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INFLUENCE OF FAT OXIDATION ON THE STABILITY OF LYSINE AND PROTEIN DIGESTIBILITY IN FROZEN MEAT PRODUCTS

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ABSTRACT

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

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

Results showed increase in lipid oxidation during storage. The highest values for applied measurement of oxidation were observed in the control sample without antioxidants. Added antioxidants markedly slowed down the fat oxidation.

In control samples the content of available lysine was reduced by 53%, while protein digestibility by 48% at the end of storage. Limitation of lipid oxidation products formation by addition of antioxidants, limited significantly lysine losses and reduction of protein digestibility.

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

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

INTRODUCTION

Lipids and proteins undergo chemical reactions, which investigations allow to qualify their changes under different conditions of storage and processing. The recognition of scale of their mutual influences becomes necessity for quality improvement of processed food and results from increase of nutritive and wholesome requirements formulated for final products.

Oxidative changes of lipids in food products are the main cause of lowering their quality reflected in worsening sensoric properties, interactions with other food components as well as unfavorable influence onto human organism.

Oxidized lipids affect not only are the main cause of rancidity formation in food products but they also interact with other food components and change nutritional value. This concern particularly where nutritional their nutritive value can be reduced. Protein represents one of the most valuable compounds of our diet and it should be protected during processing. The maintenance of protein nutritive value is particularly important during production of meat products, because meat proteins are balanced containing all essential amino acids [19]. The nutritive value of meat proteins is determined not only by quantitative and qualitative composition of amino acids, but also by their availability to digestive tract proteolytic enzymes. Reduction of digestibility as well as limitation of the amount and the degree of amino acid availability is affected by the formation of amino acid bonds with lipid oxidation products. Fat oxidation products react first of all with functional groups of proteins and amino acids, particularly with amine-, sulphydryle- and hydroxyl groups [44]. In the physiology of nutrition of principle importance are lysine and sulphur contained amino acids, which are essential amino acids and they are limiting the nutritive value of protein in majority of food products [14].

Lipid hydroperoxides attack sulphur-containing amino acid residues, e.g. cysteine or methionine. The main reaction product is a sulphoxide or a thiosulphinate in case of mathionine or cysteine, respectively, or even a sulphone [44].

Carbonylic lipid oxidation products, particularly aldehydes and vicinal hydroxyketones, can react with amine groups of lysine with formation of Schiff bases. The Schiff bases react with another aldehyde molecule forming aldolization products. Conjugated double bond systems produced by repeated aldolization are the cause of brown coloration of macromolecular reaction products. The browning is often accompanied by the formation of fluorescing substances [27]. Pyrroles formed in reactions of oxidized lipids with proteins are important precursors of both brown and fluorescing compounds [54]. The resulting products of oxidized lipids with proteins are yellow, red, or brown in color [42]. The color intensity increases rapidly with increasing unsaturation of the original lipid fraction, and correlates with loss of primary amine groups [43]. The character of brown products is similar to that of melanoidins from Maillard reactions [18]. Because fish lipids are highly unsaturated, their interaction products are dark brown. Melanoidins partially lose nitrogen by retro-aldolization, and the remaining pigments become increasingly soluble in the lipidic phase [45]. They form stains on the surface of white fish muscle, which are objectionable from the standpoint of the appearance of frozen fish. Similar problems sometimes occur with stored poultry. On storage of apples, scald-like stains are produced by reactions of membrane lipids with amino acids [4].

The application of antioxidants offers an effective prevention method of undesirable oxidative lipid changes. Limited possibility for use of synthetic antioxidants to food resulting from their toxic character [2,11,12,13,15,53], caused the increase of interest in natural compounds inhibiting oxidative reactions [3,9,34,35,46,50,52]. The general interest is connected today with the antioxidative substances in food (or in another biological materials) reducing not only amount of lipid oxidation products but also active in human organism and able to influence onto nutritive value of products.

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

MATERIALS AND METHODS

The studied material were products made from minced pork meat with addition of ethanol extract of rosemary (0.05%), soy protein hydrolyzate (2%) and BHT in quantity of 0.02% (in relation to meat). Soy protein aciditial hydrolyzate, 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 100 g 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 4 times. The filtrates were combined, and the respective solvent was evaporated. BHT (3,5-di-tert-butyl-4-hydroxytoluene) was purchased from Merck (Germany).

Meat products samples were treated thermally with steam at 105°C by 30 min in convection oven, and next frozen and stored in polyethylene bags for 14 weeks.

Water content was determined by drying method at 105°C. The content of fat was determined by ether extraction method in the Soxtec-HT6 apparatus, while the protein content was determined using the Kjeldahl method and Kjeltec 2200 apparatus from Tecator (Sweden). Oxidative changes of fat were analyzed periodically (each 7 weeks) based on measurement of peroxide value by iodometric method (PN-ISO 3960:1996), anisidine value (PN-EN ISO 6885:2001) TBARS content by distillation method with thiobarbituric acid [37]. Totox coefficient was calculated, too (PN-93/A-86926). The influence of oxidized fat onto product protein was characterized by changes of available lysine content as well as the measurement of protein digestibility under *in vitro* conditions as significant factors determining nutritional value of meat products. Available lysine was determined by the method of Hall et al. [16]. The meat sample was ground to a very fine power, which was suspended in a solution of agar and the suspension 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. Determination of protein in vitro digestibility was obtained with method of Laser-Reuterswärd [26] with the use of pepsin and pancreatin enzymes.

The results of the experiment were analyzed statistically, calculating the basic statistical measures (x, SD). Statistical analysis included qualification of lipid oxidation products influence on nutritional value determinants by a assignation of rectilinear and curvilinear regression, using a computer program *STATISTICA^{TM}PL 6.0* (StatSoft).

RESULTS AND DISCUSSION

Basic composition of studied products made from minced meat was given on Table 1.

Table 1. Chemical composition of products made from minced meat

Component	Type of additive			
	Control x ± SD	BHT x± SD	Soy hydrolyzate x ± SD	Rosemary extract x ± SD
Water [%]	59.10 ± 1.32	59.40 ± 1.21	60.30 ± 1.72	59.24 ± 1.79
Fat [%]	11.64 ± 0.41	11.81 ± 0.19	12.67 ± 0.28	11.97 ± 0.50
Protein [%]	23.10 ± 0.22	22.96 ± 1.42	23.98 ± 1.82	23.30 ± 0.42

x – mean value, SD – standard deviation

Changes of fat oxidation determinants (PV, TBARS, AV, Totox) indicate considerable increase of fat oxidation degree in meat products during storage (Fig. 1-4). Final period of storage showed that PV and AV values in a control sample was properly 2.9 and 3.9 times higher than in fresh meat (Fig. 1 and 2). As the time of storage was elongated the TBARS contents raised and it tripled in 98th day of control samples storage (Fig. 3). The oxidation process advancing of samples without additions showed high (>10) Totox value, which was noticed in a first day of storage (Fig. 4).

Figure 1. Change of peroxides content in frozen meat products made from minced pork meat with addition of antioxidants

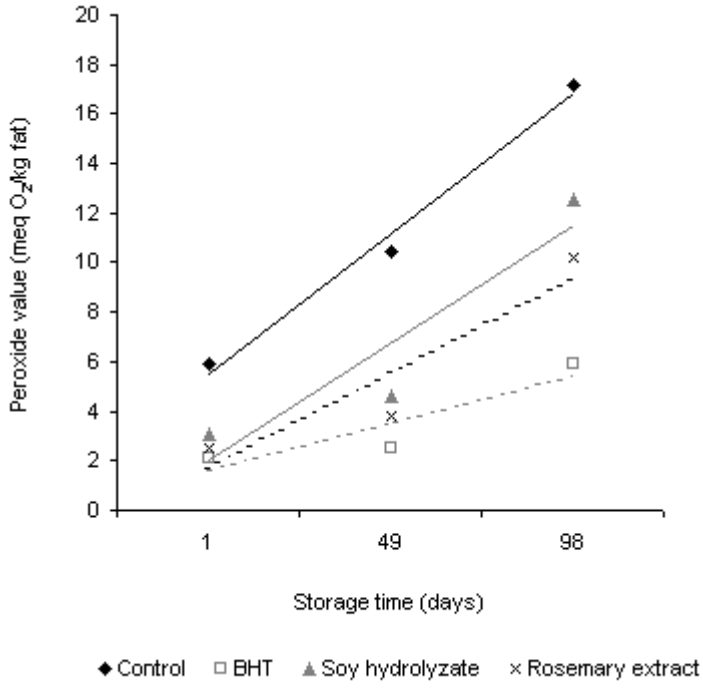


Figure 2. Change of anisidine number in frozen meat products made from minced pork meat with addition of antioxidants

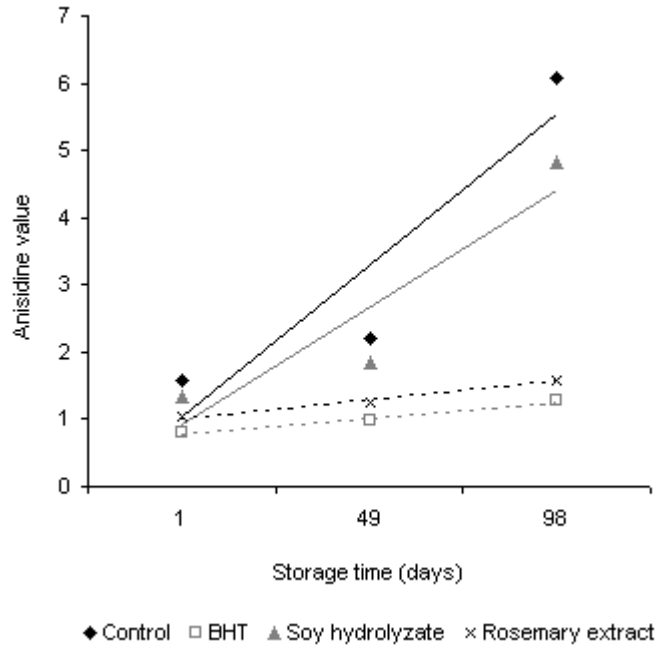


Figure 3. Change of content of TBARS in frozen meat products made from minced pork meat with addition of antioxidants

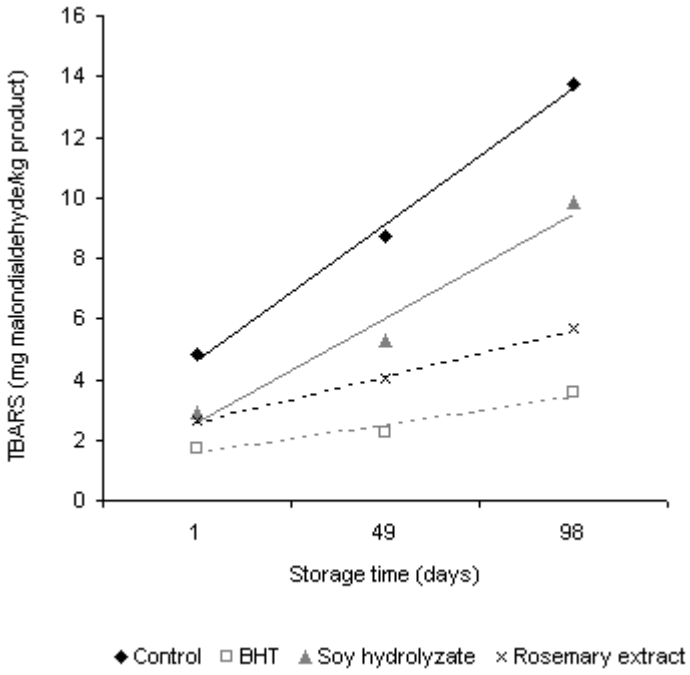
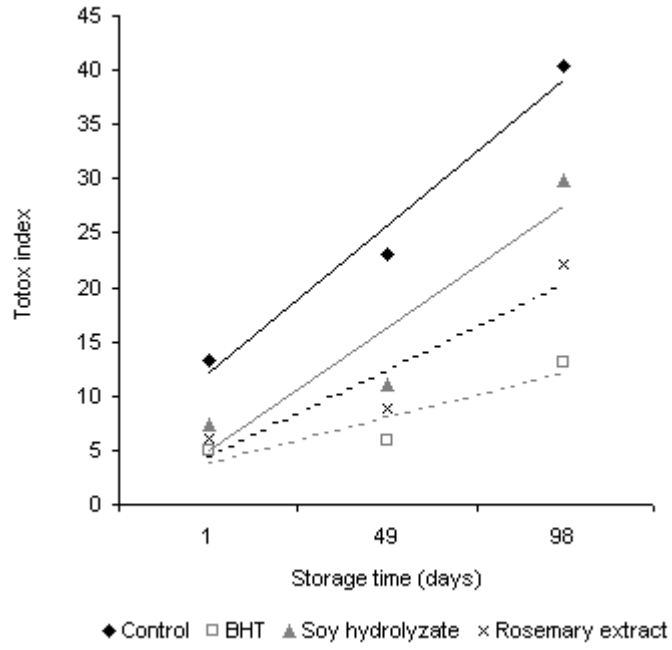
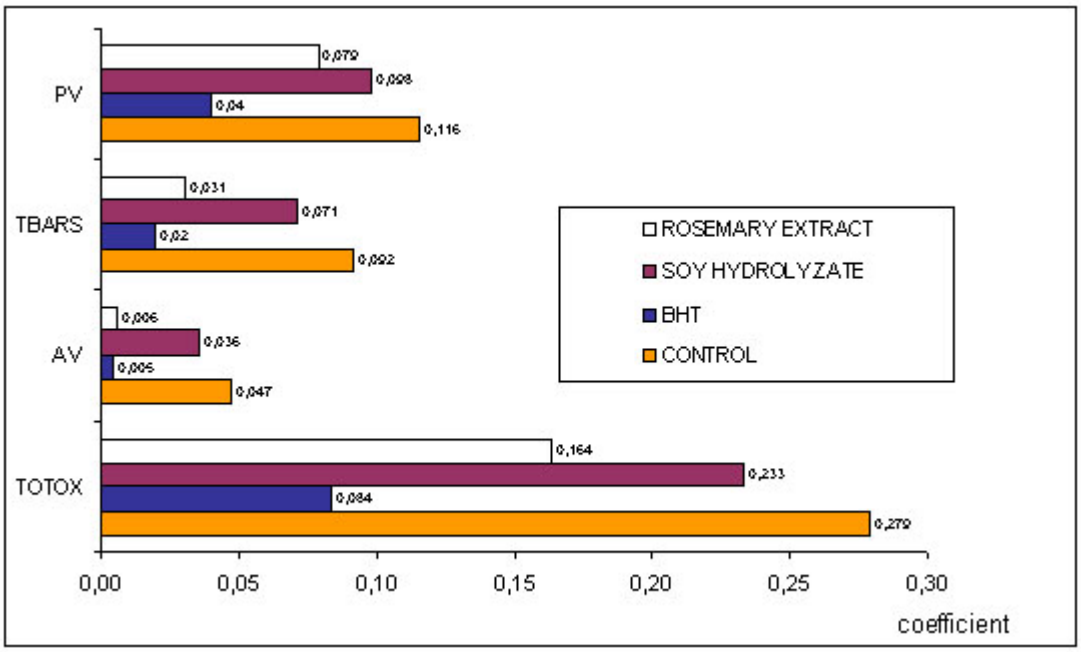


Figure 4. Change of Totox coefficient in frozen meat products made from minced pork meat with addition of antioxidants



Results unequivocally showed inhibitive antioxidants influence on lipids autooxidation progress in examined preserves. Antioxidants added to the samples clearly slowed down process of fat oxidation. Individual antioxidants demonstrated distinctly different effectiveness. In aim of determination of its antioxidant activity the directional coefficients were determined (coefficient) presenting a slope of a straight regression line drawn from the measurement points of a determinant in time. Figure 5 shows the statistical analysis results of antioxidants influence on straight lines inclination in stored minced pork meat.

Figure 5. Statistical analysis results of antioxidants influence on straight lines inclination in stored minced pork meat



The lowest total peroxides incrimination, amounted merely 0.040 per storage day of samples with BHT addition, but in control samples it was 2.9 times higher. Soy protein hydrolyzate and rosemary extract showed lower activity than BHT, but compared to control sample the rise of peroxides content was respectively 1.2 and 1.5 times lower. Similar results obtained during the estimation of lipids oxidation susceptibility expressed as TBARS value. It was stated that BHT addition caused smallest incrementation of this value, unlike in a control sample where it was 4.6 times higher. In samples with soy protein hydrolyzate and rosemary extract addition in comparison to control sample, 1.3 and 3.0 time rise of TBARS value respectively was defined. Also incrementation of AV and Totox value was significantly lower in samples with BHT than in others. Soy protein hydrolyzate, which antioxidant properties are responsible to the presence of amino acids, peptides and Maillard reaction products [1,5,6,7,25,51,55] showed weaker protective influence than rosemary. Results of published work indicate, that rosemary antioxidative properties mainly depends on carnosol, carnosic acid, and rosmanol [8,17,49]. High antioxidative activity of rosemary extract was indicated by Korczak et al. [24], where among ten herbal seasoning from Labiatae family, rosemary showed the strongest antioxidative properties. Despite, that above-mentioned researches were model, they suggest that rosemary can act similar manner in meat preserves. Addition of industrial rosemary extract in poultry meat, mechanically recovered in amount 0.01% and 0.05%, relatively to fat contents, inhibited oxidation processes during the storage period in 3°C [38]. Research over antioxidative influence on lipids in frozen minced pork meat was carried by Karpińska et al. [23]. The content of malonaldehyde in control sample and rosemary extract added in amount of 1% and 1.5%. Decrease of malonaldehyde contents was noted down after three months of storage, 28% in sample with 1% and 40% in sample with 1.5% addition of rosemary extract. These results are approximated for results introduced in the present work, where samples with rosemary extract added had lower lipid oxidation products concentration, also malonaldehyde.

Lipids stability in pork meat is also related with tocopherol contents. Antioxidative efficiency of tocopherols action is rising in a presence of other natural or synthetic antioxidants, what was proved in stored restructurised pork and beef meat [38].

All antioxidants used in this study demonstrated inhibiting influence on rate of fat oxidation corresponding with literature data reported by different authors [10,28,29,30,31,32,33, 36,47,48].

During storage of meat products manufactured without addition of antioxidants considerable lower content of available lysine and protein digestibility was found. Content of amino acid decreased about 53% (Fig. 6), while the protein digestibility about 48% (Fig. 7) in comparison to the initial value.

Figure 6. Change of available lysine content in frozen meat products made from minced pork meat kept with addition of antioxidants

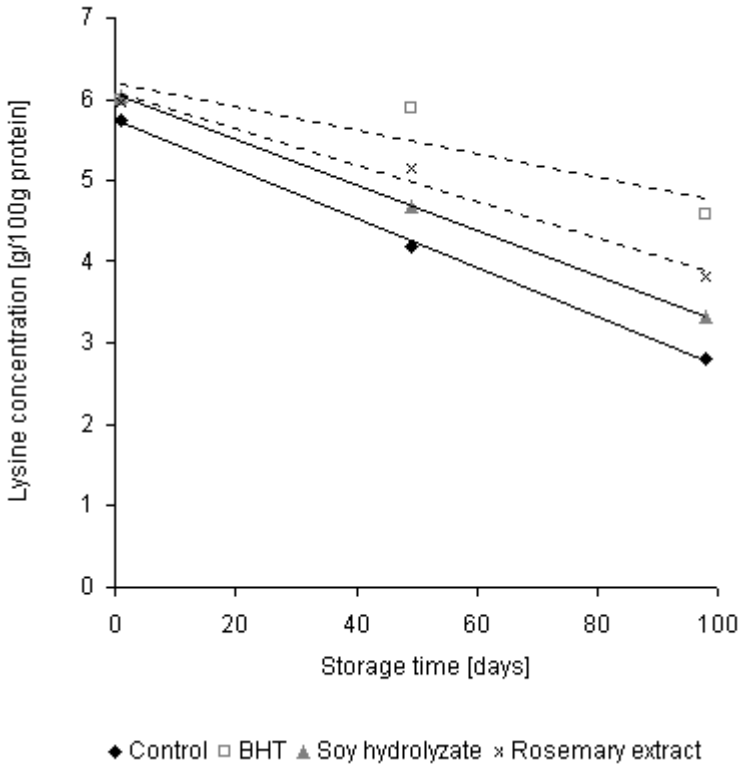
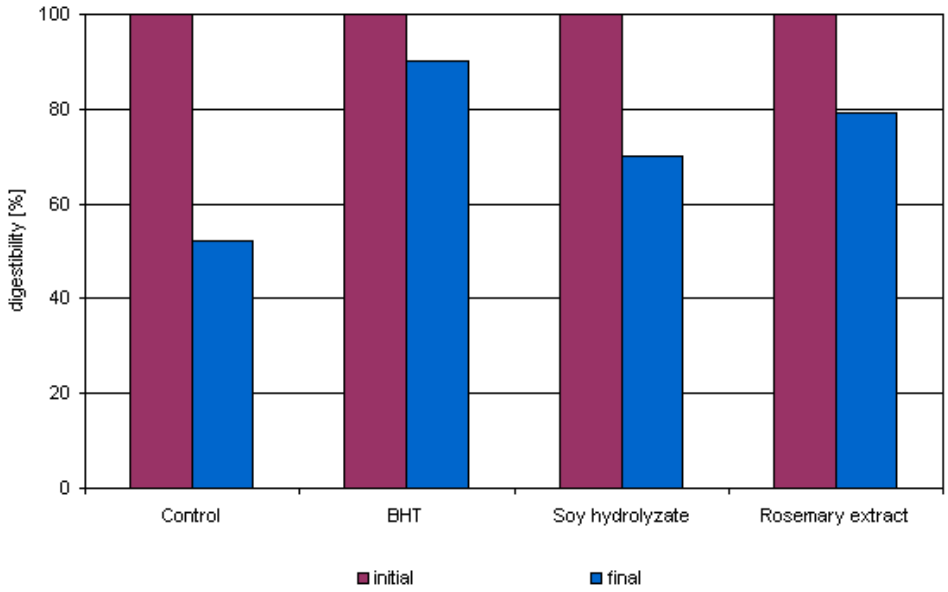


Figure 7. Influence of antioxidant addition onto reduction of protein digestibility in frozen meat products made from minced pork meat after 14 weeks of storage (initial digestibility of sample was expressed as 100%)



Limitation of lipid oxidation products formation with addition of antioxidants, reduced lysine losses significantly. In samples with BHT they carried out 23%, in samples with rosemary extract – 36%, and in samples with soy protein hydrolyzate it was 44% (Fig. 6).

Lysine half-life period (T_{IC50}) in frozen minced pork meat balls showed, that the addition of antioxidants was significantly elongated (Table 2). BHT introduction, as antioxidant interrupting the radical chain reaction, has extended the lysine T_{IC50} about 2.8 times as compared with control sample. Natural antioxidants showed weaker protective properties than BHT. Presence of rosemary extract and soy protein hydrolyzate contributed the extension amino acid T_{IC50} 1.6 and 1.2 times respectively.

Table 2. Influence of antioxidants addition on half-life period of lysine in frozen meat products made from minced pork meat

Type of additive	Half-life period T_{IC50} (days)
Control	97.4
BHT	271.5
Rosemary extract	157.3
Soy hydrolyzate	114.8

Antioxidants limited reduction of protein digestibility. In samples with BHT it was 10%, with rosemary extract and protein hydrolyzate – 21% and 30%, respectively (Fig. 7).

Content of available lysine and protein digestibility were correlated with degree of fat oxidation, the higher degree of oxidation, the lower content of this essential amino acid and lower digestibility of stored products. Investigations made in model systems by Janitz [20, 21, 22] show also, that oxidized fats make difficult the utilization of diet protein, and it resulted in reduction of their digestibility. The blocking of active protein groups by lipid oxidation products were also a part of Pokorny and Davídek research [44]. On the ground on their results it was stated that the crosslinking of protein reaction, amino acid oxidation and amino group's transformations in to imin groups are mainly initiated by hydroperoxides. Hexanal as well as other aldehydes can initiate proteins crosslinking, blocking and transformation of functional groups. Aldehydes, reacting with sulfhydrylic cysteine group, form thioacetale, and combining with lysine amin group they form Schiff's bases [44]. The kinetic of aldehyde reaction is considerably smaller relatively to hydroperoxide reaction, which reactions with proteins can cross rapidly even at ambient temperatures [20].

Therefore, products of fat oxidation react with proteins limiting their nutritive value. Natural and synthetic antioxidants inhibiting fat oxidation showed the ability of maintenance of higher biological value of meat protein. In products contained antioxidant higher content of available lysine and slower reduction of protein digestibility was observed.

CONCLUSIONS

Product of fat oxidation indicates destructive influence onto content of available form of lysine in pre-cooked frozen products made from minced pork meat.
Addition of antioxidants inhibit significantly digestibility of meat products protein as well as it diminishes quantitative losses of lysine.
Application of natural antioxidants, such as rosemary extract and soy protein hydrolyzate, permits to elongate the stability and to keep longer the biological value of protein in meat products, but they were less effective than BHT, synthetic antioxidant.

REFERENCES

1. Amarowicz R., Shahidi F., 1997. Antioxidant activity of peptide fractions of capelin protein hydrolysates. Food Chem. 58, 355-359.
2. Barlow S. M., 1990. Toxicological aspects of antioxidants used as food additives. In: Food antioxidants. B.J.F. Hudson (ed.). Elsevier, London.
3. Bonnely S., Peyrat-Maillard M. N., Rondini L., Masy D., Berset C., 2000. Antioxidant activity of malt rootlet extracts. J. Agric. Food Chem. 48, 2785-2792.
4. Burmeister D. M., Dilley D. R., 1995. A scald-like controlled atmosphere storage disorder of Empire apples- a chilling injury induced by CO_2. Postharv. Biol. Technol. 6, 1-7.
5. Chen H-M., Muramoto K., Yamauchi F., 1995. Structural analysis of antioxidative peptides from soybean β-conglycinin. J. Agric. Food Chem. 43, 574-578.
6. Chen H-M., Muramoto K., Yamauchi F., Nokihara K., 1996. Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. J. Agric. Food Chem. 44, 2619-2623.
7. Chen H-M., Muramoto K., Yamauchi F., Fujimoto K., Nokihara K., 1998. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. J. Agric. Food Chem. 46, 49-53.

8. Cuvelier M-E., Richard H., Berset C., 1996. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. J. Am. Oil Chem. Soc. 73, 645-652.
9. Duh P. D., Yen D. B., Yen G. C., 1992. Extraction and identification of an antioxidative component from edible oils. Food Chem. 14, 45-51.
10. Flaczyk E., 1997. Zalety technologiczne i żywieniowe hydrolizatów białkowych. Technological and nutritional advantages of hydrolyzed proteins. Part 2. Przem. Spoż. 4, 43-45 [in Polish].
11. ord S. M., Hook J. B., Bond J. S., 1980. The effects of butylated hydroxyanisole and butylated hydroxytoluene on renal function in the rat. Food Cosmet. Toxicol. 18, 21-26.
12. Foti M., Piatelii M., Baratta M., Ruberto G., 1996. Flavonoids, coumaris and cinnamic acids as antioxidant in a micellar system. Structure-activity relationship. J. Agric. Food Chem. 44, 497-501.
13. Frankel E. N., Waterhouse A. L., Teissedre P., 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low density lipoproteins. J. Agric. Food Chem. 43, 890-894.
14. Friedman M., 1996. Nutritional value of proteins from different food sources. A review. J. Agric. Food Chem. 44, 6-29.
15. Gazzani G., Papetti A., Massolini G., Daglia M., 1998. Antioxidative and pro-oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. J. Food Chem. 6, 4118-4122.
16. Hall R. J., Trinder N., Givens D. I., 1973. Observations on the use of 2,4,6-trinitrobenzenesulphonic acid for the determination of available lysine in animal protein concentrates. Analyst 98, 673-686.
17. Haraguchi H., Saito T., Okamura N., Yagi A., 1995. Inhibition of lipid peroxidation and superoxide generation by diterpenoids from Rosmarinus officinalis. Planta Med. 61, 333-336.
18. Hidalgo F. J., Alaiz M., Zamora R., 1999. Effect of pH and temperature on comparative nonenzymatic browning of proteins produced by oxidized lipids and carbohydrates. J. Agric. Food Chem. 47, 742-747.
19. Hoffmann K., 1993. Nutritional value of proteins and protein requirements of people with special reference to meat proteins. Mitt. Bund. Fleischforsch. 32, 422-429.
20. Janitz W., 1985. Interakcje tłuszczów i białek mięsa ze szczególnym uwzględnieniem wpływu utlenionych tłuszczów na wartość odżywczą białka (badania modelowe). Interactions of meat fats and proteins with particular consideration of the effect of oxidized fats on the nutrition value of proteins. Rocz. AR Pozn. Rozpr. Nauk. 147 [in Polish].
21. Janitz W., Pyrcz J., Grześkowiak B., Berghofer E., 1990 a. Einfluss technologischer Massnahmen auf die Reaktionen oxidierter Fette mit Fleischproteinen. I. Qualitative und quantitative Veränderungen der Fett-Proteinkomplexe. Influence of technological operation on reactions oxidized lipids with meat proteins. I. Qualitative and quantitative changes of lipid-protein complexes. Fleischwirtschaft 70, 206-209 [in German].
22. Janitz W., Grześkowiak B., Korczak J., Berghofer E., 1990 b. Einfluss technologischer Massnahmen auf die Reaktionen oxidierter Fette mit Fleischproteinen. III. Nährwertveränderungen. Influence of technological operation on reactions oxidized lipids with meat proteins. III. Changes of nutritive value. Fleischwirtschaft 70, 600-604 [in German].
23. Karpińska M., Borowski J., Danowska-Oziewicz M., 2000. Antioxidative of rosemary extract in lipid fraction of minced meat balls during storage in a freezer. Nahrung 44, 38-41.
24. Korczak J., Pazoła Z., Gogolewski M., 1990. Właściwości przeciwutleniające przypraw ziołowych z rodziny wargowych (*Labiatae*). Cz. I. Ocena aktywności przeciwutleniającej w układach modelowych. Antioxidant properties of herbal seasoning from Labiatae family. Part 1. Evaluation of antioxidant activity in model systems. Rocz. AR Pozn. 218, 61-74 [in Polish].
25. Korczak J., 1998. Czynniki warunkujące właściwości przeciwutleniające hydrolizatów białkowych soi i kazeiny. Factors affecting antioxidant activity of soybean meal and caseine protein hydrolysates. Rocz. AR Pozn. Rozpr. Nauk. 281 [in Polish].
26. Laser-Reuterswärd A., 1985. Solubilization of pigskin and bovine tendon after pepsin and pancreatin treatment. Effect of incubation conditions and age of animal. J. Food Technol. 20, 129-143.
27. Liang J. H., 1999. Fluorescence due to interactions of oxidizing soybean oil and soy proteins. Food Chem. 66, 103-108.
28. Lindberg Madsen H., Andersen L., Christiansen L., Brockhoff P., Bertelsen G., 1996. Antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in minced, cooked pork meat. Z. Lebensm. Unters. Forsch. 203, 333-338.
29. Lindberg Madsen H., Sorensen B., Skibsted L. H., Bertelsen G., 1998. The antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in dressing stored exposed to light or in darkness. Food Chem. 63, 173-180.
30. Maniak B., Targoński Z., 1996. Przeciwutleniacze naturalne występujące w żywności. Natural antioxidants in food. Przem. Ferm. Owoc. Warz. 4, 7-10 [in Polish].
31. McCarthy T. L., Kerry J. P., Kerry J. F., Lynch P. B., Buckley D. J., 2001 a. Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. Meat Sci. 57, 45-52.
32. McCarthy T. L., Kerry J. P., Kerry J. F., Lynch P. B., Buckley D. J., 2001 b. Assessment of the antioxidant potential of natural food and plant extracts in fresh and previously frozen pork patties. Meat Sci. 57, 177-184.
33. Mielnik M. B., Aaby K., Skrede G., 2003. Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. Meat Sci. 65, 1147-1155.
34. Miyake T., Shibamoto T., 1997. Antioxidative activities of natural compounds found in plants. J. Agric. Food Chem. 45, 1819-1822.

35. Nieto S., Garrido A., Sanhueza J. Loyola L. A., Morales G., Leighton F., Valenzuela A., 1993. Flavonoids as stabilizers of fish oil: an alternative to synthetic antioxidants. J. Am. Oil Chem. Soc. 70, 773-778.
36. Peña-Ramos E. A., Xiong Y. L., 2003. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Sci. 64, 259-263.
37. Pikul J., Leszczynski D. E., Kummerow F. A., 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J. Agric. Food Chem. 37, 1309-1313.
38. Pikul J., 1992. Utlenianie lipidów i powstawanie obcego zapachu oraz smaku w ogrzewanym i przechowywanym mięsie. Oxidation of lipids and generation of off flavour and taste in cooked and stored meat. Part 2. Gosp. Mięs. 8, 22-26 [in Polish].
39. PN-93/A-86926 Tłuszcze roślinne jadalne. Oznaczanie liczby anizydynowej oraz obliczanie wskaźnika oksydacji tłuszczu Totox. Edible vegetable fats. Determination of anisidine value and calculation of total oxidation value Totox.
40. PN-EN ISO 6885:2001 Oznaczanie liczby anizydynowej. Determination of anisidine value.
41. PN-ISO 3960:1996 Oleje i tłuszcze roślinne oraz zwierzęce. Oznaczanie liczby nadtlenkowej. Animal and vegetable oils and fats. Determination of peroxide value.
42. Pokorný J., El-Zeany B. A., Kołakowska A., Janíček G., 1974. Correlation of autoxidation and browning reactions in lipid-protein mixtures. Z. Lebensm. Unters. Forsch. 155, 287-291.
43. Pokorný J., Kołakowska A., El-Zeany B. A., Janíček G., 1975. Effect of free amino groups on browning reactions in lipid-protein mixtures. Z. Lebensm. Unters. Forsch. 157, 323-326.
44. Pokorný J., Davídek J., 1979. Influence in interactions of proteins with oxidized lipids on nutrition and sensory value of food. Acta Aliment. Pol. 5, 87-95.
45. Pokorný J., Davídek J., Chocholatá V., Pánek J., Bulantová H., Janitz W., Valentová H., Vierecklová M., 1990. Interactions of oxidized ethyl linoleate with collagen. Nahrung 34, 159-169.
46. Pokorný J., 1991. Natural antioxidants for food use. Trends Food Sci.Technol. 9, 223-227.
47. Pokorný J., Nguyen H. T. T., Korczak J., 1997. Antioxidant activities of rosemary and sage extracts in sunflower oil. Nahrung 41, 176-177.
48. Rižner Hraš A., Hadolin M., Knez Ž., Bauman D., 2000. Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem. 71, 229-233.
49. Schwartz K., Ternes W., 1992. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. Isolation of carnosic acid and formation of other phenolic diterpenes. Z. Lebensm. Unters. Forsch. 195, 99-103.
50. Wang H., Cao G., Prior R. L., 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44, 701-705.
51. Wu S. Y., Brewer M. S., 1994. Soy protein isolate antioxidant effect on lipid peroxidation of ground beef and microsomal lipids. J. Food Sci. 59, 702-706.
52. Yen G. C., Wu S. C., Duh P. D., 1996. Extraction and identification of antioxidant components from the leaves of mulberry. J. Agric. Food Chem. 44, 1687-1690.
53. Yen G. C., Chen H. W., Duh P. D., 1998. Extraction and identification of antioxidative component from Jue Ming Zi (*Cassia tora* L.). J. Agric. Food Chem. 46, 820-824.
54. Zamora R., Alaiz M., Hidalgo F. J., 2000. Contribution of formation and polymerization to the nonenzymatic browning produced by amino-carbonyl reactions. J. Agric. Food Chem. 48, 3152-3158.
55. Zhou S., Decker E. A., 1999. Ability of amino acids, dipeptides, polyamines, and sulfhydryls to quench heksanal, a saturated aldehydic lipid oxidation product. J. Agric. Food Chem. 47, 1932-1936.

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