Acta Sci. Pol., Technol. Aliment. 11(2) 2012, 99-109
pISSN 1644-0730 eISSN 1889-9594 www.food.actapol.net/

OXIDATIVE STABILITY OF FERMENTED MEAT PRODUCTS

Karolina M. Wójciak, Zbigniew J. Dolatowski

Department of Meat Technology and Food Quality, University of Life Sciences in Lublin
Skromna 8, 20-704 Lublin, **Poland**

ABSTRACT

Meat and meat products, which form a major part of our diet, are very susceptible to quality changes resulting from oxidative processes. Quality of fermented food products depends on the course of various physicochemical and biochemical processes. Oxidation of meat components in raw ripening products may be the result of enzymatic changes occurring as a result of activity of enzymes originating in tissues and microorganisms, as well as lipid peroxidation by free radicals. Primary and secondary products of lipid oxidation are extremely reactive and react with other components of meat, changing their physical and chemical properties. Oxidised proteins take on a yellowish, red through brown hue. Products of lipid and protein degradation create a specific flavour and aroma; furthermore, toxic substances (such as biogenic amines or new substances) are formed as a result of interactions between meat components, e.g. protein-lipid or protein-protein combinations, as well as transverse bonds in protein structures. Oxidation of meat components in raw ripening products is a particularly difficult process. On the one hand it is essential, since the enzymatic and non-enzymatic lipid oxidation creates flavour and aroma compounds characteristic for ripening products; on the other hand excessive amounts or transformations of those compounds may cause the fermented meat product to become a risk to health.

Key words: probiotic, meat products, antioxidant, oxidative stability, colour

ENZYMATIC LIPID PEROXIDATION IN RAW RIPENING PRODUCTS

Unprocessed raw meat and fats, and the resulting meat products undergo hydrolytic transformation in the presence of water and hydrolases of glycerol esters (lipases). The reaction produces mono- and diacylglycerol, glycerol and "free" fatty acids. The hydrolysis kinetics is characterised by an initially slow course, gradually intensifying until it reaches equilibrium, after which its intensity decreases again. The intensity of enzymatic hydrolytic changes is affected by factors such as water content, temperature, active acidity, water activity, and presence of cation catalysts such as magnesium and calcium salts. Navarro et al. [1997] confirmed the significant role played by ripening temperature on the content of free fatty acids, although it depended on using a starter culture. At 8°C lipolytic activity was highest in samples added with *Lactobacillus sake* L. 110 and *Staphylococcus carnosus* M. 72, while the temperature of 16°C increased lipase activity in samples without a starter culture. The rate of hydrolytic changes increases with the increasing water content. Reducing water activity below 0.9 inhibits the growth and development of the majority of microorganisms. Pikul et al. [1989] report that 35-40°C is the optimal temperature for the enzymatic hydrolysis process. In studies of Italian ripening sausages, stored loose or vacuum-packed, Summo et al.

✉karolina.wojciak@up.lublin.pl

[2006] found an increase in the acid number value in vacuum-packed samples (9.69% oleic acid). They suggest that the significantly higher acid number in vacuum-packed samples may have been the result of higher water availability in the packed sample, providing a basis for enzymatic changes. High acid number values (approx. 17 mg KOH/g of fat) have been observed in studies conducted on Spanish *salchichón* sausage by Lizaso et al. [1999] and by Martin-Sanchez et al. [2011]. The studies also found high content of peroxides in the final product (~56.84 meqO_2/kg fat). Freeing fatty acids from triacylglycerols and phospholipids by endogenic (tissue) and exogenic (presence of lactobacilli and micrococci) enzymatic apparatus resulted in their greater availability for oxidation by free radicals. During the process of sausage maturation, Lizaso et al. [1999] observed an increase in the ratio of saturated to unsaturated fatty acids, which suggests a high share of the latter in oxidative processes and as such a reduction in their amount in the final product [Lizaso et al. 1999]. However, Navarro et al. [1997] found the opposite relationship in their studies. The authors explained this by the effect of different ripening conditions on the enzymatic activity and oxidation of fatty acids. Summo et al. [2010] found a gradual increase in the acid number (from 4.12 to 7.80%) in ripening sausages during the five-month vacuum storage. Aguirrezábal et al. [2000] observed a significantly higher level of free fatty acids in samples of fat and meat and fat sausages with no added salt (approx. 5 g oleic acid/100 g sausage) in comparison with the same samples containing salt (approx. 2 g oleic acid/100 g sausage).

Enzymatic oxidation of unsaturated fatty acids in meat products is also linked to another enzyme – lipoxygenase. It catalyses the oxidation of free unsaturated or esterified polyenic fatty acids with a *cis, cis* 1,4-pentadiene arrangement to hydroperoxides and free radicals which play a part in the oxidation of vitamins, pigments, phenolic compounds and proteins [Baraniak and Szymanowska 2006]. The main reaction products are conjugated unsaturated fatty acids and hydroperoxides. Lipid peroxides undergo further changes catalysed by lyases, hydroperoxide isomerases and peroxigenases, leading to the formation of aldehydes and alcohols, ketones, n-hexanal, hexanal and hexenol giving the products a rancid flavour. Research into lipoxygenase activity in pork conducted by Jin et al. [2011] revealed the effect of temperature, acidity and salt content in meat and raw ripening meat products on lipoxygenase activity. It was found that enzyme activity increases with increasing temperature up to 35°C, after which it starts gradually decreasing. Sodium chloride concentrations of 3-4% increase lipoxygenase activity in fermented meat products. Higher sodium chloride content may cause denaturation of the protein part of the enzyme. Reducing the pH of meat and meat products to 5.0 causes a reduction in lipoxygenase activity. An increase in the enzyme's oxidative activity was noted for the pH ranging between 6.0-9.0 by Jin et al. [2011]. These pH values cause an increase in protein solubility, as well as the degree of disassociation of unsaturated acids. Disassociated fatty acids form bonds with lipoxygenase more easily which may accelerate their enzymatic oxidation [Jin et al. 2011]. Zhou and Zhao [2007] found that enzymatic oxidation is responsible for the formation of just 20% of all chemical compounds formed as a result of oxidation. In the production of traditional dry-cured *Jinhua* ham, over 80% of secondary oxidation products are formed as a result of non-enzymatic peroxidation. It is a process designed to produce a distinctive flavour and aroma bouquet of ripening products. As well as the enzymatic processes listed above, volatile aroma compounds of fermented meat products are also produced during Maillard reactions, lipid oxidation and Strecker degradation [Zhou and Zhao 2007].

NON-ENZYMATIC LIPID PEROXIDATION IN RAW RIPENING PRODUCTS

Lipids, and in particular phospholipids in membranes of muscle cells after slaughter, especially after comminuting the meat, are exposed to the main oxidation factors – oxygen and light [Fellenberg and Speisky 2006]. Lipid peroxidation is a free radical-driven process of fatty acid oxidation producing fatty acid peroxides. It occurs in three steps: initiation, propagation and termination. The lipid peroxidation initiation step involves removing a hydrogen atom from a free polyunsaturated fatty acid or fatty acid forming a part of a phospholipid molecule [Morrissey et al. 1998]. The hydrogen atom most commonly removed is located between two double bonds. The fatty acid

molecule is transformed into an alkyl free radical with a group of conjugated bonds [Fellenberg and Speisky 2006]. Factors stimulating the initiation step include hydroxyl, alkoxyl, peroxide and alkyl radicals, as well as nitrogen oxide and dioxide, ozone, and sulphur dioxide [Hęś and Korczak 2007 a, Fellenberg and Speisky 2006, Morrissey et al. 1998]. The propagation step leads to oxygen molecules binding to the alkyl free radical. The process results in the formation of peroxide free radicals which stimulate the initiation of subsequent polyunsaturated fatty acids, eventually leading to the formation of a lipid peroxide. The chain reaction of the initiation and propagation processes stops when two radical react to produce a non-radical species (termination step). Products of the termination step are lipid dimers, oxo- or hydroxy fatty acids, and other modified and damaged lipid molecules [Hęś and Korczak 2007 a, Fellenberg and Speisky 2006, Morrissey et al. 1998]. The re-initiation process is important due to high numbers of transition metal ions – haem and non-haem iron [Bartosz 2006]. Lipid peroxides are broken down to form free radical products. This occurs in the presence of transition metal ions, where Fe^{2+} ions react faster with lipid peroxides than Fe^{3+} ions [Carlsen et al. 2005]. The role of non-haem iron in accelerating lipid oxidation processes appears to be important, especially in environments with a pH of 4.5 such as raw ripening meat products [Carlsen et al. 2005, Skibsted 1996]. Re-initiation may also form the ferryl radical (Fe^{4+}). Hernández et al. [1999] report that the presence of glucose or glycosylated peptides may cause the re-initiation of lipid peroxidation.

Further changes of peroxidation products lead to the degradation of polyunsaturated fatty acid residues to shorter fragments with a dozen or so carbon atoms, including malondialdehyde (MDA), 4-hydroxyalkenals, 2-alkenals, hepta 2,4-dienal, 4-hydroxynonenal (4-HNE), 4-hydroxy-*trans*-nonenal, and hydroxyoctanal. Hernández et al. [1999] noted increased levels of peroxides in raw pork loin as compared with the cured and dried product. They explained the reduction in the peroxide number and the concurrently observed increase in the TBA value as the transformation of primary products of lipid degradation into secondary products, including MDA. However, MDA is just one of many products of lipid degradation and may be subject to further transformations (condensation, reaction with free amino acids) which leads to a reduction in the TBA value [Summo et al. 2010]. Summo et al. [2006] found significantly higher TBARS values and peroxide numbers in vacuum-packed Italian ripening sausages (4.37 mg MDA/kg) in comparison with loose-stored sausages (2.89 mg MDA/kg). However, no loss of the red colour or intensification of rancid flavour or aroma were observed in sausages during the sensory analysis. High TBARS values not correlated with the sensory analysis of the product were explained by low credibility of the research method. The studies into vacuum-packed ripening sausages stored for 5 months showed a statistically insignificant increase in the TBARS value (1.14 to 1.39 mg MDA/kg). However, the increase correlated with the percentage increase of oxidised forms of triacylglycerols and diacylglycerols. Numerous authors have noted a high TBARS value in sausages studied immediately after ripening, which they explain by intensification of mechanical processes and high availability of oxygen [Bozkurt and Erkmen 2002, Summo et al. 2006, 2010]. Bozkurt and Erkmen [2002] have found a significant decrease of the TBA value in samples of sausages added with starter cultures (*Pediococcus acidilactici*, *Lactobacillus plantarum*, *Staphylococcus carnosus*) in comparison with samples with no added microbial mix. During the 45-day storage of the samples at 20°C, the TBA value ranged between 2.00-4.22 mg/kg. The fact was explained by the presence of a microbial catalyst breaking down products of lipid autoxidation. The authors also confirmed the protective effect of antioxidants (α-tocopherol, ascorbic acid) on oxidative stability of the studied sausages [Bozkurt and Erkmen 2002]. Samples added with antioxidant were found to have lower TBA values (approx. 0.5-1 mg/kg) than control samples [Bozkurt and Erkmen 2002].

OXIDATION OF PROTEINS IN RIPENING PRODUCTS

Proteins are another meant component exposed to oxidation. Although oxidation of proteins is slower than lipid oxidation, it is equally important in the shaping the functional and health-promoting properties of meat and meat products. Primary and secondary products of lipid autoxidation reacting with proteins are less reactive than free radicals; however, they can

move within biological material [Carlsen et al. 2005]. Aldehydes formed in a lipid peroxidation reaction react mainly with triol and amine groups in proteins, amino sugars and nitrogen bases forming nucleic acids [Fellenberg and Speisky 2006, Hęś and Korczak 2007 b]. Reactions causing damage to meat proteins are frequently originated by a free radical removing a hydrogen atom. Under aerobic conditions, the protein radical may be bound by oxygen leading to the formation of a protein peroxide radical. Subsequent transformations lead to the modification of amino acid residues and fragmentation of polypeptide molecules. Damaged protein molecules aggregate under anaerobic conditions. Recombination of protein free radicals with cysteine or tyrosine residues leads to the formation of protein dimers linked by cysteine or bistyrosine bridges. Protein-lipid interactions affect the amino acid content and digestibility of proteins, loss of enzyme activity, changes in nutritive value (loss of essential amino acids) as well as cause the formation of cross-linked protein complexes which reduces their water absorption, and induces formation of antioxidant products of non-enzymatic browning [Adams et al. 2009, Fellenberg and Speisky 2006]. In nutrition an essential role is played by losses of exogenous amino acids because they reduce the nutritive value of protein in products. The most sensitive amino acids toward oxidation are heterocyclic amino acids are susceptible to oxidation. Due to their structure tryptophan, histidine, proline, lysine, cysteine, methionine and tyrosine are prone to oxidation where the hydrogen atom is abstracted either from OH-, S-, N- containing groups. The results of oxidation those amino acids are methionine sulfone, kynurenine, dityrosine originate. Viljanen [2005] bring back the research in which authors reported that the amount of tryptophan residues decreased 18% after 24 hours reaction with linoleic acid hydroperoxides. The reaction between amino acids and secondary lipid oxidation products leads to formation of carbonyl group in the proteins. Estévez and Cava [2006] found significant correlation ($R^2 = 0.77$; $p < 0.01$) between lipid and protein oxidation as measured by TBARS and protein carbonyls respectively. Hęś et al. [2007] in their research indicated that decreased in the content of available lysine during storage may be connected with changes occurring in the lipid fraction, leading to blocking ε-amino groups of protein, but a decrease in methionine content may be caused by lipid oxidations and formation of methionine sulfoxide or sulfone. Hydroperoxides and aldehydes may react with free amino groups of the protein to generate fluorescent compounds and promote polymerization. Those processes lead to create yellow and brown pigments. The protein-lipid complexes (carbonyls) due to the protein-lipid interaction are formed rapidly, they are stable and have a specific fluorescence at the excitation wavelength around 350 nm [Viljanen 2005]. Chelh et al. [2007] created model system witch demonstrated that fluorescent Schiff bases are formed in pig myofibrils during chemical oxidation. Irradiation process, storage in the presence of high level of oxygen, mincing, cooking could induce the formation of fluorescent Schiff bases [Chelh et al. 2007].

Biogenic amines are a natural component of high-protein fermented food products. They are formed as a result of decarboxylation of amino acids and tri- and dipeptides, and amination and transamination of aldehydes and ketones [Berthold and Nowosielska 2008]. In fermented meat products they are formed during microbial decarboxylation of amino acids. Biogenic amines are aliphatic (e.g. putrescine, cadaverine, spermine, spermidine), heterocyclic (histamine, tryptamine) or aromatic (phenylethylamine, tyramine). Biogenic amines are formed in fermented food products as a result of proteolysis or through the transformation of secondary products of lipid peroxidation [Berthold and Nowosielska 2008, Bartosz 2006]. In raw ripening sausages the presence of microorganisms is the main cause of biogenic amines. The formation of these compounds is closely linked with the environmental pH (a low pH, 4.5-5.5, of ripening sausages promotes the activity of amino acid decarboxylase of bacterial origin), temperature of ripening and storage, concentration of salt in the product [Berthold and Nowosielska 2008], and the environmental redox potential [Stadnik and Dolatowski 2010]. In their studies of carp meat, Kordiovská et al. [2006] found that reducing the redox potential stimulates the formation of histamine. They demonstrated that formation of biogenic amines by bacteria is most effective at temperatures between 20-37°C. A significant reduction in the content of biogenic amines was observed by Suzzi and Gardini [2003] alongside increasing salt

concentration. However, Kordiovská et al. [2006] claim that the presence of sodium chloride results in the increase of tyramine content while reducing histamine content. Bozkurt and Erkmen [2002] found a gradual increase in the content of biogenic amines in sausages up to the 31st day of storage, followed by a decrease in their levels. The authors observed higher levels of histamine in samples without added antioxidant. Similarly, higher histamine concentrations were found in samples of sausages without starter cultures [Bozkurt and Erkmen 2002]. However, Bozkurt and Erkmen [2002] found concentrations of tyramine to be above acceptable levels (1276.19 mg/kg) in samples of sausages with added starter cultures on the 31st day of storage. The authors did not observe any significant effects of using a starter culture and antioxidant on the content of tryptamine, serotonin and spermidine. Appropriately selected starter cultures can increase or decrease biogenic amine content in the final product. Literature lists species from the genera *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Lactococcus* as capable of amino acid decarboxylation. Lactic acid bacteria *Lb. brevis*, *Lb. buchnerii*, *Lb. curvatus*, *Lb. carnis*, *Lb. divergens*, and *Lb. nilgardii* have been isolated as species forming biogenic amines in meat and meat products [Berthold and Nowosielska 2008].

RELATIONSHIPS BETWEEN LIPID OXIDATION AND COLOUR CHANGE IN RAW RIPENING PRODUCTS

The earliest scientific reports on the interactions between haem pigments and lipids date back to 1941. Haurowitz et al. [1941] found that under certain conditions haemoglobin is damaged, which is accompanied by a release of iron from the porphyrin ring. The phenomenon was observed in the presence of unsaturated fatty acids and oxygen, and at increased temperatures (38°C). Szebeni et al. [1984] observed a high correlation between the TBARS value in cellular membranes and the content of oxidised haemoglobin. Similarly, Zanardi et al. [2002] noted a high positive correlation between lipid oxidation and colour change in salami-type sausage, manifested by an increased browning index in the product. Literature lists numerous factors that affect oxymyoglobin oxidation. They include temperature, acidity of the environment, partial pressure, and lipid oxidation. Oxidation of oxymyoglobin occurs at the fastest rate at increased temperatures, in an acidic environment, and in the presence of non-haem iron [Faustman et al. 1992 a]. The relationship between the process of autoxidation of meat lipids and the content and form of haem pigments is significant in terms of the degree of rancidity and colour loss in the ripening product [Skibsted 1996, Zanardi et al. 2002]. A strong relationship between the oxidation of lipids and myoglobin has been observed in beef stored under aerobic conditions [Faustman et al. 1992 b, O'Gready et al. 2001]. However, it is not fully understood when we are dealing with oxidation of haem pigments as a result of lipid peroxidation, and when the situation is opposite. Zanardi et al. [2002] list hepta-2,4-dienal, 2-nonenal, and 4-hydroxy 2-nonenal as secondary products of autoxidation of fatty acids from the n-6 family, capable of accelerating myoglobin oxidation. In raw processed meats with a pH between 4.5-6.0, green myoglobin may be formed as a result of the catalytic properties of peroxides. Sulphmyoglobin may be formed as a result of proteolytic microbial activity in the reaction between myoglobin and hydrogen sulphide in raw processed meats. Studies conducted by Faustman and Wang [2000] confirm the effect of adding vitamin E on delaying the process of myoglobin oxidation in raw sausage, partially through the inhibition of lipid peroxidation. In their studies of beef stored in packaging with varying oxygen content, Zakrys et al. [2008] found a strong correlation between changes in the oxymyoglobin content and colour parameter "a", and the TBARS value [Faustman et al. 1999, Zakrys et al. 2008]. The hypothesis of catalytic properties of products of lipid autoxidation on the oxidation of haem pigments has found high support in various publications, concerning mainly beef [Faustman et al. 1999, 2000, Zakrys et al. 2008]. It has been found that adding vitamin E delays myoglobin oxidation by inhibiting lipid oxidation. O'Gready et al. [2001] used *in vitro* models to explain the mechanism of oxymyoglobin oxidation as the result of the catalytic effect of products of oxidation of unsaturated fatty acids in cellular membranes.

On the other hand, there is good evidence supporting the oxidative action of haem pigments on lipid autoxidation. Catalytic properties of haem and non-haem

iron were confirmed by Carlsen et al. [2005]. High content of haem iron and myoglobin is closely correlated with a high degree of lipid oxidation [Faustman et al. 1992 a]. Rhee and Ziprin [1987] studied the effect of haem and non-haem iron on the degree of lipid oxidation in pork, beef and poultry. The authors found that haem iron has a greater effect on accelerating lipid oxidation processes than free iron, which they mainly observed in beef. The mechanism of oxidation of oxymyoglobin to metmyoglobin causes the formation of secondary, extremely reactive chemical compounds, which in turn are able to oxidise oxymyoglobin or residues of unsaturated fatty acids. Superoxide anions formed as a result of oxidation are quickly converted to hydrogen peroxide. Hydrogen peroxide, produced by milk fermentation bacteria, including probiotic strains, reacts with free iron (III) leading to the formation of ferryl cation radicals $[Fe(IV)OH]^{3+}$ or perferryl radicals $[Fe(V)\text{-}O^{\cdot}]$ with powerful oxidative properties. The reaction of hydrogen peroxide with haem iron in myoglobin or metmyoglobin leads to the formation of ferrylmyoglobin (Mb-Fe(IV)=O). Ferrylmyoglobin can undergo further transformations gradually leading to the formation of metmyoglobin, or react with myoglobin to form two myoglobin molecules. Haem pigments display activity similar to catalase breaking down hydrogen peroxide, and the meat product colour produced as a result of the reaction is unappealing [Kanner and Harel 1985, Skibsted 1996]. Loss of colour in ripening meat products may occur as a result of high levels of saccharides (15 g/kg or higher) and excessive growth of lactic acid bacteria in stuffing. It may also occur as a result of oxidative properties of peroxides produced by lactic acid bacteria. Their excessive growth may inhibit multiplication of *Staphylococci*, which produce enzymes breaking down peroxides (catalase, peroxidase). Kanner and Harel [1985] report that this may occur during fermentation at high temperature (25°C) and humidity. Various processes used to cure meat products make them a difficult material to study correlations between lipid oxidation and changes in haem pigments. Different curing mixtures, varying degrees of comminution and mixing, presence of various additives, and different fermentation and ripening conditions make ripening meat products susceptible to oxidative factors. Nitrates added to protect meat products against *Clostridium botulinum* growth are responsible for creating the typical colour and aroma of meat products. They also protect lipids against oxidation as a result of binding haem iron. Nitrosyl myoglobin is formed as a result of the reaction of nitric oxide with haem pigments. However, Zhang et al. [2007] and Arihara [2006] obtained the characteristic NO-Mb(Fe^{2+}) pigment without nitric compounds by adding bacterial strains *Kurthia* spp. and *Lactobacillus fermentum* (JCM1173), reducing metbyoglobin to nitrosyl myoglobin. The experiment was conducted *in vitro* and *in vivo* in smoked sausages. Similar results were obtained by Morita et al. [1998] by adding the bacterial strain *Staphylococcus xylosus* (FAX-1). The listed bacterial strains are capable of producing catalase and superoxide dismutase that inhibit the action of free radicals and the oxidation of unsaturated fatty acids. Zanardi et al. [2002] found positive correlation between the increase of the browning index and increasing indicators of lipid oxidation in their studies of *salami Milano* packed in a vacuum or modified atmosphere. A significant correlation (0.890) was found between the increase in cholesterol oxidation products (7β-hydroxy-cholesterol, 5,6α-epoxycholesterol, 7-ketocholesterol) and the browning index [Zanardi et al. 2002]. Aldehydes formed in the lipid peroxidation reaction bind the primary amine group of lysine bound in protein to form colourless Schiff bases, which obtain a brownish hue after aldolization and polymerisation, and become fluorescent. Final products of protein-lipid complexes have a yellow, red or brown colour [Adams et. al 2009, Hęś and Korczak 2007 a, b].

PROBIOTICS IN THE OXIDATION OF RAW RIPENING PRODUCTS

Probiotic bacteria are defined as 'living microoganisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition'. Health benefits for products containing live probiotic bacteria include alleviation of symptoms of lactose intolerance, treatment of diarrhea, anticarcinogenic properties, reduction in blood cholesterol and improvement in immunity. Consumption of high concentration of probiotic bacteria at 10^7 viable cells per gram or per millilitre of product is required to confer health benefits. Nevertheless probiotics may be responsible for some kinds of side-effects like for example

systemic infections, risk of gene transfer, risk of adjuvant side-effects and of immunomodulation [Kołożyn-Krajewska et al. 2011]. Some species of *Lactobacillus* are capable to forming biogenic amines. There were a few cases of histamine intoxication by foods what has been connected with activity of *Lactobacillus* spp. The histidine decarboxylase activity and the tyrosine decarboxylase activity have been observed in many species of lactobacilli. But many authors did not observe histidine decarboxylase activity in *Lactobacillus* spp. isolated from dry fermented sausages. According to Kołożyn-Krajewska et al. [2011] it seems that the most serious hazard connected with probiotic meat product consumption is because of *Enterococcus* genus, and the minimum because of *Leuconostoc.* Authors claimed that the risk is very small when daily consumption of probiotic meat products is 15 gram. Only in the case of *Enterococcus* bacteria, taking into account every day consumption, the risk can be significant. The risk of *Lactobacillus* infection is estimated at about one case per 10 milion people over a century of probiotic consumption in France. The risk of lactobacillemia was considered at below one case per million individuals [Kołożyn-Krajewska et al. 2011].

Most publications concerning incorporation of probiotic bacteria into foods have focused only on their survival during manufacture and storage and only a few studies have considered the effect of probiotic adjuncts on the oxidative stability of raw meat products.

Ong and Shah [2009] examine the influence of ripening temperatures (4 and 8°C) on the survival of probiotic bacteria, compositional changes in cheeses and the organic acid profiles after ripening for 24 wk. Authors proved that increasing the ripening temperature from 4°C to 8°C did not affect the viability of the probiotic microorganisms, the salt, fat and protein contents of the cheeses during ripening of 24 wk. Ripening temperature, however, have significantly affected the count of starter lactococci, the moisture and pH of the cheeses [Ong and Shah 2009]. Aragon-Alegro et al. [2007] conducted that chocolate mousse is an excellent matrix for the incorporation of *L. paracasei* subsp. *paracasei* LBC 82. The addition of prebiotic and probiotic strain did not interfere in the sensory preference of the product by consumers. Jaworska et al. [2011] claimed that sensory and microbiological quality of fermented pork loin was dependent on the kind of probiotic strains used for fermentation. The highest sensory quality after ripening and chilling storage was observed in samples produced with *L. acidophilus* Bauer with 0.2% glucose addition. In these samples the number of LAB bacteria was at the level 10^6 CFU g^{-1} after 6 months of chilling storage [Jaworska et al. 2011].

In their studies of meat originating from pigs fed with a traditional feed mixture, a feed mixture enriched with linseed, and a fodder mix fortified with linseed and the probiotic strain *Lactobacillus casei*, Marcinčák et al. [2009] found an increase in the TBARS value in meat originating from pigs fed with fodder fortified with *Lb. casei* during refrigerated storage. Studies of effects of the potentially probiotic strain *Lactobacillus acidophilus* CH-2 and green tea infusion on oxidative stability of ripening pork loin showed that adding the *L. acidophilus* CH-2 strain, glucose and green tea infusion increased oxidative stability of the meat product by lowering the TBARS value in comparison with the control sample, as well as improving the redness of the product [Dolatowski et al. 2010]. The authors also observed better colour durability during light exposure. This is confirmed by Nowaczyk and Dolatowski [2010] studies conducted on raw ripening pork shoulder, and by Skwarek and Dolatowski [2010] conducted on raw ripening hams. The authors obtained lower TBARS values (0.385 mg MDA/kg and 0.97 mg MDA/kg) respectively, and lower levels of oxidised myoglobin (46.3% and 43.35% respectively), in samples with added probiotic bacteria and green tea infusion in comparison with the control sample. However, the authors also found an increase in the oxidative index (0.524 mg MDA/kg) and metmyoglobin (72.5%) and a loss of redness in samples with added probiotic strain *Lactobacillus casei* ŁOCK 0900, which were not treated with antioxidants. The increase in the TBARS value and metmyoglobin was higher in potentially probiotic products compared to the control product.

METHODS OF PREVENTING OXIDATIVE CHANGES IN RAW RIPENING MEAT PRODUCTS

Methods of protecting raw ripening meat products against oxidation can be divided into direct and

indirect procedures. Indirect methods involve enriching feed mixtures with antioxidant compounds. High success rates in stabilising meat products have been obtained by feeding pigs on feed mixtures added with vitamin E. It has been found that the method preventing oxidation by indirect application of vitamin E is more effective than the method of directly adding α-tocopherol to meat products in stabilising colour and lipid oxidation.

In Bozkurt [2006] studies of traditional ripening Turkish sausage added with green tea extract or *T. spicata* oil, or a mixture of green tea and oil, the author found lower TBARS values in samples of sausage treated with antioxidants (0.2-0.6 mg/kg) in comparison with the control sample (0.2-1.0 mg/kg). A comparable level of oxidation was found in the sample added with green tea extract and synthetic BHT [Bozkurt 2006]. The author also found that adding antioxidants inhibited the production of biogenic amines like histamine, tyramine and putrescine in stored sausage. Estévez and Cava [2006] found that rosemary essential oil was effective antioxidant in frankfurters from Iberian pigs but not for frankfurters from white pigs. The addition of rosemary essential oil only had antioxidant effects at the lower concentrations (150 ppm) while higher concentrations (300 ppm, 600 ppm) led to no effect or even prooxidant effects. In comminuted meat products such as frankfurters with high oxidative instability the activity of plant phenolics could be diminished since phenolic compounds can oxidised and the oxidation products could act as prooxidants promoting oxidative reactions [Estévez and Cava 2006]. The applied antioxidants that also display some bacteriostatic properties may inhibit multiplication of bacteria from the *Enterobacteriaceae* family, responsible for forming biogenic amines. Studies by Aguirrezábal et al. [2000] into the effect of adding paprika, garlic and salt on the rancidity of traditional Spanish *chorizo* sausage were conducted during a storage period of 96 days. The authors found peroxide values to be lower by approx. 4 meqO_2/kg sausage in the sample added with paprika (3%) or a mixture of paprika (1%) and garlic (3%) in comparison with the control sample and the sample added with garlic (1%). The authors also found lower TBARS values (by approx. 4 ppm MDA/kg) in sausage with added paprika or a mixture of paprika and garlic in relation to the control sample. Aguirrezábala et al. [2000] concluded that adding a mixture of spices (paprika and garlic) and salt is more effective in protecting *chorizo* against autoxidation of lipids than adding nitrates (300 ppm) and ascorbic acid (500 ppm). Hęś et al. [2007] found that addition of antioxidants: ethanol extract of rosemary or green tea (0.05%) to the frozen pork meatballs significantly inhibited the lipid oxidation and limited quantitative losses of lysine and methionine.

By taking into account the oxidative properties of hydrogen peroxide produced by milk fermentation bacteria in ripening products, the authors attempted to use starter cultures of bacteria producing catalase. Catalase is an enzyme with antioxidant properties, able to break down hydrogen peroxide to oxygen and water. The ability to produce catalase and superoxide dismutase by *S. carnosus* and *S. xylosus* strains was described by Barriére et al. [2001]. Until the last decade, lactic acid bacteria were regarded as catalase-negative. However, Igarashi et al. [1996] demonstrated that certain bacterial strains from the *Lactobacillus*, *Pediococcus* and *Leuconostoc* genera are capable of producing catalase [Igarashi et al. 1996].

Valencia et al. [2006] studied the effect of different packaging methods (oxygen, vacuum, modified atmosphere) of ripening sausage added with linseed oil and BHA or BHT as an antioxidant on the product's oxidative stability. They found high levels of peroxides (approx. 120-200 meq O_2/kg) in the control sample without added linseed oil and stored in oxygen atmosphere. Storage of ripening sausage in vacuum or modified atmosphere has resulted in increased oxidative stability of raw sausage studied by Valencia et al. [2006], both after 2 and 5 months of storage. Packing sausage in a modified atmosphere or vacuum protected the product against the formation of toxic products such as hepta-2,4-dienal [Valencia et al. 2006].

SUMMARY

The growth and development of the saprophytic and pathogenic microflora, the enzymatic and non-enzymatic lipid peroxidation and connected with them proteine oxidation, losses of exogenous amino, as well as the presence of biogenic amine become the most important risk factors in production and storage period of raw fermented meat products.

It appears essential to carry out research not only oriented towards product sensoric assesment and probiotic strain identification but also towards the original and secondary lipid and protein oxidation markings and the content of exogenous amino acids and the attempts to determine their mutual interactions in fermented meat product.

REFERENCES

Adams A., Kitrytė V., Venskutonis R., De Kimpe N., 2009. Formation and characterisation of melanoidin-like polycondensation products from amino acids and lipid oxidation products. Food Chem. 115, 904-911.

Aguirrezábal M.M., Mateo J., Dominguez M.C., Zumalacárregui J.M., 2000. The effect of paprika, garlic and salt on rancidity in dry sausages. Meat Sci. 54, 77-78.

Aragon-Alegro L.C., Alegro J.H.A., Cardarelli H.R., Chiu M.C., Saad S.M.I., 2007. Potentially probiotic and synbiotic chocolate mousse. LWT-Food Sci. Technol. 40, 669-675.

Arihara K., 2006. Strategies for desinging novel functional meat products. Meat Sci. 74, 219-229.

Berthold A., Nowosielska D., 2008. Aminy biogenne w żywności [Biogenic amines in food]. Medycyna Wet. 64 (6), 745-748 [in Polish].

Baraniak B., Szymanowska U., 2006. Lipooksygenaza w żywności pochodzenia roślinnego [Lipoxygenase in food of plant origin]. Żywn. Nauk. Technol. Jakość 2 (47), 29-45 [in Polish].

Barriére C., Centeno D., Lebert A., Leroy-Sétrius S., Berdagué J.L., Talon R., 2001. Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. FEMS Microbiol. Lett. 201, 181-185.

Bartosz G., 2006. Druga twarz tlenu. Wolne rodniki w przyrodzie [The other face of oxygen. Free radicals in nature]. Wyd. Nauk. PWN, Warszawa [in Polish].

Bozkurt H., 2006. Utilization of natural antioxidants: Green tea extract and *Thymbra spicata* oil in Turkish dry-fermented sausage. Meat Sci. 73, 442-450.

Bozkurt H., Erkmen O., 2002. Effects of starter cultures and additives on the quality of Turkish style sausage (sucuk). Meat Sci. 61, 149-156.

Carlsen C.U., Møller J.K.S., Skibsted L.H., 2005. Heme-iron in lipid oxidation. Coordin. Chem. Rev. 249, 485-498.

Chelh I., Gatellier P., Santé-Lhoutellier V., 2007. Characterisation of fluorescent Schiff bases formed during oxidation of pig myofibrils. Meat Sci. 76, 210-215.

Dolatowski Z.J., Jachacz L., Kołożyn-Krajewska D., 2010. Stabilność oksydacyjna modelowego produktu mięsnego z dodatkiem naparu herbaty [Oxidative stability of model meat product with tea infusion]. Jakość i bezpieczeństwo żywności wyzwaniem XXI wieku. Ed. T. Sikora. Kraków, 26-35 [in Polish].

Estévez M., Cava R., 2006. Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: Contradictory effects in different types of frankfurters. Meat Sci. 72, 348-355.

Faustman C., Liebler D.C., McClure T.D., Sun Q., 1999. Alpha, beta-unsaturated aldehydes accelerate oxymyoglobin oxidation. J. Agr. Food Chem. 47, 3140-3144.

Faustman C., Specht S.M., Malkus L.A., Kinsman D.M., 1992 a. Pigment oxidation in ground veal: Influence of lipid oxidation, iron and zinc. Meat Sci. 31, 351-362.

Faustman C., Yin M., Nadeau D., 1992 b. Color stability, lipid stability and nutrient composition of red and white veal. J. Food Sci. 57 (302-304), 311.

Faustman C., Wang K.W., 2000. Potential mechanisms by which vitamin E improves oxidative stability of myoglobin. Antioxidants in muscle foods. Nutritional strategies to improve quality. Ch. 6. John Wiley, New York.

Fellenberg M.A., Speisky H., 2006. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. World Poultry Sci. J. 62, 53-70.

Haurowitz F., Schwerin P., Yenson M.M., 1941. Destruction of hemin and hemoglobin by the action of unsaturated fatty acids and oxygen. J. Biol. Chem. 140, 353-359.

Hernández P., Navarro J.L., Toldrá F., 1999. Lipolytic and oxidative changes in two Spanish pork loin products: dry-cured loin and pickled-cured loin. Meat Sci. 51, 123-128.

Hęś M., Korczak J., 2007 a. Wpływ różnych czynników na szybkość utleniania się lipidów mięsa [The influence of different factors on the kinetics of the lipid oxidation in meat]. Nauka Przyr. Technol. 1, 1, #3 [in Polish].

Hęś M., Korczak J., 2007 b. Wpływ produktów utleniania lipidów na wartość odżywczą białka [The effect of lipid oxidation products on the nutrition value of proteins]. Nauka Przyr. Technol. 1, 1, #4 [in Polish].

Hęś M., Korczak J., Gramza A., 2007. Changes of lipid oxidation degrees and their nfluence on protein nutritive value of frozen meat products. Pol. J. Food Nutr. Sci. 57, 3, 323-328.

Igarashi T., Kono Y., Tanaka K., 1996. Molecular cloning of manganese catalase from *Lactobacillus plantarum*. J. Biol. Chem. 271, 29521.

Jaworska D., Neffe K., Kołożyn-Krajewska D., Dolatowski Z., 2011. Survival during storage and sensory effect

of potential probiotic lactic acid bacteria *Lactobacillus acidophilus* Bauer and *Lactoabcillus casei* Bif3'/IV in dry fermented pork loins. Int. J. Food Sci. Tech. doi: 10.1111/j.1365-2621.2011.02772.x.

Jin G., Zhang J., Yu X., Lei Y., Wang J., 2011. Crude lipoxygenase from pig muscle: Partial characterization and interactions of temperature, NaCl and pH on its activity. Meat Sci. 87, 257-263.

Kanner J., Harel S., 1985. Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. Arch. Biochem. Biophys. 237, 314-321.

Kołożyn-Krajewska D., Dolatowski Z., Zielińska D., 2011. Risk assessment of probiotic use particularly in meat products – a review. Fleischwirtschaft International 26, 61-68.

Kordiovská P., Vorlová L., Borkovcová I., Karpíšková R., Buchtová H., Svobodová Z., Křížek M., Vácha F., 2006. The dynamics of biogenic amine formation in muscle tissue of carp (*Cyprinus carpio*). Czech J. Anim. Sci. 51, 262-270.

Lizaso G., Chasco J., Beriain M.J., 1999. Microbiological and biochemical changes during ripening of salchichón, a Spanish dry cured sausage. Food Microbiol. 16, 219-228.

Marcinčák S., Nemcová R., Sokol J., Popelka P., Gancarčíková S., Švedová M., 2009. Impact of feeding of flaxseed and probiotics on meat quality and lipid oxidation process in pork during storage. Slov. Vet. Res. 46 (1), 8-13.

Martín-Sánchez A.M., Chaves-López C., Sendra E., Sayas E., 2011. Lipolysis, proteolysis and sensory characteristics of a Spanish fermented dry-cured meat product (salchichón) with oregano essential oil used as surface mold inhibitor. Meat Sci. 89 (1), 35-44, doi: 10.1016/j.meatsci.2011.03.018.

Morita H., Sakata R., Nagata Y., 1998. Nitric Oxide Complex of iron (II) myoglobin converted from metmyoglobin by *Staphylococcus xylosus*. J. Food Sci. 63 (2), 352-355.

Morrissey P.A., Sheehy P.J.A., Galvin K., Kerry J.P., Buckley D.J., 1998. Lipid stability in meat and meat products. Meat Sci. 49, 73.

Navarro J.L., Nadal M.I., Izquierdo L., Flores J., 1997. Lipolysis in dry cured sausages as affected by processing conditions. Meat Sci. 45, 161-168.

Nowaczyk A., Dolatowski Z.J., 2010. Właściwości fizykochemiczne baleronów surowo dojrzewających z dodatkiem probiotyku [Physiochemical properties of raw-cured pork shoulder with the addition of probiotic]. In: Probiotyki w żywności. Eds D. Kołożyn-Krajewska, Z.J. Dolatowski. Kraków, 103-121 [in Polish].

O'Gready M.N., Monahan F.J., Brunton N.P., 2001. Oxymyoglobin oxidation and lipid oxidation in bovine muscles – mechanistic studies. J. Food Sci. 66, 386-392.

Ong L., Shah N.P., 2009. Probiotic Cheddar cheese: Influence of ripening temperatures on survival of probiotic microorganisms, cheese composition and organic acid profiles. LWT-Food Sci. Technol. 42, 1260-1268.

Pikul J., Leszczyński D.E., Kummerow F.A., 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J. Agr. Food Chem. 37, 1309.

Rhee K.S., Ziprin Y.A., 1987. Lipid oxidation in retail beef, pork and chicken muscles as affected by concentrations of heme pigments and nonheme iron and microsomal enzymic lipid peroxidation activity. J. Food Biochem. 11, 1-15.

Skibsted L.H., 1996. Chemical changes in meat and meat products during storage, transportation and retail display – theoretical considerations. In: Meat quality and meat packaging. Eds S.A. Taylor, A. Raimundo, M. Severini, F.J.M. Smulders. ECCEAMST Utrecht, The Netherlands, 169-181.

Skwarek M., Dolatowski Z.J., 2010. Właściwości fizykochemiczne szynek surowo dojrzewających z dodatkiem probiotyku [Physiochemical properties of raw ripening hams with the addition of probiotic]. Acta Sci. Pol. Biotechnol. 9 (1), 15-24 [in Polish].

Stadnik J., Dolatowski Z.J., 2010. Biogenic amines in meat and fermented meat products. Acta Sci. Pol., Technol. Aliment. 9 (3), 251-263.

Summo C., Caponio V.M., Pasqualone A., 2006. Effect of vacuum-packaging storage on the quality level of ripened sausages. Meat Sci. 74, 249-254.

Summo C., Caponio F., Paradiso V.M., Pasqualone A., Gomes T., 2010. Vacuum-packed ripened sausages: Evolution of oxidative and hydrolytic degradation of lipid fraction during long-term storage and influence on the sensory properties. Meat Sci. 84, 147-151.

Suzzi G., Gardini F., 2003. Biogenic amines in dry fermented sausages: a review. Int. J. Food Microbiol. 88, 41-54.

Szebeni J., Winterbourn C.C., Carell R.W., 1984. Oxidative interactions between haemoglobin and membrane lipid. Biochem. J. 220, 685-692.

Valencia I., Ansorena D., Astiasarán I., 2006. Stability of linseed oil and antioxidants containing dry fermented sausages: A study of the lipid fraction during different storage conditions. Meat Sci. 73, 269-277.

Viljanen K., 2005. Protein oxidation and protein-lipid interactions in different food models in the presence of berry phenolics (dissertation). EKT series 1342. Depart. Appl. Chem. Microbiol. Univ. Hels., Helsinki, 87+36.

Zanardi E., Dorigoni V., Badiani A., Chizzolini R., 2002. Lipid and colour stability of Milano-type sausages: effect of packing conditions. Meat Sci. 61, 7-14.

Zakrys P.I., Hogan S.A., O'Sullivan M.G., Allen P, Kerry J.P., 2008. Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. Meat Sci. 79, 648-655.

Zhang X., Kong B., Xiong L., 2007. Production of cured meat color in nitrite-free Harbin red sausage by *Lactobacillus fermentum* fermentation. Meat Sci. 77, 593-598.

Zhou G.H., Zhao G.M., 2007. Biochemical changes during processing of traditional Jinhua ham. Meat Sci. 77, 114-120.

STABILNOŚĆ OKSYDACYJNA MIĘSNYCH WYROBÓW SUROWYCH DOJRZEWAJĄCYCH

STRESZCZENIE

Mięso i jego przetwory – będące podstawową częścią naszej diety – są szczególnie podatne na zmiany jakości wynikające z procesów oksydacji. Jednym z ważniejszych założeń procesów technologicznych jest ochrona przed utlenianiem składników mięsa, głównie lipidów. Kształtowanie jakości żywności fermentowanej zależy od przebiegu różnorodnych procesów fizykochemicznych i biochemicznych. Utlenianie składników mięsnych wyrobów surowych dojrzewających może być wynikiem przemian enzymatycznych, które zachodzą na skutek aktywności enzymów pochodzenia tkankowego oraz mikrobiologicznego, jak również procesu wolnorodnikowej peroksydacji lipidów. Pierwotne i wtórne produkty utleniania tłuszczów są bardzo reaktywne i wchodzą w reakcje z innymi składnikami mięsa, zmieniając ich właściwości fizyczne i chemiczne. Białka jako związki utlenione przybierają kolor żółty, czerwony czy brązowy. Produkty degradacji lipidów i białek tworzą specyficzny smak i aromat. Jednocześnie powstają substancje toksyczne, jak aminy biogenne, lub nowe substancje w wyniku interakcji składników mięsa – np. połączenia białko-tłuszcz lub białko-białko, a także wiązania poprzeczne w strukturach białkowych. Utlenianie składników mięsa w wyrobach surowo dojrzewających jest procesem szczególnie trudnym. Z jednej strony jest on pożądany, gdyż w wyniku enzymatycznego i nieenzymatycznego utleniania lipidów powstają związki smakowo-zapachowe charakterystyczne dla wyrobów dojrzewających, z drugiej jednak strony nadmiar i przekształcenia tych związków mogą prowadzić do zagrożenia bezpieczeństwa zdrowotnego mięsnej żywności fermentowanej.

Słowa kluczowe: probiotyki, wyroby mięsne, przeciwutleniacze, stabilność oksydacyjna, barwa

Received – Przyjęto: 22.07.2011 Accepted for print – Zaakceptowano do druku: 15.11.2011

For citation – Do cytowania

Wójciak K.M., Dolatowski Z.J., 2012. Oxidative stability of fermented meat products. Acta Sci. Pol., Technol. Aliment. 11(2), 99-109.