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The influence of natural antioxidants on the quality and storage capacity of semi-finished horse meat products

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ABSTRACT

The article is devoted to the influence of natural antioxidants on the quality parameters of meat semi-finished products from horse meat and the microbiological stability of the product during its storage. In solving the set tasks, the standard, generally accepted methods of research were used. Statistical processing of the obtained results and assessment of data reliability were carried out by statistical methods using Microsoft Excel and Statistica (version 12.0). A new antioxidant of natural origin, dihydroquercetin, has been proposed and tested. Effective doses of its application have been established: 0.05 - 0.075% for raw horse fat and 0.075% for semi-finished meat products from horse meat produced with its addition. It has been shown that the administration of dihydroquercetin (Dx) together with ascorbic acid (AA) and tocopherol (Tp) is 10-30% more effective. The effect of dihydroquercetin on the indicators of oxidative spoilage in the technological process, from the production of horse meat to finished products using the latter, has been studied. The organoleptic parameters (appearance, odour, colour) of the control sample and the sample with 0.025% Dx by fat weight corresponded in their characteristics to the experimental samples with 0.05%, 0.075% and 0.1% by 45 days of storage. The introduction of dihydroquercetin, ascorbic acid, and tocopherol also makes it possible to slow down the microbiological spoilage of the semi-finished products that have been obtained during storage.

Keywords: semi-finished meat product, horse meat, raw horse fat, dihydroquercetin, ascorbic acid, tocopherol, peroxide value, acid value, thiobarbituric value

INTRODUCTION

Horse meat is used as a source of meat in Asia and some European countries. Horse meat is especially popular in Kazakhstan, Mongolia, and Kyrgyzstan, but it is also consumed in European countries, such as Sweden, Belgium, and France. Horsemeat has a high content of substances important for the human body, particularly amino acids, vitamins (A, group B, E and PP) and minerals (phosphorus, sodium, copper, iron, potassium, magnesium). Compared to the meat of other animals, horse meat contains more complete protein, organic acids and water. Horse meat is distinguished by a high content of complete, high-quality protein balanced in amino acid composition. Absorption of horse meat in the body occurs approximately eight times faster than beef absorption. According to nutritionists, horse meat fats differ from fats from other meat types in their high polyunsaturated fatty acids content. Due to its ability to lower cholesterol in the blood, horse meat helps regulate metabolic processes in the body, and its low-fat content allows it to be used in weight loss diets **[1]**.

The Republic of Kazakhstan has great opportunities to increase the industrial processing of horse meat into various meat products, which have recently become increasingly popular worldwide, especially in Western Europe. After epidemics of foot-and-mouth disease and “mad cow disease”, the popularity of horse meat in European countries is growing. In France, where residents previously consumed horse meat as a delicacy, consumption of this meat has increased by more than 60 %. They plan to replace beef with horse meat in the menu

of school canteens in Italy. In European countries, horse meat costs are high **[2]**. In this regard, producing high-quality export-oriented meat products from horse meat is promising for the Republic of Kazakhstan **[3]**.

The leaders in the number of horses worldwide are the USA, Mexico, Brazil, Mongolia, and China. Kazakhstan has more than 3 million horses, and the population growth in recent years has been about 10 % **[4]**.

Nevertheless, the shortage of raw materials and the predominance of imported meat, which is not always of acceptable quality, determine the tasks for scientists' research in creating technologies for new meat products using domestic raw materials of animal and plant origin.

The increasing demand for chopped semi-finished meat products from the modern consumer has determined the direction of current research based on the use of raw domestic meat materials and components of plant origin in the technology of products with high nutritional value and low cost **[5]**.

Widely popular among the Republic of Kazakhstan's population, cutlet meat is formed after isolation, consisting of pieces of meat pulp of various sizes and weights obtained from trimmings from different parts of the carcass. Scientific literature analysis and patent searches showed that horse meat cutlets are limited due to the need for more technology based on scientific research **[6]**.

At the same time, horse meat products are produced in small quantities at meat processing plants, which is explained by the specifics of the raw material and the limited scientifically based recommendations for its integrated use in producing meat products. Important The production and social task is stabilising oxidative processes in horsemeat lipids through additives to increase the shelf life of minced semi-finished meat products **[7]**, **[8]**.

Oxidative changes in fats are inevitable during storage of any food product. Scientists have researched lipid oxidation and its mechanism, and much attention has been given to the problem of fat oxidation in meat products because oxidative processes influence the formation of the quality and safety of meat and meat products. Oxidative processes cause a decrease in the content of essential fatty acids and vitamins, deterioration in colour, taste, consistency and the appearance of foreign odours, and also reduce the shelf life of products **[9]**. Preventing fat oxidation is paramount to the meat industry, helping to increase the production of quality, flavorful products and extend their shelf life **[10]**.

In this regard, it is of interest to search for innovative methods of processing horse meat, such as the use of natural stabilisers, which allow you to regulate the properties of raw materials, increase shelf life, and expand the range while simultaneously improving the quality of the finished product.

The purpose of these studies is to study the effect of antioxidants of natural origin on the quality indicators and shelf life of semi-finished horse meat products.

Scientific Hypothesis

The use of antioxidants of natural origin during storage inhibits lipid oxidation processes without reducing the quality and safety indicators in meat semi-finished products.

MATERIAL AND METHODOLOGY

Samples

The following raw materials were used to produce experimental samples of meat semi-finished products from horse meat: horse meat of 1 grade, raw horse fat, dihydroquercetin, ascorbic acid, and tocopherol.

Chemicals

Hexane (for HPLC ≥ 95%), chloroform (anhydrous ≥ 99%, contains 0.5–1.0% ethanol as a stabiliser – $CHCl_3$), methanol (anhydrous 99.8% – CH_3OH), ethyl alcohol (C_2H_5OH), potassium hydroxide (KOH), phenolphthalein, sodium chloride (BioXtra ≥ 99.5% (AT) – NaCl), nitrogen ≥ 99.998% (N_2), barium chloride ($BaCl_2$), ferrous sulfate heptahydrate/iron (II) sulfate heptahydrate/($FeSO_4$), hydrochloric acid reagent grade, 37% (HCl), ammonium thiocyanate (NH_4SCN), iron (II) chloride – anhydrous, beads −10 mesh, 99.99% trace metals basis ($FeCl_2$), trichloroacetic acid, 25% alcohol solution (AS), butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), malondialdehyde – $CH_2(CHO)_2$(MDA) and ascorbic acid (AA), tocopherol (Tp) were purchased from Sigma-Aldrich, Inc. (Merck KGaA, Darmstadt, Germany). Dihydroquercetin (Dx) with purity ≥ 96% was purchased from Flavitlife Bio JSCo (Sofia, Bulgaria) **[10]**.

Instruments

A Camspec model M550 dual-beam UV-VIS spectrophotometer (Camspec Ltd, Kembridge, UK) was used to determine peroxide number (POV) and 2-thiobarbituric acid reactive substances (TBARS).

Laboratory Methods

Laboratory studies of raw materials were conducted at JSC "Almaty Technological University" (Almaty, Kazakhstan) and in the meat processing training center at the "University of Food Technologies" (Plovdiv, Bulgaria).

The POV was determined spectrophotometrically based on the oxidation of Fe2+ to Fe3+ in hydroperoxides. The formation of a colour compression between the Fe3+ and SCN was obtained by the method of Stine et al. **[11]** and refined by Schmedes and Holmer **[12]**. The absorption was measured at 507 nm.

The extracted lipids' acid value (AV) was determined according to EVSEN ISO 660:2009 procedure **[13]**. The extracted lipids were dissolved in ethyl alcohol (99%) and heated for about 2 min before being titrated while still hot against 0.1 M KOH using phenolphthalein as an indicator.

TBARS were determined by Botsoglou et al. **[12]**.

Organoleptic evaluation establishes whether products comply with the standard's requirements regarding the main qualitative indicators (appearance, colour, odour, aroma, taste, and consistency) **[14]**.

Determination of microbiological parameters according to SST 9958-81 **[15]**. Studies were conducted to determine the number of mesophilic aerobic and facultative-anaerobic microorganisms, *E. coli* bacteria (coliforms), sulfite-reducing clostridia, and *S. aureus*. Meat-peptone agar was used for all microbiological analyses. (BioMedia, Russia).

Description of the Experiment

Sample preparation: A chilled to 1 °C horse shoulder (first category of fatness) was used. The horse meat was in the shape of semi-circular pieces, not heavier than 0.2 kg, and had a thickness of about 10 cm. The meat pieces were ground in a meat mincer (wolf machine) with holes that were 3 mm in diameter and separated into 9 samples of 100 g each. Then add raw horse fat and mix. Weigh dihydroquercetin, ascorbic acid and tocopherol on a bench scale. In one part of the test samples we add dihydroquercetin, ascorbic acid and tocopherol in dry form in different dosages. In the other part of the experimental samples, we add dihydroquercetin in 25% alcohol solution at room temperature until complete dissolution after adding ascorbic acid and tocopherol. The ready minced meat is mixed in a mincer.

Number of samples analyzed: A total of 13 samples were analysed.

Number of repeated analyses: All instrument readings were measured three times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: Standard methods were used to determine the effect of storage time on lipid oxidation, organoleptic studies, and microflora content during the storage of semi-finished meat products from horse meat.

Statistical Analysis

To analyse the test parameters of meat semi-finished products from horse meat, statistical analysis of the obtained data was carried out, and the reliability of the obtained data was assessed by statistical methods using Microsoft Excel and Statistica program (version 12.0). Statistical functions of mean value and standard deviation were used to describe continuous variables. Graphical interpretation of the results was performed using Microsoft Excel. The statistical analysis results are presented in Figures 10-19, with each value being the mean of at least 9 determinations. We then calculated each measurement's error and the squares of the errors to calculate the absolute error of the measurement. We chose a reliability value of p = 0.95 **[16]**.

RESULTS AND DISCUSSION

Research has been carried out on studying the influence of dihydroquercetin, ascorbic acid, and tocopherol on quality and safety indicators of meat semi-finished products from horse meat during storage at their joint use.

In several scientific research works, scientists have experienced the introduction of dihydroquercetin in poultry meat **[17]**, ground beef **[18]**, semi-finished broiler meat **[19]**, semi-finished products from moose meat **[20]**, and pork fat **[21]**.

Samples were developed from raw horse fat to determine application methods and select an effective dosage of dihydroquercetin. Dx was added in dry form to one part of the experimental samples in dosages of 0.01%, 0.025%, 0.05%, 0.075, 0.1%, and 0.2%. The minimum study dosage of 0.01% was chosen according to the recommendations of the Dx manufacturer.

From an analysis of literary sources **[22]**, it is known that Dx is highly soluble in a water-spirit solution; therefore, samples of raw horse fat with similar dosages of Dx but previously dissolved in a 25% alcohol solution (AS) were also produced.

The control sample without adding Dx. After production, the prototypes were stored at t =4 ±2°C.

The results obtained on the accumulation of peroxides (Figure 1) clearly show that in the control sample, from the fourth day of storage, there was an active accumulation of primary fat oxidation products.

In samples with a Dx content of 0.01%, the peroxide value by 12 days of storage was only 1.5% and 4.5% lower than that of the control, indicating an insufficient Dx dosage. Dosages of Dx starting from 0.05% and up to

0.075% were more effective in reducing peroxides. As seen in Figure 1, an alcohol solution of Dx is much more effective in reducing the accumulation of peroxides.

Dynamics of changes in acid value also showed (Figure 2) that a dosage of 0.01% is not enough. The acid value for the control sample and samples with a dosage of 0.01 - 0.025% remained almost the same throughout the entire storage period. At the same time, the acid value of the remaining samples was 1 mg/kg less than that of the first three samples. The acid value values for samples containing an alcohol solution of Dx were significantly lower than samples with the same amount of Dx but added in dry form.

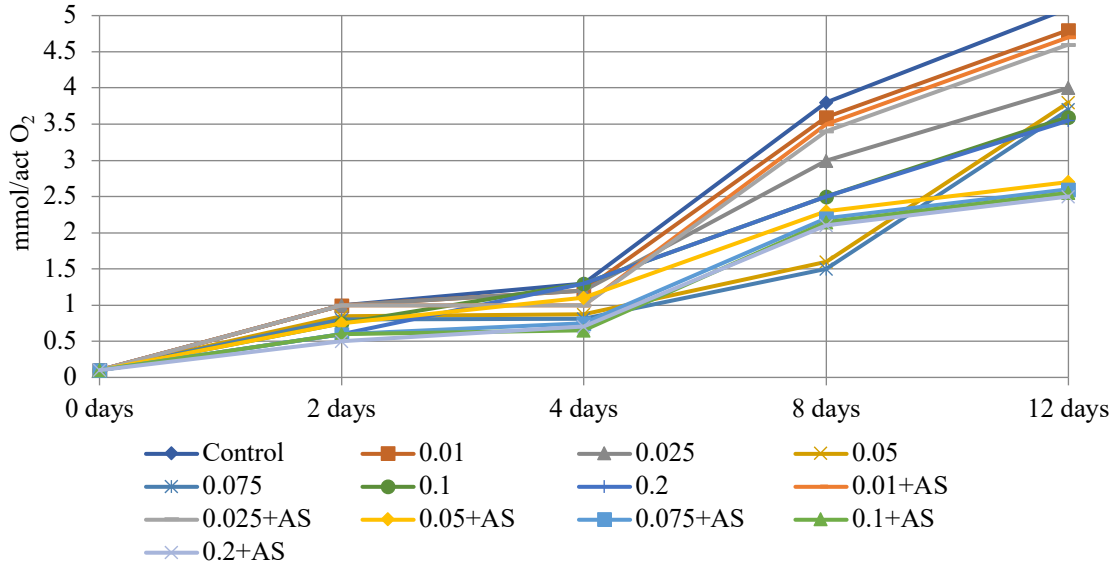


Figure 1 Dynamics of changes in the peroxide value of horse fat during storage (mmol/act O_2).

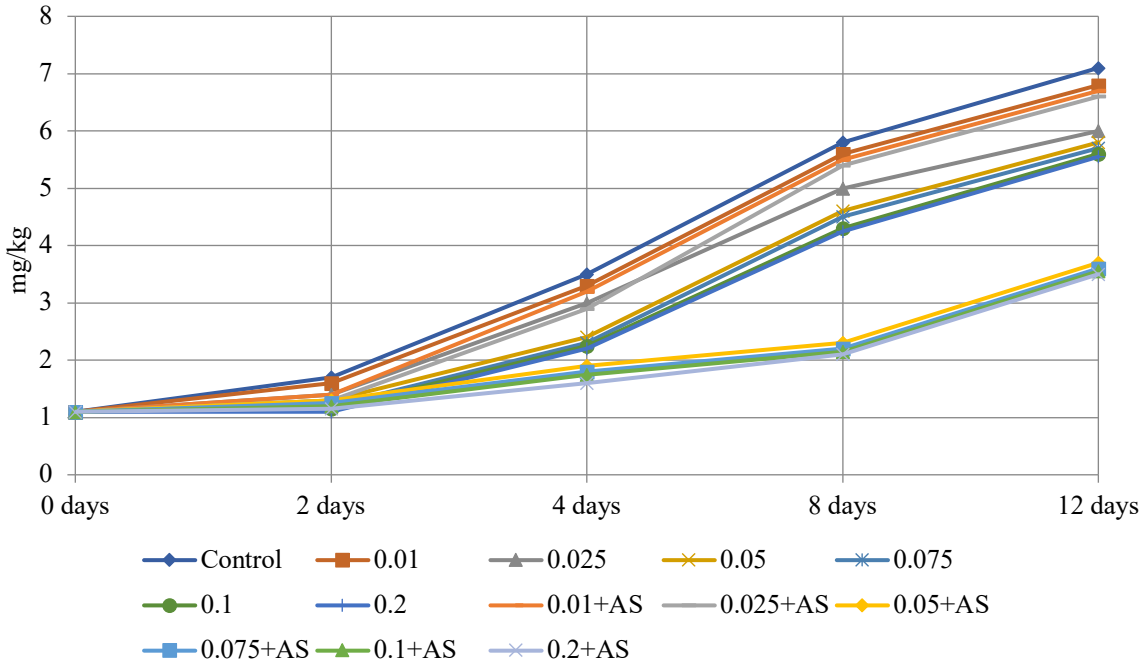


Figure 2 Dynamics of changes in the acid value of horse fat during storage (mg/kg).

The accumulation of products of secondary breakdown of fats occurred very intensively in the control sample (Figure 3). It exceeded the established norm for thiobarbituric value of 0.5 mg/kg already on the 8th day of storage. In contrast, not a single test sample with Dx content had the normalised value for thiobarbituric value was exceeded. However, in samples with a Dx content of more than 0.05%, the accumulation of secondary fat oxidation products occurred two times slower.

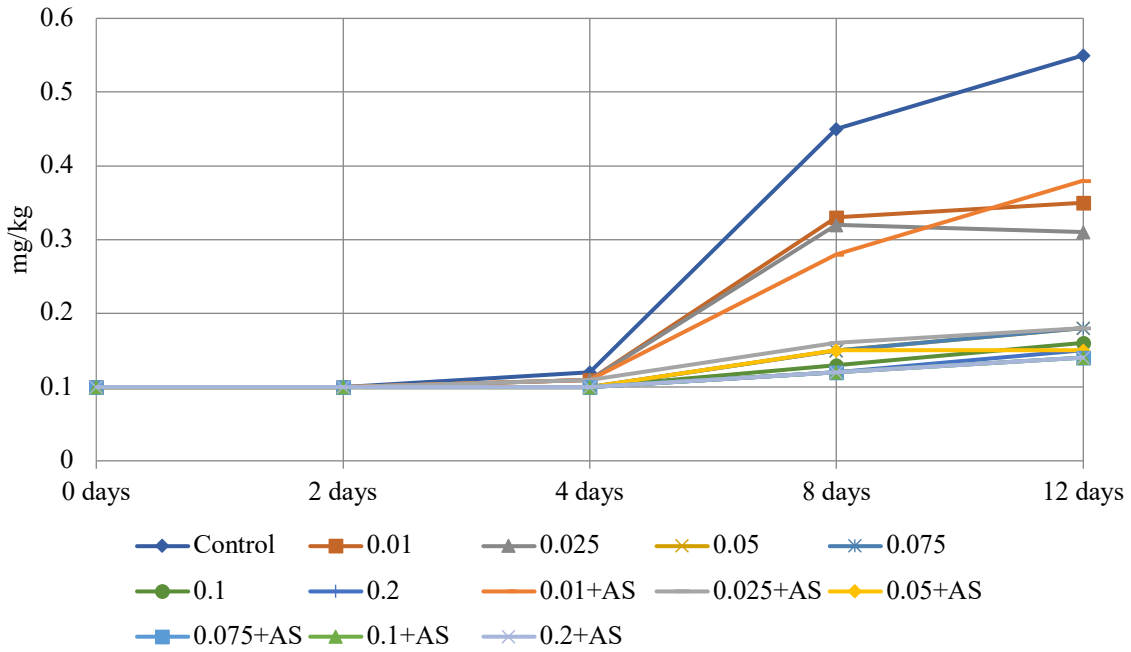


Figure 3 Dynamics of changes in the thiobarbituric value of horse fat during storage (mg/kg).

Studies conducted on raw horse fat have shown that a dosage of 0.01% Dx is ineffective in reducing fats' oxidative spoilage. Dosages of Dx from 0.05% to 0.2% suppressed the development of oxidative spoilage in test samples, and increasing the dosage fourfold (up to 0.2%) slightly reduced the values of peroxide, acid, and thiobarbituric.

The results obtained also clearly show that introducing Dx in the form of an alcohol solution was more effective, obviously due to its more uniform distribution over the fat mass.

Thus, a dosage of Dx from 0.05% to 0.075% by weight of fat in an alcohol solution is most effective for maintaining the quality and safety of raw horse fat.

In meat products technology, ascorbic acid is added to the recipes of semi-finished meat products in the recommended dosage of 0.05% and tocopherol—0.02%. Thus, it is advisable to study the effect of the above components on the antioxidant capacity of dihydroquercetin and, if necessary, adjust the dosage in meat systems.

To select an effective dosage of dihydroquercetin, experimental samples were developed from raw horse fat, into which 0.01%, 0.025%, 0.05%, and 0.75% by weight of the dihydroquercetin raw material were added in the form of an aqueous-alcohol solution together with the addition of ascorbic acid and tocopherol in dosages of 0.05% and 0.02%, respectively. The control was a sample without the addition of ascorbic acid and tocopherol.

Changing the amount of tocopherol and ascorbic acid added is undesirable since their purpose in meat-containing products is to prevent oxidative spoilage of fats and stabilise colour characteristics.

The results obtained on the accumulation of peroxides (Figure 4) clearly show that the combined addition of tocopherol and ascorbic acid with Dx slows the accumulation of primary fat oxidation products.

In all samples, the peroxide value was 15-30% lower after 12 days of storage when adding tocopherol and ascorbic acid.

The dynamics of changes in acid value (Figure 5) also showed that adding tocopherol and ascorbic acid helps prolong shelf life without losing organoleptic characteristics.

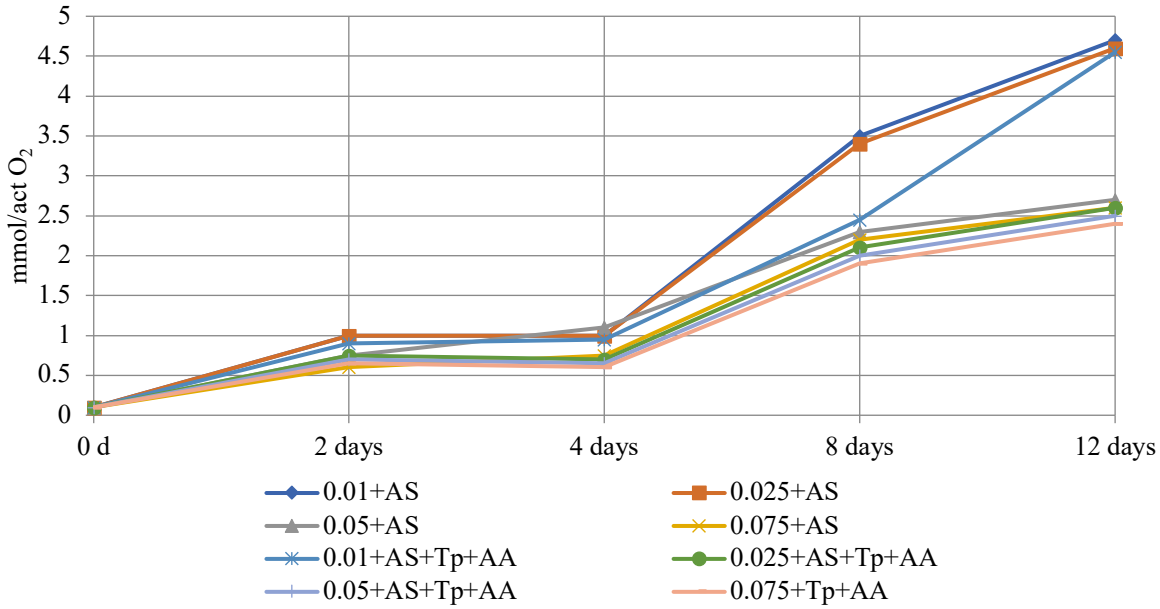


Figure 4 Dynamics of changes in the peroxide value of horse fat during storage (mmol/act O_2).

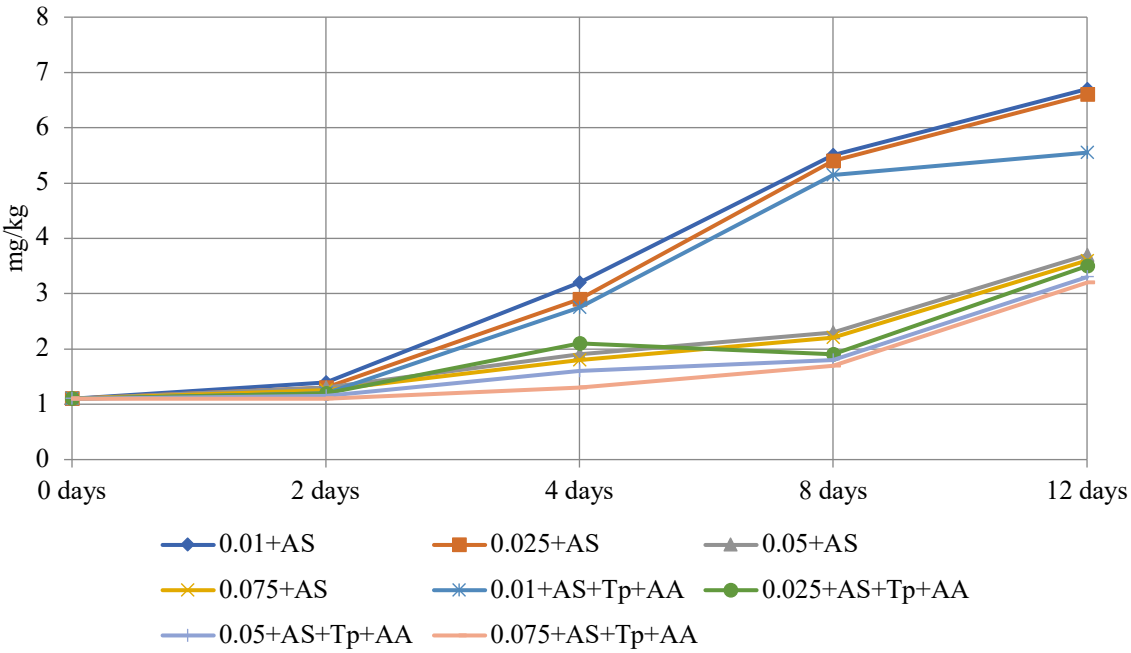


Figure 5 Dynamics of changes in the acid value of horse fat during storage (mg/kg).

The accumulation of products of secondary fat breakdown did not occur intensively in all samples; in control samples (Figure 6), it exceeded the established norm for thiobarbituric value of 0.5 mg/kg 24 days of storage, while in test samples with dosages of 0.025%—0.075%, the normalised value for thiobarbituric value was exceeded only on the 28th day of storage.

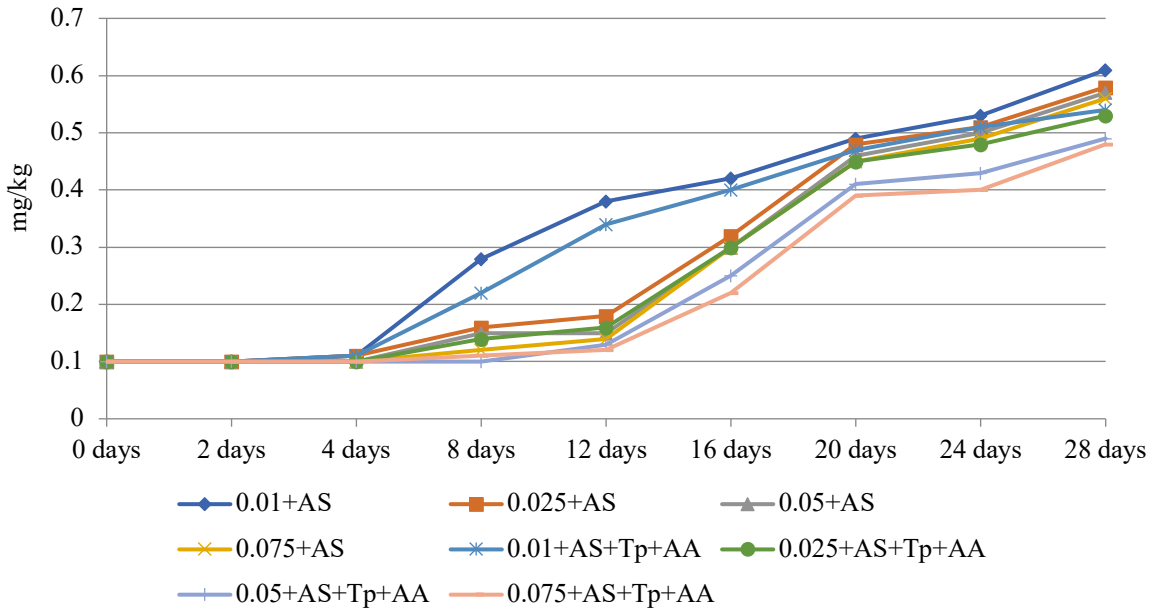


Figure 6 Dynamics of changes in the thiobarbituric value of horse fat during storage (mg/kg).

Fats deteriorate as volatile fatty acids and carbon dioxide accumulate, resulting in a change in aromatic characteristics. In addition, compounds with strong oxidising properties are formed: peroxides, hydroperoxides, free radicals, and atomic oxygen.

To confirm the selected dosage of Dx in raw horse fat, experimental studies were conducted on the storage of horse meat. To solve this problem, test samples were developed: control - without adding Dx, and test samples into which an alcohol solution was added containing Dx to the fat mass of 0.025%, 0.05%, 0.075%, 0.1%, respectively. Ascorbic acid and tocopherol were added to each test sample in the recommended dosages - 0.05% and 0.02%, respectively.

Horse meat with a fat content of 15±1% was used for the research. Horse meat was chosen as a research object for testing the selected dosage of Dx because it contains a large amount of moisture—about 70%—and Dx is poorly soluble in water.

After processing, all obtained samples were separated and stored at -18 ±2°C.

The results of studying the dynamics of accumulation of oxidative spoilage products (Figures 7, 8 and 9) showed that in the control sample, there was an intensive accumulation of peroxides from 3.1 to 22.0 mmol of active 02, i.e. increased over 60 days of storage, by more than 7.1 times the original value. At the same time, an increase in acid value was also observed in the control sample, and by 30 days of storage, the established norm was exceeded by 2 g KOH/kg. The thiobarbituric value value increased 14 times compared to the initial level.

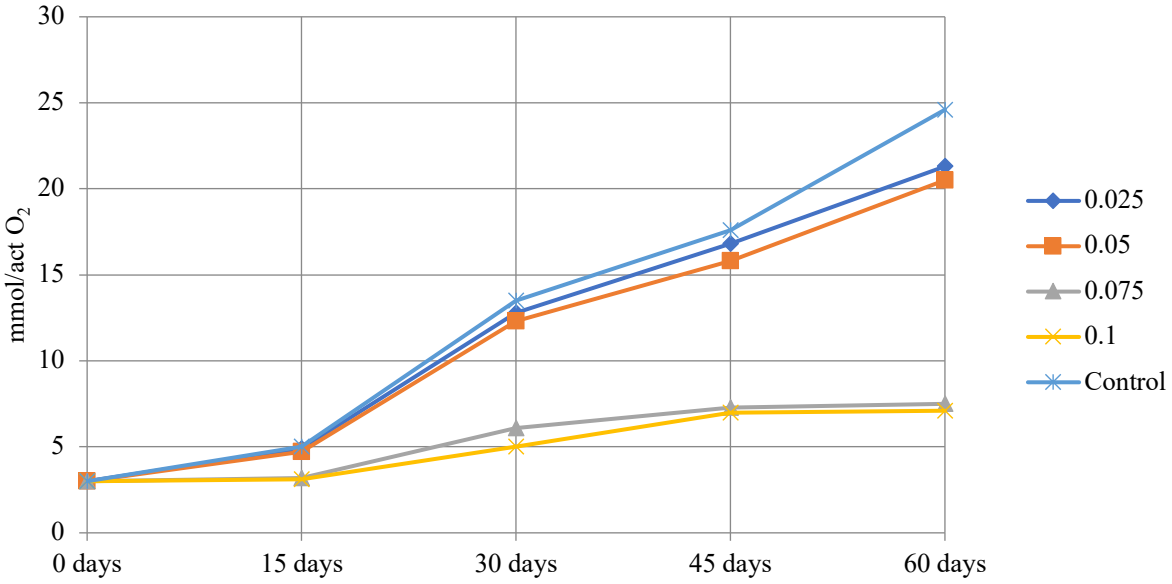


Figure 7 Dynamics of changes in the peroxide value of horse meat during storage (mmol/act O_2).

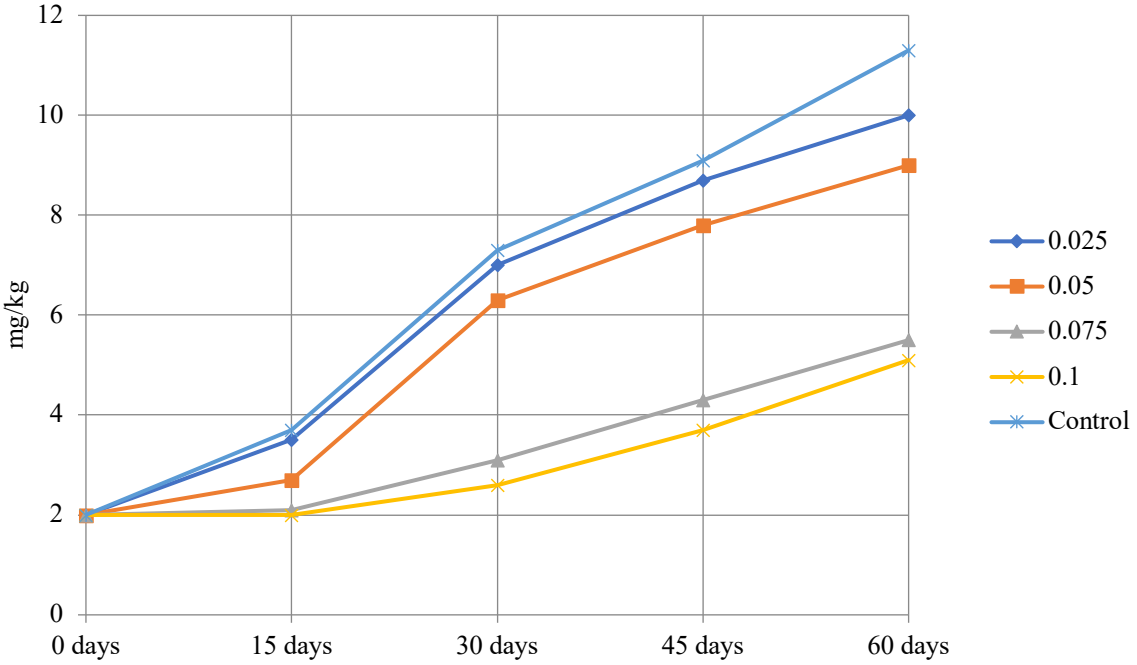


Figure 8 Dynamics of changes in the acid value of horse meat during storage (mg/kg).

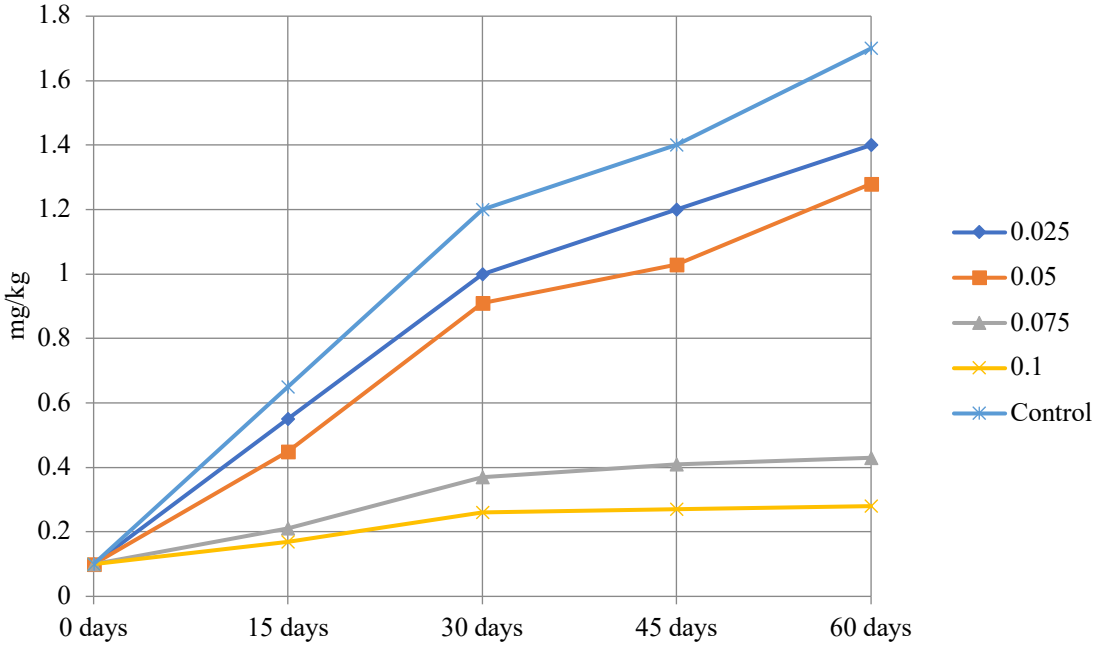


Figure 9 Dynamics of changes in the thiobarbituric value of horse meat during storage (mg/kg).

As can be seen from the results obtained, dosages of 0.025% and 0.05% Dx by fat weight are not effective enough to inhibit the accumulation of oxidation products. By 60 days of storage, samples with a dosage from 0.025% to 0.05% practically did not differ from the control for all studied indicators.

In test samples with a Dx dosage of 0.075% to 0.1%, the accumulation of peroxides proceeded much slower than in the first two samples and at approximately the same rate.

Hydrolytic breakdown of fat in model samples of horse meat during storage led to the accumulation of free fatty acids, which increased the acid value. Samples containing 0.075% and 0.01% Dx by fat weight were resistant to the accumulation of free fatty acids and did not significantly exceed the permissible limit on the 60th day of storage.

According to the organoleptic indicators (appearance, smell, colour) presented in Table 1, on the thirtieth day of storage, the control sample and the sample with 0.025% Dx by weight of fat corresponded in their characteristics to the experimental samples with 0.05%, 0.075% and 0.1 % to 45 days of storage. Already on the 30th day of storage, a pronounced foreign odour was noted in the first two samples, which was associated with the accumulation of fat oxidation products and microbiological spoilage.

The effects of dihydroquercetin, tocopherol, and ascorbic acid on the preservation of hydrolytic and oxidative spoilage rates during the storage of fats and meat products have been documented in several studies. Dihydroquercetin has been shown to improve the stability of ghee fats, with higher concentrations correlating with lower oxidative spoilage rates, thereby extending shelf life **[23]**. Tocopherol, especially α-tocopherol and its acetate form, has been found to protect against lipid oxidation in various meat products by preserving the colour and reducing volatile aldehyde formation **[24]**, **[25]**, **[26]**, **[27]**. Ascorbic acid has demonstrated efficacy in preventing a colour change in irradiated beef and reducing lipid oxidation. However, its effectiveness may vary depending on the age of the meat and storage time after irradiation **[24]**, and ascorbic acid used in combination with rosemary extract effectively retards lipid oxidation in pork meat without affecting other quality parameters **[28]**. Similarly, tocopherol and ascorbic acid have been demonstrated to reduce oxidative reactions in frozen fish **[29]**.

Interestingly, although ascorbic acid generally acts as an antioxidant, it has been observed to stimulate lipid oxidation in certain contexts, such as in cooked pork cutlets **[26]**. Moreover, combining ascorbic acid with tocopherol may have a synergistic effect, as shown in the protection against stress-induced lipid oxidation in chicken meat **[30]**. However, in some cases, ascorbic acid alone did not protect against oxidation, and no synergism was observed between α-tocopherol acetate and ascorbic acid in chicken meat **[25]**.

Table 1 Organoleptic studies of minced horse meat.

Name semi-finished product	Type of packaging	Reaction with copper sulfate	Appearance, color, smell	Broth clarity and aroma
		0 days storage		
Minced horse meat	Control 0.025% 0.05% 0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of fresh meat.	Transparent, fragrant
		15 days of storage		
Horse meat	Control 0.025% 0.05% 0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant
		30 days storage		
Minced horse meat	Control 0.025%	Broth with flakes of questionable freshness	Pink colour. Smell with a hint of mustiness.	Cloudy with a slight unpleasant odor
	0.05% 0.075% 0.1%	Clear broth fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant
		45 days storage		
Minced horse meat	Control 0.025%	A small amount of flaked meat of questionable freshness	Pink colour.. The smell is slightly musty.	Cloudy with a slight unpleasant odor
	0.05% 0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant
		60 days storage		
Minced horse meat	Control	The broth is cloudy, the meat is stale	–	–
	0.025% 0.05%	A small amount of flaked meat of questionable freshness	Pink colour. The smell is slightly musty.	Cloudy with a slight unpleasant odor
	0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant

Thus, the optimal dosage for adding an antioxidant component is the addition of an alcohol solution containing Dx to the fat mass from 0.075% to 0.1%, respectively, with the additional addition of ascorbic acid and tocopherol in the recommended dosages - 0.05% and 0.02%, respectively.

As emphasised by **[31]**, adding dihydroquercetin to semi-finished products from meat of broilers enhances their biological activity. It positively affects the quality and yield of finished products without affecting the organoleptic characteristics.

Dihydroquercetin has antioxidant properties that can inhibit the oxidation process in various meat products. Studies have shown that the addition of dihydroquercetin to ground meat can slow down lipid oxidation, thereby increasing oxidative stability and potentially extending the shelf life of the product. These findings are consistent with researchers Bozhko et al. **[32]** and by Bee Cheah et al. **[33]**.

Studies show that dihydroquercetin maximises the consumer properties of chopped semi-finished products in developing oxidative spoilage processes **[34]**. Previous studies of antioxidants in fat storage **[21]** also confirm that it slows the decomposition rate of fats resistant to oxidative deterioration.

Experiments were conducted to determine the optimal ratio of ascorbic acid and dehydroquercetin to be added to horse meat products. The experiment was carried out as follows: dehydroquercetin and ascorbic acid were added to the horse meat product in different proportions, the samples were encrypted, and nothing was added to the control sample. The samples were examined for acid, peroxide and thiabarbituric values.

Below are the results and data processed in the Statistica 12.0 program—determination of acid value. The acid value indicates the degree of hydrolytic breakdown of lipids, in this case, a horse meat product.

The results obtained are presented in the form of response surface graphs. Figure 10 shows the response surface of the acid value depending on the dose of Dx and AA.

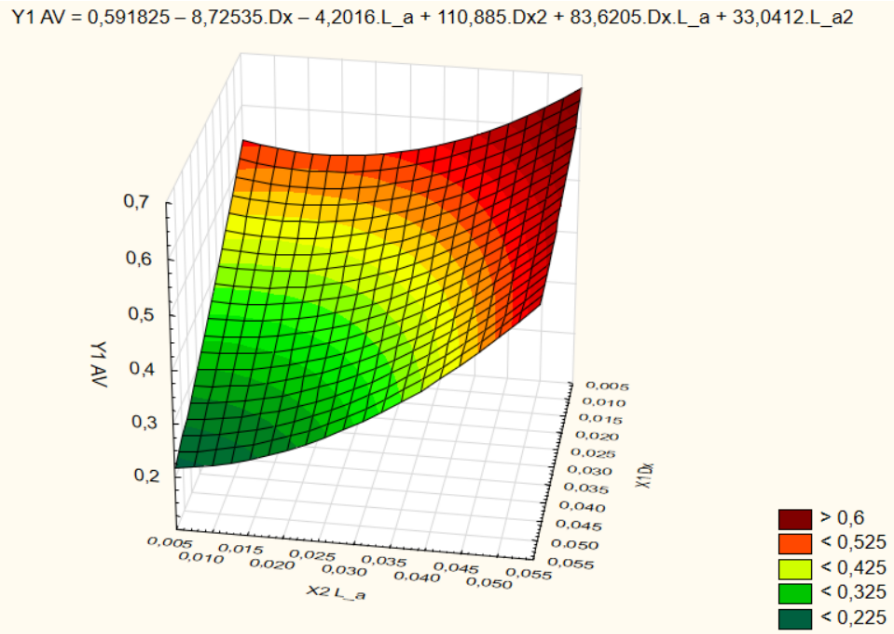
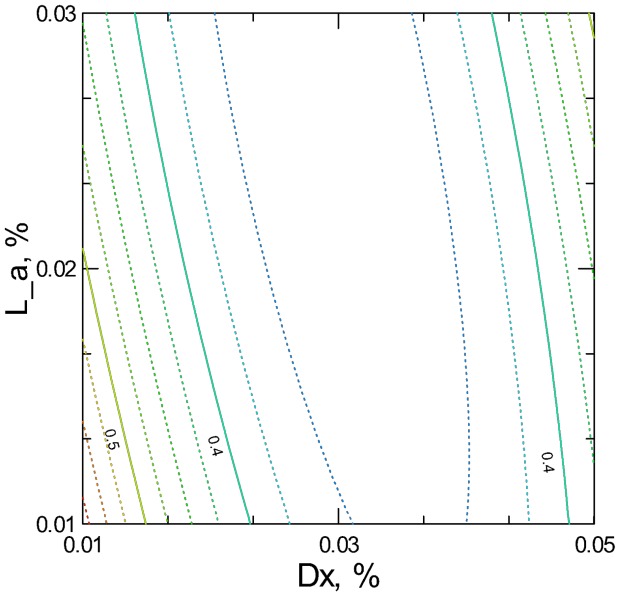


Figure 10 Response surface of the acid value depending on the dose of Dx and AA.

The following mathematical and statistical indicators were obtained when processing experimental data: R-squared = 96.9135%, R - squared (adjusted ford. f.) = 94.7089%, Standard Error of Est. = 0.006, Mean absolute error = 0.0026, Durbin-Watson statistic = 1.84348 (P=0.3264), Lag 1 residual autocorrelation = 0.0780817.

Based on the results of mathematical and statistical analysis with a reliability of 94.7%, it can be said that the acid value is optimal for horse meat with a concentration of ascorbic acid (AA = 0.020%) and concentration of dehydroquercetin (Dx = 0.035%). Figure 11 shows the data processing results, which shows the optimum values.



Where: concentration of AA, %
concentration of Dx, %

Figure 11 Minimum value of AV at concentration AA (AA=0.020%) and concentration Dx (Dx=0.035%).

When processing data in the Statistica 12.0 program, a second-degree equation was obtained indicating the dependence of the AV on the concentration of two antioxidants:

$AV = 0.591825 - 8.72535.Dx - 4.2016.AA + 110.885.Dx^{2} + 83.6205.Dx.AA + 33.0412.AA^{2,}$ cm ^{3} KOH/g

The reliability of the model was checked statistically, and the following data were obtained:

r^2CoefDet	DF Adj r^2	Fit Std Err	F-val
0.97	0.94	0.006158807	43.96

Figure 12 shows the critical limit of the AV, based on the Fisher criterion (blue line) and the degree of significance of various factors:

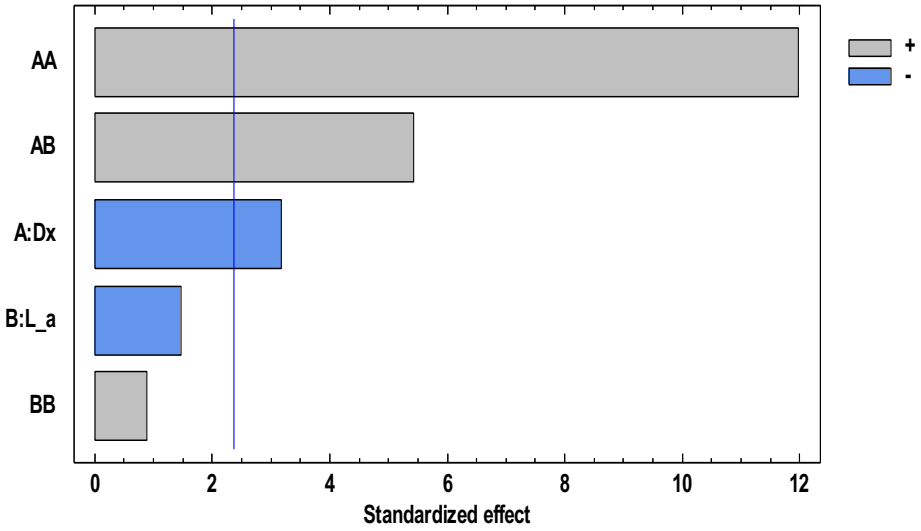


Figure 12 Critical limit of acid value.

The influence of the selected factors on the peroxide value (POV) is shown in Figure 13, as a peroxide value response surface.

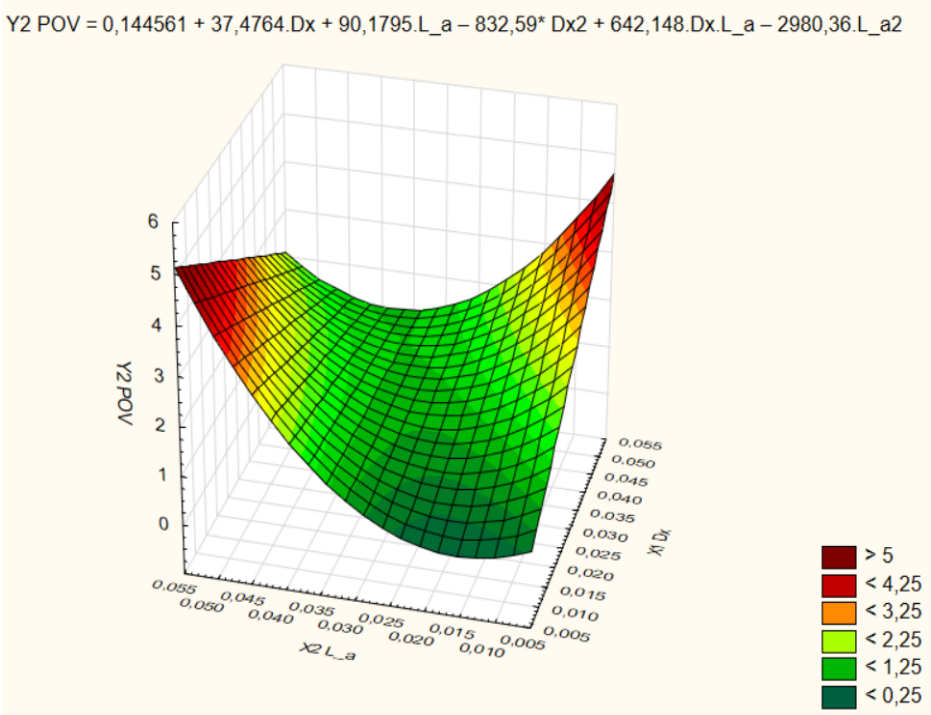


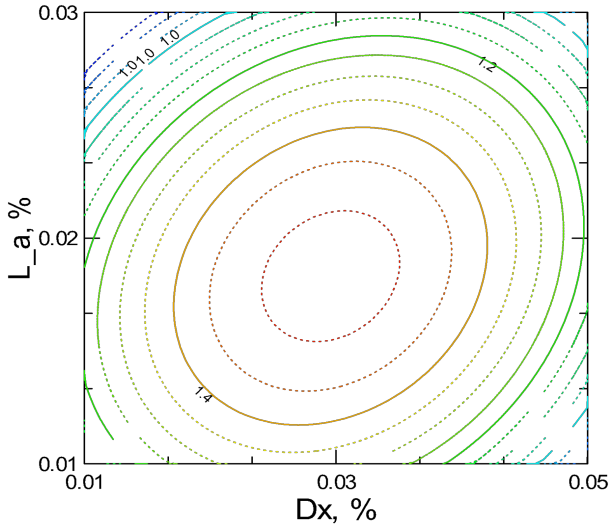
Figure 13 Surface response of peroxide value depending on the dose of Dx and AA.

When determining the peroxide value, which indicates the degree of primary oxidation of lipids and the production of primary oxidation products - hydroperoxides, data were obtained with the following mathematical and statistical indicators:

R-squared = 99.5%, R-squared (adjusted for df) = 99.13%, Standard Error of Est. = 0.0272, Mean absolute error = 0.0166366, Durbin-Watson statistic = 2.53 (P = 0.7746).

Lag 1 residual autocorrelation = -0.321102.

The mathematical and statistical analysis results show that the model is described with an accuracy of 99.5%.



Where : concentration of AA, %
concentration of Dx, %

Figure 14 Minimum values of POV.

As can be seen from Figure 14, the minimum values of the peroxide value are achieved at the concentration of ascorbic acid (AA = 0.030%) and the concentration of dehydroquercetin (Dx = 0.010%) or at the concentration of ascorbic acid (AA = 0.010%) and the concentration of dehydroquercetin (Dx = 0.050%)

Figure 15 shows the critical limit of the peroxide value based on the Fisher criterion (blue line) and the degree of significance of various factors:

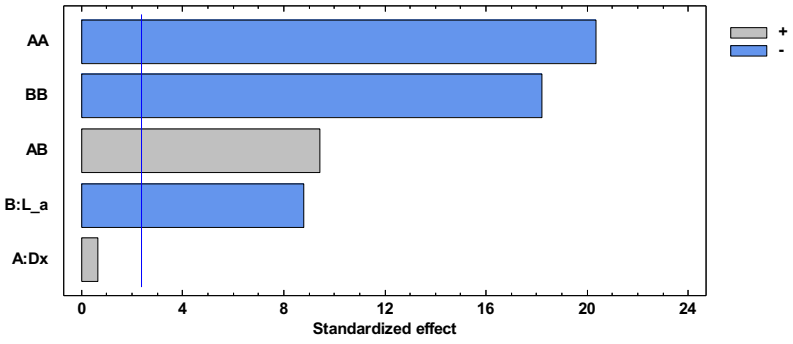


Figure 15 Critical limit of peroxide value.

A second-degree equation is derived, indicating the dependence of the peroxide value on the concentration of two antioxidants:

$$POV = 0.144561 + 37.4764.Dx + 90.1795.AA - 832.59^{*} Dx^{2} + 642.148.Dx.AA - 2980.36.AA^{2,}\ meqvO_{2}/kg$$

Reliability has been statistically proven by the following results:

r^2CoefDet	DF Adj r^2	Fit Std Err	F-val
0.994915	0.991283	0.00166366	273.9187

Thiobarbituric value was also determined, which indicates the degree of secondary oxidation of lipids and the production of secondary oxidation products—malonaldehyde.

Figure 16 shows a thiobarbituric value response surface showing the effect of selected factors on the thiobarbituric value.

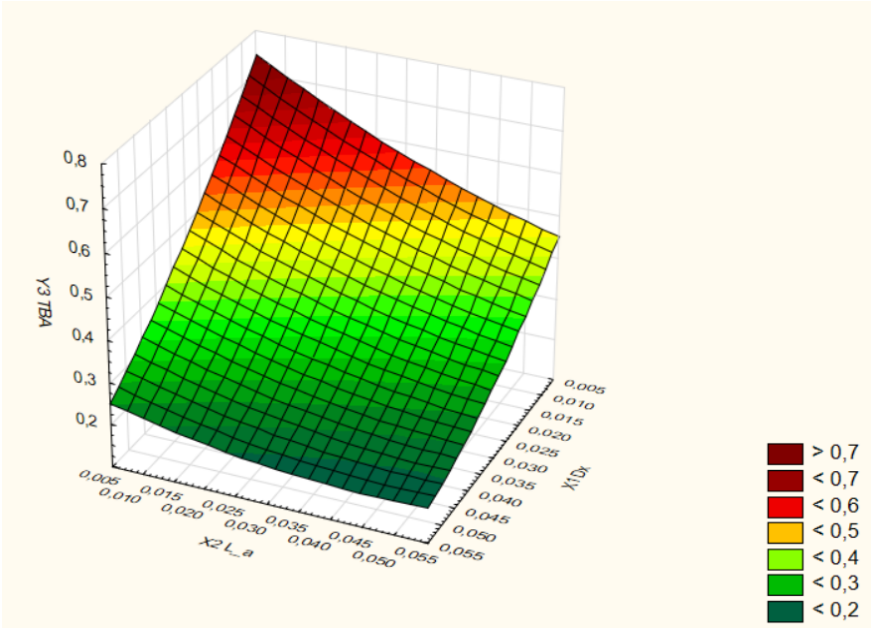
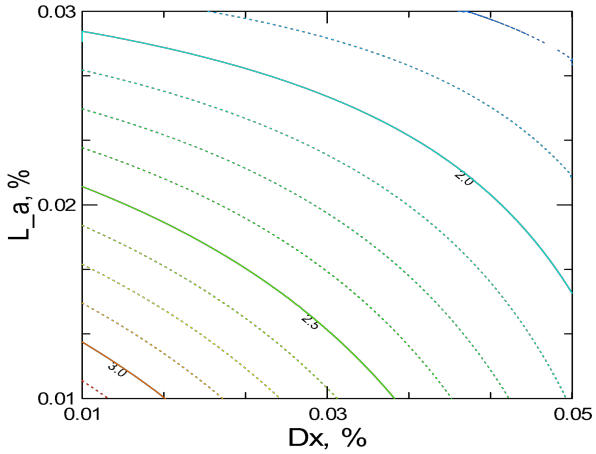


Figure 16 Response surface of the thiobarbituric value depending on the dose of Dx and AA.

When processing the experimental results, the following mathematical and statistical indicators were obtained:

R-squared = 97.0878%, R-squared (adjusted for df) = 95.3404%, Standard Error of Est. = 0.088, Mean absolute error = 0.0509082.

According to mathematical and statistical analysis, the resulting model is 97% reliable, and the standard error is 0.08.



Where: concentration of AA, %
concentration of Dx, %

Figure 17 Thiabarbituric number values.

The minimum values of thiabarbituric value are shown at the concentration of ascorbic acid (AA = 0.030%) and the concentration of dehydroquercetin (Dx = 0.050%)

Figure 18 shows the critical limit of the thiabarbituric value, based on the Fisher criterion (blue line) and the degree of significance of various factors:

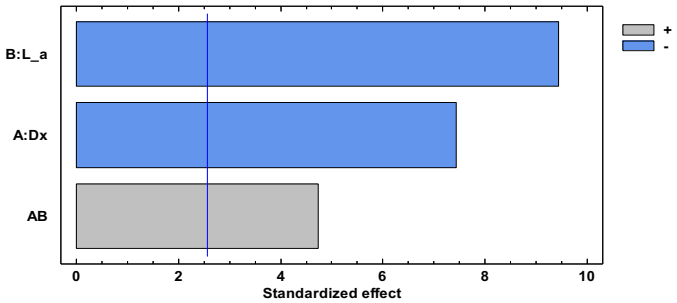


Figure 18 Critical boundary thiabarbituric value

As a result of the research and the results obtained, the Triple Optimum of the problem was obtained (yellow area) which is located at concentration of ascorbic acid (AA = 0.027-0.030%) and dehydroquercetin concentrations (Dx = 0.024 – 0.035%) is shown in Figure 19.

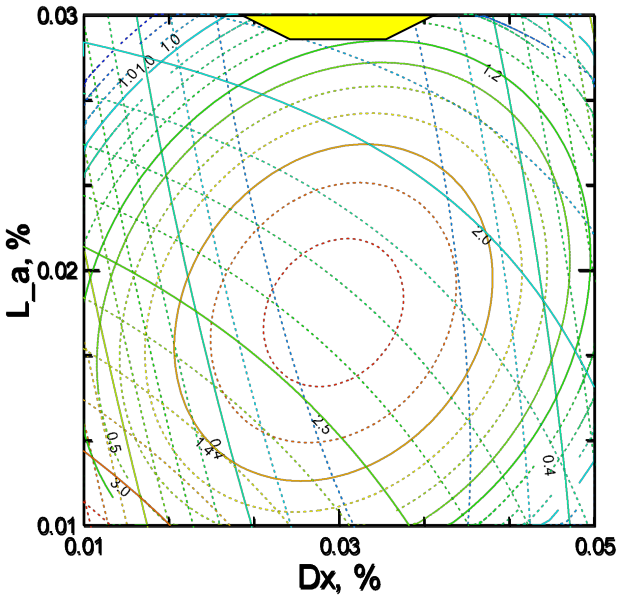


Figure 19 Triple optimum for finding the concentration of two antioxidants.

Plant-based derivatives not only improve some of meat's technological characteristics but can also help reduce the perception of meat products as unhealthy by allowing consumers to include functional compounds beneficial to human health in their daily diet.

In practice, the optimal solution is to add two antioxidants to the semi-finished horse meat product in the following concentration: 0.05-0.02 % ascorbic acid (0.05-0.02 g/kg) and 0.075-0.01 % dehydroquercetin (0.075-0.01 g/kg) **[35]**.

Manufacturers add horse meat to semi-finished meat products to reduce costs and rationally use raw materials.

Horse meat is subject to rapid spoilage, so it is important to study the influence of the proportion of horse meat added on the storage capacity of semi-finished meat products (minced meat).

4 prototypes were compiled:

– control (horse meat without horse fat) with the addition of ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%;

– experiment 1 (horse fat with the addition of 15%) with the addition of ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%;

– experiment 2 (horse fat with the addition of 25%) with the addition of ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%;

– experiment 3 (horse fat with the addition of 35%) with ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%.

The produced semi-finished products were frozen and stored at -18°C for 60 days.

The results of a study of the dynamics of the accumulation of oxidative spoilage products (Figures 20, 21, 22) showed that in the control sample, the accumulation of peroxides occurred somewhat more slowly, which is explained by the lower moisture content. However, all processed samples remained of good quality throughout the entire storage period, and the accumulation of breakdown products of fatty acids and peroxides was within normal limits.

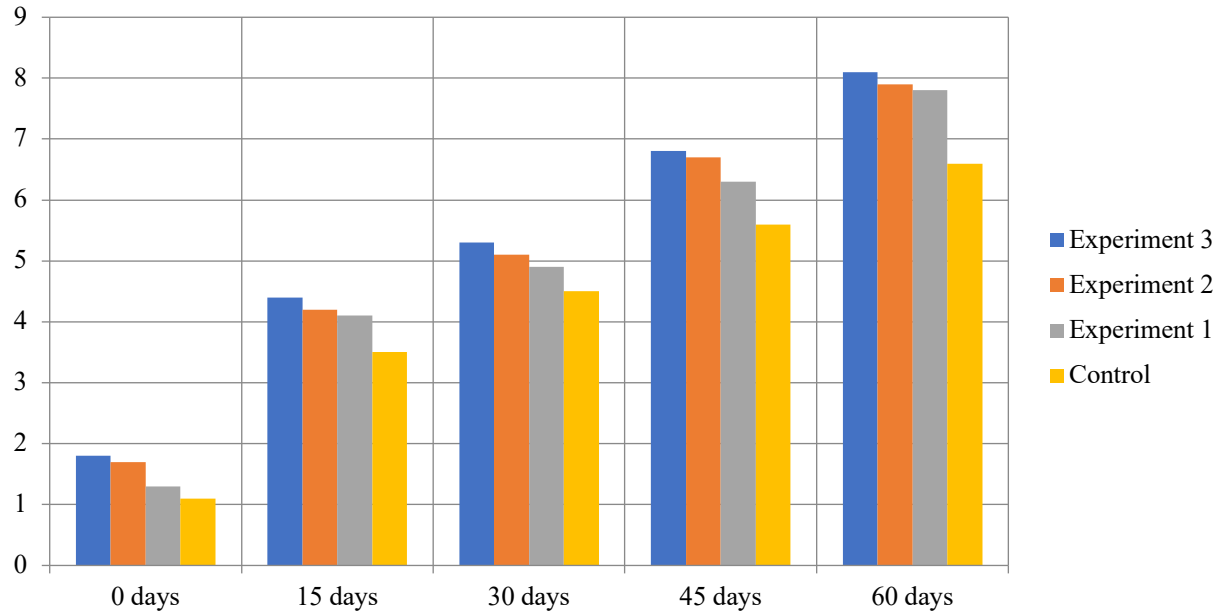


Figure 20 Dynamics of changes in the peroxide value of semi-finished meat products during storage (mmol/act O_2).

The beneficial effect of natural antioxidants on the inhibition of oxidative reactions during storage of both meat semi-finished products and finished products was noted in **[36]**.

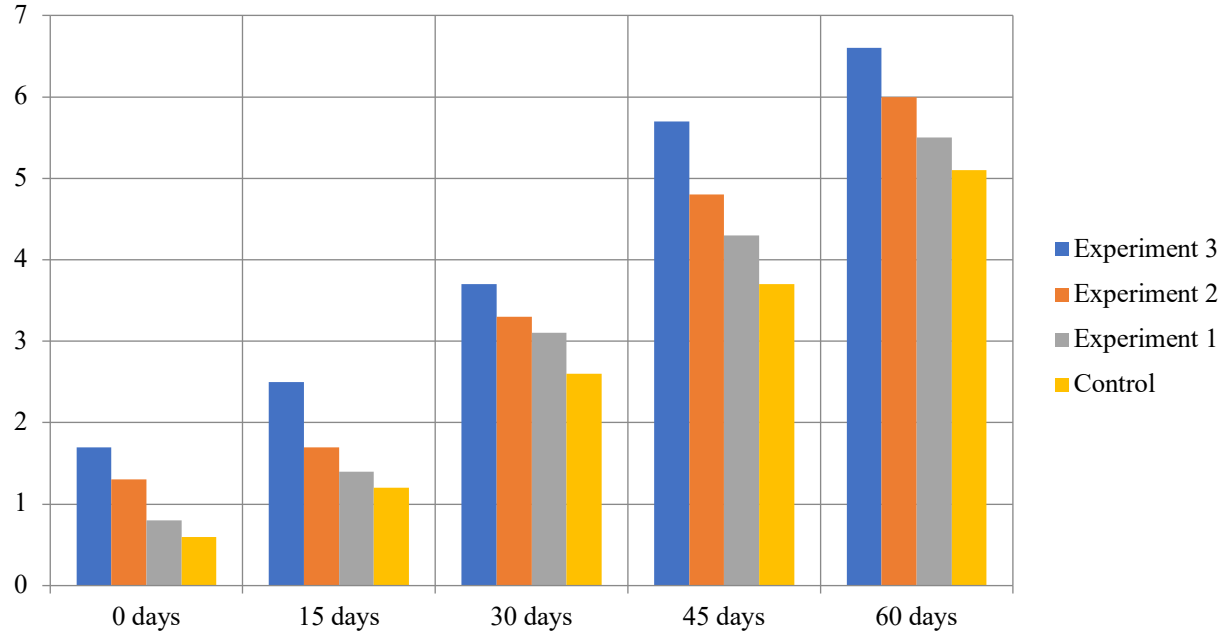


Figure 21 Dynamics of changes in the acid value of semi-finished meat products during storage (mg/kg).

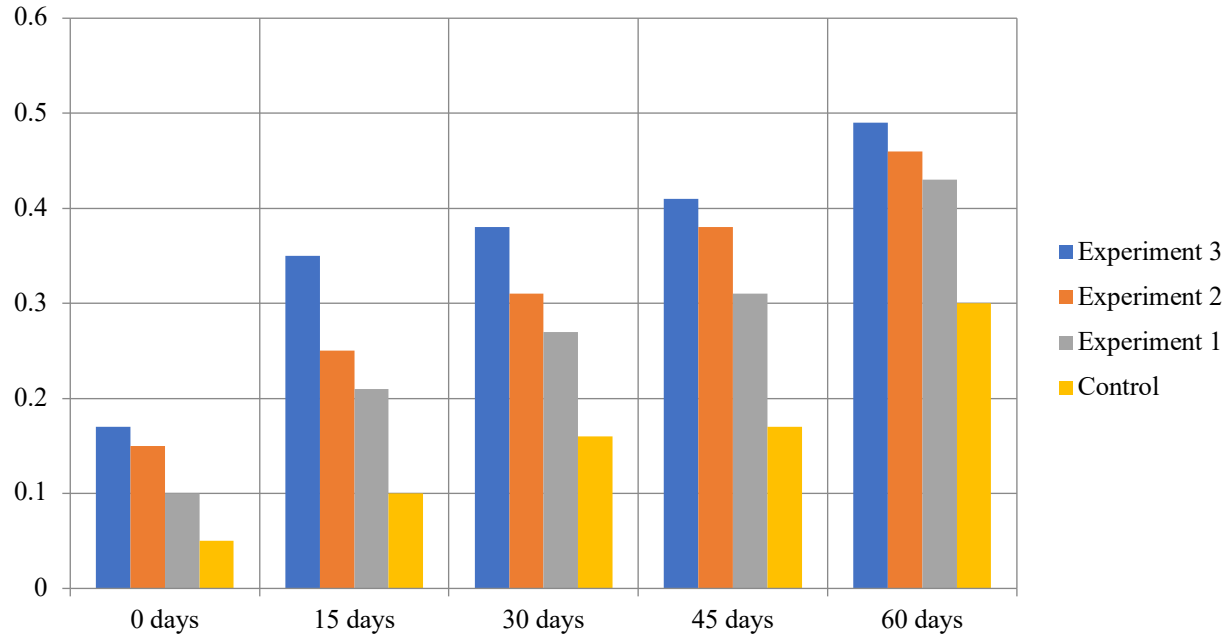


Figure 22 Dynamics of changes in the thiobarbituric value of semi-finished meat products during storage (mg/kg).

The conducted research **[34]** testifies about the achievement of positive technological effects from the application of the natural antioxidant - dihydroquercetin due to the retardation of the development of oxidative spoilage processes. Also, it shows that its use allows keeping consumer properties of minced meat semi-finished products within 6 months and more.

Cold-smoked pork sausage improved the hygienic quality of the sausage with no significant effect on the growth of lactic acid bacteria **[37]**. According to Kuz'mina et al. **[38]** objectively established that dihydroquercetin exhibits strong antioxidant activity, thereby reducing oxidative spoilage of semi-finished products and creates an opportunity to manage its qualitative characteristics during storage. It is noted that using food additives allows the preservation of organoleptic and microbiological properties of semi-finished products for a long period of storage due to the inhibition of oxidation product formation **[39]**.

The results obtained confirm similar data available in the literature. For example, the addition of grape seed powder and green tea extract to meat semi-finished products **[40]**, dihydroquercetin **[41]**, **[42]**, **[43]**, tiger nuts and quinoa **[44]**, milk thistle meal **[45]**, grape seed powder, green tea extract and amaranth/flaxseed flour **[46]**, as well

as rosemary **[47]** prevents the accumulation of oxidation products and protects semi-finished products from oxidative damage.

The introduction of Dx, ascorbic acid, and tocopherol also makes it possible to inhibit microbiological spoilage of the resulting semi-finished meat products during storage (Table 2). The research object was minced meat produced from horse meat with the addition of 35% since the presence of a large amount of raw fat accelerates the development of not only oxidative processes but also microbiological spoilage.

Table 2 Dynamics of microflora content during storage of semi-finished meat products at a temperature of -18°C.

Index	Unit measurements	Limit	Changes in microflora									
			control sample					experimental sample				
			0	15	30	45	60	0	15	30	45	60
NMAFAM	CFU	n/m than1.0 10^4 / g	1.8 10^2	9.2 10^2	3.7 10^3	7.9 10^3	1.9 10^4	1.6 10^2	6.9 10^2	1.1 10^3	1.5 10^3	3.8 10^3
Escherichia coli	CFU	n/a in 2 g	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
L. monocytogenes	CFU	n/a in 25 g	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Pathogenic, including *Salmonella*	CFU	n/a in 25 g	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

Note: NMAFAM (Number of mesophilic aerobic and facultative-anaerobic microorganisms), n/m – not more, n/a – not allowed, n/d – not detected.

Based on the results of microbiological studies, it was established that the use of Dx together with tocopherol and ascorbic acid leads to inhibition of microflora growth and has a bacteriostatic effect, which helps to increase the shelf life of semi-finished meat products compared to control samples.

Figure 23 Semi-finished meat products from horse meat.

CONCLUSION

The use of Dx in the composition of fat mixtures and horse meat allows you to slow down the development of oxidative processes. The dosage of Dx from 0.05% to 0.075% by weight of fat in an alcoholic solution is most effective for maintaining the quality and safety of raw horse fat. When applying AA and Tp in dosages of 0.05% and 0.02%, respectively, it allows reducing the dosage of Dx to 0.025% without losing the qualitative characteristics of raw fat in for 24 days of storage. Organoleptic studies of minced horse meat the optimal dosage for the introduction of an antioxidant component is the introduction of an alcoholic solution with a Dx content by fat weight from 0.075% to 0.1%, respectively, with additional addition of AA and Tp in recommended dosages – 0.05% and 0.02%, respectively. The introduction of Dx from 0.075% to 0.01% by weight of fat in an alcoholic solution together with AA and Tp allows you to extend the shelf life of horse meat and meat semi-finished products (at a temperature of -18°C) up to 60 days.

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