

Effect of a compound bio-preservative on microbiological indicators and shelf life of fresh pork chops

Keywords: preservative, antioxidant, fresh meat products, total viable count, yeast, shelf life, storage, microbiology

1. SUMMARY

The article deals with the study of the effect that a compound preservative produces on microbiological indicators and shelf life of fresh pork products. The effect of various preservatives on the total viable count and yeast growth in fresh meat during storage was studied. Experimental studies have shown that the compounds of additive a preservative mixture* actively inhibits microorganism growth during the fresh pork chops storage. In the control sample, the number of microorganisms on the seventh day of storage was $12 \cdot 10^4$ CFU/g, and, in the sample with the compound additive preservative mixture added, it amounted to $0.1 \cdot 10^4$ CFU/g. The usage of the ready to use preservative mixture allows actively suppressing the yeast reproduction during long-term storage (seven days) of coarsely chopped fresh pork products (250 CFU/g). The optimal method for applying the preservative to fresh pork chops has been determined. Applying the preservative to coarsely chopped fresh meat by simply mixing and massaging (for example, together with spices or marinades) is the most rational method for this product type. Primary and secondary lipid degradation products are considered, and the peroxide and acid numbers of fresh meat products during 30-day storage are determined. After 30 days of storage, a noticeable increase in oxidative processes in the control sample is observed, whereby the end point of the shelf life of coarsely chopped fresh pork products has been chosen.

**We have to negligate the trade mark name of the preservative mixture by the law of advertisement (The Ed.)*

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³ LLC "Antey"

2. Introduction

The problem of efficient preservation of food and raw materials at all stages of their production, storage, transportation and trade, including home food preservation, appears highly relevant today. According to some estimates, up to 25% of the world's food produced is susceptible to the damaging effects of mold alone [1].

Current methods for preserving food products and preventing their microbiological spoilage are divided into three groups: physical, chemical, and biological ones. Physical methods include temperature (thermal and refrigeration) exposure, drying, vacuuming, etc. Chemical methods comprise salting, smoking, brining, the use of preservatives, etc. Biological ones consist in the treatment with starter and bioprotective cultures, the use of bactericides, enzyme preparations, etc [2, 3]. Each of these methods has certain limitations in the production of a particular product due to their impact on organoleptic properties and nutritional values as well as technical feasibility (for example, need for the required equipment, scarcity of the substances or preparations used). Of all the known methods for preventing microbiological spoilage, chemical preservatives are considered the most easily applicable, quickly feasible, not requiring special equipment and/or changing the manufacturing method [4, 5]. However, the meat industry is rather conservative in terms of the use of food additives, due to the fact, that chemical preservatives are allowed in the production of meat products only in limited quantities, mainly in the manufacture of jellied products and for surface treatment [6]. In addition, consumers overwhelmingly have a negative attitude to meat, labelled as containing preservatives. In this regard, the use of chemical preservatives in the production of meat products is significantly limited and cannot be regarded as universal means to prevent microbiological spoilage.

In Russia, the consumption level and production of pork has been growing rapidly recently. At that, the meat industry is dominated by pork nowadays. Pork production increased by 23% in 2020.

Pork is also a source of complete animal protein and has a high nutritional value. In addition, pork meat contains vitamins, macro- and microelements necessary for a comprehensive development of the human body [7].

For all health benefit properties to be maximally preserved, the rules of processing, transportation and storage of meat have to be observed. According to the Sanitary Rules and Norms SanPiN 2.3.2.1078-01 and Technical Regulations of the Customs Union TRCU 034/2013 "On the safety of meat and meat products", pork belongs to the category of perishable goods.

If the storage conditions and terms are violated, the growth and reproduction of microorganisms in fresh pork significantly accelerates, which leads to an increase in bacterial contamination. Under favorable conditions, microorganisms accumulate on the surface and gradually penetrate deep into the meat, causing the product spoilage. During storage, meat loses its positive properties, its organoleptic, physical and chemical parameters deteriorate significantly, and the risk of harm to human health increases due to the vital activity of pathogenic microbial flora. There are several types of meat spoilage: putrefaction, slime production, mold formation, acid fermentation (meat souring), etc. The intensity of these processes depends on temperature, relative humidity, microorganism type, and the degree of initial meat contamination [8].

Putrid spoilage is most often found when storage conditions are violated. Putrefactive microflora causes meat spoilage. Putrefactive microorganisms can be both aerobic and anaerobic. They are able to secrete protease enzymes that break down proteins. These microorganisms include aerobic bacilli (*B. pyocyaneum*, *B. mesentericus*, *B. subtilis*, *B. megatherium*), anaerobic clostridia (*Cl. putrificus*, *Cl. histolyticus*, *Cl. perfringens*, *Cl. sporogenes*) and facultative anaerobic cocci. The end products of aerobic putrefaction are ammonia, carbon dioxide, hydrogen sulfide, and mercaptans. Each of these compounds can cause harm to a human body, which manifests as a serious intoxication [9].

Anaerobic putrefaction of pork can occur without oxygen. Therefore, even vacuum packing will not protect the meat from spoilage if storage temperature requirements are violated. The end products of anaerobic putrefaction are the products of decarboxylation of amino acids causing the formation of off-odour substances, such as indole, skatol, phenol, cresol, diamines. Their derivatives are cadaveric poisons (cadaverine, putrescine, etc.); they are toxic to humans and can cause death [10].

Slime production is a result of slime-forming microorganisms (lactic acid bacteria, yeast, and micrococci) proliferating and partially dying off on the pork meat surface. The meat storage at a temperature of 18 to 25 °C and high humidity contribute to slime production. However, some microorganisms that cause slime formation can develop even at sub-zero temperatures. During sliming, the meat surface becomes sticky, acquires a gray-green hue and a stale off-odour, the pH of the meat surface layers is 5.2 to 5.3. It is important to distinguish between slime production and the initial stage of putrefaction, as each is caused by a completely different microflora [11].

Another equally dangerous type of meat spoilage is mold formation, which occurs when microscopic fungi develop on the surface during long-term storage of the product. When mold grows, the meat quality decreases because of protein hydrolysis and deamination of amino acids. The fungi most often found on the meat surface are *Mucor*, *Penicillium*, *Aspergillus* and *Cladosporium*. They are able to grow at low temperatures (in the refrigerators). These fungi produce mycotoxins, cause food spoilage, allergic reactions and various diseases in humans [12, 13].

The goal of this paper is investigating the effect of the compounds of food additives in the preservative mixture (detailed in the section 3.1.) on the resistance of fresh pork to microbiological spoilage during storage.

3. Materials and methods

3.1. Research objects

The research objects in this paper are the follow items:

- Coarsely chopped fresh pork (with a fat content of not more than 15% by weight)
- The compounds of food additive preservative mixture. The content of ready to use mixture are potassium sorbate (E202), sodium acetate (E262), sodium benzoate (E211), glycerin (E422), carboxymethylcellulose (E466) and an antioxidant (dihydroquercetin). The additive is manufactured by a research and manufacturing association Russia
- Lactic acid
- Acetic acid
- Sodium acetate (E262)

3.2. Research methodology

The total viable count and the amount of yeast were determined by plating the product onto agar plates with culture media, allowing microorganisms to grow and counting all individual colonies.

The peroxide value and acid value were found using the standard methods [14, 15]. Method for determining the peroxide value is based on the reaction of the oxidation products of animal fats (peroxides and hydroperoxides) with potassium iodide in a solution of acetic acid and isooctane or chloroform, followed by quantitative determination of the released iodine with a solution of sodium thiosulfate using a titrimetric method. The method for determining the acid value is based on the dissolution of a sample in a mixed solvent, and titration of free fatty acids with a solution of potassium hydroxide.

All analyses were repeated in triplicate unless otherwise stated and the average values were calculated. The results are expressed as the mean value \pm standard deviation. Significant differences between the mean values at significance level $p < 0.05$ were identified using the one-way analysis of variance and Student's test. Microsoft Excel version 2010 was used as the statistical analysis software.

4. Results and discussion

To identify the functional properties of preservative mixture, tests were carried out on chilled pork in comparison with control samples and the most common substances having a preservative effect (lactic acid, acetic acid and sodium acetate). A comparative assessment of microbiological indicators in meat products was carried out, wherefore the quantities of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM) were monitored for 7 days at a temperature of 8 to 10 °C. The experimental results are shown in **Figure 1**.

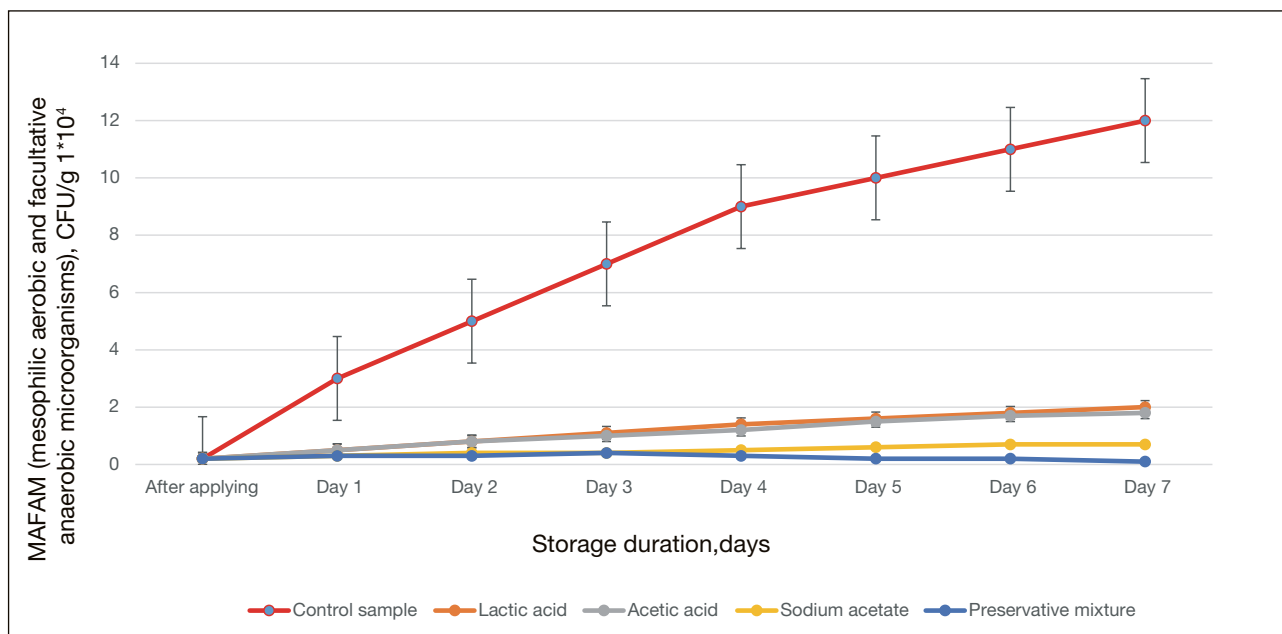


Figure 1. Effect of various preservatives on the total viable count of fresh pork chops during storage

The most common indicator of meat chops spoilage is natural acid fermentation. As a rule, acid fermentation develops in muscle tissue rich in glycogen. The main signs of the process are a sour off odour, gray or greenish hue, a decrease in the tissue elasticity and, as a result, a loose consistency. The causative agents of the defect are psychrotrophic lactic acid bacteria and yeast fungi, which ferment carbohydrates to form organic acids, as well as gases (carbon dioxide and, in some cases, hydrogen). In addition to meat carbohydrates, chopped meat products also contain carbohydrates that come from onions, marinades and other ingredients. These carbohydrates located in the brine between pork cuts are a favorable medium for pathogens of acid fermentation to develop.

Our experimental studies have shown that the selected preservatives actively inhibit microbial growth during storage. Thus, on day 7, the microbial content in the control sample was $12 \cdot 10^4$ CFU/g¹, in the sample with lactic acid added was $2 \cdot 10^4$ CFU/g, in the sample with acetic acid added was $1.8 \cdot 10^4$ CFU/g, in the sample with sodium acetate added was $0.7 \cdot 10^4$ CFU/g, and in the sample with the compounds of preservative mixture added was $0.1 \cdot 10^4$ CFU/g.

Preventing the yeast development in meat chops is an important component of raw meat manufacturers' success, as it directly relates to the shelf life of the product and guarantees its safety for the consumer [16]. The contamination of meat products results from contaminated workers' hands, storage containers, unsterilized spices and onions. **Figure 2** shows the effect that various preservatives produce on the yeast growth in raw meat during storage.

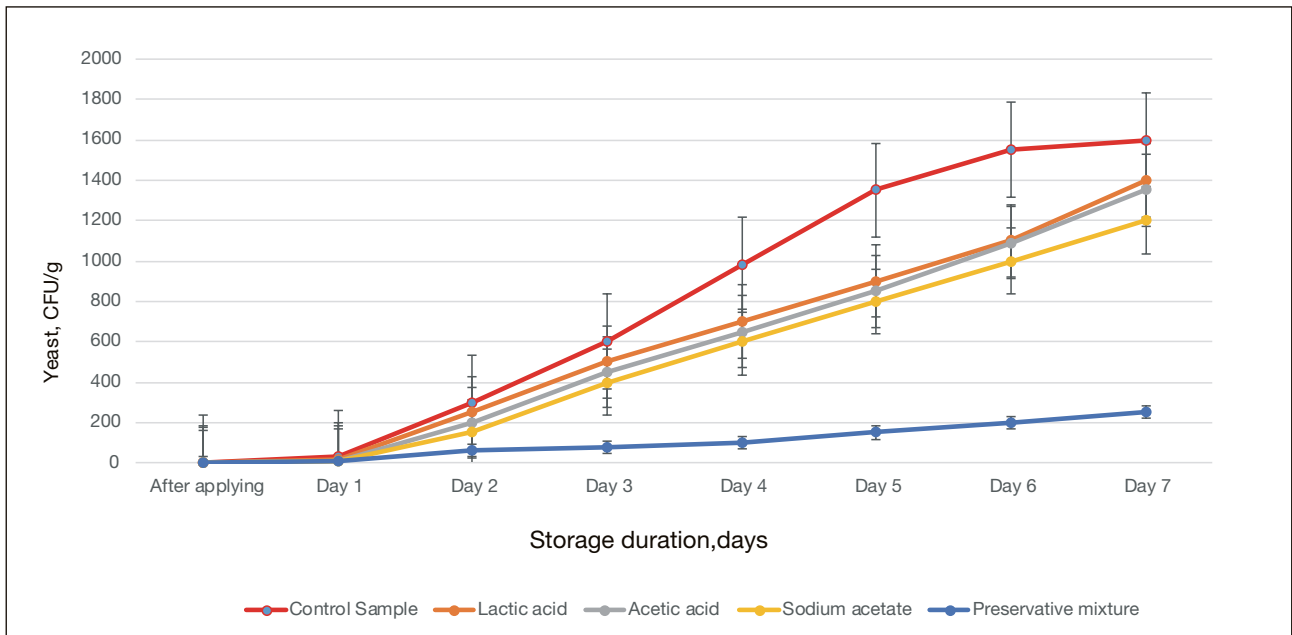


Figure 2. Effect of various preservatives on the yeast growth in fresh pork chops during storage

According to the research results, the classical preservatives (lactic acid, acetic acid and sodium acetate) have a weak effect on the growth and reproduction of yeast during raw pork storage. The fast growth of yeast in fresh pork chops starts on day 2 and reaches its peak value of 1600 CFU/g (control sample) on day 7. However, the use of the compounds of the preservative mixture allows actively suppressing the yeast reproduction during long-term storage of fresh pork products (250 CFU/g).

The data obtained confirm that the preservative mixture not only effectively inhibits the growth of yeast, but also exhibits a clear antimicrobial activity against a wide range of microorganisms.

On studying the microbiological indicators of coarsely chopped fresh pork, it was found that the yeast content on the brined meat cuts surface is hundreds of times higher than in the internal tissue. Given this fact, it is obviously the meat surface as well as the ingredients in the brine that should mostly be exposed to preservatives. In order to verify this statement, the microbiological indicators of raw pork prepared according to the same recipe, but using different methods for applying the preservative, were compared. In one sample, the preservative was applied with a syringe solution; in another one, it was added in a liquid form, being mixed with onions and marinade and then massaged. The control sample was prepared without any preservatives. The experimental results are shown in **Figure 3**.

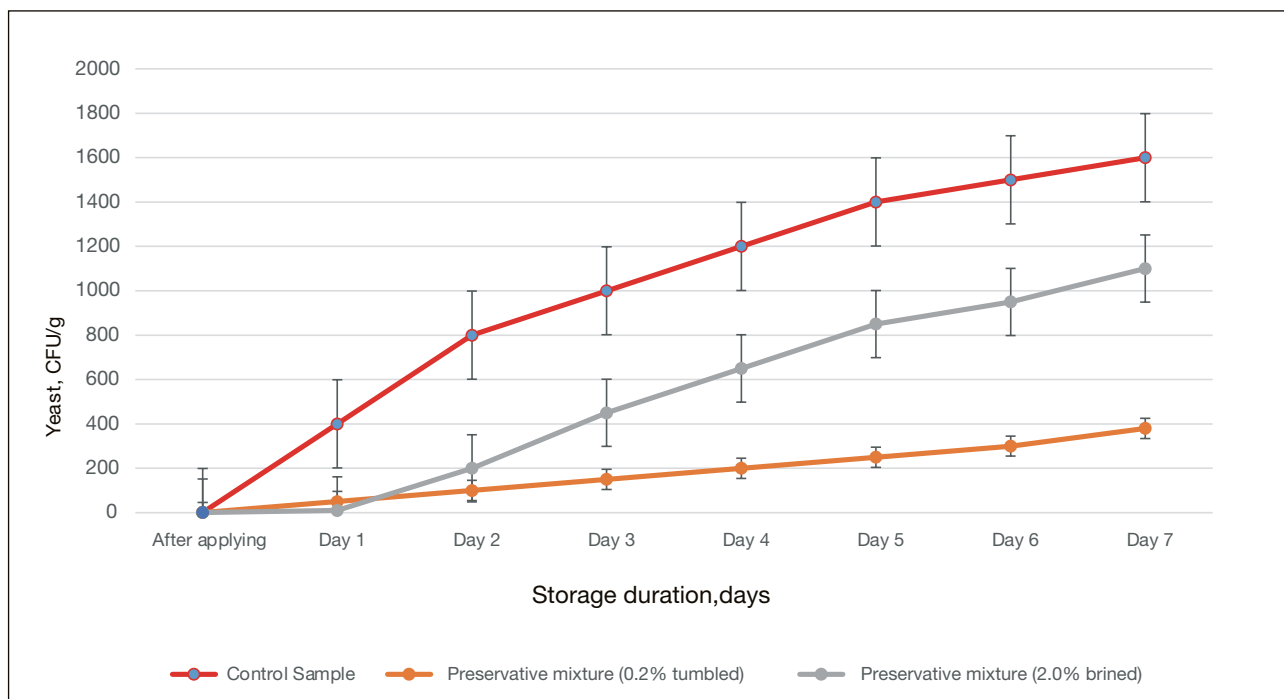


Figure 3. Effect of the method of applying a preservative on the yeast growth in fresh pork chops

Massaging was carried out in a meat tumbler “Metat master” in accordance with the tumbling program selected. Tumbling parameters are shown in **Table 1**.

Table 1. Tumbling parameters

Parameter	Value
Tumbling duration	2–3 hours
Tumbling mode (rotation / pause)	20/10 min
Cylinder rotation rate	8 rpm
Maturation	10 hours
Load factor of the tumbler	0.6
Vacuum depth	80–90%

Thus, applying a preservative to raw meat chops by simply mixing and massaging (for example, together with spices or marinades) is the most rational method for this product type. In this case, two positive effects are achieved simultaneously: the preservative concentration in the area affected by yeast increases and the total amount of the preservative in the product decreases, which provides advantages from the points of view of both product safety for consumer health and economic benefit.

The study of the increase in the shelf life of the product preserved using the preservative mixture was carried out at various time periods (from 5 to 30 days), every 5 days the amounts of primary and secondary lipid degradation products being measured and peroxide and acid numbers being determined [17].

The food additive was applied with different mass concentrations (0.1%, 0.4%, 0.6% and 1%).

Based on the data obtained, the dependence of the peroxide number (PN) values of the samples on the product storage duration was determined (**Figure 4**).

Depending on the dosage of the preservative mixture, the PN values vary, but in all options the increasing dynamics is observed over time. The highest rate is detected in the control sample. The lowest oxidative processes are observed in the samples containing 0.6% and 1% of the preservative and having significant differences with the control sample.

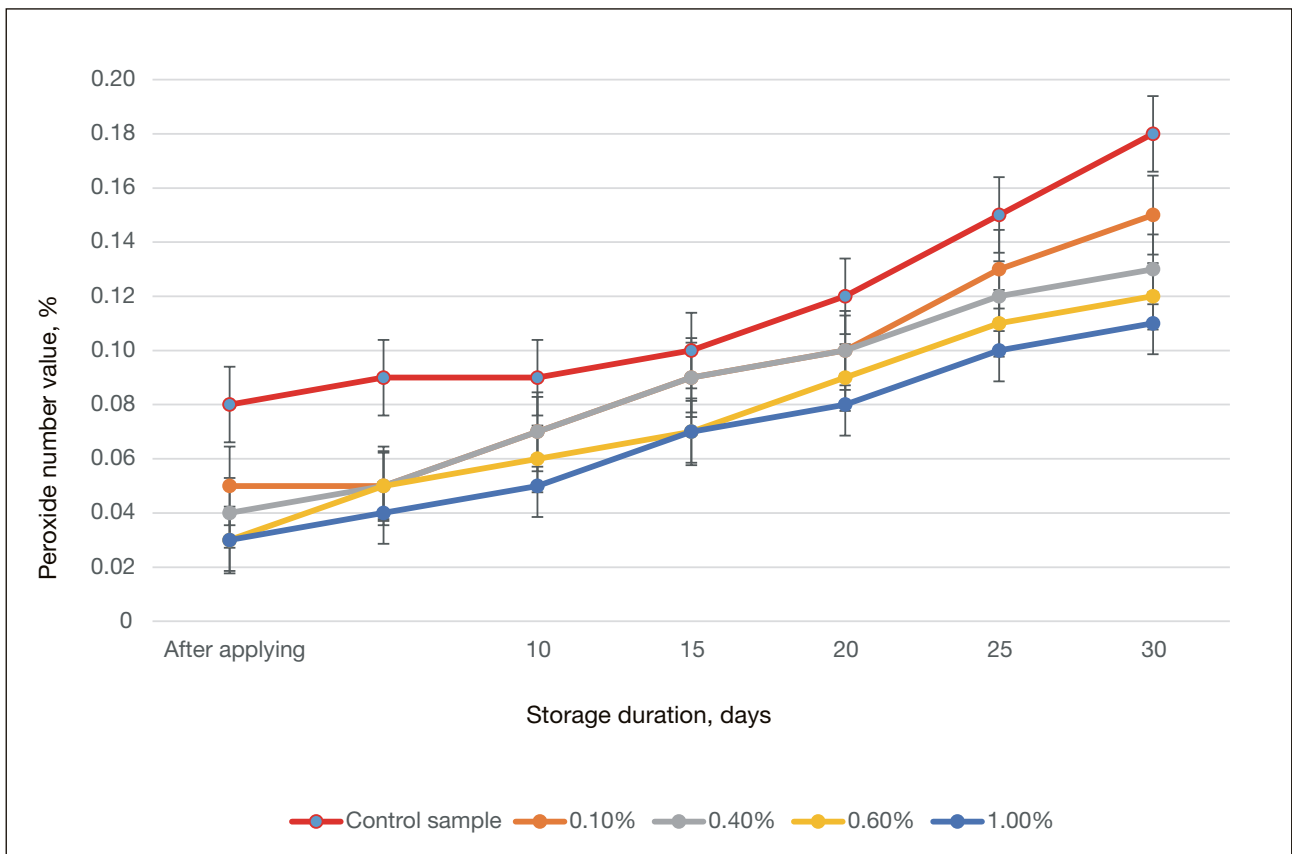


Figure 4. Accumulation of primary oxidation products in fresh pork chops during storage

On day 10, all samples containing preservative mixture exhibit a sharp increase in the PN values (1.6 times on average), which causes the interaction of acetic acid contained in the marinade with the antioxidant (dihydroquercetin), accompanied by a shift in acidity towards the alkaline side. On day 15 of storage, the PN value continues to gradually increase, and the active substance of the food additive begins inhibiting lipid peroxidation throughout the storage period, demonstrating significant differences with the control sample. After 30 days of storage, a noticeable increase in oxidative processes in the control sample is observed, whereby the end point of the shelf life of fresh meat has been chosen. However, the antioxidant activity allows increasing the product storage stability.

Similar dynamics are observed in the acid number variation (**Figure 5**).

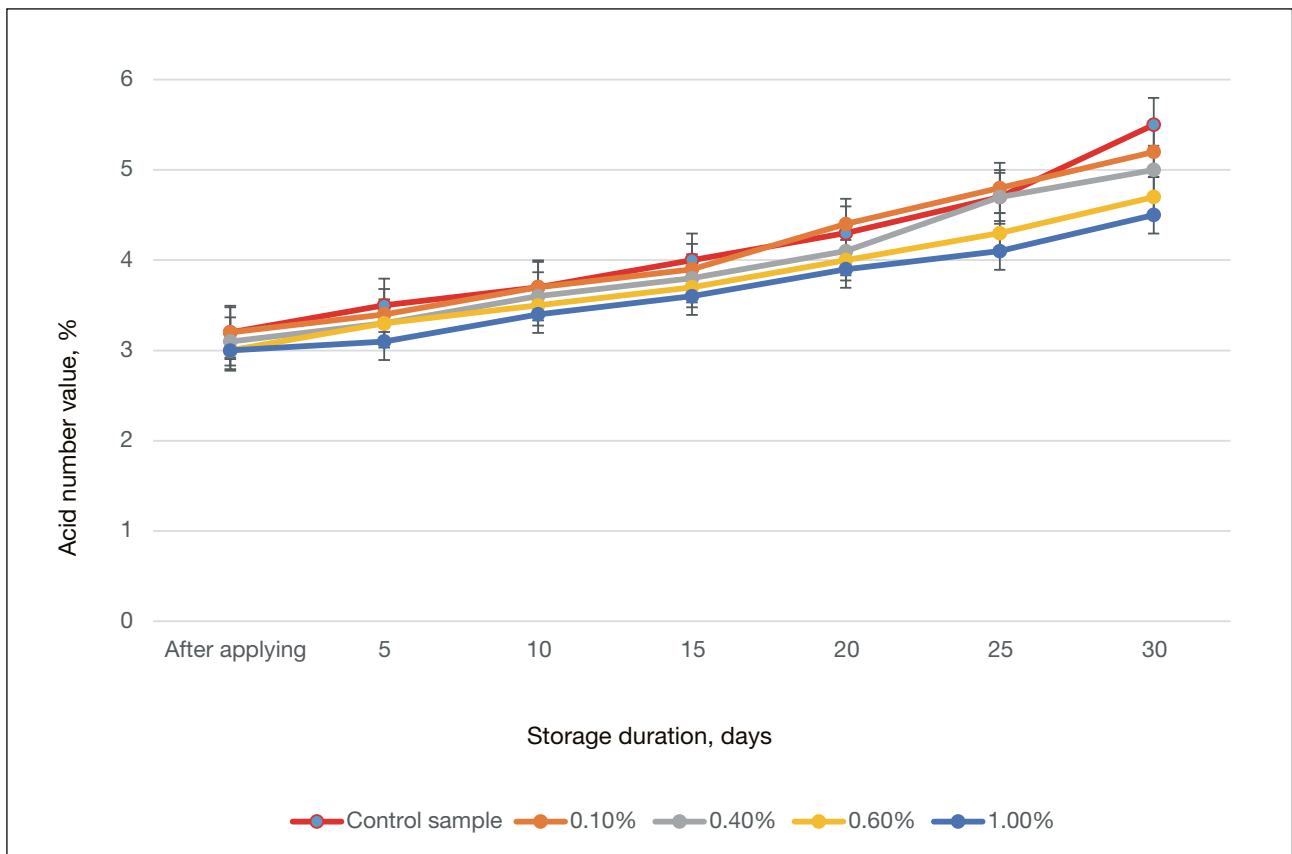


Figure 5. Acid number variation over the entire product shelf life

Oxygenated products with excessive acidity reduce meat quality due to moisture loss. In turn, moderate acidity of meat products reduces the product quality to a lesser degree, so it remains juicy. In addition, the antioxidant contained in preservative mixture allows increasing the shelf life.

5. Conclusions

The novelty of this research is theoretically justified, and a high performance of the compounds of food additive preservative mixture in manufacturing raw pork is experimentally confirmed. Applying the additive allows reducing losses during heat treatment and storage of the meat products, increasing yield and improving consistency, as well as reducing the cost of production and increasing the shelf life of up to 30 days. This result is ensured by the use of the latest broad-spectrum antimicrobial preparation of preservative mixture. The preparation has a significant bactericidal effect and inhibits the growth and development of yeast. The most rational way to apply the preparation into a meat system is to add it to the product by simply mixing and then massaging (for example, with spices or marinades). As the preservative contains the natural antioxidant dihydroquercetin, the preparation can be used to good advantage for products with a high fat content to prevent the lipid fraction oxidation during storage. The optimal concentrations of the preparation in the meat system is from 0.6% to 1% by weight. A higher concentration will lead to a higher price of the end product. Meanwhile, a concentration of the bio-preservative less than 0.6% by weight will reduce the product storage stability and its resistance to microbial spoilage.

6. Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

7. Acknowledgement

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