



Effects of lingonberry extract on the antioxidant capacity of meat paste

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Abstract:

Introduction. Modern meat scientists are currently interested in combining meat raw materials with plant ingredients to enrich products with biologically active substances of natural origin, including antioxidants. In this regard, we studied the antioxidant capacity of a dry lingonberry marc extract introduced into meat paste and analyzed its effects on the product's color and stability during storage.

Study objects and methods. Our objects of study were a dry lingonberry marc extract originating in the Republic of Buryatia, forcemeat, and a ready-made paste in a casing. The extract was obtained by water-alcohol extraction using microwave irradiation. We investigated the physicochemical characteristics of the dry extract, including its contents of phenolic compounds, benzoic acid, and antioxidants.

Results and discussion. During the experiment, we analyzed the extract's effect on the paste's total antioxidant capacity, coloring, and shelf life. The results showed that increasing the extract's amount from 0.1% to 0.4% changed the color of the paste from gray-brown to purple-brown, respectively, due to anthocyanins. In further tests, we used a 0.2% concentration of lingonberry extract – the optimal amount that retained the usual brown color of the paste while increasing the content of antioxidant substances. Then, we analyzed the degree of fat oxidation in the paste samples made with and without sodium lactate during storage. According to the results, the lingonberry marc extract used without the acidity regulator and with it inhibited lipid oxidation by 12.7% and 20%, respectively, by neutralizing free radicals. Finally, we tested the presence of pathogenic microorganisms in the end products. We detected no *E. coli* bacteria in the samples and found an inhibited growth of mesophilic anaerobic and facultative anaerobic microorganisms due to the extract's bactericidal effect established in our earlier studies.

Conclusion. Thus, our results indicated that the dry lingonberry marc extract introduced into meat paste increased the product's total antioxidant capacity and improved its stability during storage.

Keywords: Meat products, berry extract, lingonberry, paste, phenolic compounds, antioxidants, oxidation, peroxide value

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INTRODUCTION

Introducing slaughter by-products into food formulations and technology is a promising direction in the meat industry that ensures a rational use of protein raw materials. Modern meat scientists are developing new products using heat-treated offal of farm animals [1–4].

Such products include liver sausage, paste, head cheese, and jellied meat. Meat pastes are especially popular. They have a spreading consistency and can be packaged in a casing or container. According to

the standards, pastes are classified into “meat pastes” (category A) with at least 20% of muscle tissue and “meat-containing pastes” (category B) with 0 to 20% of muscle tissue. Pastes are affordable meat products due to a lower cost of liver, skirt, lungs, kidneys, and meat trimmings compared to meat. Our meat market traditionally offers pastes in a casing that are popular among students, schoolchildren, tourists, and passengers on trains and planes. These products are made from inexpensive protein-containing ingredients with a high nutritional value and are packaged in small portions.

According to literature, modern meat scientists are interested in combining meat products (including pastes) with plant ingredients to enrich the product with biologically active substances of natural origin (micro- and macroelements, vitamins, amino acids, antioxidants, etc.) and increase its functional, technological, and other properties.

For example, Gurinovich *et al.* formulated a meat paste by combining animal protein with that of pine nut oilcake. This way, the authors improved the functional properties of meat systems and enriched the end product with a plant-origin ingredient [5].

Bazhenova *et al.* mixed forcemeat with wheat flour containing selenium, an essential trace mineral. The authors described how they selected their method of introducing selenium-enriched flour into the forcemeat. They concluded that a 10–15% protein-fat emulsion with selenized flour increased the functional and technological parameters of forcemeat and provided 50–70% of our daily requirement of selenium [6].

Giro and Chirkova proposed enriching paste with iron [7]. They aimed to develop functional meat-based products for people predisposed to, or suffering from, iron-deficiency anemia. Their study showed that offal-based pastes enriched with chickpea could be used to prevent disturbed hematopoiesis caused by iron deficiency. These products contain highly bioavailable microelements that help the body to quickly mobilize its compensatory reactions.

Another study by Okuskhanova *et al.* looked into the composition and properties of maral deer pastes fortified with beans and protein. The authors developed three formulations with varying amounts of the protein fortifier and beans: no protein fortifier or beans; 15% protein fortifier and 20% beans; and 25% protein fortifier and 10% beans. The study showed that the third formulation had a higher content of essential and non-essential amino acids compared to the first two variants [8].

Pastes from hypoallergenic horse meat and lamb were formulated by Lyakh *et al.* with the addition of dried dill and Polisorbovit-95, a biologically active dietary supplement. According to their results, this combination of ingredients improved the product's sensory and physicochemical properties [9].

As we can see, meat scientists have created various formulations of pastes with plant ingredients rich in biologically active substances.

Further, modern scientific literature shows increased interest in studying antioxidant capacities of natural plant ingredients in order to introduce them into food products to improve their functional properties and inhibit fat oxidation processes [10–21]. Antioxidants can neutralize the destructive effects of free radicals on a human body. Our antioxidant system is one of the main mechanisms for stabilizing our adaptive potential. This is especially important for people who live in adverse

environmental conditions and have an unbalanced diet containing synthetic ingredients.

For example, Lisitsyn *et al.* studied the antioxidant activity of aromatic plant extracts (black pepper, rosemary, sage, and thyme) at the GORO Research Center for Ecological Resources (Rostov-on-Don, Russia). The scientists commercialized a new technology for processing aromatic raw materials – supercritical CO₂ extraction. This technology produces extracts with a significantly different composition from those obtained in traditional ways. Supercritical extracts contain a variety of terpene compounds, as well as waxes, pigments, high molecular weight saturated and unsaturated fatty acids, alkaloids, vitamins, and phytosterols. These substances have high biological, antimicrobial, and antioxidant activities. According to the results, the highest and the lowest contents of antioxidants were found in sage and black pepper extracts (3.1% and 0.07%, respectively). It is generally accepted that natural extracts with an antioxidant content of at least 0.1% can be considered as a dietary supplement with antioxidant properties. Therefore, the authors recommended using sage, rosemary, and thyme extracts as antioxidant ingredients for meat products [10].

Another group of researchers, Zabalueva *et al.*, looked at antioxidant contents in water-alcohol infusions of medicinal plants, depending on the method of their preparation. They found that the concentration of water-soluble antioxidants in infusions obtained by maceration did not differ significantly from those prepared by ultrasound and an ultra-high frequency electromagnetic field. The study showed the potential of using water-alcohol infusions from rose hips and barberry fruits as antioxidant supplements in the production of meat products [15].

A wide range of plant materials (vegetables, fruits, berries, and herbs), including wild plants, are introduced into meat products in the natural form or as extracts, infusions, and decoctions treated in various ways. Edible and medicinal plants are collected, processed, and utilized almost without waste. However, waste from processing wild plants is not always used rationally, being an environmentally friendly, renewable raw material that could be used as a source of biologically active natural substances. These wild plants include lingonberries growing in Transbaikalia (east of Lake Baikal) that are rich in biologically active compounds with medicinal properties. Lingonberry leaves contain phenolic glycosides (arbutin and methylarbutin), vaccinine, lycopene, hydroquinone derivatives, acids (ursolic, tartaric, gallic, quinic, and ellagic), tannin, hyperoside, and other flavonoids. Lingonberries are rich in sugars, ascorbic acid, carotene, and organic acids [22].

The chemical composition of lingonberry leaves and fruits indicates a high antioxidant capacity of respective products. In fact, lingonberries are processed in large

quantities for juice production. However, their by-products – such as husks, pulp or marc – could also be used as a source of biologically active substances. Some authors propose enriching meat products (e.g. liver paste) with fresh or dried lingonberry and cranberry pulp [18, 19].

For example, Bitueva and Ayusheeva introduced dried cranberry or lingonberry pulp, pre-crushed and reconstituted, into ground meat products. The powdered pulp was added at the stage of forcemeat preparation, replacing 13–15% of bread. This method enriched the meat products with biologically active substances [18].

In another study, Ivanova and Izosimova proposed a formulation of meat paste with 19% polyfunctional additives – lingonberry or cranberry marc. The marc contains citric and malic acids that shift the medium's pH away from the isoelectric point, enhancing the dissociation of the main and acid groups of protein, as well as increasing bound moisture and the yield of the end product. A high content of low-ester pectins in the berry marc also contributed to the system's stabilization. Biologically active substances and antioxidants increased the microbiological resistance of the meat and plant paste. The product also had an improved vitamin and mineral composition [19].

In our previous work, we prepared a water-alcohol extract of lingonberry marc which was then dried [23]. The dry extract is a powder rich in biologically active nutrients that is easy to store and transport. Due to a high concentration of dry substances, the extract can be introduced in small amounts into the formulation of meat products, providing them with functional properties and eliminating negative effects on the product's sensory and physicochemical characteristics.

Thus, it is extremely relevant to create products based on a combination of meat and plant materials to enrich them with micro- and macroelements, vitamins, amino acids, and antioxidants. Many types of plant materials contain a variety of compounds with an antioxidant effect. Even low concentrations of antioxidants in the human body can slow down or prevent oxidation processes which are known to cause premature aging and disease. Thus, we can inhibit fat oxidation by introducing antioxidants into food products.

In view of the above, we aimed to develop a meat paste formulation with an extract from lingonberry marc (originating in Transbaikalia), as well as evaluate the total content of antioxidants and effect on the color and stability of the paste in the casing during storage.

STUDY OBJECTS AND METHODS

The objects of the study included a dry lingonberry marc extract (DLME), forcemeat, and a ready-made paste in the casing.

A dry extract of lingonberry marc was obtained in accordance with Patent No. 2626565. Lingonberries were pressed for juice and the remaining marc was placed on

a baking sheet in a 5–8 mm layer and then dried in an oven with infrared radiation at 35–40°C for 40–50 min to 10–15% moisture. The dried marc was crushed to a powder state and subjected to extraction with a water-alcohol solution using a microwave electromagnetic field (700 W, 2450 MHz).

The water-alcohol extracts were filtered and concentrated on a rotary evaporator under vacuum in a water bath at temperature below 45°C until a syrup consistency was reached (40–50% dry matter). The syrup was then vacuum-dried at a temperature below 50°C for 3.5–4.5 h to obtain a powder with a residual moisture of < 5%. Such process parameters do not cause any qualitative changes in thermolabile substances. They preserve maximum biological activity of active substances and ensure high quality of the extract.

The main ingredients in the formulation of paste samples were cheek meat, beef liver, meat trimmings, soy isolate, and semolina. To evaluate the DLME effects on forcemeat characteristics, samples were made with 0.1, 0.2, 0.3, and 0.4% DLME previously dissolved in water in a ratio of 1:5. The paste was made in a casing according to the traditional technology.

A DLME-free paste sample was used as a control. To evaluate the shelf life of the paste, we conducted two experiments. For the first experiment, we prepared control and DLME samples and stored them for 14 days. For the second experiment, we used a 0.2% acidity regulator – sodium lactate (E325) and stored the samples for 18 days.

To evaluate the samples' antioxidant activity, we performed amperometric measurement of the total content of antioxidants in terms of quercetin. The test samples were subjected to extraction with bidistilled water to isolate water-soluble compounds with an antioxidant effect. The total content of antioxidants was measured on a Tsvet Yauza-01-AA analyzer. Quercetin solutions were used to construct calibration graphs [24]. The extraction efficiency was determined by the amount of phenolic compounds isolated spectrophotometrically using the Folin-Ciocalteu reagent. The content of benzoic acid was measured by the HPLC method.

The optical density of the colored aqueous extracts of the test and control samples was determined by the photolorimetric method on a KFK-3-01 ZOMZ photometer. This method is based on measuring the polychromatic radiation of the visible part of the spectrum. The dependence between light absorption and the radiation wavelength is expressed by a curve (spectrum) of light absorbed by this solution. In the graph, wavelengths are plotted along the abscissa, while optical densities are plotted along the ordinate.

The sensory evaluation of the paste samples was carried out on a nine-point scale according to State Standard 9959. The peroxide value was determined by a method based on the interaction between fat oxidation products (peroxides and hydroperoxides) and potassium

Table 1 Qualitative indicators of dry lingonberry marc extract

Indicator	Meaning
Appearance	loose mass
Taste and smell	sweet and sour, tangy, with lingonberry flavor
Color	burgundy
Acidity, pH units	3.24 ± 0.08
Moisture, %	4.52 ± 0.08
Phenolic compounds, %	6.63 ± 0.04
including anthocyanins	3.58 ± 0.04
Benzoic acid, %	1.34 ± 0.02
Total antioxidants, mg/g	382.60 ± 8.70

iodide in a solution of acetic acid and chloroform, followed by the quantification of iodine released in a sodium thiosulfate solution by the titrimetric method (State Standard R 51487-99^I).

The microbiological parameters of the paste samples were assessed according to State Standards R 50454-92^{II} and 9958-81^{III}.

The experiments were performed in triplicate. Statistical processing of the data was carried out in Microsoft Excel.

RESULTS AND DISCUSSION

First, we analyzed the chemical composition of the lingonberry marc extract originating in Transbaikalia (Table 1).

As we can see, the main component of the lingonberry marc extract is a group of phenolic compounds (6.63%), including water-soluble pigments, anthocyanins, and benzoic acid (1.34%). In a preliminary study [25], we used the disk diffusion method and found that the extract had antimicrobial activity, partly due to the presence of benzoic acid with bactericidal properties. Thus, introducing the lingonberry marc extract into food products, namely meat, can inhibit the growth of microorganisms.

Table 1 also shows a high total content of antioxidants, including polyphenols, anthocyanins, vitamin C, and other compounds (382.6 mg/g). Anthocyanins (3.58%), accounting for half of all phenolic compounds, give lingonberries bright red or burgundy coloring. They include malvidins and peonidins (polyphenolic compounds from the flavonoid group) which contain mono- and diglycosides decomposing into sugar and aglycon (anthocyanidins) upon hydrolysis.

^I State Standard R 51487-99. Vegetable oils and animal fats. Method for determination of peroxide value. Moscow: Standartinform; 2008. 6 p.

^{II} State Standards R 50454-92. Meat and meat products. Detection and enumeration of presumptive coliform bacteria and presumptive *Escherichia coli* (Reference method). Moscow: Standartinform; 2010. 7 p.

^{III} State Standards 9958-81. Sausage products and meat products. Methods of bacteriological analysis. Moscow: Izdatel'stvo standartov; 2001. 14 p.

Anthocyanins are widely used in the food, medical, pharmacological, and cosmetic industries. A daily intake of brightly colored berries (160–2000 mg) leads to the absorption of anthocyanins (0.005–0.1%), which can have an antioxidant effect. Solutions of anthocyanins neutralize almost all radical forms of oxygen and nitrogen four times as efficiently as ascorbate or α -tocopherol. Even low concentrations of antioxidant substances can slow down or prevent oxidative processes. For example, adding only 0.001–0.01% of antioxidants to oil can slow down its oxidation for a long time [26].

When dissolved, the lingonberry marc extract retains its dark red color. When it is added to gray non-nitrite forcemeat, the latter acquires a purple hue. Anthocyanins are known to act as pigments and the color of plants depends on their concentration, as well as the medium pH. They are red in acidic media, purple in neutral and blue in alkaline media. In this regard, we studied how the concentration of the dry lingonberry extract affected the forcemeat pH (Fig. 1).

The forcemeat pH decreased with the introduction of the dry extract, while remaining closer to the neutral region. The extract's acidity was quite high (3.24, see Table 1) due to the use of marc, whose biologically active substances are better extracted into the solution than those of the fruit juice. Therefore, the marc extract is rich in acids (about 2.5%) – citric, malic, benzoic, oxalic, acetic, glyoxylic, pyruvic, hydroxypyruvic, ketoglutaric, ascorbic, and others, with the highest content of benzoic acid (1.34%). This high concentration of acids provides the extract with a low pH, so even very small amounts of the dry extract can significantly decrease the forcemeat pH.

Before the extract was introduced into the forcemeat, it was pre-hydrated for uniform distribution. The DLME concentrations of 0.1, 0.2, 0.3, and 0.4% reduced the forcemeat pH by 1.49, 2.98, 3.7, and 4.47%, respectively. However, the absolute value of the forcemeat pH remained close to neutral, which did not affect the functional and technological properties of the forcemeat system.

Adding the DLME in concentrations from 0.1 to 0.4% affected the forcemeat color. To select the optimal

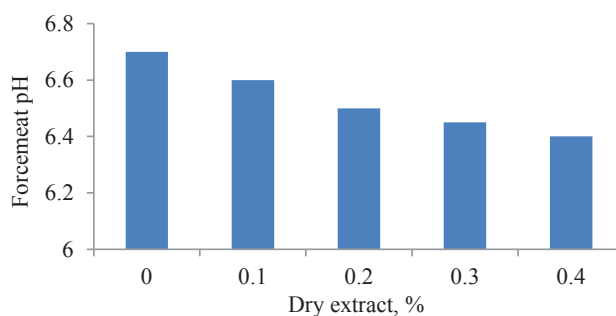


Figure 1 Amounts of dry lingonberry marc extract vs. forcemeat acidity

Table 2 Paste color with different concentrations of dry lingonberry marc extract

Paste samples	Color in section
Control (without DLME)	Gray-brown
Test (with DLME):	
0.1%	Gray-brown
0.2%	Dark gray-brown
0.3%	Brown with a light purple hue
0.4%	Brown with a light violet hue

concentration, we analyzed the color characteristics of ready-made pastes in the casing after heat treatment (Table 2).

As we can see, 0.1% and 0.2% DLME concentrations did not change the habitual sectional color of paste, gray-brown or slightly darker. However, larger amounts of the extract (even 0.3%) gave the paste a purple hue. This change is associated with the presence of anthocyanins, water-soluble plant pigments, in the extract.

Lingonberries may contain such anthocyanins as cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-arabinoside, cyanidin-3-glucoside, and others. However, their qualitative composition depends on the growth conditions. Of great importance for the color of plant pigments is the pH of vacuoles where pigments accumulate. The same pigment in different media can exhibit varying colors: yellow-green in an alkaline medium, purple in a neutral, and red in an acidic medium [27].

In the DLME, just like in lingonberries, the medium is strongly acidic (pH 3.24), so the color is bright red. When the extract is introduced into the forcemeat, whose medium is close to neutral, it acquires a purple tint from water-soluble anthocyanins. The color, however, depends on the concentration of pigments in the forcemeat.

For the quantitative and qualitative analysis of water-soluble compounds, we determined the optical density of the paste samples with different DLME concentrations, using the spectrophotometric method (Fig. 2).

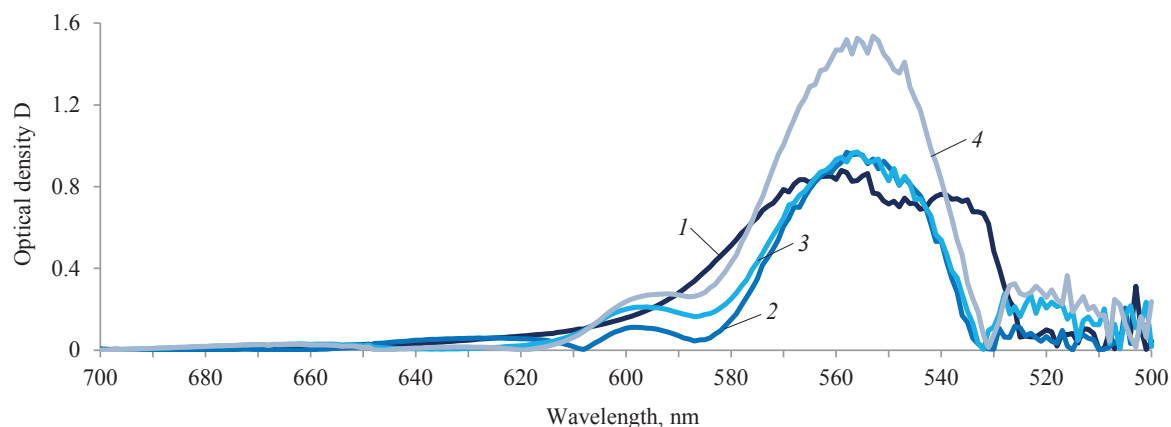
Optical density is known to be directly proportional to the concentration of compounds in a solution. The scale of the abscissa did not show a significant difference in the samples. However, a thorough analysis indicated that the highest peaks for the control sample (curve 1), 0.1% DLME sample (curve 2), 0.2% DLME sample (curve 3), and 0.3% DLME sample (curve 4) were at wavelengths of 554 nm, 557 nm, 557 nm, and 559 nm, respectively (Fig. 2). As we know, the spectral range from 500 to 560 nm corresponds to purple, while that from 560 to 575 nm to purple. Our study showed the same results: the sample with 0.3% DLME had a violet hue (Table 2).

Optical density along the ordinate axis characterizes the color intensity. It means that the height of the peaks corresponds to the concentration of dissolved substances (polyphenols) in the samples. As we can see, the optical density values for the control sample (curve 1), 0.1% DLME sample (curve 2), 0.2% DLME sample (curve 3), and 0.3% DLME sample (curve 4) were 0.86, 0.92, 0.93, and 0.93, respectively. The results show that larger amounts of the extract led to higher concentrations of water-soluble compounds, having reached a maximum on curve 4 (0.3% DLME sample).

Thus, we found that increasing the DLME concentration to 0.3% provided the paste with a high content of antioxidants, but added a purple hue to its color due to the presence of anthocyanins, which might spoil the product's appearance.

Antioxidants, including phenolic compounds, neutralize lipid peroxidation, all radical forms of oxygen and nitrogen. Therefore, we analyzed the samples for the total content of antioxidants (Fig. 3).

Figure 3 shows a correlation between increased amounts of lingonberry marc extract and higher total antioxidant capacity of the paste. According to the results, the total antioxidants in the test samples with 0.1, 0.2, 0.3%, and 0.4% DLME was higher than that of the control by 1.3, 1.5, 1.8, and 2.05 mg/g, respectively. The extract antioxidant complexes were rich in polyphenols



1 – control, 2 – 0.1% DLME sample, 3 – 0.2% DLME sample, 4 – 0.3% DLME sample

Figure 2 Optical density of paste samples with different concentrations of dry lingonberry marc extract

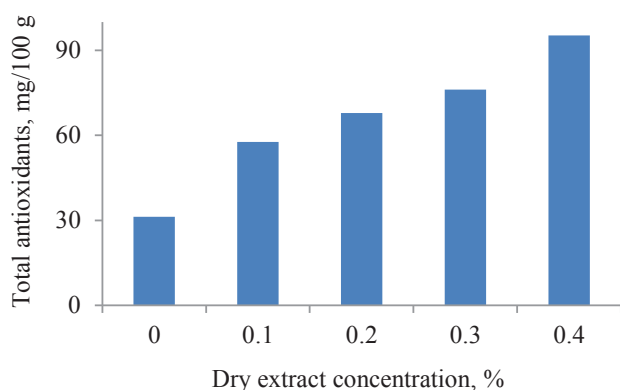


Figure 3 Total content of antioxidants in paste samples with different concentrations of dry lingonberry marc extract

(6.63%; Table 1). They also contained organic acids, vitamins, lycopene, and other antioxidant compounds.

Further, we performed a sensory evaluation of the paste on a nine-point scale to establish how the extract affected the product's consumer appeal (Table 3).

According to the results, small amounts of the dry lingonberry marc extract did not significantly affect the texture, smell, or taste of the end product. However, its concentrations above 0.2% had a negative effect on the paste color in section. As we could see in Table 2, the sample with 0.3% DLME acquired a light purple hue and that with 0.4% DLME, a light purple tint. The sensory evaluation showed a concentration of 0.2% as optimal since it did not spoil the characteristics of the end product while enriching it with antioxidant compounds (Fig. 3). Therefore, we used this concentration in further studies. For a physicochemical analysis, we prepared a control and a test sample with 0.2% DLME (Table 4).

The results showed that the dry lingonberry marc extract did not affect the quality of the paste, but it doubled the total content of antioxidants. The content of phenolic compounds in the extract is the most important indicator of its biological value, which determines its antioxidant activity.

To assess the extract's antioxidant capacity, we studied the process of fat oxidation. For this, we prepared a control and a test paste samples and determined the peroxide value which characterizes the accumulation of primary lipid decomposition products during storage.

Table 3 Sensory characteristics of paste samples with dry lingonberry marc extract

Characteristic	Control	Test samples with DLME			
		0.1%	0.2%	0.3%	0.4%
Appearance	8.5 ± 0.2	8.5 ± 0.1	8.5 ± 0.2	8.3 ± 0.1	8.2 ± 0.1
Texture	8.4 ± 0.2	8.5 ± 0.2	8.4 ± 0.1	8.4 ± 0.2	8.3 ± 0.2
Color and appearance in section	8.6 ± 0.2	8.6 ± 0.1	8.6 ± 0.1	8.0 ± 0.1	7.4 ± 0.1
Smell and taste	8.7 ± 0.2	8.7 ± 0.1	8.7 ± 0.2	8.3 ± 0.2	8.1 ± 0.2

Table 4 Physicochemical characteristics of paste in casing (0.2% DLME)

Characteristics	Paste in casing	
	Test	Control
Content, %		
moisture	71.5 ± 0.1	71.8 ± 0.03
protein	13.6 ± 0.1	13.3 ± 0.1
fat	11.9 ± 0.05	12.1 ± 0.2
minerals	1.4 ± 0.02	1.2 ± 0.1
salt	1.6 ± 0.02	1.6 ± 0.02
Total antioxidants, mg/100 g	67.84	31.23

Storage periods were selected in accordance with State Standard R 55334-2012^{IV} (10 days for pastes in polyamide casings and 15 days for pastes with acidity regulators). Our experiment consisted of two tests. In the first test, the control and the test samples (without DLME and with 0.2% DLME, respectively) were made without acidity regulators and stored for 14 days (Fig. 4). In the second test, the control and the test samples (without DLME and with 0.2% DLME, respectively) contained 0.2% sodium lactate as an acidity regulator and were stored for 18 days (Fig. 5).

The reason for this experiment was that acidity regulators are necessarily used in production, especially in summer, to increase the shelf life of perishable meat products. Thus, the experiment could show the DLME role in the inhibition of peroxidation of animal lipids. The samples were stored under identical conditions, in the dark at 2 ± 2°C.

Figure 4 shows the effect of DLME on the process of fat oxidation in the paste.

In Fig. 4, we can see an irreversible process of fat oxidation with the accumulation of primary fat decomposition products. Animal fats contained in the paste undergo auto-oxidation or peroxidation. The polyamide casing cannot completely prevent oxidation, since it is caused by a complex of factors: oxygen, light, positive temperature, unsaturated fatty acids, etc.

According to regulatory documents, the peroxide value for a high-quality fat product, low-oxidized raw materials, and fat raw materials should not exceed 0.5, 3.5, and 10 mmol of active oxygen per 1 kg of fat.

As we can see in Fig. 4, the peroxide value in the control and test samples immediately after preparation was 0.71–0.75 mmol O/kg. After six days of storage, it increased 3.17 times in the control and 2.98 times in the test sample, reaching 2.38 and 2.12 mmol O/kg, respectively. We found that on day 6, the peroxidation process in the test sample slowed down by 10.9% compared to the control. After 10 days (the shelf life for this type of product), the process of lipid oxidation continued to intensify and the peroxide value increased to 3.1 mmol O/kg in the control sample (6.2 as high as

^{IV} State Standard R 55334-2012. Meat and meat containing pate. Specifications. Moscow: Standartinform; 2014. 34 p.

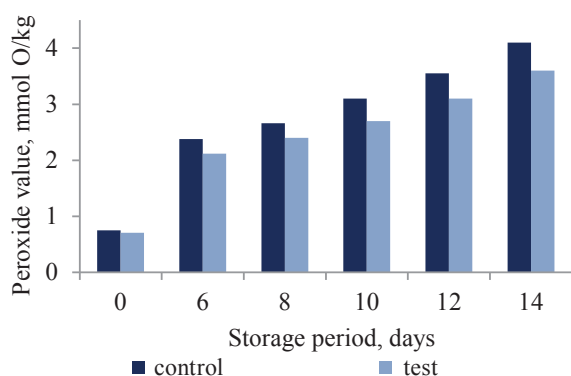


Figure 4 Effect of dry lingonberry marc extract on the peroxide value of paste without acidity regulators during storage

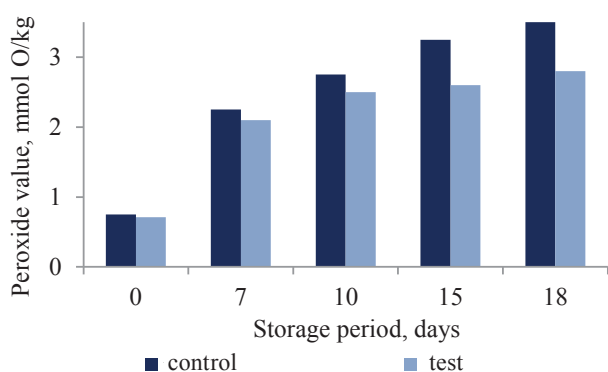


Figure 5 Effect of dry lingonberry marc extract on the peroxide value of paste with sodium lactate during storage

the initial value) and to 2.7 mmol O/kg in the test sample (3.8 times as high as the initial value). On day 10, the difference between the control and the test samples reached 12.9%.

Further analysis of the oxidation process showed that after 12 days, the peroxide values in the control and the test samples were 3.55 and 3.1 mmol O/kg, respectively. After 14 days, the process accelerated and the values reached 4.1 and 3.7 mmol O/kg, respectively. The difference between the control and the test samples was 12.7% on day 12 and 9.7% on day 14. Thus, the steadily lower peroxide value in the test sample, compared to the

control, indicated a decreased rate of lipid peroxidation reactions throughout the whole storage period. This result can be explained by the presence of DLME rich in antioxidant compounds that can neutralize the effects of free radicals playing a significant role in chain reactions of lipid oxidation.

The first test showed that introducing DLME into the paste made without acidity regulators helped to slow down fat oxidation and increase the shelf life by two days (total of 12 days without signs of oxidative damage).

In the second test, the control (without DLME) and test (0.2% DLME) samples contained 0.2% sodium lactate as an acidity regulator (Fig. 5).

The growth of peroxide values indicated the accumulation of fat oxidation products in the paste samples with sodium lactate throughout storage. However, we found a certain inhibition of the oxidation process compared to the first test, in which the samples were made without an acidity regulator. For example, on day 10, the peroxide values of the DLME samples without and with sodium lactate were 2.7 and 2.5 mmol O/kg, respectively. Thus, we can see a synergistic effect of sodium lactate and DLME antioxidant compounds during fat oxidation in the paste.

Further, we compared the peroxide values in the control and test samples with sodium lactate. We found that after 15 and 18 days of storage, the peroxide value of the test samples was 20% lower compared to the control, which was significantly higher than in the samples without sodium lactate (12.9%).

At this stage, we concluded that a combination of lingonberry extract with sodium lactate produced a more pronounced antioxidant effect. At the end of the storage period (18 days), the peroxide values of the control and test samples were 3.5 and 2.7 mmol of active oxygen per 1 kg of fat. This means that the paste’s shelf life could be extended by three days.

Thus, our experiment showed that although DLME contributed to the inhibition of lipid oxidation, its synergism with sodium lactate could significantly slow down these reactions.

The shelf life of paste containing a large amount of water (71–72%) is affected by not only oxidative

Table 5 Microbiological indicators of control and test samples during storage

Indicator	Storage of paste without sodium lactate, days							
	DLME test samples				Control			
	0	6	10	12	0	6	10	12
QMAFAnM, CFU/g	1.4×10 ²	4.1×10 ²	6.2×10 ²	7.3×10 ²	1.4×10 ²	5.8×10 ²	7.5×10 ²	8.5×10 ²
Coliforms in 1 g	not detected							
Indicator	Storage of paste with sodium lactate, days							
	DLME test samples				Control			
	0	7	15	18	0	7	15	18
QMAFAnM, CFU/g	1.4×10 ²	4.5×10 ²	8.4×10 ²	1.6×10 ³	1.4×10 ²	6.1×10 ²	1.0×10 ³	2.1×10 ³
Coliforms in 1 g	not detected							

processes, but also by the growth of microorganisms. According to Table 1, the dry lingonberry extract is rich in benzoic acid (1.34%) that has strong antimicrobial, antiseptic, and bactericidal effects inhibiting decay and fermentation processes. As a result, lingonberries last quite a long time without canning. Also, previous studies have proven the antimicrobial activity of DLME added to bakery products [25].

In our study, we investigated a possibility of inhibiting microorganisms in the DLME paste samples with and without sodium lactate (Table 5).

As we can see, all the samples showed a growth of microorganisms. However, it was less intensive in the DLME test samples with and without sodium lactate, compared to the controls. Thus, the presence of benzoic acid with strong bactericidal action slowed down the growth of microorganisms and had a positive effect on the test samples' shelf life.

As for pathogens, no *E. coli* bacteria were detected in any of the test samples, which might be due to the preliminary heat treatment of the raw materials and the use of a polyamide casing that excludes the product's contact with air, containers or equipment.

CONCLUSION

Thus, our study showed that 0.2% of dry lingonberry marc extract was the optimal amount to be introduced into paste forcemeat. This amount increased the

nutritional and biological value of the paste and maintained high consumer appeal. We found that the extract provided the product with a high content of polyphenols with antioxidant properties, including anthocyanins. Rich in antioxidant compounds, the extract inhibited fat oxidation in the paste and, in combination with sodium lactate, produced a synergistic effect on lipid peroxidation processes. In addition, the dry lingonberry marc extract slowed down the growth of microorganisms due to a high content of benzoic acid with antimicrobial and bactericidal properties. The integrated effect of the extract's components extended the shelf life of the paste in a casing by two or three days.

CONTRIBUTION

Authors are equally related to the writing of the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. Antipova LV, Glotova IA. Osnovy ratsional'nogo ispol'zovaniya vtorichnogo kollagensoderzhashchego syr'ya myasnoy promyshlennosti [Fundamentals for rational use of recycled collagen-containing raw materials in the meat industry]. Voronezh: Voronezh State Technological Academy; 1997. 246 p. (In Russ.).
2. Lafarga T, Hayes M. Bioactive peptides from muscle meats and by-products: generation, functionality and application as functional ingredients. *Meat Science*. 2014;98(2):227–239. DOI: <https://doi.org/10.1016/j.meatsci.2014.05.036>.
3. Henchion M, McCarthy M, O'Callaghan J. Transforming beef by-products into valuable ingredients: which spell/recipe to use? *Frontiers in Nutrition*. 2016;3. DOI: <https://doi.org/10.3389/fnut.2016.00053>.
4. Uzakov YaM, Kaimbaeva LA. Using meat and offal of marals in production of meat products. *Meat Industry*. 2015;(8):40–43. (In Russ.).
5. Gurinovich GV, Gargaeva AG, Kudryashov LS. Investigation of the emulsifying ability of meat systems containing plant components for the production of pates. *All about meat*. 2018;(6):54–56. (In Russ.).
6. Bazhenova BA, Balzhinimaeva SK. Pâté forcemeat with a biologically active additive. *Food Processing: Techniques and Technology*. 2011;23(4):19–23. (In Russ.).
7. Giro TM, Chirkova OI. Mediko-biologicheskaya otsenka myasorastitel'nykh pashtetov dlya korrektsii zhelezodefitsitnykh sostoyaniy [Biomedical evaluation of meat and plant pastes for correcting iron deficiency]. *Food Processing: Techniques and Technology*. 2009;12(1):50–53. (In Russ.).
8. Okuskhanova EK, Asenova BK, Rebezov MB, Omargalieva NK, Yesimbekov ZS. Aminoacid composition of pâté based on maral meat and protein enricher. *Food Processing: Techniques and Technology*. 2015;39(4):71–79. (In Russ.).
9. Lyakh VA, Fedyanina LN, Smertina ES. Development and evaluation of consumer properties of hypoallergenic meat pastes. *Food Processing: Techniques and Technology*. 2016;40(1):32–38. (In Russ.).
10. Lisitsyn AB, Semenova AA, Gundyreva MI. Issledovanie antiokislitel'nykh svoystv sverkhkriticheskikh CO₂-ehkstraktov [A study of the antioxidant properties of supercritical CO₂ extracts]. *Meat Industry*. 2006;(3):30–35. (In Russ.).

11. Yendonova GB, Antsupova TP, Bazhenova BA, Zabaluyeva YuYu, Gerasimov AV. Antioxidant activity of the extract of chickweed (*Stellaria media*). *Chemistry of plant raw material*. 2018;(4):141–147. (In Russ.). DOI: <https://doi.org/10.14258/jcprm.2018043749>.
12. Zhirkova EV, Skorokhodova MV, Martirosyan VV, Sotchenko EF, Malkina VD, Shatalova TA. Chemical composition and antioxidant activity of corn hybrids grain of different pigmentation. *Foods and Raw Materials*. 2016;4(2):85–91. DOI: <https://doi.org/10.21179/2308-4057-2016-2-85-91>.
13. Golubtsova YuV. Physical and chemical indicators and merchandasing assessment of wild strawberry, gooseberry, cherry, raspberry, banana, wild rose and kiwi. *Foods and Raw Materials*. 2017;5(1):154–164. DOI: <https://doi.org/10.21179/2308-4057-2017-1-154-164>.
14. Gougoulis N. Evaluation of antioxidant activity and polyphenol content of leaves from some fruit species. *Oxidation Communications*. 2015;38(1):35–45.
15. Zabalueva YuYu, Meleshkina NV, Bazhenova BA, Danilov MB. One of the ways of enrichment of meat products by natural antioxidants. *All about meat*. 2017;(2):12–15. (In Russ.).
16. Shah MA, Bosco SJD, Mir SA. Plant extracts as natural antioxidants in meat and meat products. *Meat Science*. 2014;98(1):21–33. DOI: <https://doi.org/10.1016/j.meatsci.2014.03.020>.
17. Turgut SS, Işıkçi F, Soyer A. Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. *Meat Science*. 2017;129:111–119. DOI: <https://doi.org/10.1016/j.meatsci.2017.02.019>.
18. Bitueva EhB, Ajusheeva EE. Meat cutlets production method. Russia patent RU 2410981C1. 2011.
19. Ivanova GV, Izosimova IV. Meat-vegetable paste. Russia patent RU 2385652C2. 2010.
20. Oswell NJ, Thippareddi H, Pegg RB. Practical use of natural antioxidants in meat products in the U.S.: A review. *Meat Science*. 2018;145:469–479. DOI: <https://doi.org/10.1016/j.meatsci.2018.07.020>.
21. Kebede M, Admassu S. Application of antioxidants in food processing industry: options to improve the extraction yields and market value of natural products. *Advances in Food Technology and Nutritional Sciences*. 2019;5(2):38–49. DOI: <https://doi.org/10.17140/AFTNSOJ-5-155>.
22. Sokolov SYa, Zamotaev IP. *Spravochnik po lekarstvennym rasteniyam (Fitoterapiya) [A handbook of medicinal plants (Phytotherapy)]*. Moscow: Meditsina; 1988. 464 p. (In Russ.).
23. Zambulaeva ND, Zhamsaranova SD. The antioxidant activity of extracts from bagasse of cowberry and cranberry. *Problems of Biological, Medical and Pharmaceutical Chemistry*. 2015;(11):54–55. (In Russ.).
24. Yashin AYa. Inzhektionsionno-protokhnaya sistema s amperometricheskim detektorom dlya selektivnogo opredeleniya antioksidantov v pishchevykh produktakh i napitkakh [The injection-flow system with an amperometric detector for the selective determination of antioxidants in foods and beverages]. *Russian Journal of General Chemistry*. 2008;52(2):130–135. (In Russ.).
25. Zambulaeva ND, Zhamsaranova SD. Investigation of the antioxidant and antimicrobial properties of juice processing by-products for use as ingredients for the enrichment of food products. *Proceedings of Universities. Applied Chemistry and Biotechnology*. 2018;8(1)(24):51–58. (In Russ.). DOI: <https://doi.org/10.21285/2227-2925-2018-8-1-51-58>.
26. Yashin YaI, Ryzhnev VYu, Yashin AYa, Chernousova NI. *Prirodnye antioksidanty. Soderzhanie v pishchevykh produktakh i vliyaniye ikh na zdorov'e i starenie cheloveka [Natural antioxidants. Their content in food products and effect on human health and aging]*. Moscow: TransLit; 2009. 212 p. (In Russ.).
27. Polina SA, Efremov AA. Determination composition of anthocyanins in bilberry, cowberry and cranberry with HPLC. *Chemistry of plant raw material*. 2014;(2):103–110. (In Russ.).

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