*, 2004, 67, 237-244.
- [2] Ansorena D., Astiasarán I.: The use of linseed oil improves nutritional quality of the lipid fraction of dry-fermented sausages. *Food Chem.*, 2004, 87, 69-74.

- [3] Balasundram N., Sundram K., Samman S.: Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.*, 2006, 99, 191-203.
- [4] Barbut S., Josephson D.B., Maurer A.J.: Antioxidant properties of rosemary oleoresin in turkey sausage. *J. Food Sci.*, 1985, 50, 1356-1363.
- [5] Barlow S.M.: Toxicological aspects of antioxidants used as food additives. In: *Food Antioxidants* – ed. Hudson B.J.F., Elsevier, London 1990, pp. 253-307.
- [6] Branen A.L.: Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *JAOCS*, 1975, 52, 59-63.
- [7] Decker E.A., Xu Z.: Minimizing rancidity in muscle foods. *Food Technol.*, 1998, 52, 54-59.
- [8] Estévez M., Cava R.: Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: Contradictory effects in different types of frankfurters. *Meat Sci.*, 2006, 72, 348-355.
- [9] Georgantelis D., Ambrosiadis I., Katikou P.: Effect of rosemary extracts, chitosan and  $\alpha$ -tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. *Meat Sci.*, 2007, 76, 172-181.
- [10] Gray J.I., Gomaa E.A., Buckley D.J.: Oxidative quality and shelf life of meats. *Meat Sci.*, 1996, 43, 111-123.
- [11] Heś M., Korczak J., Gramza A.: Changes of lipid oxidation degrees and their influence on protein nutritive value of frozen meat products. *Pol. J. Food Nutr. Sci.*, 2007, 3, 323-328.
- [12] Hidalgo F.J., Zamora R.: Modification of bovine serum albumin structure following reaction with 4,5(E)-Epoxy-2(E)-heptenal. *Chem. Res. Toxicol.*, 2000, 13, 501-508.
- [13] Hoffman K.: Nutritional value of proteins and protein requirements of people with special reference to meat proteins. *Mitteilungsbl. Bundesanst. Fleischforsch.*, 1993, 32, 422-429.
- [14] Janitz W.: Interactions of meat fats and proteins with particular consideration of effect of oxidized fats on the nutrition value of proteins. *Roczniki Akademii Rolniczej w Poznaniu. Rozprawy Naukowe*, 1985, Zeszyt 147.
- [15] Korczak J.: Factors affecting antioxidant activity of soybean meal and caseine protein hydrolysates. *Roczniki Akademii Rolniczej w Poznaniu. Rozprawy Naukowe*, 1998, Zeszyt 281.
- [16] Korczak J., Heś M., Gramza A., Jędrusek-Golińska A.: Influence of fat oxidation on the stability of lysine and protein digestibility in frozen meat products. *EJPAU*, 2004, 7, 1-13.
- [17] Nassu R.T., Goncalves L.A.G.: Oxidative stability of fermented goat meat sausage with different levels of natural antioxidant. *Meat Sci.*, 2003, 63, 43-49.
- [18] Pokorný J.: Effect of lipid degradation on taste and odor of foods. *Nahrung*, 1990, 34, 887-897.
- [19] Pokorný J., Davídek J.: Influence in interactions of proteins with oxidized lipids on nutrition and sensory value of food. *Acta Aliment. Pol.*, 1979, 5, 87-95.
- [20] Pokorný J., Kołakowska A.: Lipid – protein and lipid – saccharide interactions. In: *Chemical and functional properties of food lipids* – eds. Sikorski Z.E., A. Kołakowska A., CRC Press LLC, 2003, pp. 345-362.
- [21] Pyrcz J., Heś M., Kowalski R.: Einfluss ausgewählter Antioxidantien auf die Qualität von Rohwurst. *Fleischwirtschaft*, 2007, 9, 115-118.
- [22] Ressurrecion A.V.A., Reynolds A.E.: Evaluation of natural antioxidants in frankfurters containing chicken and pork. *J. Food Sci.*, 1990, 55, 629-631.
- [23] Wu S.Y., Brewer M.S.: Soy protein isolate antioxidant effect on lipid peroxidation of ground beef and microsomal lipids. *J. Food Sci.*, 1994, 59, 702-706.
- [24] Yanishlieva-Maslarova N.V., Heinonen I.M.: Sources of natural antioxidants: vegetables, fruits, herbs, spices and teas. In: *Antioxidants in food* – eds. Pokorný J., Yanishlieva N., Gordon M., CRC Press, Boca Raton FL, 2001, pp. 210-263.
- [25] Zamora R., Alaiz M., Hidalgo F. J.: Contribution of pyrrole formation and polymerization to the nonenzymatic browning produced by amino-carbonyl reactions. *J. Agric. Food Chem.*, 2000, 48, 3152-3158.

**WPLYW STABILIZOWANIA LIPIDÓW NA ZACHOWANIE DOSTĘPNEJ LIZYNY I METIONINY W PRZECHOWYWANEJ KIELBASIE TYPU „POLSKA SUROWA”****Streszczenie**

W pracy określono wpływ dodatku przeciwutleniaczy na stabilność dostępnej lizyny i metioniny w kielbasie typu „polska surowa”, jako istotnych czynników determinujących wartość żywieniową produktów mięsnych.

Materiałem badawczym były cztery warianty wędlin doświadczalnych. Jeden wariant stanowiła próba bez dodatku przeciwutleniacza (próba kontrolna), do pozostałych dodano odpowiednio: BHT (0,02%), etanolowy ekstrakt rozmarynu (0,05%) i hydrolizat sojowy (2%). Okresowo oznaczano liczbę nadtlenkową (PV), liczbę anizydynową (AV), zawartość substancji dających reakcję barwną z kwasem 2-tiobarbiturowym (TBARS), indeks Totox oraz zawartość dostępnej lizyny i metioniny.

W celu określenia aktywności przeciwutleniającej badanych dodatków wyznaczono współczynniki kierunkowe (Wsp.a/24 h), stanowiące kąt nachylenia krzywej regresji wykreślonej z punktów pomiarowych danego wskaźnika w czasie. Wpływ przeciwutleniaczy na stabilność dostępnej lizyny i metioniny analizowano na podstawie wyznaczonego czasu połowicznego rozpadu aminokwasów ( $T_{IC50}$ ).

Przeciwutleniacze wykazywały hamujący wpływ na postęp reakcji autooksydacji lipidów w badanych wędlinach oraz w istotny sposób zmniejszały straty ilościowe dostępnej lizyny i metioniny. Przeciwutleniacze naturalne wykazywały mniejszą zdolność obniżania strat wartości odżywczej białka niż BHT.

**Słowa kluczowe:** wędliny surowe, utlenianie tłuszczu, przeciwutleniacze, lizyna, metionina 