THE STUDY OF FACTORS AFFECTING THE ACTIVITY OF MEAT ANTIOXIDANT SYSTEM

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Abstract: Oxidation of lipids and myoglobin in raw meat are interrelated processes that affect the overall meat quality. The intensity of oxidation processes in meat raw material is regulated by its own antioxidant system (catalase, peroxidase, glutathione, etc.), the activity of which should be considered in the development of new technological solutions. Oxidation of lipids and myoglobin, directly affect the quality and safety of meat products, and reducing of the intensity of these processes contributes to the life time of raw meat, as well as that of finished products. The paper presents the study results of the salt curing mixture, including combination with yeast extract, affecting on the activity of the antioxidant system of the main types of raw meat - pork and beef. The basic systems, minced pork and beef being subjected to salting with curing salt (sodium chloride) and curing mixture consisting of 70% sodium chloride and 30% of composition KCl + $CaCl_2$ at the ratio of 1:1 are investigated. The influence of curing mixture on the intensity of oxidation of lipids and myoglobin of raw meat of different species is stated. It is found, that reducing the amount of sodium chloride in curing composition of the mixture brings down oxidative changes of heme pigments and meat lipids. Introduction of yeast extract into raw meat, in the amount of 2% enhances the inhibitory effect on oxidation in raw minced meat.

Keywords: Antioxidant system, antioxidant enzymes, catalase, peroxidase, meat, myoglobin, metmyoglobin, lipids, oxidation, yeast extract

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INTRODUCTION

The degree of lipids oxidation has a considerable influence on the formation of sensory, functional characteristics, nutritional value and safety of meat products. As a result of lipid oxidation, firstly, the accumulation of peroxides occurs, and also that of aldehydes and ketones, the presence of which adversely affects the security of raw materials; secondly, the degree of digestibility and protein content of essential fatty acids, amino acids, vitamins is reduced, affecting biological value; thirdly, the decrease of protein solubility, change of color, taste and odor is stated [1, 2, 6].

Peroxidation is the result of interaction of organic compounds, and molecular oxygen to form hydroperoxides and reactive free radicals. In muscle tissue polar and nonpolar lipids are subjected to oxidation and to a greater extent - phospholipids in membranes of muscle fibers, the composition of which contains the polyunsaturated fatty acids. The process of lipid oxidation begins immediately after slaughter and it is the result of the imbalance between pro-oxidant and antioxidant systems of raw meat [3, 4, 5, 8].

The proper antioxidant meat system includes enzymatic and non-enzymatic systems. Catalase, glutathione peroxidase, superoxide dismutase are distinguished from endogenous antioxidant enzymes. Their activity depends on the presence of antioxidants such as tocopherols, ascorbic acid, ubiquinone, glutathione, etc. [6].

Endogenous antioxidant enzymes, especially catalase and glutathione peroxidase can potentially inhibit the development of oxidation processes during storage of raw meat. Glutathione peroxidase is selenium-containing enzyme able to recover almost all types of organic hydroperoxides, as well as to prevent the accumulation of secondary peroxidation products [17, 18]. Catalase - heme-containing enzyme - is able to use one molecule as an electron donor, and another one - as an oxidizer, i.e. electron acceptor. It is a basic primary antioxidant, which catalyzes the decomposition of hydrogen peroxide to water, by combining this function with glutathione peroxidase. Protoheme is presented in peroxidase prosthetic group that, unlike most of the hemeproteins of heme groups is very weakly bound to the apo-enzyme. In the reaction catalyzed by peroxidase, hydrogen peroxide is restored by the compounds serving as electron donors, such as ascorbate, or quinines or cytochrome C. This enzyme has a high specificity and effectively neutralizes several hydroperoxide compounds: methyl and etilgidroperoxide, methyl, ethyl and other aliphatic alcohols. The mechanism of peroxidase and the action of glutathione peroxidase is to supplement each other, providing protection from the effects of lipid peroxidation at the stage of chain reactions branching and the formation of secondary peroxide products [18]. Both enzymes implement detoxification of enzyme active oxygen radicals, the formation of hydrogen peroxide from superoxide being catalyzed.

In addition to differences in their substrate specificity, these two enzymes differ in substrate affinity. At low concentrations of hydrogen peroxide, organic peroxides are preferably catalyzed by peroxidase, whereas at high concentrations catalases work [19].

Activity of antioxidant endogenous enzymes depends on several factors: the type of animal raw material, its localization, physiological function, on which in its turn depends the amount of heme pigment (myoglobin, hemoglobin) being active prooxidants. This is confirmed by the results of both domestic and foreign scholars. According to the data obtained by O.L. Golostyuhina, I.V. Golovina, and T.B. Vakhtina, the highest antioxidant enzyme activity is observed in gills, liver and red muscles of flounder, while the white muscles of observed fish are characterized by minimal activity of catalase and peroxidase. P. Hernandez, A. Lopez, M. Marco, A. Blasco have stated that the activity of catalase in the tested rabbit meat is lower than that in beef, pork and chicken thigh, but higher than in chicken breast [20, 21, 18]. Thus, it may be confirmed, that antioxidant endogenous enzyme activity is closely correlated with the concentration of heme pigments in muscle tissue and provides the stability of oxidation and restoration processes, greatly influencing oxidation changes which occur in meat.

Myoglobin is a complex protein – heme protein which plays an important role in the provision of animals with oxygen necessary for muscle metabolism and is the main meat pigment responsible for color formation. In the process of energy generation and in relation to oxygen, heme proteins perform such functions as oxygen transportation and tissue depositing, catalytic oxidation of organic compounds, decomposition of hydrogen peroxide and transfer of electrons [15].

Quantitative myoglobin content depends on the species, breed, sex and age, keeping and feeding conditions of an animal, type of muscles and their level of muscle activity. The ratio of such forms of myoglobin as dezoximioglobin (Fe^{2+}), oxymyoglobin (Fe^{2+}) and metmyoglobin (Fe^{3+}) is presented in meat synchronically, thus determining its color. In the presence of atmospheric oxygen myoglobin is oxidized to form oxidized oxymyoglobin - MbO_2, which gives the meat a nice bright pink-red color. However, this compound is unstable and transferred from ferrous to ferric state, thereby metmyoglobin of brown- gray color is formed (MetMb). When salting the meat in the presence of salt, which is an additional pro-oxidant factor, myoglobin or oxymyoglobin is oxidized and transformed into metform. As a result, then salted meat loses its natural color and becomes brown with different shades.

Myoglobin is a predominant iron compound in muscle tissue. Thus iron content in the heme myoglobin is 73.3%, 47.0% of the total concentration of iron in beef and pork, respectively, to the share of low molecular weight fraction of non-heme iron, and depending on the type of meat, it fits 2.4–3.9% of the total iron content, on average.

Organic and inorganic iron compounds act as catalysts in various stages of lipid peroxidation. It should be noted that the mechanism of catalytic effect and free iron ions involved in action, bound iron and heme iron in the oxidation of lipids, remains controversial. Though Kanner et al argued that the main catalysts for the oxidation of lipids are meat-free iron ions. At the same time during the research of Johns it was found that all inorganic forms of iron have low pro-oxidant activity compared to the heme iron [1, 4, 7, 8, 9, 10, 11].

Nowadays, myoglobin is one of the most powerful catalysts of lipid oxidation in meat raw material, which occurs in a certain sequence. At the initial stage oxidation of oxymyoglobin takes place and it is transformed into metmyoglobin with hydrogen peroxide, while with the increase of metmyoglobin in raw meat amount the rate of oxidation of lipids in muscle tissue increases. Baron et al have found that the maximum metmyoglobin pro-oxidant effect is manifested at low pH of raw meat and in the presence of hydroperoxides, whereas at physiological meaning of pH in the presence of lipids neutralization of metmyoglobin is observed, non-catalytic heme pigments being formed [8, 14]. On the next stage metmyoglobin is activated by hydrogen peroxide to parfirrylkation-radical containing tetravalent iron that turns into a short-lived parfirryl-myoglobin, and then into the long-life ferryl-myoglobin under the influence of which, at the final stage an oxidation process is initiated by lipids:

$$\text{Metmyoglobin} + H_2O_2 \rightarrow {}^{\circ}\text{MbFe IV} = O \rightarrow$$
$$\rightarrow \text{MbFe IV} = O.$$

It should be noted that pro-oxidant effect of ferryl-myoglobin, unlike metmyoglobin does not depend on pH and concentration of raw meat lipids [1, 6, 11, 12]. This scheme allows us to say that the metmyoglobin is able to exercise "pseudoperoxidase" activity to form erryl-myoglobin, being peroxidase analog which is an active oxidizer with respect to ascorbate, glutathione, cysteine, tyrosine.

There are evident data [3, 4], indicating the influence of physical condition, the degree of stress of an animal prior to slaughter, rigor characteristics, changes in pH, temperature, duration and conditions of maturation, which may occur at the intensive exposure, such as electrical stimulation of the antioxidant reactivity of the pro-oxidant meat systems. The rate of oxidation of meat lipids and meat products is also significantly affected by the composition of raw meat, the fatty-acid composition of lipids, the content of endogenous two-valent ferrum, technological processing methods, including grinding, emulsification and heat treatment.

At the same time, the question about the impact of processing additives on the activity of the antioxidant system of meat remains underinvestigated. Among the additives conventionally used in the technology of meat, special attention deserves salt (sodium chloride), which promotes the formation of organoleptic characteristics of the finished products and increase of their storage capacities. However, it is known that, depending on the concentration of salt used, it can have both pro-oxidant and also antioxidant effect.

It should be noted that the mechanism of sodium chloride pro-oxidant effect is not understood enough, to assume that its oxidative effect may be the result of the influence of reactive chlorine ion on lipids and heme pigments of muscle tissue. Pro-oxidant activity of sodium chloride is associated with its ability to disrupt the integrity of cell membranes, thereby ensuring the free access of heme and non-heme iron to lipids in muscle tissue. Besides, sodium chloride may promote the formation of metmyoglobin, which reacts with hydrogen peroxide to form ferrylmioglobin, being the catalyst of lipid peroxidation.

Analysis of available publications has revealed that the impact of sodium chloride on oxidation of heme pigments, which indirectly affect the antioxidant enzymes, has been studied to more extent. However, available data suggest that the catalyzing effect of sodium chloride - depends on its concentration in raw meat. Some scientists have found, that the pro-oxidant effect of sodium chloride concentrations up to 3% on the unit weight of raw material has a profound effect, whereas increasing it above 3% has, in contrast, an inhibitory effect on lipid oxidation [4, 7]. As regards to the effect of sodium chloride on antioxidant enzymes there is a well- known work of Lee S. K. et al, who found that the activity of catalase, glutathione peroxidase, and superoxide dismutase in its presence is reduced by 8%, 32%, 27%, which may be a prerequisite for the acceleration of lipid peroxidation [25]. Therefore, one of the possible ways to reduce the pro-oxidant effect of sodium chloride the decrease of its introduction into the technology of meat products should be considered.

Production of meat foodstuffs with reduced salt, and therefore sodium, fully meets the modern trends in healthy eating. A modern consumer among a wide variety of food products gives increasing preference to products, which are able to exert a positive effect on health, which ones are the functional foods. Production of functional foods involves the enrichment of both a traditional product by functional food ingredients during the manufacturing process and the decrease in product content of the components, being able to have a negative impact on human health [30]. One of these components, in accordance with the recommendations of the WHO, the sodium chloride has been declared, excessive intake of which may contribute to the development, primarily, of cardiovascular diseases [27].

By reducing the amount of sodium chloride added into meat products it is necessary to consider accompanying aspects related to the possible decrease of functional properties in meat systems, stability of meat products against the development of microorganisms.

The results of the analysis of domestic and foreign scientific publications suggest that the problem of effect of low-level introduction of sodium chloride, including its combination with different classes of commonly used food products, the activity of the antioxidant system of meat in relation to its oxidation processes of heme pigments remains poorly understood.

The aim of the research was to study the activity of beef and pork antioxidant enzymes, depending on the duration of salting, curing of ingredients composition as factors influencing the ratio of concentrations of oxidized and non-oxidized forms of heme pigments and intensity of lipid oxidation processes.

OBJECTS AND METHODS OF RESEARCH

Taking into account that oxidation intensity of lipid muscular tissue depends on the activity of the antioxidant enzyme systems of meat, and endogenous factors such as heme pigment number, points for investigation included: total pigment content and their oxidized form - metmyoglobin, the activity of catalase and peroxidase enzymes.

The objects of the study were beef of the first grade and half-fat pork meat, stored frozen up to 3 months. Raw meat was thawed to the temperature of (-1÷+1)°C, minced and mixed with 3% of sodium chloride (Sample K). In its prototypes sodium chloride in amount of 30% was replaced by the compound KCl + $CaCl_2$ at the ratio of 1 to 1 (Sample A1). In order to enhance the effect of antioxidant in the test sample yeast extract has then been added in an amount of 2% to the amount of raw materials (Sample A2).

Yeast extract is a natural source of natural antioxidant - glutathione (Table 1).

Table 1. Characteristics of yeast extract (Group of companies "Protein Ingredients Technologies ", Moscow)

Index type	Index value
Appearance	Powder, yellow and dark-brown color of varying intensity
Smell and taste	Yeast extract, characteristic without outward odor and taste
Extraneous admixtures	Not allowed
Mass fraction of protein, %, not less	37.5
Ash mass fraction, %, not more	15 (contains no salt)
Moisture mass fraction, %, not more	6
Nitrogen content (Kjeldahl method),%, not less than	6
Protein nitrogen content,% not less	2.5
Solubility of a 2% solution, %	100
pH of a 2% solution	4.5–6.5

Glutathione serves as a cofactor of glutathione peroxidase and antioxidant of primary cells having low molecular weight, it can be considered as one of the major players of the antioxidant system, rather active against a wide range of free radicals and lipid peroxidation products (hydrogen peroxide, organic radicals and reactive hydroxyl radical) [22].

The prepared samples were kept in salting at the temperature of (0–4)°C for 48 hours with sampling after 24 hours. Depth of oxidative changes in the raw meat was determined in accordance with the change of peroxide and thiobarbituric number during salting.

Research methods

The content of total amount of the pigments according to the method of Lee B.J., Hendricks D.G., & Cornforth D.P., is based on the extraction of meat pigments by aqueous solution of acetone and by consequent measuring of the extract optical density on SF PE 5400UF spectrophotometer at a wave length of 640 nm against acetone hydrochloride [31].

Total pigment content was determined by the formula

$$X = A640 * 680, \quad (1)$$

where A640 - optical density of solutions at wave length of 640 nm.

Determination of metmyoglobin by method of Krzywicki et al. is based on the extraction of pigments by ice phosphate buffered solution with consequent measurement of the optical density of the solution at the wave lengths of 525, 545, 565, 572 nm [32].

Metmyoglobin content, % relative to the total pigment content was calculated by the formula

$$X = [-2.51*(A_{572}/A_{525}+0.777*(A_{565}/A_{572})+ +0.8*(A_{545}/A_{572})1.098]*100, \quad (2)$$

where A_{572} - optical density of solutions at a wave length of 572nm; A_{525} - optical density of the solution at a wave length of 525nm; A_{565} - optical density of the solutions at a wavelength of 565 nm; A_{545} - optical density of the solution at a wave length of 545 nm.

Determination of peroxidase activity by a colorimetric method is based on determining the rate of the oxidation reaction to benzidine oxidation, to formation of a blue color dyeing in the presence of peroxide and peroxidase [26]. A weighed sample of meat was minced in a porcelain mortar with cold acetate buffer (pH 5.0). The homogenate was centrifuged for 5 minutes. The resulting supernatant liquid was filtered through filter paper and the filtrate was used for the reaction. The reaction mixture consisted of 0.2 M Na-acetate buffer (pH 5.0), 0.01% hydrochloric acid solution of benzidine extract, 0.3% of hydrogen peroxide. For the obtained solutions the optical density was determined at a wave length of 590 nm during 120 sec. on the spectrophotometer SF PE 5400 UF.

Calculation of peroxidase activity in relative units per 1 mg of protein was performed by the formula

$$A = ((\Delta D/T)*X)/(L*C), \quad (3)$$

where A is the activity of enzyme; ΔD - change in optical density (optical density to be subtracted at the end of the reaction from the optical density at the initial time point); X - final dilution in the cell extracts (reaction mix volume divided by the volume of inserted extract); T-reaction time, sec.; L - thickness, cm; C - protein content in the sample, mg.

Determination of catalase activity by spectrophotometry is based on determining the rate of decomposition of hydrogen peroxide by catalase of the tested sample to form water and oxygen [13].

A weighed sample of muscle tissue was triturated in a mortar with chilled extraction buffer (50 mM of K, Na-phosphate buffer (pH 7.8)). The homogenate was centrifuged for 5 minutes. The supernatant liquid was filtered through a paper filter, and the filtrate was used for the reaction process. The reaction mixture consisted of 50 mM of K, Na-phosphate buffer (pH 7.0), of extract and 0.6 M of hydrogen peroxide. Catalase activity was measured due to the change of optical density at the wave length of 240 nm every second for 100 sec. on the spectrophotometer SF PE-5400UF

Calculation of catalase activity in relative units on 1 mg of protein was performed by the formula (3).

Oxidative damage of raw meat lipids by definition of peroxidation number (PN) was performed by a standard method using chloroform extract obtained by the method of Piulskaya, B. [28], and by determining of thiobarbituric number (TBN) by the modified distillation method of Tarlagie, B. using sulfanilic reagent [29].

RESULTS AND DISCUSSION

The degree of activity of antioxidant enzymes is one of the key factors influencing the rate of lipid oxidation in meat. At the initial stage the evaluation of raw meat antioxidant system activity has been presented. The research results are shown in Table 2.

Table 2. Indices of the antioxidant activity of raw meat ($p < 0.05$)

Raw material	Catalase activity, U/g	Activity of peroxidase, U/g	Total pigments mg/100g	Number of metmyoglobin, % of total pigments	PN, mmol ½ O_2/kg	TBN
Beef	324.8 ± 6.4	12.5 ± 0.45	568.6 ± 4.2	30.7 ± 0.98	2.25 ± 0.18	0.207 ± 0.045
Pork	195.0 ± 3.8	7.5 ± 0.31	329.6 ± 5.6	22.5 ± 0.65	2.42 ± 0.13	0.342 ± 0.034

Due to the reported data, the activity of antioxidant enzymes, namely catalase and peroxidase in beef is higher than in pork by an average of 60%. The results can be explained by the whole complex of various factors.

Firstly, higher enzyme activity in beef may be attributed to higher protein content, generally, sarcoplasmic proteins in particular, which are the enzymes under investigation. Secondly, the activity of antioxidant enzymes depends on the types of muscle fibers. Muscle fibers, depending on their chemical composition and activity of enzymes can be divided into two metabolic types: oxidative (red) and glycolytic (white). Oxidative muscles are characterized by high content of mitochondria and their myoglobin content is higher than in muscles of glycolytic type. Oxidation is

used mainly by muscles fatty acids as a substrate having low activity of AT phase and phosphorylase, and glycolytic muscles use primarily glycogen as a source of energy and obtaining greater activity of the latter enzymes. It is generally believed that the muscle oxidative type shows higher antioxidant activity of enzymes such as catalase than that of glycolytic muscles [17].

Thirdly, high catalase and peroxidase activity may be the result of greater hydrogen peroxide content in the meat of beef. The process of converting heme pigments makes some contribution to the accumulation of hydrogen peroxide. Nowadays, there is the hypothesis that the oxidation of myoglobin increases the amount of metmyoglobin, which by showing pseudoperoxidase activity is converted into ferryl-mioglobin, actively involved in the oxidation of lipids. This results in the formation of accumulated peroxide and hydroperoxide - a compound being a substrate for catalase and peroxidase [17]. The total amount of pigments in beef is found to be 568.6 mg/100g, while the content of metmyoglobin is 30.7% of the total amount of pigments that corresponds to 174.6 mg/100g. Based on this result the amount of myoglobin (the total amount of myoglobin, oxymyoglobin and dezoksimioglobin) is 394 mg/100g. While the content of heme pigments in pork is 329.6 mg/100g at the content of 22.5%, i. e. metmyoglobin - 74.16 mg/100g and myoglobin - 255.4 mg/100g, respectively. These results are consistent with the published data describing the content of myoglobin in different kinds of raw meat [14].

High catalase activity in both types of materials explains the fact, that enzymes have a low affinity to hydrogen peroxide, and thus play a leading role in its inactivation at high concentrations. In contrast to catalase, peroxidase has high affinity with peroxide, which explains the lower activity index values. However, higher peroxidase activity in beef is the result of the fact, that metmyoglobin participates in the process of utilization of hydrogen peroxide, the former having pseudoperoxidase activity.

Obviously, the number of primary (peroxide number - PN) and secondary products of lipid oxidation (thiobarbituric number – TBN) determines the greatest antioxidant activity in beef. It is stated, that in beef peroxidation and thiobarbituric numbers value is lower than those in pork, by 7% and 65%, respectively.

Subsequently, we studied the effect of curing mixture and duration of salting on the catalytic ability of peroxidase and catalase.

It has been established (Table 3) that the addition of sodium chloride for 24 hours and 48 hours of salting, reduces the peroxidase activity to 16% and 21.5% in beef, respectively, relative to raw unsalted meat. A similar relationship has been established in relation to pork, the loss of peroxidase activity is 20.3% and 24%, respectively, after 24 and 48 hours of salting.

Table 3. Change of catalase and peroxidase activity, depending on the composition of curing mixture and salting duration ($p < 0.05$)

Sample	Beef		Pork	
	24 hours	48 hours	24 hours	48 hours
Peroxidase activity, U / g				
sample K	10.50 ± 0.14	9.81 ± 0.64	5.98 ± 0.31	5.70 ± 0.24
sample A1	10.42 ± 0.36	9.95 ± 0.72	6.01 ± 0.28	5.86 ± 0.32
sample A2	10.54 ± 0.41	9.98 ± 0.51	6.10 ± 0.39	5.92 ± 0.18
Catalase activity, U / g				
sample K	319.0 ± 4.5	311.3 ± 6.9	181.4 ± 2.6	179.5 ± 2.5
sample A1	320.1 ± 3.9	316.5 ± 5.4	187.5 ± 3.7	185.5 ± 3.4
sample A2	322.6 ± 4.6	318.7 ± 3.8	1916.0 ± 4.8	189.7 ± 5.7

Reduced activity of catalase in beef treated with sodium chloride is stated from 1.8% to 4.3% with respect to unsalted raw meat, respectively, after 24 and 48 hours of salting, in salted pork it is stated 7.5% and 8%.

Replacing 30% of sodium chloride in the composition on premix ($KCl + CaCl_2$) of curing mixture (sample A1) has a positive effect on the activity of the enzymes studied. Thus in sample A1 of beef increasing of peroxidase activity by 0.7% and 1.4% is observed relative to sample K at 24 hours and 48 hours, respectively. In samples A1 of pork peroxidase activity increased under salting relative to sample K in 24 hours and 48 hours, respectively from 0.5% to 2.8%.

A more pronounced increase of activity of catalase relative to sample K is observed in the pork sample A1, so the activity increase after 24 hours has been stated - 3.36%, after 48 hours - 3.34%, whereas in the beef sample A1 increased activity relative to the sample treated with sodium chloride, is 0.34% and 1.6% after 24 and 48 hours, respectively. Despite this, in general, catalase activity in samples of beef remains at a higher level as compared with pork.

Enhance of antioxidant enzyme reactivity in raw meat is facilitated by the use of yeast extract during salting, which is confirmed by the results obtained (Table 3). So peroxidase activity in beef has increased in sample K to 0.4% and 1.7% in the period under salting. In turn, the increase in catalase activity is 1.1% and 2.3% relative to sample K at 24, and 48 hours of salting. A similar dependence is observed for samples A2 of pork. The resulting dependence is explained by the fact that glutathione, as a substrate, in particular of true peroxidases, enhances their activity. [6]

The results of studies suggest a greater stability of catalase in the presence of chlorine-containing salts, which is consistent with available data in the literature [16].

Taking into account, that the heme pigments may have both an enhancing effect on the antioxidant system of raw meat, and be a catalyst for lipid peroxidation, the effect of salting on processes of meat pigments transformation has been studied (Table 4).

Table 4. Change in the number of heme pigments in meat according to curing composition of mixture and salting duration ($p < 0.05$)

Sample	Beef		Pork	
	24 hours	48 hours	24 hours	48 hours
Total pigments mg/100g				
sample K	565.8 ± 8.6	561.0 ± 7.1	318.5 ± 6.6	316.8 ± 7.6
sample A1	567.3 ± 7.3	562.3 ± 6.5	321.1 ± 5.9	319.6 ± 6.4
sample A2	567.9 ± 6.9	563.0 ± 6.8	320.6 ± 6.3	318.1 ± 7.8
Number of metmyoglobin,% of total pigments				
sample K	46.1 ± 1.3	50.8 ± 1.6	34.0 ± 1.2	36.7 ± 1.7
sample A1	45.1 ± 2.1	49.9 ± 1.4	33.7 ± 1.8	35.8 ± 1.2
sample A2	43.6 ± 1.6	48.6 ± 1.9	32.4 ± 1.6	34.6 ± 1.5

It is found that the composition of curing mixture and salting period does not affect the content of the total number of pigments, which remains almost constant throughout the period of salting for all the samples, the changes in the content of common pigments are within the limits of experimental error. However, it should be noted, that with the constant amount of total pigments, the quantitative changes are observed at the ratio of various forms of myoglobin.

According to the presented results, salting period for 24 hours is followed by an increase in the amount of metmyoglobin in sample A1 of beef and is stated 15.4% and 20.1% after 48 hours relative to that of raw meat unsalted, that of salted pork - from 11.5% to 14.2%, respectively. The data obtained are the result of influence of chlorine ions on myoglobin.

Reducing of the amount of sodium chloride in the composition of curing mixture on 30% decreases the amount of irreversibly oxidized form of myoglobin. So in sample A1 of beef regarding to sample K the number of metmyoglobin increases by 1% and 0.9% in the studied period of salting, and in samples of pork - by 0.3% and 0.9%, respectively.

In the systems observed the presence of the yeast extract has an additional inhibitory effect on the oxidation action of myoglobin. It has been found that in samples A2 of beef metmyoglobin number decreases by 2.5% and 2.2%, relative to the sample K - after 24 and 48 hours of salting. Similar values of metmyoglobin content are characteristic to the system of samples being used on the basis of pork. Reducing of the amount of metmyoglobin in test samples A1 and A2 may be the result of a greater activity of catalase and peroxidase.

Reducing of the amount of metmyoglobin is a positive precondition for the stabilization of lipid peroxidation. There is an assumption that the intensity of the lipid oxidation depends on the level of heme pigments in raw material. Since high content of oxymyoglobin and metmyoglobin promotes formation of hydrogen peroxide, and as a consequence, of ferrylmioglobin, these compounds have a strong pro-oxidant action. In this connection it will be of interest to study the effect of antioxidant enzyme activity and content of heme pigments on lipid peroxidation, depending on the composition of curing mixture.

The intensity of the formation of primary oxidation products has been evaluated by the change in peroxide number (PN).

Table 5. Dynamics of peroxide number changes in the process of salting ($p < 0.05$)

Duration of salting	PN mmol ½ O_2/kg		
	Sample K	Sample A1	Sample A2
Pork			
24 hours	4.02 ± 0.05	3.75 ± 0.02	3.53 ± 0.09
48 hours	4.69 ± 0.08	3.53 ± 0.06	4.02 ± 0.06
Beef			
24hours	3.98 ± 0.06	3.69 ± 0.04	3.41 ± 0.07
48 hours	4.53 ± 0.03	4.12 ± 0.07	3.94 ± 0.04

According to the study (Table 5) sodium chloride catalyzes the oxidative processes of lipids, while introduced in raw meat, since the value of PN relative to raw unsalted meat after 24 hours in samples K increases by 76.8% and 101.3%, for beef and pork - in 48 hours after salting by 66.1% and 93.8%, respectively.

Replacement of 30% of sodium chloride on premix ($KCl + CaCl_2$) in the curing mixture composition, helped to reduce the rate of oxidation. So in samples A1 of beef value PN relative to animal raw material increased by 64.0% and in pork - 54.9%. Over the next 24 hours of salting the number of primary oxidation products increased by 83.1% and 75.1% for beef and pork, respectively.

It has been found that the introduction of the minced yeast extract into the composition helps to reduce the intensity of accumulation of oxidation products. Thus, in sample A2 value of PN in beef decreased after 24 hours relative to the samples K and A1 - by 14.3% and 7.5%, to pork samples, - 12.1% and 5.8%, respectively. A similar dependence is retained after 48 hours of salting. The results are explained by the fact that yeast extract is a source of glutathione, which in turn is a low molecular weight antioxidant, able to carry out an independent antioxidant effect.

On the whole, the findings suggest the more intensive process of oxidation in the samples of beef, that may be the result of a greater heme pigment content in raw material [6, 11, 18]. In this case, the

absolute values of the test index for pork samples throughout the process of salting remain higher than in beef samples, which is the result of a greater content of fatty tissue. At the same time at the end of the term of salting the PN of all the samples taken under conditions is consistent with hygienic standards - no more than 10 mmol O_2/kg.

The transformation of primary products of oxidation into secondary oxidation products causes a further acceleration of oxidation. The intensity of secondary oxidation products formation was assessed by determining the number of thiobarbituric (TBN), which reflects the amount of malondialdehyde formed (Fig. 1).

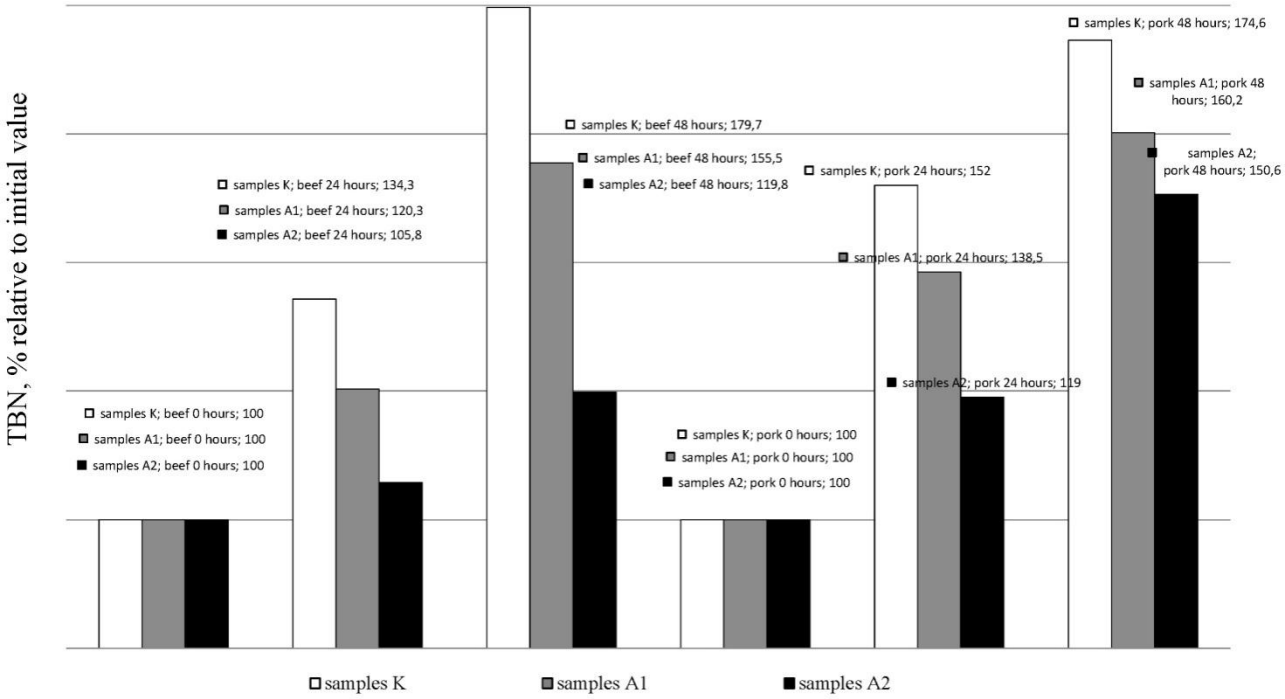

Fig. 1. Dynamics of TBN, of the samples in the process of salting.

According to the study results, the accumulation of products of secondary fat breakdown in the control samples is more intensive. Thus, after 24 hours of salting TBN increased by 34.3%, after 48 hours - 79.7% in the samples of beef and - 52.0% and 74.6% in the samples of pork, respectively.

Reducing the amount of salt in the composition of salting mixture and the presence of yeast extract helped to reduce the rate of formation of secondary oxidation products in the samples being based on beef and pork, which is consistent with the results of determination of TBN.

Oxidative stability of raw meat is the result of lipids and of myoglobin oxidation affecting each other. The intensity of these processes is regulated by its own antioxidant system, the activity of which depends on many factors, including the conditions of salting.

Analysis of the results suggests that beef is characterized by a more balanced ratio of active antioxidant complex. Peroxidase and catalase activity of beef is higher than that of corresponding enzymes in pork - by 60%. Reduction in traditional curing mixture of sodium chloride content by 30% contributes to the stabilization of antioxidant activity of the enzymes studied, which is reduced with respect to the initial values of 20.4% and 2.5% after 2 days of ripening in salting medium, whereas the use of sodium chloride is up to 21.5% and 4.1%, respectively. Combining of the curing mixture with a reduced content of sodium chloride and yeast extract does not lead to increase of antioxidant enzyme activity relative to the animal raw material, but contributes to further stabilization of the antioxidant system and reducing the intensity of lipids and processes of meat pigments oxidation. The obtained dependences are similar for both types of raw materials.

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