In order to prevent oxidative damage, an experiment was conducted to determine the effectiveness of berries extracts (Aronia melanocarpa Elliot and Ribes nigrum L.) in the production of half-smoked sausages. The recipe of half-smoked sausages with a poly-component composition of raw materials includes semi-fat pork with muscle tissue, tendon-free lean pork, tendon-free Muscovy duck meat, side pork, hydrated bamboo fiber.

Berry extracts (Aronia melanocarpa Elliot and Ribes nigrum L.) at concentrations of 0.2–0.5 % to the weight of crude minced meat were added to the examined samples of minced meat. Sample No. 1 was a control, that is, made without the addition of extracts of berries.

During the storage of products with extracts, an acidic number, a peroxide number, a thiobarbituric number, and the predefined indicators of microbiological safety were determined.

The addition of chokeberry extract in the amount of 0.2–0.5 % to the minced meat weight significantly slows down the hydrolytic oxidation of lipids in finished products, effectively inhibits the peroxide oxidation of fat. The use of blackcurrant extract also has an antioxidant effect but is weaker. Stabilizing the peroxide oxidation of lipids in half-smoked sausages has the effect of inhibiting the formation of secondary oxidation products, which is confirmed by the results reported here. The amount of secondary oxidation products was the smallest at the end of the shelf life of the product with a concentration of chokeberry extract of 0.5 % and was 0.197±0.001 mg MA/kg, which is 3.74 times lower than that in the control.

The addition of extracts of chokeberry and black currant reduces microbiological contamination and has a bacteriostatic effect. The most effective is the introduction of chokeberry extract in the amount of 0.05 %, which reduces the oxidative damage to fat by more than three times.

Keywords: chokeberry extract, blackcurrant extract, half-smoked sausages, natural antioxidants

STUDYING THE INFLUENCE OF BERRY EXTRACTS ON THE QUALITY AND SAFETY INDICATORS OF HALF-SMOKE SAUSAGES

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1. Introduction

The issue of quality and safety of meat products with the maximum shelf life was and remains relevant for scientists and employees in the meat processing industry. Meat systems are quite unstable and undergo rapid microbiological, hydrolytic, and oxidative changes, lose moisture, nutrients, lose color when pigments of meat are oxidized. It is impossi-
ble to prevent these processes but they can be slowed down by correctly selecting formulation components, processing techniques, and storage modes.

Oxidation is one of the main reasons for the deterioration of the quality of meat. Meat becomes sensitive to oxidative damage due to the high concentration of unsaturated lipids, heme pigments, and complex physicochemical processes in muscle tissue [1, 2]. Therefore, the issue related to spoilage of meat products during storage is relevant and requires scientific research and technological solutions.

2. Literature review and problem statement

Hydrolytic and oxidative processes in the lipids of meat products can significantly affect their quality and shelf life. Due to the development of oxidative processes in products with a high content of lipids, which is characteristic of sausages, there is an accumulation of peroxide compounds, which gives them the taste and smell of bitterness. As a result, food products lose their nutritional value, which leads to the destruction of fat-soluble vitamins, reduces the content of unsaturated fatty acids while toxic and carcinogenic substances can accumulate in the human body [3].

The use of antioxidants in the food industry is a very popular technological method of extending the shelf life of meat products. In most cases, synthetic antioxidants that are not completely safe for the human body are used to prevent and suppress oxidative spoilage of products. These include substances obtained from oil refining, namely: tert-butylhydroquinone (E319), butylhydroxyanisole (E 320), butylhydroxytoluene (E 321), and others. Synthetic antioxidants are a modern solution for stabilizing the oxidative process and extending the shelf life of such products. However, adverse health effects can pose a risk to consumers. The authors of [4] show that exogenous antioxidants of natural origin reduce the content of free radicals in the human body that damage muscles. At the same time, paper [5] found that chronic toxicity of BOT can act as a potential tumor stimulator when used in large quantities. The use of bioactive compounds contained in plants can improve the shelf life of meat, slow down the progress of oxidative processes, change color, reduce sensory quality in the implementation of thermal processes during the production and storage of food products.

The authors of [6] conducted a comparative study of the effectiveness of chestnut extracts and BOT at different concentrations on the shelf life of beef cutlets over 18 days of storage in the refrigerator at (2±1 °C). The action of natural antioxidants, in terms of efficiency, was not inferior to synthetic and inhibited the peroxide oxidation of lipids. On the other hand, work [7] confirms that plant extracts from rosemary, thyme, ginger maintain oxidative stability, stabilize color parameters, and improve the technological and sensory properties of fresh meat products. The authors of [8] demonstrated that grape seeds and pine extracts significantly improve the oxidative stability of boiled beef, reducing the amount of secondary oxidation products.

Recent studies have confirmed the positive effect of natural antioxidants on food quality, human health, new mechanisms of antioxidative action of natural plant antioxidants and their positive effect on the human body. In [9], it has been confirmed that natural antioxidants can be used to prevent several non-infection diseases such as cancer, coronary heart disease, and mountain sickness. They can be a sustainable substitute for chemical preservatives in a variety of functional foods to reduce the chemical danger to human health. In [10], it is confirmed that natural antioxidants are subjected to intensive metabolism in vivo, which changes their reductive potentials. The cellular effects of natural antioxidants can also be mediated by their interaction with specific proteins. Such proteins are central to intracellular signal cascades, their modulation of expression and activity of key proteins, their effect on epigenetic mechanisms or their modulation of the intestinal microbiota. The authors of [11] proved that endogenous antioxidants do not always stop the production of reactive metabolites. Active inhibitors of oxidative stress in the human body can be plant antioxidants from foods.

Polyphenols and essential oils are the main fractions that are contained in plants and can be used in the meat industry. These compounds can be obtained from different parts of plants, such as seeds, leaves, and fruits.

The authors of [12] found that polyphenols (such as coffee acid, quercetin, luteolin-7-O-rutinoside, and epigallocatechin-3-gallate) and essential oils (D-3-karen and ß-pinen) are contained in spices. It was investigated in [13] that polyphenols made of black pepper, oregano, and sage, green tea leaves, and guarana can slow down the oxidative reaction, color change, and modification of the sensory properties of meat products.

For example, black pepper (Piper nigrum L.) is the spice widely used all over the world, mainly cultivated in the tropics. Due to the pleasant sensory effects in cooking (causticity and characteristic taste), black pepper is considered a high-value spice. The predominant components of black pepper essential oil are D-3-karen, caryophyllene, limonene, ß-pinene, and ß-pinene [14].

Plant extracts can be used as an ingredient and as a component of packaging during long-term storage (for example, for fresh meat, cutlets, sausages) [15]. As the use of plant extracts becomes more common and reaches industrial levels, it is important to combine them with other healthier strategies: reducing or replacing fat, salt, and nitrates, using innovative processing technologies [16]. It was investigated [17] that cranberry extract inhibited oxidative processes in meat-containing boiled sausages with duck meat and poultry meat of mechanical turkey treatment with a fat content of more than 20 %. In work [18], it is reported that rosemary extract has a positive effect on the course of oxidative processes in minced duck meat during long-term storage. It is established that its introduction in the amount of 0.12–0.36 % contributes to a slowdown in hydrolytic oxidation of minced meat lipids by 10.5–32.5 %. When the composition is introduced, the peroxidation of unsaturated fatty acids is stabilized. It is proved that the addition of extracts of rosemary and cranberries inhibits the oxidation of lipids during the storage of meat-containing breads with a combined raw material composition [19]. The general disadvantages of the described extracts include a high price, different efficiency on the course of oxidative processes at different stages of their development, insufficient antimicrobial effect, etc.

Given this, the task of finding new, more effective, and affordable natural sources of antioxidants is not completely solved. In addition, the effectiveness of antioxidants depends on the composition and formulation of sausage products, especially when the ingredient composition of products is polycomponent and combined from raw materials of various origins. Therefore, the selection and study of the effective-
ness of various antioxidant preparations of natural origin is a relevant task for the meat industry. One possible natural source of antioxidants may be extracts of berries such as chokeberry and blackcurrant. It is established [20] that the leaves and berries of these plants are rich in polyphenols, namely phenolic acids and flavonoids, which have strong antioxidant properties.

3. The aim and objectives of the study

The aim of this study was to investigate the effects of chokeberry extract (CBE) and blackcurrant extract (BCE) in the technology of half-smoked sausages with a polycomponent composition of raw materials. This could make it possible to extend the shelf life of half-smoked sausages by slowing down the oxidative spoilage of products and decreasing the concentration of oxidation products that reduce the quality and consumer value of sausages.

To accomplish the aim, the following tasks have been set:
- to study the influence of berry extracts on hydrolytic processes of lipid oxidation in half-smoked sausages with multicomponent raw material composition;
- to investigate the influence of extracts of berries on the peroxide oxidation of lipids in half-smoked sausages with a polycomponent composition of raw materials;
- to investigate the influence of extracts of berries on the accumulation of secondary products of oxidation of lipids in half-smoked sausages;
- to investigate the effect of berry extracts on the microbiological indicators of half-smoked sausages during storage.

4. The study materials and methods

Preparation of half-smoked sausages.

We have devised a recipe for half-smoked sausages with the following ratio of components: semi-fat pork – 30 %, lean pork – 10 %, duck meat (Anas platyrhynchos) – 30 %, pork fat – 25 %, hydrated bamboo fibers. Spices and additional materials were used in the formulation. In order to effectively use the local raw materials, we replaced beef in the recipe with duck meat, which increased the content of the lipid fraction. To prevent a negative impact on the functional and technological properties of the system, hydrated bamboo fiber was added.

For the preparation of sausages, the meat was crushed in a laboratory meat grinder (Philips, Germany). Pork fat was hand-cut into cubes measuring 4×4 mm. The crushed ingredients were mixed for 8 minutes. Minced sausages were stuffed into the shells of mutton sausages. Sausages were aged at a temperature of 4–8 °C for 2 hours, then dried in a drying chamber at t=90±10 °C for 30–40 minutes.

Smoking was carried out in the smoking chamber at an initial temperature of 43 °C, every 30 minutes the temperature was increased by 8–10 °C until the temperature in the center of the sausage was 70±2 °C. After smoking, the sausage was cooled to a temperature not higher than 8 °C.

Extracts of berries (manufactured by “Food Ingredients Mega Trade”, USA) were added to the samples of minced meat in the following concentrations: sample No. 1 – control, without antioxidants; No. 2 – 0.2 %; No. 3 – 0.3 %; No. 4 – 0.5 % of chokeberry extract (Aronia melanocarpa Elliot) to the mass of raw minced meat; No. 5 – 0.2 %; No. 6 – 0.3 %; No. 7 – 0.5 % of blackcurrant extract (Ribes nigrum L.). Recommended doses of antioxidants ranging from 0.01 to 1.0 % were used to determine the dose in meat products technology [21, 22].

Boiled sausages were stored for 25 days at a temperature of +4 °C and relative humidity of 75–78 %. During the storage of sausages, the controlled parameters were the acid number (AN), peroxide number (PN), thiobarbituric number (TBN), microbiological indicators [23, 24].

Determining the acid number.

The acid number was measured by titration with sodium hydroxide in the presence of an alcohol solution of phenolphthalein [25]. In a conical flask with a volume of 150–200 cm³, 3–5 g of the prototype were weighed. The batch was heated in a water bath. We added 50 cm³ of neutralized essential alcohol mixture and shook it. Then we added 3–5 drops of alcohol solution of phenolphthalein with a mass fraction of 1 %. The solution at constant shaking was quickly titrated with a solution of potassium hydroxide with a concentration of 0.1 mol/dm³ until the appearance of a pink color, which does not disappear for 1 minute. The acid number was calculated as the volume of sodium hydroxide with a molar concentration of 0.1 mol/dm³ spent on titrating the batch of the prototype.

Determining the peroxide number.

Determining a peroxide number is based on the extraction of a mixture of chloroform and icy acetic acid and subsequent titration with a solution of sodium hyposulphite with a pre-added solution of starch [26]. In a flask with a cork we placed 0.8–1 g of the batch, heated in a water bath, and added 10 cm³ of chloroform and 10 cm³ of icy acetic acid. We then quickly added 0.5 cm³ of saturated freshly prepared solution of potassium iodide. The flask was closed with a cork, the contents were mixed. 1 cm³ of starch solution of 1 % concentration and 100 cm³ of distilled water were added. Then the flask was put in a dark place for 3 minutes. The contents were titrated with a 0.01 mol/dm³ solution of sodium hyposulphite to eliminate blue coloration.

To check the transparency of the reagents, a control determination was performed without a test sample. The peroxide value was calculated as the difference between the volume of sodium hyposulphite solution with a concentration of 0.01 mol/dm³ spent on the titration of the sample and the volume of sodium hyposulphite solution (0.01 mol/dm³) spent on the control titration based on the weight of the batch.

Determining the thiobarbituric number.

TBN was determined by measuring the color intensity of the distillate mixture of the study sample with a solution of thiobarbituric acid (1:1) after being aged in a water bath for 35 minutes at the Spectol-11 spectral photo colorimeter (Germany) at a wavelength of 535 nm [23].

50 g of crushed sausage was mixed with 50 cm³ of distilled water to a homonymous state. The prepared mass was quantitatively transferred to the Kjeldahl flask, the remains were washed from a mortar of 47.5 cm³ of distilled water; we added 2.5 cm³ of hydrochloric acid. Distillation in the Kjeldahl apparatus was carried out by collecting 50 cm³ of distillate in a measuring flask. We selected 5 cm³ of the distillate, added 5 cm³ of thiobarbituric acid, and put the flask in a boiling water bath for 35 minutes. The control test was carried out using 5 cm³ of distilled water instead of the distillate. After cooling the samples for 10 minutes, optical density was measured at a wavelength (535±10) nm relative to the control solution.
TBN, mg MA (malonic aldehyde)/kg of product, was calculated as the optical density of the solution multiplied by 7.8 – the coefficient of proportional dependence of the density of MA on its concentration in the solution.

Determining the microbiological indicators.

The analysis of Table 1 shows that at the beginning of the shelf life of 25 days, in control sample the AN in all samples was almost the same and amounted to 0.019–0.021 mg/KOH. This indicates a small amount of free fatty acids and a low intensity of triacyl glyceride hydrolysis.

Our analysis of Table 1 shows that at the beginning of the storage of sausages, the AN in all samples was almost the same and amounted to 0.019–0.021 mg/KOH. This indicates a small amount of free fatty acids and a low intensity of triacyl glyceride hydrolysis.

The results of studying the influence of extracts of berries on the accumulation of secondary oxidation products in half-smoked sausages

Table 2 shows that at the end of the shelf life of 25 days, the concentration of free fatty acids in all the samples studied was much lower than in the sample without the addition of antioxidants.

5.2. Results of studying the effect of berry extracts on the peroxide oxidation of lipids in half-smoked sausages

Table 2 gives the results of studying the accumulation of secondary peroxides in half-smoked sausages.

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage time, day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0.021±0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.019±0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.019±0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.019±0.002</td>
</tr>
<tr>
<td>5</td>
<td>0.019±0.002</td>
</tr>
<tr>
<td>6</td>
<td>0.019±0.002</td>
</tr>
<tr>
<td>7</td>
<td>0.019±0.001</td>
</tr>
</tbody>
</table>

On day 25 of storage, the AN of sausages had the lowest value in sample 4; it was equal to 0.391±0.06 mg KOH, which is 39 % lower than that in the control. At the end of storage, the concentration of free fatty acids in all the samples studied was much lower than in the sample without the addition of antioxidants.

5.3. The results of studying the influence of extracts of berries on the accumulation of secondary oxidation products of lipids in half-smoked sausages

To establish the degree of accumulation of secondary oxidation products, on the last day of storage of sausage samples we examined the TBN: the results are shown in Fig. 1.
The results of microbiological studies of the experimental samples are given in Table 3.

Table 3 demonstrates that the microbiological indicators of all samples met the norm or the state standard for half-smoked sausages. The difference between the experimental samples and the control one was observed in terms of the QMAFAnM indicator. The lowest total contamination was registered in the sample with the highest concentration of chokeberry extract – 0.5 % in sample 4. The downward trend in QMAFAnM has been noted in all experimental samples. The intensity of this decrease depended on the concentration of extracts.

6. Discussion of results of studying the effect of berry extracts on the oxidative processes in half-smoked sausages

According to studies [28, 29], synthetic antioxidants are potentially harmful, so the use of natural preparations is an affordable alternative in meat production technology. According to [30], extracts of berries, especially dark in color, contain polyphenolic compounds that have a high antioxidant effect. In addition, due to the antioxidant and antibacterial effects of phenolic compounds, plant extracts are an alternative to chemical preservatives used in the meat industry, especially nitrates (III). They can inhibit the growth of pathogenic microflora, oxidation of meat ingredients (lipids and proteins), and prevent color changes [31–33]. When choosing the appropriate additive, they take into consideration the concentration of active ingredients, the ingredient composition of the meat product, the fat content, the ratio of saturated and unsaturated fatty acids. The effectiveness of an antioxidant preparation is determined by its ability to inhibit the rate of hydrolysis, primary and secondary oxidation of meat lipids during storage [34].

The AN study showed that during storage there is a gradual accumulation of triglyceride decay products, which, by the end of the shelf life, reach the maximum value consistent with data reported in [35]. A comparative analysis of the effectiveness of various antioxidants revealed that the greatest positive effect on suppression of the primary stage of oxidation is provided by the extract of chokeberry at a concentration of 0.5 %. The antioxidant and antimicrobial efficacy of chokeberry extract was confirmed by the authors of [36] when used in pork products.

In the second stage of oxidative damage, further oxidation of released free fatty acids occurs. It was established that lipid hydroperoxides do not harm the quality of food since they have no smell and taste [37]. However, hydroperoxides are unstable compounds, so they tend to decompose into alkyl and peroxide radicals [38]. These radicals are further broken down into secondary compounds that are responsible for sensitivity disorders, such as odors and tastes associated with lipid oxidation. In the early stages of oxidation, there is an increase in hydroperoxides since the level of formation is higher than the level of decomposition. However, since these compounds are unstable, at deeper stages of oxidation, the process of decomposition of hydroperoxides is more intensive than the formation process [39]. Suppression of triacyl glyceride hydrolysis and a decrease in the concentration of free fatty acids to 0.391–0.813 mg/KOH in experimental samples is possible due to the high oxidative potential of flavonoids of berry extracts, which is confirmed by studies [40].
Our analysis of the dynamics of PN in the experimental samples shows that when adding extracts of chokeberry and black currant, the suppression of peroxidation is observed after the first 5 days of storage. This is due to the fact that the components of extracts make it impossible to attach active oxygen to the radicals of fatty acids and thus interrupt free radical oxidation [41]. That becomes possible due to the fact that chokeberry contains high concentrations of phenolic compounds, proanthocyanins, anthocyanins, and phenolic acids with high antioxidant activity [42].

In the early stages of oxidation, the use of peroxides as an indicator of oxidative damage leads to an underestimation of the oxidation state [43], so this parameter is not guaranteed to be reliable in meat with a high degree of oxidation [44]. In this regard, although the amount of peroxidation is a widely used parameter for determining the degree of oxidation, it is effective only in the initial stages of oxidative processes [45]. Accordingly, to assess the stages of deep oxidation, the concentration of mono- and dialdehyde reacting with thiobarbituric acid is determined.

As a result of our authentic research, it was found that the introduction of extracts of berries contributes to a slowdown in the accumulation of secondary oxidation products. The authors of [46, 47] proved that secondary oxidation products are carriers of unpleasant taste and smell of oxidized fats. In contrast to [48], where a decrease in the concentration of aldehydes is achieved due to polyphenols of seaweed, it was found that the use of polyphenols of chokeberry can reduce PN by almost 40 %. Of particular importance is the effectiveness of berry extracts for inhibition of the accumulation of secondary oxidation products when using them as part of multicomponent meat-containing products, including ingredients of various origins [49].

In the study of microbiological safety indicators, it was found that in all experimental samples there was a decrease in QMAFAnM. The intensity of this decrease depended on the concentration of extracts. It is proved that the high concentration of phenolic compounds of extracts of chokeberry and blackcurrant provide the level of total inactivation of half-smoked sausages in the range of 0.96–1.64×10³, which is consistent with the research reported in [50]. The addition of extracts of chokeberry and black currant at the same time makes it possible to ensure the microbiological safety of perishable products and extend the shelf life, unlike the use of other plant products [51].

The practical use of extracts of chokeberry and black currant in concentrations of 0.2–0.5 % to the mass of raw minced meat ensures the suppression of oxidative and microbiological processes in ready-made semi-smoked sausages. However, this effect is limited to a shelf life of 25 days at a temperature of +4 °C and a relative humidity of 75–78 %. Therefore, further studies are planned to extend the shelf life of half-smoked sausages with berry extracts under different temperature regimes.

### 7. Conclusions

1. Our study has confirmed the antioxidant activity of extracts of chokeberry, compared with blackcurrant extract, when used in the technology of half-smoked sausages. It has been established that the introduction of chokeberry extract in the amount of 0.2–0.5 % to the mass of minced meat can significantly slow down the hydrolytic oxidation of the lipids of finished products, effectively suppress the peroxide oxidation of fat.

2. It has been confirmed that the stabilization of peroxide oxidation of lipids in half-smoked sausages using extracts of chokeberry and black currant leads to inhibition of the formation of primary oxidation products. At the end of the storage period, the PN of the experimental samples was at least 0.017 mg/KOH, which is 63.04 % less than that in control.

3. We have proven the possibility of reducing by 3.74 times, in the process of storing half-smoked sausages, the accumulation of secondary oxidation products reacting with thiobarbituric acid when chokeberry extract is used in the recipe in the amount of 0.5 %.

4. It has been confirmed that the introduction of chokeberry and black currant extracts in the formulation of half-smoked sausages can reduce microbiological contamination and achieve a bacteriostatic effect. The greatest effect on reducing the oxidative damage to fat is achieved by introducing to the raw minced sausages of chokeberry extract in the amount of 0.5 %.

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