STUDY OF PROCESSES OF OXIDATION OF LIPIDS AND PROTEINS OF HALF-SMOKED SAUSAGES AT THE STAGES OF TECHNOLOGICAL PROCESSING DEPENDING ON THE COMPOSITION OF CURES

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Abstract: The formation of qualitative characteristics of sausages is significantly effected by the oxidizing processes of fatty and protein fraction of meat raw materials. The orientation and intensity of processes depends both on the type of used raw materials and nutritional supplements and the parameters of each stage of technological process. The decrease in intensity of processes of peroxide oxidation of lipids is aimed at the increase in safety of ready-made products and lengthening of terms of their storage. This article presents the results of researches of effect of composition of cures on the anti-oxidizing potential of meat raw materials at the stage of salting and on the dynamics of oxidizing processes in half-smoked sausages in the course of cold storage. The properties of source raw materials, pork and beef and the properties of the combined mincemeat subjected to salting by salt and cures consisting of 70% of chloride of sodium and 30% of the composition of $KCl+CaCl_2$ in the ratio of 1 : 1 and also with the addition of yeast extract are studied. The effect of conditions of salting on the intensity of oxidizing changes of lipid fraction and haem pigments in half-smoked sausages within 20 days of storage at a temperature of (2–6)°C is established. It is established that the decrease in the amount of salt as part of cures provides an increase in the activity of antioxidant enzymes of meat raw materials and, as a result, a decrease in the intensity of processes of oxidation of lipids and haem pigments. The addition of yeast extract to the weight of raw materials in the amount of 2% provides the strengthening of inhibiting effect on oxidation processes.

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

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INTRODUCTION

The oxidation of lipids of muscular tissue and proteins is the interconnected processes that have an effect on the organoleptic characteristics and nutrition value of meat and meat products. It is possible to claim that the decoloration of meat and the duration of storage are considerably caused by the complex of reactions between lipids and pigments. The rate of oxidizing changes of lipids of meat depends on a lot of external factors, including the parameters of technological processing, a packaging method, storage temperature and the use of antioxidants. The antioxidant system which consists of antioxidant enzymes and also of such components as glutathione, ascorbic acid, tocopherols, carotenoids, coenzyme Q 10 and others belongs to the internal protective factors which effectively protect the lipids of muscular tissue from oxidation. The most significant antioxidant enzymes of muscular tissue are catalase, peroxidase, superoxide dismutase and glutathione peroxidase which form an intracellular barrier for free radicals [1, 2, 3, 4, 5].

Catalase is a haem-containing enzyme which catalyzes hydrogen peroxide disintegration according to the following reaction $H_2O_2 \rightarrow 2H_2O+O_2$. The disintegration of hydrogen peroxide inhibits the processes of oxidation of oxymyoglobin and prevents the formation of H_2O_2-activated methmyoglobin which is considered as one of the important factors of lipidic oxidation of meat raw materials during storage [9].

The enzyme peroxydase has the same mechanism of action as catalase and is characterized by high specificity concerning such hydroperoxide compounds as methyl- and ethylhydroperoxide and methyl, ethyl and other alcohols. A protohaem which, unlike the haem groups of the majority of haemproteins, is very poorly bound with an apoenzyme is a prosthetic group in peroxidase. In the reaction catalyzed by the peroxidase $2H_2O_2+AH_2 \rightarrow 2H_2O+A$, hydrogen peroxide is reduced due to the bonds acting as the

donors of electrons, such as an ascorbate, quinones or cytochrome C [9, 15].

Peroxidase, as well as catalase, also performs the detoxication of active oxygen radical, with the formation of hydrogen peroxide of superoxide, at the same time they differ with the affinity to a substratum. In case of a low content of hydrogen peroxide, organic peroxides are mainly catalyzed by peroxidase, whereas catalases act at high concentrations [16].

Superoxide dismutase is a tetrameric protein consisting of four identical subunits, each of which contains one residue of selenocysteine which catalyzes the metabolism of hydrogen peroxide and a series of organic hydroperoxides, including cholesterin. Superoxide dismutase is an enzyme conjugated with catalase. There are conformational changes of oxymyoglobin in meat raw materials during the postlethal period as a result of change of pH and development of peroxide oxidation, as a result of which an anion radical with the formation of methmyoglobin is formed. The formed superoxide-anion is neutralized effected by superoxide dismutases according to the following reaction: $O_2+2H_2O \rightarrow H_2O_2$ [5, 7, 8, 10].

In turn, according to the mechanism of action, peroxidase and glutathione peroxidase supplement each other, providing the protection against the effect of peroxide oxidation of lipids at the stage of branching of chain reactions and formation of secondary peroxide products [17].

Glutathione peroxidase is a selenium-containing enzyme capable to inhibit and prevent oxidizing reactions in muscular tissue both during lifetime and during the postlethal period by the control of formation of free radicals of the hydroperoxides contained in raw materials [13]. The enzyme glutathione peroxidase provides the disintegration of active forms of oxygen including that of hydrogen peroxide, as a result of several consecutive reactions. At the first stage there is the oxidation of selenium-anions E-Se or E-SeH which are two catalystically active forms of selenocysteine contained in glutathione peroxidase (Reaction 1).

$$E\text{-}SeH + H_2O_2 \rightarrow E\text{-}SeOH + H_2O. \quad (1)$$

After the oxidation of active center of glutathione peroxidase, an enzyme reacts with a reduced glutathione with the formation of the enzyme-glutathione complex (Reaction 2).

$$E\text{-}SeOH + GSH \rightarrow E\text{-}Se\text{-}SG + H_2O. \quad (2)$$

Further, the formed complex reacts with another molecule of glutathione which, being oxidized, reduces the enzyme glutathione peroxidase (Reaction 3).

$$E\text{-}Se\text{-}SG + GSH \rightarrow E\text{-}SeH + GSSG. \quad (3)$$

In turn, glutathione peroxidase reduces once again the oxidized glutathione in the presence of NADP [14].

Glutathione, being a cofactor of glutathione peroxidase, is capable to have an independent antioxidant effect as the main low-molecular antioxidant of cell. Glutathione (GSH) is a tripeptide γ-glutamylcysteinylglycine which contains an unusual peptide bond between an amino group of cysteine and a carboxygroup of side chain of glutamate. Meat raw materials are characterized by a sufficiently high content of glutathione from 50 mg/kg to 200 mg/kg (175–600 μmol/kg) which is present both in the oxidized and reduced form [1, 17, 19]. The sufficient solubility in water solutions provides the fast penetration of glutathione through a cellular membrane. It is active in relation to a wide range of free radicals and products of peroxide oxidation of lipids – hydrogen peroxide and organic radicals ROO, single oxygen and also an extremely reactive hydroxyl radical OH [15, 18].

Endogenous antioxidant enzymes, especially catalase, glutathine peroxidase and peroxidase, are potential inhibitors of peroxide oxidation of lipids of meat. The activity of antioxidant enzymes is determined by a lot of factors, including the type of meat, type of muscles, duration of cold storage, and also the presence of various nutritional supplements both decreasing and increasing their antioxidant potential.

The type of meat, type of muscles and their anatomic origin have an effect on the content of lipids in general and on the fatty acid composition of meat raw materials, as well. The scientific researches testify that phospholipids play an important part in the development of peroxide oxidation of lipids both in raw and thermally processed meat [20, 21, 22]. Thus, in case of storage of raw beef and pork in a frozen state, the value of thiobarbituric number was higher than in the frozen fowl, which is the result of high content of haem iron in it, as well. At the same time, it is established that thermally processed chicken meat is more susceptible to peroxide oxidation of lipids than beef and pork are. Thus, 90% of malonic aldehyde are formed of polynonsaturated fatty acids of phospholipid fraction of chicken meat and provide the formation of taste of rancidity. On this basis, it is possible to say that the intensity of peroxide oxidation of raw meat, first of all, effect the content of haem pigments and activity of catalase whereas the content of polynonsaturated fatty acids is the main factor that determines the lipid oxidation in meat products [3, 23, 24, 25].

The nutritional supplements which are traditionally applied in technology of meat products can decrease the antioxidant potential of enzymes. One of such supplements is salt (sodium chloride) which has a positive effect on the formation of functional and technological and flavoring characteristics of meat products, and also inhibits the development of spoilage microorganisms. At the same time, sodium chloride in the concentration, applied in the technology of meat products, accelerates the oxidation of myoglobin, having a negative impact on the color of raw meat, and also provides the oxidation of lipids [26]. The results of works by Lee S.K. and others testify that in the presence of 2% of salt the activity of catalase, glutathione peroxidase and superoxide dismutase in its presence decreases by 8%, 32% and 27%, which can provoke the peroxide oxidation of lipids [35]. Sarraga, Karreras and Regueiro, studying the effect of concentration of salt on the activity of glutathione peroxidase, established that in the samples with 2% of

sodium chloride the activity of enzyme was higher than that in the samples with 3% of salt [33]. Therefore, a decrease in the level of addition of sodium chloride in meat products should be considered as one of the possible methods of decrease in prooxidant effect of sodium chloride.

It is especially urgent in view of the fact that the overconsumption of table salt as a sodium source provides the development of cardiovascular diseases [28, 29]. Today the production of foodstuffs, including meat products, with the lowered content of sodium is one of the priority directions of food technology. This tendency can be realized by such methods as the decrease in the formulation quantity of salt, the partial or complete replacement of salt by other substitutes and the use of intensifiers of flavor and spices. Chlorides of potassium, calcium, more rarely – chloride of magnesium and ammonium [30, 31, 32] gained the widest spread as substitutes of salt.

The positive effect of substitutes of salt on the quality and safety of meat products can be due to their capability to affect on the activity of antioxidant enzymes in meat. The researches by Hamid Reza Gheisari and Hossien Motamedi established that the replacement of salt by potassium chloride has no significant effect on the activity of catalase and glutathione peroxidase. The most significant factor is the value of ionic force. Thus, during the increase in ionic force from 0.175 to 0.7 the activity of catalase in beef and fowl decreases, respectively, by 3.2% and 7.5%, whereas the activity of glutathione peroxidase decreases by 14.6% and 32%, respectively. Along with it, it is established that the absolute values of activity of antioxidant enzymes were higher in the samples of beef and fowl processed by potassium chloride [27]. Similar results are received by Hernandez and others [34].

Nevertheless, the analysis of available information testifies that the data about the factors effecting the activity of antioxidant enzymes in meat raw materials are not enough.

The purpose of their own researches was the study of effect of composition of salting mixtures on the activity of antioxidant enzymes as the factor of regulation of intensity of oxidation of lipids of sausage mincemeat in the course of salting and during the storage of sausages.

OBJECTS AND METHODS OF STUDY

The objects of research were second-grade beef and semifat pork stored in the frozen state no more than 3 months. The meat raw materials were defrozen up to the temperature (-1 ÷ +1)°C, crushed and mixed in the ratio of 1 : 1 with the addition of sodium chloride 3% (the control sample is Sample K). In the test samples 30% of sodium chloride were replaced by the mixture KCl+$CaCl_2$ in the ratio of 1 : 1 (test sample A1). For the purpose of strengthening the antioxidant effect, yeast extract in the amount of 2% was added to the weight of raw materials as a source of glutathione in another test sample (Sample A2). The prepared samples were cured at a temperature of (0–4)°C for 48 hours with sampling in 24 hours. Half-smoked sausages with the formation of artificial protein coat of mincemeat were produced of cured mincemeat. The depth of oxidizing changes was estimated in sausages by the value of thiobarbituric number within 20 days of cold storage.

Determination of activity of peroxidase using the colorimetric method based on the determination of rate of reaction of oxidation of benzidine before the formation of blue coloring of its oxidation in the presence of peroxide and peroxidase [36].

Determination of activity of catalase using the spectrophotometric method based on the determination of rate of disintegration of hydrogen peroxide by the catalase of studied sample with the formation of water and oxygen [37].

Determination of total of pigments using the method of Lee B.J., Hendricks D.G. and Cornforth D.P. based on the extraction of meat pigments by water solution of acetone and the subsequent measurement of optical density of extract using the spectrophotometer SF PE-5400UF with the wavelength of 640 nanometers concerning muriatic acetone [38].

Determination of content of methmyoglobin using the method of Krzywicki and others based on the extraction of pigments by ice phosphatic buffer solution with the subsequent measurement of optical density of solution with the lengths of waves of 525, 545, 565 and 572 nanometers [39].

Oxidizing spoilage of lipids of meat raw materials by the determination of thiobarbituric number (TBN) using the distillation modified method of Tarlagis B. with the use of a sulfanilic reagent [40].

Color characteristics of products in the system Lab using the non-destructive testing method with the use of spherical color comparator KTs-3, which can work in the mode of comparator and spectrophotometer. The preparation of samples consisted in the cutting of slices of samples of the correct form from 3 to 5 mm thick with a plain surface of cutoff and without emptiness. The measurement of intensity of light reflection from the measured samples throughout the whole visible range of wavelengths and the summing of intensity of light reflection in case of the selected ordinates during the operation in the mode of spectrophotometer are performed, which is achieved by the use of integrating light filters – orange, green and blue – which are fixed in the instrument. When operating automatically, the instrument calculates the values of chromaticity coefficients (X, Y, Z) in the presence of source C reproducing the conditions of day lighting. Taking into account the coordinates of chromaticity of the source (X_0, Y_0, Z_0), the calculation of color indicators in the system of Lab is performed using the following formulas:

$$L = 116 \cdot (Y/Y_0)^{0.33} - 16,$$

$$a = 500 \cdot \left[(X/X_0)^{0.33} - (Y/Y_0)^{0.33} \right],$$

$$b = 200 \cdot \left[(Y/Y_0)^{0.33} - (Z/Z_0)^{0.33} \right].$$

The color saturation or brightness is calculated using the following formula:

$$S = \left[(a)^2 + (b)^2 \right]^{0.5}.$$

Hue is determined using the formula:

$$H = arctg(b/a).$$

The integrated assessment of identity of coloring is performed on the basis of indicator of full color distinctions that are calculated using the following formula:

$$\Delta E = \left[(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2 \right]^{0.5},$$

where *L* is the lightness, *a* is the redness degree, *b* is the blueness degree.

The content of nitric oxide pigments is determined using the method based on the extraction of pigments by water solution of acetone of homogenized sample weight and the subsequent measurement of optical density of extract using a Spekol spectrophotometer in case of wavelength of 540 nanometers concerning an 80% water solution of acetone [43].

RESULTS AND DISCUSSION

The oxidation of lipids is a chain reaction which consists of the initiation, development and break of chain. The mechanism of oxidation proceeds under the influence of both internal and external factors, such as the concentration of prooxidants, endogenous bivalent iron, myoglobin, enzymes, pH, temperature, ionic force and the reaction of consumption of oxygen and fatty acid composition of meat.

The intensity of process of oxidation in meat raw materials is regulated by various endogenous antioxidatic factors, such as reducing bonds (for example, ascorbic acid), natural antioxidants (carnosine, anserine, glutathione, α-tocopherol, etc.) and antioxidatic enzymes, including catalase and peroxidase.

The antioxidant potential of meat raw materials was estimated by the activity of catalase and peroxidase, and also by the content of glutathione and haem pigments in raw materials (Table 1). According to the obtained data, the activity of catalase and peroxidase of beef is on average higher than in pork by 30% and is 325 U/g and 12.4 U/g for beef and 255 U/g and 7.5 U/g for pork, respectively.

The high activity of enzymes in beef is, on the one hand, a consequence of a higher content of contained proteins, including sarcoplasmic proteins which are antioxidant enzymes. The hyperactivity of catalase in the studied raw materials, unlike peroxidase, is due to the high affinity of this enzyme concerning a substratum which hydrogen peroxide is. The formation of hydrogen peroxide in meat raw materials also occurs as a result of the oxidation of haem pigments, in particular, of myoglobin which being oxidized, turns into ferrylmyoglobin and initiates the peroxide oxidation of lipids with the formation of peroxides and hydroperoxides. On the other hand, the higher content of muscle fibers of oxidizing type in beef characterized by a high quantity of myoglobin also provides a higher activity of antioxidant enzymes [41].

Glutathione peroxidase, except catalase and peroxidase, belongs to the antioxidant enzymatic system of meat. An enzyme is one of the main antioxidants of cell that allows to consider it as one of the important participants of antioxidant system, active in relation to a wide range of free radicals and products of peroxide oxidation of lipids (hydrogen peroxide, organic radicals and a reactive hydroxyl radical). The main cofactor of glutathione peroxidase is glutathione the quantity of which is 10.42 mg/100g in beef and 8.06 mg/100g which is coordinated with the available literary data [42].

According to the obtained experimental data the total of pigments in beef is 240.04 mg/100g, at the same time the content of methmyoglobin is 42.4% of total of pigments that corresponds to 101.7 mg/100g. Proceeding from it, the total quantity of myoglobin is 138.34 mg/100g. The total of haem pigments in the studied pork at the initial point of time was 108.3 mg/100g with the content of methmyoglobin of 49.9%, that is 54.1 mg/100g, and the quantity of myoglobin is 54.2 mg/100g. The obtained results are coordinated with the available literary data concerning the muscular tissue of different types of meat raw materials, including beef, broilers and flounder [3, 5, 6, 15].

The assessment of effect of conditions of salting on the activity of antioxidant enzymes of meat raw materials was performed at the following stage of researches (Table 2). The research was performed using combined forcemeat consisting of beef and pork in the ratio of 1 : 1.

Table 1. Characteristics of the antioxidant potential of meat raw materials

Name of food raw material	Activity of enzymes, U/g of protein		Glutathione, mg/100g	Pigments		
				Total, mg/100g	Methmyoglobin	
	catalase	peroxidase			%	mg/100g
Beef	325.0 ± 11.3	12.40 ± 0.61	10.42 ± 0.49	240.04 ± 11.40	42.4	101.70 ± 5.24
Pork	255.0 ± 9.2	7.50 ± 0.28	8.06 ± 0.18	108.30 ± 8.42	49.9	54.10 ± 2.87

Table 2. Effect of conditions of salting on the activity of catalase, peroxidase and the content of haem pigments

Test sample	Activity of peroxidase, U/g	Activity of catalase, U/g	Total of pigments, mg/100g	Quantity of methmyoglobin, mg/100g
unsalted mincemeat	10.10 ± 0.34	295.00 ± 11.40	139.40 ± 6.87	59.60 ± 1.45
24 hours of brine treatment				
Sample K	7.40 ± 0.28	282.00 ± 10.30	139.60 ± 5.48	67.66 ± 2.31
Sample A1	7.70 ± 0.26	289.00 ± 9.25	138.70 ± 6.12	56.36 ± 2.97
Sample A2	7.80 ± 0.34	291.00 ± 9.65	139.70 ± 5.67	53.96 ± 1.97
48 hours of brine treatment				
Sample K	6.10 ± 0.42	276.00 ± 8.75	139.20 ± 4.27	69.34 ± 2.54
Sample A1	6.60 ± 0.37	281.00 ± 9.24	138.40 ± 5.74	58.96 ± 1.82
Sample A2	6.90 ± 0.21	288.00 ± 7.42	138.70 ± 6.54	57.55 ± 1.77

It is established that the salting of mincemeat by sodium chloride (Sample K) during 24 hours and 48 hours provides a decrease in the activity of peroxidase by 26.7% and 39.6%, respectively, as compared to the unsalted mincemeat. The decrease in the activity of catalase in Sample K was 4.4% and 6.4% as compared to the unsalted raw materials which is the result of effect of table salt on sarcoplasmic proteins which are the studied enzymes.

The decrease in the quantity of salt as part of cures provides some increase in the activity of enzymes. The replacement of 30% of chloride of sodium as part of cures by premix ($KCl+CaCl_2$) (Sample A1) has a positive effect on the activity of studied enzymes. Thus, an increase in the activity of peroxidase by 4.05% and by 8.2% occurs in Sample A1, as compared to Sample K, in 24 hours and 48 hours, respectively. The strengthening of activity of catalase, as compared to the control sample (Sample K) occured in Sample A1, thus, the increase in the activity was 2.48% in 24 hours and 1.81% in 48 hours as compared to Sample K.

The strengthening of reactive capacity of antioxidant enzymes of meat raw materials was provided by the use of yeast extract during salting, which is confirmed by the obtained results. Thus, the activity of peroxidase in Sample A 2 increased by 5.4% and 13.1% as compared to Sample K during the studied periods of salting. In turn, the increase in the activity of catalase was 3.19% and 4.34%, as compared to Sample K, in 24 and 48 hours of salting. The increase in the activity of peroxidase was 1.30% and 4.55%, as compared to the sample with a lower content of salt, in 24 hours and 48 hours, respectively. The obtained relation is explained by the fact that glutathione, being the substratum of true peroxidases, provides an increase in their activity. The results of researches allow to judge about a higher stability of catalase in the environment of chlorine-containing salts which is coordinated with the available literary data [27].

In view of the fact that haem pigments can act both as synergists and prooxidants in relation to the antioxidant system, the effect of salting on the processes of transformation of meat pigments were studied. It is established (Table 2) that the composition of cures and the duration of salting does not effect the total of pigments which remains almost the same for all the samples throughout the studied salting period, the revealed changes are within the limits of test error.

At the same time, it should be noted that against the background of constant presence of common pigments, changes in the ratio of various forms of myoglobin have been revealed. According to the obtained data, the curing of meat (the control sample) is followed by an increase in the content of methmyoglobin by 13.5% and 16.3% within 24 hours and 48 hours, respectively.

The decrease in the amount of chloride of sodium as part of cures provides a decrease in the amount of irreversibly oxidized form of myoglobin by 30%. The quantity of methmyoglobin in Sample A1 decreased by 16.7% and 14.97% as compared to the control sample during the studied salting periods. It should be explained by the decrease in the quantity of ions of chlorine in the test cures, which, according to the calculations, is 0.0427 mol in the control cures whereas it is 0.038 mol in the test cures. The addition of yeast extract, as the source of glutathione, to the studied systems, has an additional inhibiting effect on the process of oxidation of myoglobin. It is established that the quantity of methmyoglobin in Sample A2 decreased by 20.2% in 24 hours of salting and 17.0% in 48 hours of salting as compared to the control sample. The decrease in the quantity of methmyoglobin in Samples A1 and A2 should be regarded as occuring due to a higher activity of catalase and peroxidase. The decrease in the quantity of methmyoglobin is a positive prerequisite for stabilization of the processes of peroxide oxidation of lipids. There is an assumption that the intensity of oxidation of lipids depends on the level of haem pigments in raw materials, the high content of oxymyoglobin provides the formation of methmyoglobin and hydrogen peroxide, and, as a result, of ferrylmyoglobin, a compound which is a strong prooxidant.

Thus, the use of cures with the lowered content of salt at the stage of salting provides an increase in the activity of antioxidant enzymes and a decrease in the content of methmyoglobin as a prooxidant factor, at the same time the effect amplifies in the presence of yeast extract.

Further, the effect of composition of cures on the stability of lipid and protein fraction of half-smoked sausages in the course of cold storage was studied, as from the point of view of the process of hydrolysis and oxidation the quantity of initiating factors in meat products is much higher than that in the source raw materials. Haem iron, free fatty acids, free moisture, salt and also the modes of thermal treatment and storage conditions belong to them.

The sausages were cooled and stored in the refrigerator at a temperature of (2–6)°C within 20 days after thermal treatment.

The intensity of formation of primary products of oxidation was estimated by the dynamics of peroxide number (Fig. 1) as regards the reference value. The value of peroxide number of the control sample (Sample K) increased by 26.5% in 10 days of storage at low positive temperatures, and by 41.9% in 20 days. In the sausages with the partial replacement of salt (Sample A1) the value of peroxide number increased by 24.5% and by 36.1%, as compared to the reference value, in 10 days and 20 days, respectively. In the sausages with the addition of barmy extract (Sample A2) the increase in the peroxide number was 23.2% in 10 days of storage, as compared to the reference value, and 31.0% in 20 days.

It should be noted that the values of peroxide number for all the studied samples throughout all the process of storage remain in the values which do not exceed the established norm and hygienic standards of safety equal to in the course of storage no more than 10 mmol of O_2/kg.

The intensity of formation of secondary products of oxidation in sausages was estimated by the change of thiobarbituric number (TBN) that reflects the amount of formed malonic aldehyde (Fig. 2).

According to the obtained data, the accumulation of products of secondary disintegration of fats proceeds more intensively in the control sample, than in the test samples. Thus, the value of peroxide number increased by 13.5% in 10 days and by 36.5% in 20 days of storage, as compared to the reference value. The decrease in the amount of salt as part of cures and the addition of yeast extract provided a decrease in the rate of formation of secondary products of oxidation in the samples. It is established that in Sample A1 the increase in the value of peroxide number was 9.7% in 10 days and 15.3% in 20 days of storage, as compared to the reference value, whereas in Samples A2 the increase was 5.4% and 11.2% in 10 and 20 days, respectively, as compared to the reference value.

The relation of change of forms of pigments in half-smoked sausages in the course of storage (Table 3) is coordinated with the results of determination of activity of catalase and peroxidase depending on the composition of cures.

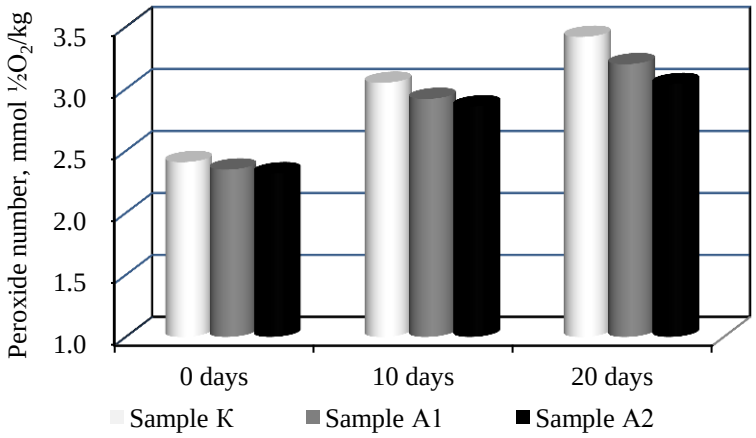

Fig. 1. Change of peroxide number in the course of storage of sausages.

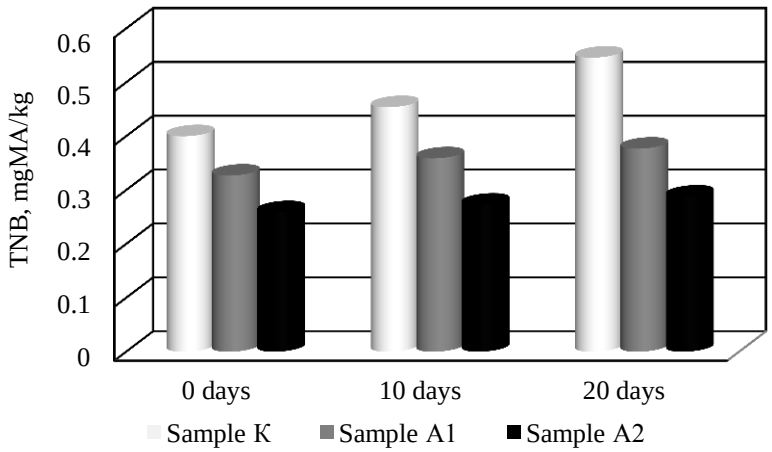

Fig. 2. Change of TBN in the course of storage of sausages.

Table 3. Dynamics of change of quantity of haem pigments in the course of storage half-smoked sausages

Test sample	Storage duration, days		
	0	10	20
Total of pigments, mg/100g			
Sample K	127.24 ± 6.35	126.96 ± 5.23	129.88 ± 6.12
Sample A1	124.72 ± 6.21	125.60 ± 4.89	126.00 ± 5.95
Sample A2	126.24 ± 5.62	124.30 ± 4.62	126.40 ± 5.58
Quantity of methmyoglobin, mg/100g			
Sample K	49.47 ± 1.68	52.38 ± 2.12	56.57 ± 1.97
Sample A1	45.47 ± 1.62	49.78 ± 1.97	50.61 ± 1.82
Sample A2	41.82 ± 1.72	43.00 ± 1.83	46.86 ± 1.79
Quantity of nitric oxide pigments, mg/100g			
Sample K	77.77 ± 2.54	74.58 ± 2.16	73.31 ± 2.34
Sample A1	79.25 ± 2.89	75.82 ± 2.45	75.39 ± 2.63
Sample A2	84.42 ± 2.71	81.30 ± 2.86	79.54 ± 2.45

According to the results of researches, the total of pigments in the studied samples of sausages was within the limits of 124–129 mg/100g irrespective of the duration of storage and the composition of cures.

The content of methmyoglobin in the control sample (Sample K) increased by 5.88% and 14.3% in 10 days and 20 days of storage, respectively. The increase in the content of methmyoglobin in half-smoked sausages containing combined cures (Sample A1) was 9.47% and 11.3%, as compared to the initial content, during the studied storage periods, which is less than in the control product with salt (Sample K) by 4.9% and 10.5%. In the sausages with combined cures and yeast extract (Sample A2) an increase in the content of methmyoglobin by 2.8% and 12.0%, as compared to the initial content, was revealed on the 10th and 20th days of storage, which is less than in Sample K by 17.9% and 17.1%.

The results of determination of nitric oxide pigments testify to the positive effect of decrease of amount of salt as part of cures in the combination with yeast extract on the stability of color of half-smoked sausages in the course of cold storage. It is established that in the control sample (Sample K) the quantity of nitric oxide pigments decreased by 4.1% and 5.73% in 10 days and 20 days of storage, respectively, whereas in the sausages with combined cures (Sample A1) the decrease in the amount of nitric oxide myoglobin was 4.3% and 4.8% during the studied storage periods, as compared to the initial content. This is 1.6% and 2.8% more than in the control product with salt (Sample K). The combination of cures with yeast extract as the source of glutathione (Sample A2) provided the stabilization of coloring of sausages. According to the obtained data, the content of nitric oxide myoglobin in Sample A2 decreased by 3.6% and 5.7% on the 10th and 20th days of storage, as compared to the initial content, and was higher than in the control sample by 9.0% and 8.5%. At the same time the coloring of Samples A1 and A2 with the studied compositions of cures was more stable and attractive after 20 days of cold storage. The objective confirmation to that were the data of study of coloring by a nondestructive method.

The color indicators are presented by integrated characteristics in the CIE system recommended by the International Organization for Standardization and related to the three-component theory of the colored sight according to which the perception of color with an eye of the person is caused by the existence of three types of cones in the retina: red, green and blue sensitive. The main color indicators in the CIE system are lightness (the amount of color), "*a*" and "*b*" – the degree of redness and blueness indicating the quality of color. These data are initial for the determination of more evident indicators – saturation (*S*) and hue (*H*).

Table 4 gives the results of determination of indicators of coloring of the studied samples.

Table 4. Indicators of color of test samples

Sample	Lightness (*L*)	Saturation (*S*)	Hue (*H*)	Redness index *a*/*b*	Indicator of full color distinctions Δ*E*
0 days of storage					
Sample K	55.07 ± 2.72	28.67 ± 1.23	0.56 ± 0.03	1.59 ± 0.07	–
Sample A1	55.84 ± 2.43	29.15 ± 1.43	0.56 ± 0.01	1.61 ± 0.05	1.37 ± 0.06
Sample A2	57.01 ± 2.01	30.49 ± 1.27	0.53 ± 0.04	1.70 ± 0.04	2.57 ± 0.02
10 days of storage					
Sample K	53.95 ± 2.86	28.42 ± 1.54	0.58 ± 0.02	1.52 ± 0.03	–
Sample A1	54.71 ± 1.97	28.72 ± 1.34	0.58 ± 0.04	1.54 ± 0.02	0.79 ± 0.04
Sample A2	56.62 ± 1.68	30.06 ± 1.74	0.54 ± 0.70	1.65 ± 0.05	3.25 ± 0.03
20 days of storage					
Sample K	53.08 ± 1.23	27.95 ± 1.26	0.60 ± 0.02	1.46 ± 0.03	–
Sample A1	53.98 ± 1.87	28.48 ± 1.32	0.59 ± 0.01	1.51 ± 0.04	1.13 ± 0.07
Sample A2	55.87 ± 1.67	29.43 ± 1.42	0.57 ± 0.03	1.57 ± 0.06	3.30 ± 0.05

The main color indicators in the CIE system are lightness, which characterizes the intensity of coloring and is a quantitative assessment of color. According to the obtained results, the intensity of coloring of test samples A1 and A2 had been higher by the beginning of storage by 1.4% and 3.52%, respectively, than that of control sample K.

In the course of storage a decrease in the value of indicator of lightness for all the studied samples of sausages was revealed. In 10 days of storage the decrease of lightness, as compared to the reference value, was 2.03% for control sample K, 2.02% for Sample A1, and 0.68% for Sample A2. In 20 days of storage there is still a tendency of change of the indicator, at the same time the test samples have a higher value of indicator of intensity of coloring.

An increase in the indicator of saturation in the test samples, as compared to the control sample, testifies to the improvement of color quality, that is to its higher purity with the almost equal color tone. The full color distinctions of test samples A1 and A2 are 1.4 and 2.5 of color sensitivity threshold, respectively, which suggests that the distinctions can be revealed visually.

The indicators of color tone and saturation suggest the condition of pigments in sausages. The color tone in the values up to $\pi/4$ characterizes the color of products as belonging to the red area, to the orange area in the range from $\pi/4$ to $\pi/2$ and to the yellow area when it approximates $\pi/2$.

According to the results of determination, the color tone of half-smoked sausages is 0.53, 0.53 and 0.56 for control sample K and test samples A1 and A2, respectively, by the beginning of storage, which corresponds to the red and orange area. At the same time, the values of indicator of color saturation allow to regard the coloring of all the samples of sausages as the saturated and pleasing one.

In the course of storage an insignificant decrease in the saturation of red color and a tendency to the shift of color tone to the orange area of range was revealed for all the samples. Thus, the color saturation of control sample K and test samples A1 and A2 decreased by 0.87%, 1.47% and 1.12%, respectively, as compared to the reference value, in 10 days of storage. The similar relation remains in 20 days of storage. It should be noted that the decrease in the amount of salt as part of cures, including that with barmy extract, provided the improvement of color characteristics of half-smoked sausages. The obtained results are coordinated with the experimental data of determination of quantity of haem pigments of meat.

For better understanding about the color of sausages the indicator "redness index" – the relation of degree of "redness" to the degree of "blueness" - has been determined. The higher the value of the indicator, the redder the color. According to the obtained data, the redness index for control sample K was 1.52 in 10 days of storage, which is lower than the reference value by 4.4%. The decrease in the index of redness for test sample A1 was 4.34%, as compared to the reference value, in 10 days of storage, and, respectively, 2.9% for Sample A2. This is 1.3% and 8.5% higher than in control sample K. In 20 days the decrease in the redness index, as compared to the reference value, was 6.2% and 7.6%, in samples A1 and A2, respectively, which is 3.4% and 7.5% higher than in control Sample K.

On the basis of analysis of obtained experimental data, it is possible to claim that the replacement of 30% of salt as part of cures by the premix $KCl+CaCl_2$ in the ratio of 1 : 1 and the addition of yeast extract to the formulation in the amount of 2% to the weight of raw materials provided an increase in the antioxidant potential of meat raw materials at the stage of salting and, as a result, the inhibition of processes of oxidation of lipids and pigments in half-smoked sausages in the course of storage and the improvement of their color characteristics.

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