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CHANGES OF LIPID OXIDATION DEGREES AND THEIR INFLUENCE ON PROTEIN NUTRITIVE VALUE OF FROZEN MEAT PRODUCTS*

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Key words: meat, lipid oxidation, antioxidants, nutritive value, lysine, methionine, protein digestibility

The aim of the study was to determine the influence of oxidation degree on available lysine and methionine content and protein digestibility in meat products with added antioxidants (rosemary extracts, green tea extracts, BHT) stored under frozen conditions.

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

Results showed an increase in lipid oxidation during storage. The highest values for the applied measurement of oxidation were observed in the control sample without antioxidants. The added antioxidants slowed down lipid oxidation to a significant extent.

In control samples, the content of available lysine and methionine was reduced by 53% and 75% respectively, whereas protein digestibility by 12.5% at the end of storage. Inhibition of lipid oxidation products formation by the addition of antioxidants, limited significantly lysine and methionine losses and reduction of protein digestibility.

The application of antioxidants extended stability and protected biological protein value of the meat products examined.

INTRODUCTION

Reactions of oxidative character occurring in meat and meat products are one of the major causes of deterioration in their quality. They are responsible for degradation of colour, flavour and texture, as well as losses of their nutritive value [Pokorný, 1990; Gray *et al.,* 1996]. A deterioration of nutritive value may be a consequence of interactions between very reactive products of lipid oxidation with protein, some of the most valuable components of our diet and those which need to be especially carefully protected in all processes connected with technological processing. Nutritive value of meat protein is determined by the quantitative and qualitative composition of amino acids and susceptibility of protein to hydrolysis by digestive enzymes [Hoffmann, 1993]. A deterioration of digestibility and availability of amino acids results from the formation of crosslinking bonds in the protein-lipid complexes and reactions of functional groups of amino acids with products of lipid oxidation. This pertains especially to amino, sulfhydryl and hydroxyl groups [Pokorný & Davídek, 1979]. In nutrition an essential role is played by losses of lysine and amino acids containing sulphur, since they are exogenous amino acids and at the same time they reduce the nutritive value of protein in most products.

Reduction or inhibition of negative oxidative changes in lipids of meat and meat products may be the effect of the addition of antioxidants [Decker & Xu, 1998]. The application of synthetic compounds, *i.e.* BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), is limited in many countries due to results of toxicological tests and the attitude of consumer organizations [Branen, 1975; Barlow, 1990; Nunn *et al.,* 1991; Verhagen *et al.,* 1990]. In relation to this, there is a problem of searching for alternative solutions of lipid stabilization. One of them may be the utilization of effective and cheap natural antioxidants. The most important group of substances with antioxidative properties is represented by polyphenolic compounds belonging to secondary metabolites common in plant materials. A rich source of these substances are vegetables, fruit, seeds of various plants, some cereals, as well as wines, tea, coffee, fruit juices and many spices [Yanishlieva-Maslarova & Heinonen, 2001; Moure *et al.,* 2001; Basaga *et al*., 1997; Minussi *et al*., 2003]. These natural compounds preventing oxidation may at the same time modify the nutritive value of food [Korczak *et al*., 2004]. This makes it possible to provide consumers with safe food products exhibiting extended shelf-life and improved nutritive value.

The aim of the study was to evaluate the antioxidant properties of natural substances in frozen meat products, and their influence on protein nutritional value by retardation of lipid oxidation products-protein interactions.

MATERIALS AND METHODS

The studied material were products made from minced pork meat with addition of ethanol extract of rosemary (0.05%), ethanol extract of green tea (0.05%) and BHT in quantity of 0.02% (in relation to meat). Dried rosemary

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leaves (*Rosmarinus off*. L.) were of Polish origin (Polish Herbs "Pharma" Poznań). An extract was prepared by mixing 100 g of dried material with 1 L of 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 combined, and the respective solvent was evaporated. Tea extracts were prepared by 24-h maceration of tea leaves (100 g) with 250 mL of 95% ethanol, under ambient conditions; then they were filtered (procedure was repeated three times). The collected extracts were filtered and centrifuged. Ethanol was evaporated on a rotary evaporator. BHT (butylated hydroxytoluene) was purchased from Merck (Germany).

Pork (shoulder blade) bought from an anonymous producer was used as experimental material. Meat material was ground using a meat grinder with mesh size of 2 mm, next all the other ingredients were introduced and thoroughly mixed. In order to maintain identical conditions determining the kinetics of the thermal process, round samples were formed with an identical weight (50±1 g).

Meat product samples were treated thermally with steam at 105°C for 30 min in a convection oven, and next frozen and stored in polyethylene bags for 180 days.

Water content was determined with the drying method at 105°C. The content of fat was determined with ether extraction method in a Soxtec-HT6 apparatus, while the protein content was determined using the Kjeldahl method and a Kjeltec 2200 apparatus from Tecator (Sweden). The proximate composition of products made from ground meat is typical of this kind of products (17.40% – protein, 7.08% – fat, 63.94% – water).

Oxidative changes of lipid were analysed periodically (each 60 days) based on measurement of peroxide value with the iodometric method [PN-ISO 3960:1996], anisidine value [PN-EN ISO 6885:2001], TBARS content with the distillation method with thiobarbituric acid [Pikul *et al*., 1989]. Totox coefficient was calculated, as well [PN-93/A--86926]. The influence of oxidized lipid onto product protein was characterised by changes in available lysine and methionine contents as well as the by measurement of protein digestibility under *in vitro* conditions as significant factors determining the nutritional value of meat products. Available lysine was determined by the method of Hall *et al*. [1973]. The meat sample was ground to a very fine powder, which was suspended in a solution of agar and the suspension was mixed with sodium hydrogen carbonate solution. A solution of trinitrobenzenesulphonic acid was added, which reacts with the free epsilon-amino group present in the lysine combined within the intact protein. ε-Trinitrophenyllysine (ε-TNP-lysine) was then released by hydrolysis of the reaction mixture with hydrochloric acid and determined spectrophotometrically. Interfering substances, such as free picric acid, were removed by extraction into diethyl ether and the absorbance of the remaining yellow solution of ε-TNP-lysine was measured at 415 nm. Pure DL-lysine monohydrochloride was used as a standard. The available methionine contents of proteins were determined by chemical methods after preliminary enzymic hydrolysis [Pieniążek *et al*., 1975]. The proteins were hydrolysed with pancreatopeptidase. After enzymic hydrolysis of the protein, the hydrolysate was reacted with sodium nitroprusside as described by McCarthy & Sullivan [1941]. The extinction at 520 nm of the coloured complex formed was measured using a spectrophotometer. Pure DL-methionine was used as a standard. Determination of protein *in vitro* digestibility was carried out with method of Sheffner *et al*. [1956] with the use of pepsin and tripsin enzymes.

Statistical determination results were subject to the analysis of variance and Tuckey's test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The results obtained distinctly showed the inhibitory effect of antioxidants on the course of self-oxidation of lipids in the processed products analysed. In the final period of storage, all the monitored indices of lipid oxidation increased more slowly in products with their addition. However, the effectiveness of action of individual antioxidants varied considerably. The strongest antioxidative activity at the inhibition of primary lipid oxidation products (PV) was found for BHT and ethanol rosemary extract (Figure 1). The addition of green tea extract to meatballs did not initially inhibit lipid oxidation processes; however, it also had an effect on the reduction in peroxide contents in the final stage of storage.

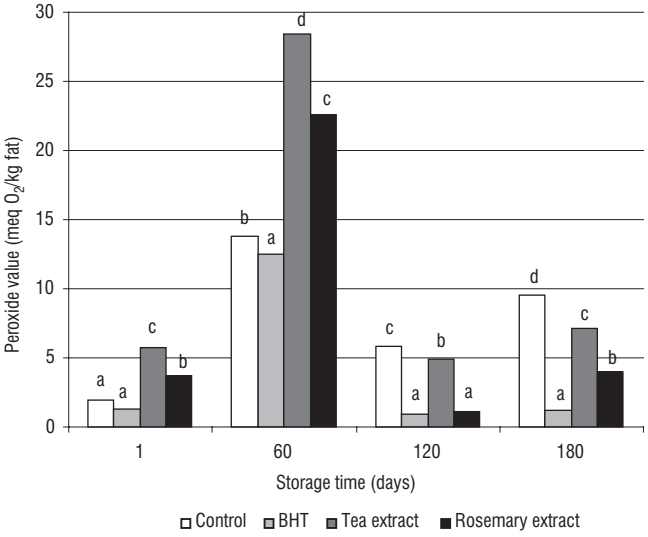

FIGURE 1. Change of peroxide value in frozen meat products made from minced pork meat with addition of antioxidants.
a, b, c, d – mean values with different letters differ statistically between bars ($p<0.05$)

Progressing lipid oxidation processes contributed also to an increase in the amounts of secondary oxidation products, as it was manifested by an increase in the TBARS content (Figure 2) and AV value (Figure 3). High activity in the reduction of TBARS formation was found for natural antioxidants. Rosemary extract showed a stronger protective action than green tea extract, but only towards the end of storage time. BHT showed lower effectiveness than both natural antioxidants; however, in comparison to control samples it also considerably limited the accumulation of TBARS. Taking into consideration the AV value, samples with the addition of BHT were of good quality until the end of the experiment. High activity was also reported for rosemary extract (Figure 3).

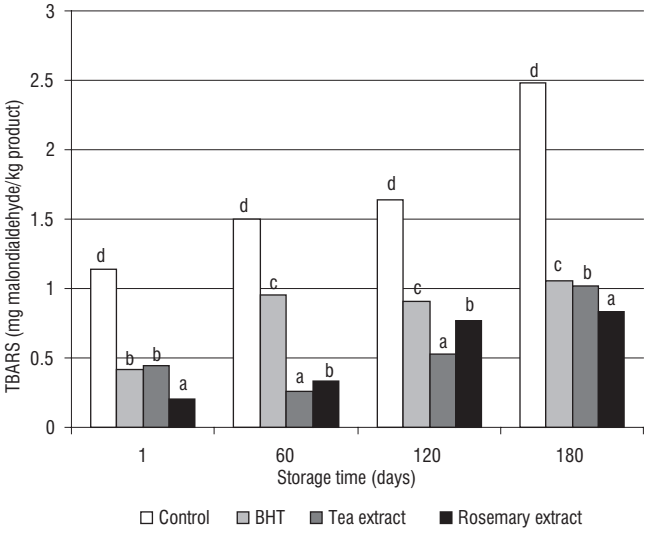

FIGURE 2. Change of content of TBARS in frozen meat products made from minced pork meat with addition of antioxidants.
a, b, c, d – mean values with different letters differ statistically between bars ($p<0.05$)

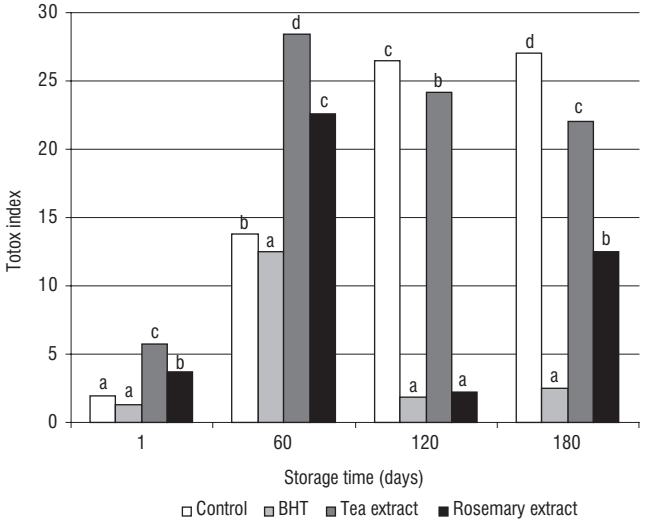

FIGURE 4. Change of Totox index in frozen meat products made from minced pork meat with addition of antioxidants.
a, b, c, d – mean values with different letters differ statistically between bars ($p<0.05$)

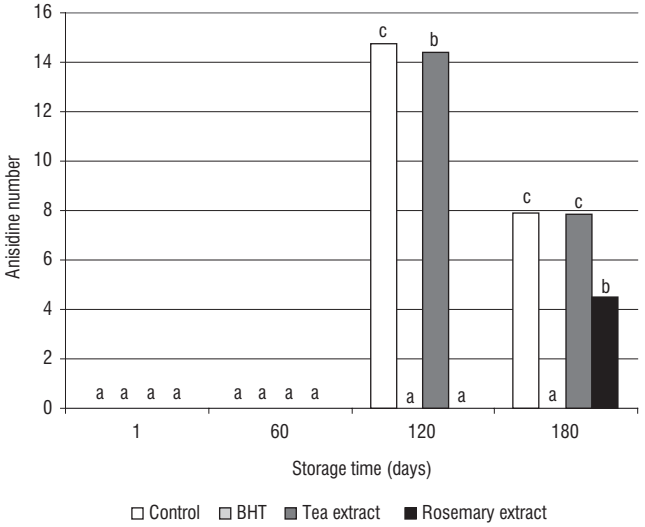

FIGURE 3. Change of anisidine number in frozen meat products made from minced pork meat with addition of antioxidants.
a, b, c – mean values with different letters differ statistically between bars ($p<0.05$)

The calculated value of the Totox index, after preparation and after 60-day storage was analogous to the determined PV value, since AV was assayed to be zero. High values of this index in samples with added natural antioxidants at this time of storage indicate a lack of their antioxidative activity. Inhibition of oxidation processes by natural extracts was observed only after 120-day sample storage, while BHT exhibited high activity throughout the whole storage time (Figure 4).

The antioxidant properties of the tea and spices are related to their phenolic compounds. The antioxidant activity of rosemary (*Rosmarinus officinalis*) has been known for many years, and their active compounds have been identified. The main active substance is carnosol; and the active diphenolic diterpenes epirosmanol and isorosmanol have also been isolated from rosemary leaves. When added to lard, the antioxidant activity of isorosmanol is comparable with the activities of the synthetic butylated phenolic antioxidants hydroxyanisole (BHA) and hydroxytoluene (BHT) [Pokorný, 1991]. Other compounds are rosmarinic acid and carnosic acid. These compounds are phenolic-type antioxidants with very good antioxidant properties [Löliger, 1983].

In tea leaves (*Camelia sinensis*) three basic polyphenol groups can be distinguished: catechin, theaflavines and thearubigens. Both green and black tea contains a similar quantity of flavonoids, differing in respect of their chemical structures. Green tea is characterised by a higher content of simple flavonoids – catechins which become transformed to more complex compounds (theaflavines and thearubigens) during the fermentation of tea leaves [Gramza *et al*., 2005].

Green tea has been acclaimed for its antioxidant properties, attributed to the presence of tea catechins (TC) including epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). It was found that tea leaves are the only food product containing EGCG, an active compound with eight free groups (OH), which determine its high antioxidant activity [Wilska-Jeszka, 1999].

The inhibitory effect of natural antioxidants on the self-oxidation rate of meat lipids was shown by the other authors. Strong antioxidative properties of rosemary were reported by Karpińska *et al*. [2000], who investigated the effect of this additive on lipid oxidation processes in pork meatballs under frozen storage conditions. That author determined the content of malonic aldehyde in the control sample and in samples with a 1% or 1.5% addition of rosemary extract. After 3-month storage of meatballs, the content of malonic aldehyde in samples with the addition of the antioxidant deceased by 28% and 40%, respectively. A similar effect of the rosemary and other spices addition was observed by Korczak *et al*. [2004, 1988]. In turn, Tang *et al*. [2001] and Mitsumoto *et al*. [2005] showed a high antioxidative activity of catechins extracted from green tea in beef and chicken meat.

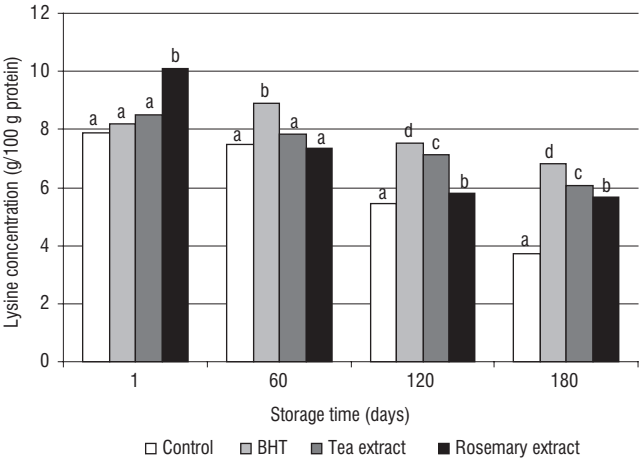

FIGURE 5. Change of available lysine content in frozen meat products made from minced pork meat kept with addition of antioxidants.
a, b, c, d – mean values with different letters differ statistically between bars ($p<0.05$)

The effect of lipid oxidation rate on protein in the processed products analysed was determined by assaying the content of available lysine and methionine and *in vitro* protein digestibility, as significant factors determining the nutritive value of meat products.

During storage of meat samples, contents of the analysed amino acids were found to decrease. Contents of lysine and methionine in the control sample decreased by 53% and 75%, respectively, in comparison to the initial value (Figures 5 and 6). A decrease in the content of available lysine may be connected with changes occurring in the lipid fraction, leading to blocking ε-amino groups of proteins. In turn, a decrease in methionine content may be caused by oxidation reactions which lead to the formation of methionine sulfoxide or even sulfone [Davídek *et al*., 1983, Janitz, 1985]. The addition of antioxidants significantly limited the decrease in amino acid contents. In samples with BHT, lysine losses reached 17%, whereas for those with rosemary and tea extracts they amounted to approx. 29% (Figure 5). The antioxidants applied showed also a protective activity towards methionine, but lower than lysine. Amino acid contents in samples with the addition of rosemary and tea extracts decreased by 67% and 73%, respectively, whereas in those with BHT - by 68% (Figure 6).

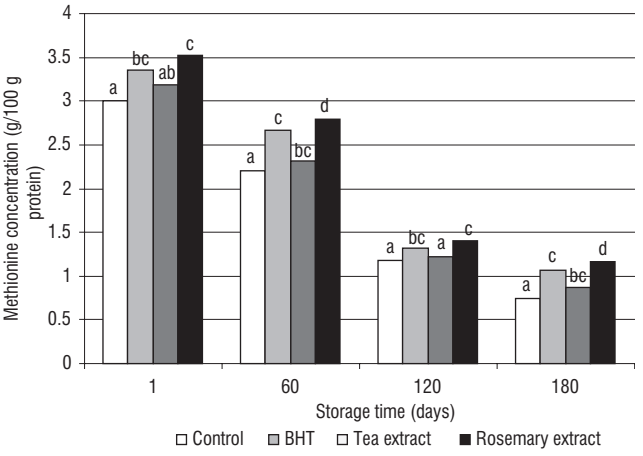

FIGURE 6. Change of available methionine content in frozen meat products made from minced pork meat kept with addition of antioxidants.
a, b, c, d – mean values with different letters differ statistically between bars ($p<0.05$)

Storage of samples with no addition of antioxidants decreased protein digestibility by 12.5% in comparison to samples directly after preparation. Antioxidants reduced the decrease in protein digestibility of meatballs. It was approx. 7% in the sample with BHT and 10% and 5%, respectively, in samples with the addition of rosemary and green tea extracts (Figure 7).

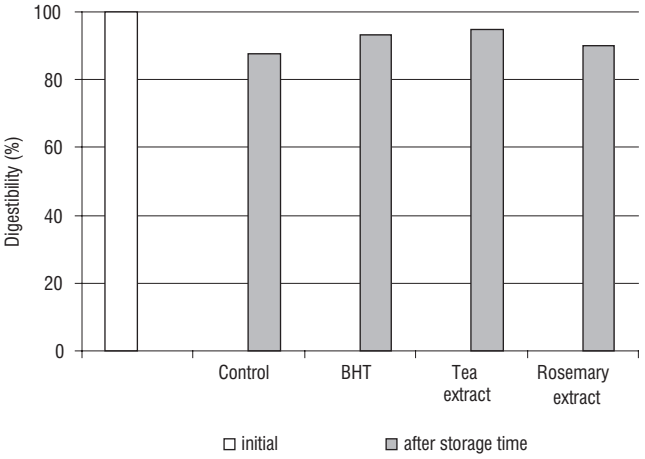

FIGURE 7. Influence of antioxidant addition onto reduction of protein digestibility in frozen meat products made from minced pork meat (initial digestibility of sample was expressed as 100%).

The phenomenon of blocking active protein groups by lipid oxidation products was studied by Pokorný & Davídek [1979], 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. In turn, hexanal, similarly as other aldehydes, may initiate protein crosslinking and blocking and transformation of functional groups. Aldehydes, reacting with the sulfydryl group of cysteine, form thioacetale, and by bonding with the amino group of lysine they form Schiff's bases [Pokorný & Davídek, 1979]. Kinetics of aldehyde reactions is much smaller in relation to hydroperoxides, whose reactions with protein may occur rapidly even at room temperature [Janitz, 1985].

The destructive effect of lipid oxidation products on contents of available forms of lysine in frozen pork meatballs was also shown by Korczak *et al*. [2004]. Results of their studies indicated as well that the addition of antioxidants significantly reduced quantitative losses of available lysine in stored processed meat products.

CONCLUSIONS

1. Changes of available forms of lysine and methionine contens in frozen pork meatballs were accompanied by lipid oxidation processes.

2. The addition of antioxidants significantly inhibited the decrease in protein digestibility of meat products and limited quantitative losses of lysine and methionine.

3. The application of natural antioxidants enabled extending the shelf-life and maintaining the biological value of protein in meat products.

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ZMIANY STOPNIA UTLENIENIA LIPIDÓW I ICH WPŁYW NA WARTOŚĆ ODŻYWCZĄ BIAŁKA MROŻONYCH PRODUKTÓW MIĘSNYCH

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Celem pracy było określenie wpływu zmian oksydacyjnych w lipidach na dostępność lizyny i metioniny oraz strawność białka w produktach mięsnych przechowywanych z dodatkiem przeciwutleniaczy (ekstrakt rozmarynu, ekstrakt zielonej herbaty, BHT) w warunkach zamrażalniczych.

Okresowo badano stopień utlenienia tłuszczu (liczba nadtlenkowa, liczba anizydynowa, TBARS, wskaźnik Totox), zawartość dostępnej lizyny i metioniny oraz strawność białka.

Rezultaty przeprowadzonych badań wykazały znaczny wzrost stopnia utlenienia tłuszczu mięsa w czasie przechowywania. Największe wartości wskaźników oksydacji zaobserwowano w próbie kontrolnej (rys. 1–4). Dodane przeciwutleniacze wyraźnie hamowały proces utleniania tłuszczu.

W czasie przechowywania prób mięsa bez dodatku przeciwutleniaczy stwierdzono znaczne obniżenie zawartości dostępnej lizyny i metioniny oraz strawności białka (rys. 5–7). Zawartość aminokwasów obniżyła się odpowiednio o 53% i 75%, a strawność białka o 12,5% w stosunku do wartości początkowej. Ograniczając powstawanie produktów utlenienia tłuszczu dodane przeciwutleniacze zmniejszały straty ilościowe dostępnej lizyny i metioniny oraz hamowały obniżenie strawności białka w przechowywanych produktach mięsnych (rys. 5–7).

Zastosowanie przeciwutleniaczy pozwoliło przedłużyć trwałość i zachować wartość biologiczną białka wyrobów mięsnych.