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Effects of bioactive compounds upon the deterioration of the chilled mechanically deboned poultry meat during storage

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Abstract

Mechanically deboned poultry meat is a valuable protein containing raw material widely used for the production of meat products. However, it does not have a high resistance to oxidation; therefore, various antioxidants including those of natural origin are used in its composition. The article provides information on the advisability of using rosemary extract and dihydroquercetin to stabilize lipids and interrupt hydrolytic and chain oxidative processes in mechanically deboned poultry meat. The permissible storage time for mechanically deboned poultry meat using the rosemary extract and dihydroquercetin in a chilled state is 96 hours. Research has been carried out on the oxidative processes of the fatty complex of mechanically deboned poultry meat during the refrigerated storage period. Antioxidants prevent the accumulation of peroxides: in the samples with their use, the peroxide number reaches critical values on 6^{th}–7^{th} day of storage, without their use – on 3^{th} day of storage. For all samples, a gradual increase in the acid number has been observed; however, for samples without antioxidants its values reach a critical level on the 4^{th} day of storage, with the use of antioxidants – on the 7^{th} day. The active formation of secondary oxidation products has begun from the second day of storage and reached the limit of permissible values in samples without antioxidants after 3 days of storage. In samples with antioxidants, the thiobarbituric number reaches a critical value on the 6^{th} day. It has also been shown that the use of antioxidants contributes to the preservation of sensorial indicators (colour, odour) improving the quality of products. The efficiency of using the rosemary extract and dihydroquercetin as inhibitors of the oxidation of mechanically deboned poultry meat has been confirmed.

For citation

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Влияние биологически активных соединений на предотвращение порчи мяса птицы механической обвалки при хранении

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Реферат

Мясо птицы механической обвалки является ценным белковым сырьем, широко используемым для производства мясных продуктов. Однако оно не отличается высокой стойкостью к окислению, для решения этой проблемы используют различные антиоксиданты, в том числе природного происхождения. В статье приведена информация о целесообразности применения экстракта розмарина и дигидрокверцетина для стабилизации липидов и прерывания гидролитических и цепных окислительных процессов в мясе птицы механической обвалки. Допустимые сроки хранения мяса птицы механической обвалки с применением экстракта розмарина и дигидрокверцетина в охлажденном состоянии составили 96 ч. Проведены исследования окислительных процессов жирового комплекса мяса птицы механической обвалки в течение срока хранения в охлажденном состоянии. Антиоксиданты препятствовали накоплению перекисей: в образцах с их применением перекисное число достигло критических значений на 6–7 сутки хранения, без их применения – на 3 сутки. Для всех образцов наблюдалось плавное повышение кислотного числа, однако для образцов без применения антиоксидантов его значения достигли критического уровня на 4 сутки хранения, с применением антиоксидантов – на 7 сутки. Активное образование вторичных продуктов окисления началось со вторых суток хранения и достигло предела допустимых значений в образцах без антиоксидантов после 3 суток хранения. В образцах с антиоксидантами показатель тиабарбитурового числа достиг критического значения на 6 сутки. Доказано, что применение антиоксидантов способствует сохранению органолептических показателей (цвет, запах), что повышает качество продукции. Подтверждена эффективность использования экстракта розмарина и дигидрокверцетина как ингибиторов окисления мяса птицы механической обвалки.

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Introduction

Worldwide, the problem of providing consumers with protein products of animal origin is urgent (*Elmadfa et al., 2017*), and for this purpose, the products of poultry processing including mechanically deboned poultry meat (MDPM) are used increasingly (*Guerra et al., 2003*). In terms of its physical and chemical properties, MDPM is similar to the manually separated from the bones and then chopped poultry meat, but differs from it in the high content of bone tissue, bone marrow, connective tissue and fat (*Guerra et al., 2007; Bondar et al., 2018*). Regulation EC No. 853/2004[1] defines different requirements for mechanically separated meat obtained using methods that do not alter the structure of meat and bone, and methods that change the specified structure and thus significantly increase the calcium content in comparison with hand-deboned meat. According to a normative document of Ukraine[2], all the types of meats of the mechanical separation are called "meat separated with the use of mechanical means". If the said separation is fulfilled without changing the structure of the tissues, such a mass is called "mechanically separated meat", if technical means do not allow such a change in the structure to be avoided, the mass is called "mechanically deboned meat" (*Yeresko et al., 2017; Sychevskyi et al., 2021*).

Along with such positive properties as nutritional value and technological acceptability, MDPM has a low resistance to oxidation, a specific red (from bright to dirty) colour, due to technological factors of obtaining and the transition of lipids to hemoproteins of the bone marrow (*Sharygina et al., 2011a*). MDPM is the most vulnerable among fat-containing foods in terms of oxidative lipid spoilage. For this reason, experts recommend stabilizing or inhibiting lipid oxidation in general and, in particular, MDPM lipids, by using biologically active substances of natural origin, characterized by high antioxidant activity (*Püssa et al., 2009; Perkovets, 2013*). A large number of compounds are known to be used to prevent oxidative processes in fats and fat-containing products. Among them, there are antioxidants, which inhibit the process of fat oxidation, and synergists, which enhance the stabilizing effect of individual antioxidants or their mixtures. By their nature, they are natural or synthetic. Today, more attention is paid to natural antioxidants, which include a large number of compounds. Most of them are in a form accessible for assimilation, they increase the nutritional value of products, and some of them show medicinal properties (*Denisovich, 2007; Lyucheva, 2008; Reshetnik, 2007*).

In the last century, the natural ingredient dihydroquercetin (DHQ) was extracted from Siberian larch wood. Laboratory studies of DHQ have revealed a number of beneficial properties inherent in flavonoids with antioxidant activity – P-vitamins, as well as properties not observed in most other flavonoids. DHQ is an antioxidant of the flavonoid group of P-vitamins, deservedly considered a model one, its antioxidant effect significantly exceeds the level of activity of quercetin and known vitamins A, C and E. The main purpose of introducing DHQ into foods is due to its properties, which include: slowing down oxidative reactions, the ability to strengthen blood vessels and P-vitamin activity. As an antioxidant, DHQ slows down lipid oxidation and makes it possible to produce products with an increased shelf life, and as a capillary protector and a variety of P-vitamins, it allows the production of health-improving products. In the Russian Federation, DHQ is officially recognized as an antioxidant[3], for which adequate and upper permissible levels of DHQ consumption are established in the amount of 240 mg per day[4].

The use of natural antioxidants to preserve the quality of products in the domestic meat processing industry has not yet become widespread. However, scientists are actively involved in this issue.

Rosemary (*Rosmarinus officinalis* L.) from the *Lamiaceae* family is a plant with a pronounced antioxidant and antimicrobial effect. Studies have shown that the complex of rosemary biologically active substances has an inhibitory effect on a wide range of bacteria, including *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Klebsiella pneumoniae* (*Dimitrijevic et al., 2007; Zhang et al., 2009; Hać-Szymańczuk et al., 2009; 2017*). The introduction technology and methods of industrial application of the rosemary extract in the meat processing industry have been studied in detail and described in (*Sharygina et al., 2011a; 2011b; Baidalinova et al., 2008a; 2008b; 2009*). In food stuffs and products of the meat processing industry, the rosemary extract limits the rate of lipid peroxidation and the formation of metmyoglobin in raw materials. Acting as a trap for free radicals, carnosic acid and carnosol block oxidation processes and thus increase shelf life, expand the range of natural colours, and improve the texture and taste profile of the finished product.

At the same time, only a few sources report on the features of the use of herbal preparations to ensure storage stability of MDPM (*Hassan et al., 2005; Hać-Szymańczuk et al., 2017*). In (*Hać-Szymańczuk et al., 2017*)

[1] Council E.U. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin // Eur. Union. 2004. P. 55–205.

[2] Order on Approval of Hygienic Requirements for Poultry Meat and Certain Indicators of Its Quality dated 06 Aug. 2013 № 694 / Ministry of Health of Ukraine. URL: http://zakon.rada.gov.ua/laws/show/z1379-13.

[3] Enactment of the Chief State Sanitary Physician of 14 November 2001 No 36 "Concerning the Implementation of Sanitary Regulations and Norms 2.3.2.1078-01".

[4] Recommended Practice of the State Sanitary and Epidemiological Rationing of the Russian Federation No 2.3.1.1915-04 of 2004 "Recommended levels of consumption of food and biologically active substances".

the study of various rosemary preparations for microbiological parameters and oxidative stability of MDPM amid freezing storage is described. Rosemary was used in the form of a dried mass (2.0 %), water and alcohol extracts (2.0 %), and essential oil (0.2 %). During the entire storage period, the use of rosemary preparations did not significantly affect the number of *Enterobacteriaceae*, but significantly limited the growth of *E. coli*. Based on the thiobarbituric value, it was concluded that rosemary preparations, with the exception of the aqueous extract, reduced lipid oxidation in MDPM during freezing storage for 4 months.

The positive effect of DHQ and the rosemary extract as bioflavonoids on human health has been proven by many years of experimental and clinical studies of medical institutions (*Kostyrya et al., 2015*). The published research results confirm the advisability of using selected natural antioxidants for long-term storage of fat-containing products, primarily meat, since their presence in products is perceived by consumers positively (*Semenova et al., 2006*).

Oxidative spoilage of meat and meat products is mainly caused by lipid peroxidation generated by reactive oxygen species; therefore, the degree of oxidative changes in fats is considered by the magnitude and dynamics of changes in acid number (AN), which is determined by free fatty acids contained in fat. The acidity of the fat is an indicator of its freshness. An increase in AN values of fat during storage indicates the onset of hydrolytic deterioration of fat. Another important, in this sense, indicator – the peroxide number (PN) – characterizes the content of peroxides in fat. Fatty acids during long-term storage of fat are oxidized by atmospheric oxygen with the formation of intermediate peroxide compounds. Therefore, by the accumulation of peroxides, one can evaluate the initial stage of oxidative deterioration. The effect of antioxidants on the formation of secondary oxidation products can be estimated by analyzing the dynamics of the thiobarbituric number (TBN). The TBN index reflects the development and depth of oxidative changes in the fat phase of meat products by the quantitative content of malonic aldehyde, the formation of which is considered to be one of the adverse effects of lipid peroxidation. Absolute TBN values less than 0.5 mg/kg indicate the stability of the lipid fraction during storage, an increase in concentration to values over 0.5 mg/kg points out some oxidation, and a value above 1.0 mg/kg means deep oxidative changes (*Voitsekhivska et al., 2020*).

Purpose and tasks of work

The purpose of this work is to carry out a set of studies on the use of known natural antioxidants to ensure the proper resistance of MDPM to oxidation during cold storage. The achievement of this goal is due to the solution of such basic tasks as:

- carrying out completed pilot workings of MDPM using the rosemary extract and DHQ to stabilize lipids and interrupt hydrolytic and chain oxidative processes;
- determination of the optimal amounts and methods of introducing biologically active compounds into the MDPM;
- research of indicators of quality and safety of MDPM, as well as rational values of its physical and chemical parameters;
- study of the resistance of MDPM to oxidation.

Materials and methods

The objects of research were:

- fat soluble rosemary (*Rosmarinus officinalis*) extract produced by Danisco (Denmark);
- DHQ – antioxidant for food products obtained from Siberian larch wood;
- pilot industrial samples of MDPM using the rosemary extract and DHQ, manufactured in industrial conditions by LLC Khodorovsky Myasokombinat (Lviv region) and LLC IKO IF (Ivano-Frankovsk).

The following parameters were determined:

- mass content of moisture – by the standard method[5];
- mass content of fat – by the standard method[6];
- mass content of protein – by the standard method[7];
- AN and PN values – by the standard method[8];
- TBN – by the method presented in (*Zhuravskaya et al., 1985*).

The antioxidant effect of the investigated extracts on meat systems was tested on MDPM samples during their storage. For better distribution in the studied samples, antioxidant extracts were added during stirring in a high-speed cutter. The dosage of the rosemary extract was 0.1 % and 0.025 % – DHQ. The control samples were without the addition of extracts. To accelerate biochemical processes, the prepared samples were stored at

[5] DSTU ISO 1442:2005. Meat and meat products. Determination of moisture content (reference method).

[6] DSTU 8380:2015. Meat and meat products. Method of measurement of mass fraction of fat.

[7] DSTU ISO 937:2005. Meat and meat products. Determination of nitrogen content (Reference method).

[8] GOST 31470-2012. Poultry meat, edible offal and semi-processed products. Methods for organoleptic and physico-chemical examinations.

a temperature of 4 ± 2 °C, AN and PN values were determined[9] in it with the release of fat by chloroform[10], TBN and the content of volatile fatty acids.

Statistical processing of the results obtained was carried out on the basis of calculating the arithmetic mean values and the root mean square error. All experimental data are the results of 3–5 parallel determinations.

Results and discussion

We found it appropriate to apply to use antioxidant additives in the following ways based on the analysis of foreign experience and according to the results of our own research.

1. The rosemary extract is added to the chopped meat obtained from manually deboned poultry meat and to the mass obtained from mechanical deboning of the carcasses and the dorsal part according to the standard technological scheme in the amount of 0.1 % to the mass of raw materials and DHQ in the amount of 0.025 % to the mass of raw materials. The mixing of chopped meat with additives is carried out in a mixer, where the chopped meat is being sequentially introduced, and then DHQ – during the rotation of the mixer blades.

2. The components of the minced meat or MDPM are mixed for 4–6 minutes until a homogeneous mass is formed. Antioxidant additives (DHQ and/or rosemary) are added to the chopped meat during the rotation of the mixer blades. DHQ is added having previously dissolved in alcohol with the concentration of 40 %.

To determine the compliance of the MDPM samples used for research with the specifications, industrial workings have been carried out using biologically active compounds. The results of physical and chemical studies are shown in Table 1.

Table 1. Physical and chemical parameters of the MDPM samples manufactured in the industrial facilities
Таблица 1. Физико-химические показатели образцов МПМО, изготовленных в промышленных условиях

Manufacturer of MDPM	Sample	Mass content, %				Mass content of bone inclusions, %	Mass content of calcium, %	Mass content of phosphorus, %
		protein	moisture	fat	ash			
LLC Khodorovsky Myasokombinat	Control 1	12.75 ± 0.09	69.68 ± 0.56	15.84 ± 0.33	1.73 ± 0.08	0.301 ± 0.08	0.097 ± 0.010	0.24 ± 0.04
	DHQ added	13.36 ± 0.10	69.42 ± 0.63	15.23 ± 0.26	1.99 ± 0.09	0.292 ± 0.07	0.092 ± 0.009	0.23 ± 0.06
LLC IKO IF	Control 2	14.61 ± 0.08	67.35 ± 0.42	16.48 ± 0.35	1.56 ± 0.11	0.183 ± 0.05	0.071 ± 0.010	0.22 ± 0.05
	Fat soluble extract of rosemary added	14.32 ± 0.06	67.48 ± 0.35	16.84 ± 0.41	1.36 ± 0.08	0.200 ± 0.06	0.075 ± 0.008	0.22 ± 0.05

The quality and safety indicators of MDPM were determined in control samples produced at each of the enterprises (Control 1 and Control 2). From these batches, samples of MDPM were made with the addition of antioxidants, DHQ and fat-soluble rosemary, in the above dosage. For these samples, quality and safety indicators were also determined in parallel with the control samples. As can be seen from the data presented in Table 1, the addition of these antioxidants did not affect the quality indicators of the experimental samples of the MDPM. Based on the analysis of the data and taking into account the standards in force, the most acceptable values of the quality and safety indicators of MDPM were determined, which are shown in Table 2.

[9] GOST 7702.1-74. Poultry meat. Methods for chemical and microscopic analysis of meat freshness.
[10] DSTU 8380:2015. Meat and meat products. Method of measurement of mass fraction of fat.

Table 2. Physical, chemical and safety parameters of MDPM with the use of biologically active compounds
Таблица 2. Физико-химические показатели и показатели безопасности МПМО с применением биологически активных соединений

Parameter	Limit	Norm
Mass content of moisture, %	max	70
Mass content of protein, %	min	12
Mass content of fat, %	max	18
Mass content of calcium[11], %	min	0.1
Mass content of phosphorus[11], %	max	0.25
Mass content of bone inclusions[11], %	max	0.6
Mass content of bone inclusions to 500 μm[11], %	max	98
Mass content of bone inclusions from 500 μm to 750 μm[11], %	max	2
Content of volatile fatty acids, mg/100 g	max	8
PN, % of iodine	max	0.25
AN, mg KOH/1 g of fat	max	3
Temperature of the mass at the press outlet, °C[11]	max	8

Further, a series of experiments was carried out confirming the effectiveness and usefulness of application, as antioxidants, extracts of rosemary and DHQ. To select effective antioxidants, a number of experiments were carried out comparing the antioxidant activity of biologically active compounds, which are used in practice as antioxidants. At the same time, it was taken into account that the level of their input into the formulation should not influence the taste, physical and chemical properties of raw meat. For DHQ, this level is in the range of 0.02 g per 100 g, that is, 0.02 %. The maximum permissible concentration the excess of which can affect the formation of odour and aroma, as well as the taste and colour of the product, is 0.025 %. The study of the effectiveness of the antioxidant properties of the selected antioxidants (DHQ and the rosemary extract) was carried out by measuring the amount of free fatty acids formed, which is determined by acidity or AN. An increase in the AN values in the samples under study at different stages of the experiment indicates the beginning of hydrolytic oxidation (spoilage) of fat. The content of the peroxides formed in the test samples, which is characterized by the PN, was also determined. Fatty acids during storage of fat are oxidized by atmospheric oxygen with the formation of intermediate peroxide compounds, therefore, by the accumulation of peroxides, that is, by an increase in the peroxide value one can evaluate the initial stage of oxidative deterioration. In preliminary studies, these preparations were added in the maximum amounts recommended by manufacturers for universal use. Our task was to determine the dosage limits of biologically active substances, with which their effective use as antioxidants for MDPM is ensured. It has been found and confirmed in industrial facilities that effective concentrations are 0.1 % for the fat-soluble rosemary extract and 0.025 % for DHQ. Exceeding these concentrations does not significantly affect the antioxidant effect of the studied antioxidants.

Studies have been carried out on the oxidative processes of the fatty complex of the MDPM during the refrigerated storage period. The course of the oxidation process in the studied samples was monitored by the change (growth) of PN during storage, the dynamics of this indicator is described in the diagram shown in Fig. 1. The dashed line in the diagram indicates the limit of permissible values.

In the cooled state, the dynamics of the oxidation process of the control and experimental samples is almost proportional, the effect of antioxidants is observed to reduce the accumulation of peroxides. On the fourth day, the average values of PN of the prototypes are half the average values of the control ones. In control samples, the amount of formed peroxides reaches a critical value already on the third day – in particular, in Control 2 with a higher mass fraction of fat (Table 1). In experimental samples, a noticeable increase in the oxidation process begins after five days and reaches critical values only on the sixth–seventh days.

The dynamics of hydrolytic processes in the fatty complex of MDPM during storage is presented by diagrams of changes in AN in Fig. 2.

The analysis of the dynamics of the process of chilled MDPM fats hydrolytic decomposition (Fig. 2) shows a gradual increase in the AN level within three days. However, in the samples with the addition of antioxidants, a decrease in the level of formation of free fatty acids is noted in comparison with the control samples already after the first day and is 28 % on the third day. On the fourth day, the AN values of the control samples reach the critical level, while for the experimental samples for the fourth day, the AN value is 50 % lower than the critical one, that is, the limit is reached only on the seventh day.

[11] TU U 10.1-00419880-155:2020. Mechanically deboned poultry meat. Specifications.

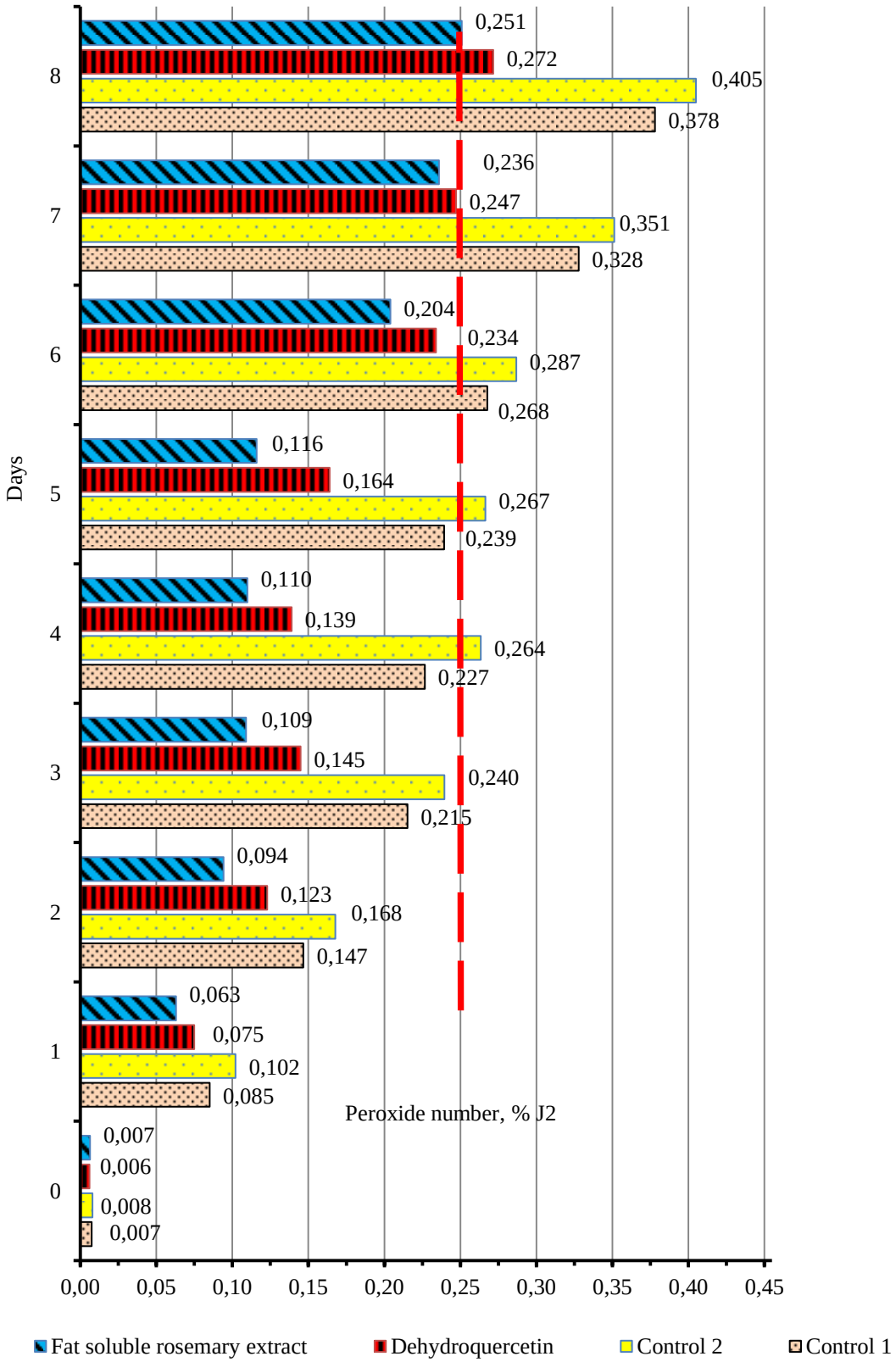

Fig. 1. Dynamics of the fat oxidation process of MDPM during storage in the chilled state
Рис. 1. Динамика окислительного процесса жиров МПМО
во время хранения в охлажденном состоянии

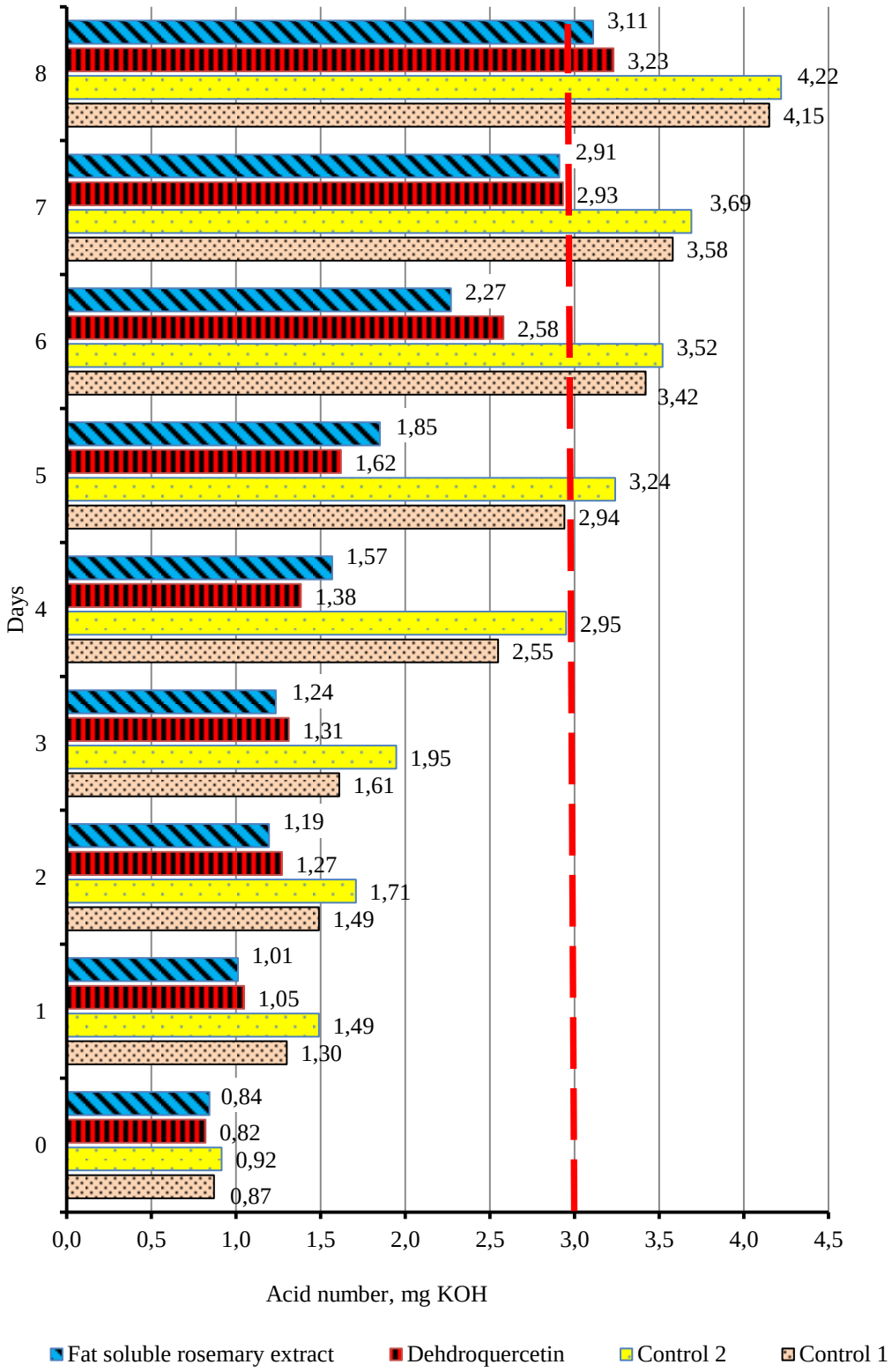

Fig. 2. Effects of biologically active compounds on the indicators of hydrolytic decomposition of the MDPM fatty complex during storage in a chilled state

Рис. 2. Влияние биологически активных соединений на показатели гидролитического распада жирового комплекса МПМО во время хранения в охлажденном состоянии

In fat-containing products and raw materials during storage as a result of further oxidation of the primary oxidation products – unstable hydroperoxide compounds – secondary oxidation products are formed. Some by-products of fat oxidation are toxic and have a negative physiological effect on the human body. In addition, they cause the deterioration in sensorial characteristics, in particular odour. Therefore, it is very important to study the formation of secondary oxidation products during storage of MDPM and the effect of antioxidants to reduce the intensity of this process. The formation of secondary fat oxidation products (aldehydes, ketones, etc.) is monitored by the malonic aldehyde index – TBN – a conventional value characterizing the content of malonic aldehyde (MA) and determined by the colour of the interaction of aldehyde groups with 2-thiobarbituric acid. The TBN value is expressed in milligrams of MA per 1 kg of product. The results of studies of the dynamics of the secondary oxidation products formation in MDPM in a cooled state are shown in Fig. 3.

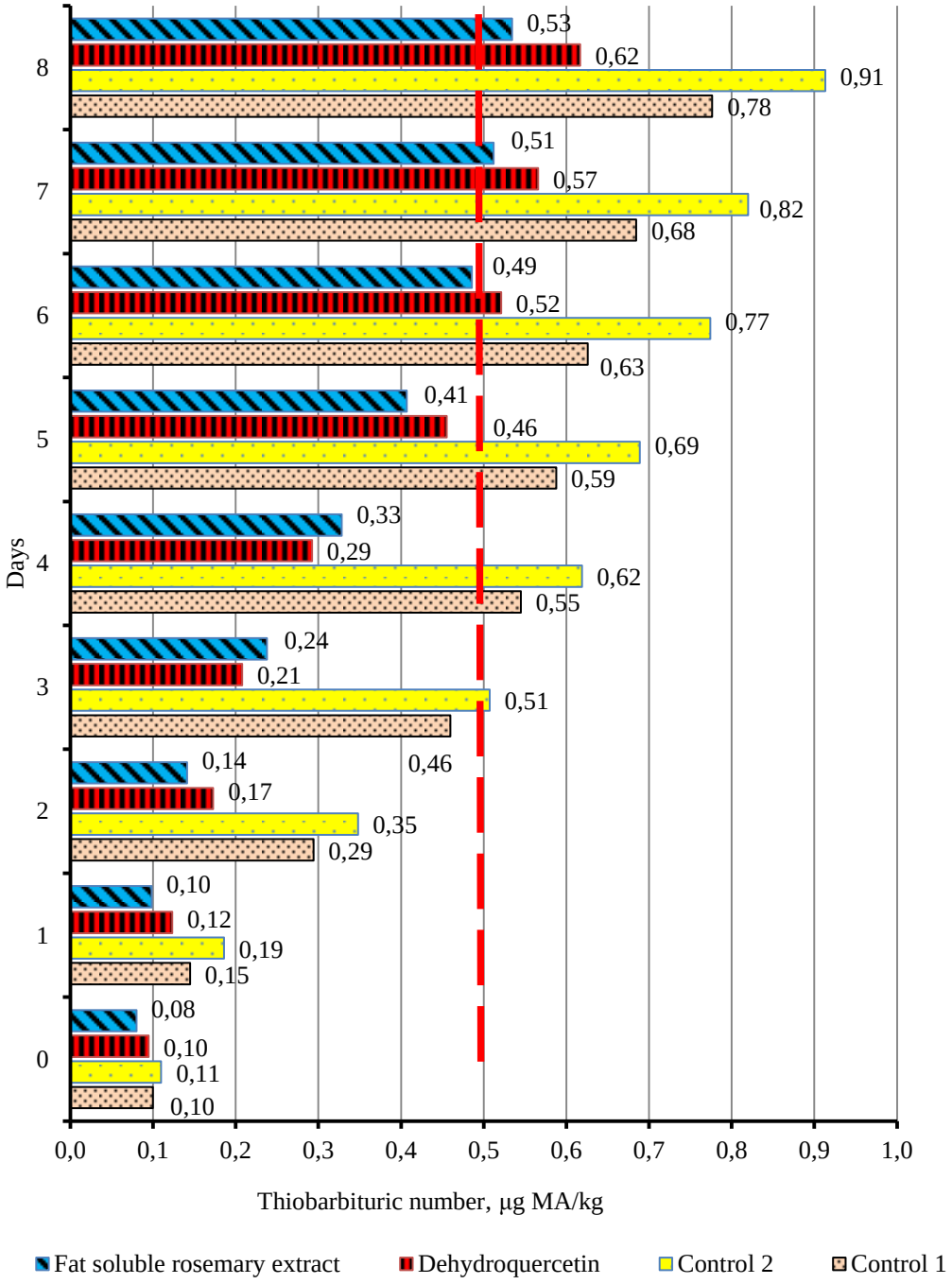

Fig. 3. Effects of biologically active compounds on the indicators of the formation of secondary products of oxidation of the MDPM fatty complex during storage in a chilled state

Рис. 3. Влияние биологически активных соединений на показатели образования вторичных продуктов окисления жирового комплекса МПМО во время хранения в охлажденном состоянии

In a cooled state, the active formation of secondary oxidation products began from the second day of storage and reached the limit of permissible values in the control samples after the third day of storage. In the experimental samples, the level of secondary oxidation products on the third day was half that in the control, on the fifth day it was 14 % less than the permissible value of 0.5 mg MA per kg and reached a critical value on the sixth day.

Conclusion

Prevention of oxidative deterioration of MDPM, which is a mass product of the poultry processing industry in order to extend its permissible shelf life, is of great national economic importance, since it contributes to the rational use of valuable protein raw materials. One of the technologically effective and nutritionally acceptable methods of slowing down oxidative reactions in these meat masses is the use of natural antioxidants from plant raw materials.

The completed pilot workings of the MDPM elaborated with the use of the rosemary extract and DHQ to stabilize lipids and interrupt hydrolytic and chain oxidative processes made it possible to determine rational acceptable amounts and methods of introducing biologically active compounds into the meat of mechanical deboning: for the rosemary extract it is 0.1 %, for DHA – 0.025 %.

The study of AN, PN and TBN as parameters for controlling the oxidative process of MDPM lipids during storage in a refrigerated state confirmed the effectiveness of using DHQ and the fat-soluble rosemary extract as antioxidants. The antioxidant and stabilizing effect of both drugs is not significantly different, the difference in the level of slowing down of oxidative processes is explained not by the greater or lesser activity of this or that preparation, but by the different composition of raw materials and, therefore, by differences in the lipid composition, for example, the content of bone marrow fats, heme pigments or other factors that affect the course of oxidation and hydrolytic processes.

The use of the studied biologically active preparations, provided that they are introduced during the technological process or immediately after the manufacture of MDPM in the recommended dosage, guarantees the stability of the fat complex of MDPM in a refrigerated state for 4 days of storage. In addition, the use of these antioxidants significantly contributes to the preservation of sensorial parameters (colour, odour), which increases the quality of products.

As a result of the research being carried out, the permissible shelf life of MDPM with the use of the rosemary extract and DHQ in a refrigerated state has been determined as 96 hours.

Based on the analysis of the literature and the results of the studies performed, it seems advisable to continue the research of the MDPM focusing on the bacterial safety of this meat raw material, as well as on the possibility of using other preparations of natural origin that can be used as antioxidants.

Conflict of interest

The authors declare no conflicts of interest.

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