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EFFECT OF BLUEBERRY (VACCÍNIUM MYRTÍLLUS) LEAVES EXTRACT OBTAINED BY MICROWAVE HEATING ON THE DYNAMICS OF ANIMAL FAT OXIDATION PROCESSES

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Keywords: *vaccínium myrtíllus, biologically active substances, chemical composition, microwave heating power, total content of antioxidants, peroxide value*

Abstract
The potential of antioxidant properties of blueberry leaves extract (vaccínium myrtíllus) that grows in the Trans-Baikal region has been studied in this work. In order to increase the efficiency of extraction of biologically active substances with antioxidant properties, this extract was obtained with the help of microwave field. The optimal parameters for the extraction of active substances from the blueberry leaves with the help of electromagnetic microwave field have been defined. The influence of the power of the electromagnetic field and the duration of the process of blueberry leaves extraction on the efficiency of extraction of biologically active polyphenolic substances has been studied. The following parameters of the technology for blueberry leaves extraction were obtained: extraction with a water-alcohol solution with a concentration of 40% in the ratio of raw materials to extractant as 1 to 5, with duration of stirring as long as 30 minutes, then the application of an electromagnetic field of microwave heating with a power of 850W for 8–10 minutes long. Blueberry leaves extract is a clear liquid with a high content of polyphenols, of rich brown color, tart taste, without bitterness. The antioxidant potential of the obtained extract has been studied. To do this, the extract has been added into the finely ground animal fat and left for storage in a closed dark container. During storage, the dynamics of the peroxide number has been measured, as this value characterizes the degree of lipid oxidation. It has been found that blueberry leaves extract inhibits the process of animal fat oxidation due to the action of biologically active substances that feature antioxidant properties.

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Introduction

Blueberry (*vaccínium myrtíllus*) is a low shrub of 20–30 cm high, which is widely spread in the taiga zone of the Trans-Baikal region. This plant is characterized by a creeping underground rootstock and numerous shoots. Its bluish-black berries are consumed. The berries are rich in tannins, anthocyanins, tannins, flavonols and other substances [1,2]. In addition to berries, the blueberry leaves and shoots are used as a remedy, which also contain various biologically active substances and are recommended against diseases of the eyes, gastrointestinal tract, diabetes, in gerontology, in the treatment of skin burns, stomatitis, etc. [3].

Blueberry leaves are a renewable raw material. These leaves are of high value due to the availability of biologically active substances, they can be dried and stored for a long time; therefore they are of interest for consuming as an antioxidant component [4,5].

The analysis of references and specialized literature showed that blueberry leaves contain a fairly large amount of antioxidants from bioflavonoids group, such as anthocyanins and polyphenols [6,7]. In addition, the flavonoids like rutin, hyperoside, isoquercetin, etc. were found there too. A number of authors in their studies underline that there are related substances like sugars and tannins up to 20%, organic acids (citric, oxalic, malic, succinic, quinic, lactic up to 7% [8], vitamin C up to 250 mg%, vitamin B, carotene, phenolic compounds — hydroquinone (1%), arbumin (1–2%), neomyrtillin (up to 2%), myrtillin (up to 1%) and many others [9].

The high antioxidant characteristics of blueberry leaves opens up an opportunity of its using in the composition of fat-containing foods to inhibit oxidative processes in lipids. Most meat products contain a large amount of saturated and unsaturated lipids, which undergo oxidation during their storage, which in its turn negatively affects the organoleptic characteristics and shelf life of the finished food [10].

To prevent oxidative spoilage, manufacturers use various antioxidant and antimicrobial food additives. The most prominent and rife antioxidants used in the meat industry are as follows: ascorbic acid (E300), sodium ascorbate (E301), butylhydroxytoluene (E321), sodium lactate (E325) and others [11,12]. So, E. V. Hardina et al. describe studies on the storage stability of chilled pork treated with rosemary extract and dihydroquercetin [13].

The authors of the research [14] have studied the effect of sage extract on the functional and technological properties, quality and shelf life of chopped semi-finished food products. It has been established that it is expedient to add the sage extract in the amount of 0.1% of raw materials weight into the recipe of minced meat semi-finished food products.

In their study, Lorenzo et al. [15] also note that oxidative reactions can reduce the quality of meat food. The authors considered the protective effects of active rosemary against oxidative degradation in meat food. Adding the rosemary essential oil or its extract can slow down the development of oxidative reactions and preserve redness pf food surface, reduce the accumulation of primary and secondary lipid and protein oxidation products, and slow down the increase of perceived rancidity in sensory analysis of foods. Consequently, more people want to use herbal extracts [16] because they are generally recognized as safe [17,18]. When cooking meat, natural extracts should be used to protect the food from external influences and extend its shelf life [19].

Reddy et al. evaluated the effectiveness of various natural antioxidants in improving the quality and shelf life of meat products [20]. Natural antioxidants keep food resistant to oxidation, and also effectively control microbial growth. However, further research is needed to explore how different duration of exposure and volumes of added extract, as well as interactions with other natural antioxidants and nutritional supplements, will affect the results.

The authors of the study [21] found that elderberry contain a significant amount of monosaccharides; citric acid dominates among organic acids. Berries also contain succinic acid, which is an antioxidant involved in the metabolic reactions in a human body. Elderberry features a hepatoprotective, anti-stress, adaptogenic effect. In addition, succinic acid reduces the formation of excess amounts of cholesterol, thus preventing the loss of calcium by cells. Antioxidants belong to the category of inhibitors — substances that slow down the oxidative processes [22]. Due to their interaction with reactogenic oxidizing agents and reactive forms of oxygen, as well as with other free radicals, they can lead to partial or complete inactivation of oxidizers. The substances of polyene group are particularly active. Some of the plants species are rich in these substances characterized by several unsaturated bonds and the mechanism of action which is associated with their ability to easily oxidize and thereby prevent oxidation of the molecules.

For more efficient use of the complex of biologically active substances in the recipe of food products several methods of their extraction are used, like herbal infusions, herbal decoctions and extracts. Various extractants are used to facilitate the extraction of useful components from the raw materials: water, acids, alkalis, alcohols, organic solvents, etc. With regard to floral and plant raw materials, a water-alcohol solution is most preferrable, since it is a food component and has a high extractive capacity in regards to the plant polyphenols [23].

As a result of the analysis of available methods of extraction, the method of extraction with a water-alcohol solution with a concentration of 40% in a ratio of 1:5 has been chosen [24]. This method is widely used in industrial food production. To intensify the extraction process, various methods are used: maceration, fractional extraction, exposure to thermal energy, microwave field, ultrasound, etc. For example, in the work [25], the authors present data on the study of the effect of microwave extraction modes on the yield of flavonoids from the leaves of the common gromwell (*Lithospermum officinale L.*). It was noted that the highest yield of the desired components is achieved at a microwaves generator power of 100W (2.5 min) at a field frequency of 2,450 MHz, which is comparable to the yield when using traditional convective heating for extraction by infusion at elevated temperature. Microwave extraction reduces the process time by 16 times and increases the yield of flavonoids by 23%. The authors state, that microwave energy destroy the plant tissues, which facilitates the efficient extraction of biologically active substances.

The authors in the work [26] present test data on the study of the effect of microwave radiation (100W, 20 min) on the degree of extraction of *Alceanudiflora*. It has been proven that the chosen conditions of the extraction, due to the ability of the substance to convert microwave energy into heat, made it possible to significantly reduce the duration of the process and obtain extracts enriched with new compounds. 13 acids were found, including 6 aromatic acids and 7 unbranched monobasic acids of unsaturated and saturated series, as well as 11 new neutral compounds, including 7 naphthalene derivatives.

The authors in the publication [27] described a method run on the sample of eucalyptus leaves. This method has proven the increase in the efficiency of extracting components from the plant materials by using microwave energy of 1 kW (microwave supply for about 5 minutes). The method allows obtaining a concentrated extract and high quality products through the use of microwave energy.

Thus, based on the analysis of the literature, it was found that herbal infusions, extracts and decoctions are used in the composition of food products to use biologically active substances with antioxidant properties from plant materials. To increase the efficiency, microwave heating is used for the preparation of herbal extracts.

It was noted that blueberry leaves and berries are rich in biologically active substances with antioxidant properties, including polyphenolic group. There are no studies on extraction of the active substances from blueberry leaves to find and confirm their antioxidant effect on animal fats. In connection with the above, the purpose of the work was to substantiate the modes of extraction from blueberry leaves growing in the Trans-Baikal region with the help of microwave field and to study its effect on the dynamics of animal fat oxidation.

Objects and methods

The objects of research were blueberry leaves and raw animal fat.

The blueberry leaves (*vaccínium myrtíllus*) were picked up in 2020 during the blossoming of the blueberry in Zaigraevsky area in the territory of Angirsky natural reservation of the Republic of Buryatia (latitude: 52.098721; longitude: 108.562931). The collected plant material — blueberry leaves — were sorted by their size and quality, yellow and damaged leaves were disposed. The usable leaves were washed with cold water, dried in room conditions at a temperature of 22 °C to 25 °C and packed in paper bags. The finished material was stored at a temperature from 4 °C to 5 °C.

The samples of animal fat were raw horse fat and raw pork fat obtained from the slaughter of healthy livestock. Raw fat was ground in a household meat grinder MFW4 (Bosch, Germany), with grate diameter of 2–3 mm. The obtained leaves extract was added to the test samples in amount of 5% of the fat weight. The amount was determined in accordance with the recommended amount of antioxidants added to minced meat — 0.05–0.075%. Based on the content of antioxidants in the leaves extract, usually they introduce blueberry leaves extract into animal fat in amount of 5%. After adding the extract, the ground fat was well mixed and the dynamics of the peroxide value was analyzed in comparison with control samples, without any extract. Control sample and test sample were sealed in jars with a tight lid and stored for 120 hours at a temperature of 2 °C to 6 °C. those jars were sampled after 24, 48, 72, 96 and 120 hours of storage, and the peroxide value was analyzed by a method based on the interaction of the oxidation products in raw fats with potassium iodide in a solution of acetic acid and chloroform, followed by a quantitative determination of the released iodine with sodium thiosulfate solution with the help of the titrimetric method (GOST R51487–991).

At the initial stage, control and test samples of the extract from dried blueberry leaves were made. To run the extraction process, dried blueberry leaves were ground in a coffee grinder (Bosch MKM6000 with a capacity of 75 grams, 180 W) (Bosch, Germany). After grinding the leaves powder was placed in a beaker flask of 100 ml with a tight lid, poured with a water-alcohol solution, which is traditionally used in as an extractant for vegetable raw materials. For extraction, the flasks with control and test samples were placed under the same conditions: they were stirred in a shaker (Elpan Bath Shaker type 357) (Elpan, Poland) for 30 min at room temperature (20–22 °C), 120 shakes/min. After extraction under the constant stirring in a shaker, the test sample was subjected to microwave waves in order to increase the efficiency of the process of extracting biologically active substances. To do this, a tightly closed flask with the liquid was placed in a Samsung GW712BR microwave oven (Samsung, Vietnam). During the experiment, the parameters of the extraction process were selected and defined: the duration and power of microwave exposure in regards to blueberry leaves. The obtained control and test samples of blueberry leaves extract were filtered through lavsan cloth and filter paper.

During the experiment, the total content of polyphenols was determined with the help of the Folin-Ciocalteu reagent. The samples were taken from the obtained extract, with a volume of 0.075 cm_3. The Folin-Ciocalteu reagent in volume of 0.075 cm^3 was diluted 5 times, added to the samples, stirred. In 3 minutes 0.15 cm^3 of 20% sodium carbonate solution and 1.2 cm^3 of distilled water were added. The mixture was covered with a lid, stirred and left at room temperature. After 1 hour of the optical density of the obtained tungsten blue (TB) was measured at a wavelength of 725 nm with the KFK-3–01 photoelectric photometer (Russia, OJSC ZOMZ), at the optical path length of 1 cm. The total content of TB is expressed in mg-equivalents of gallic acid per g of fresh weight of raw materials. The content of organic acids and water-soluble vitamins was determined by capillary electrophoresis with the Kapel-105M instrument (Russia, LLC Lumeks-Marketing) with indirect detection at a wavelength of 190 nm [28]. The acidity of the medium was determined by the potentiometric method. The obtained extract was organoleptically evaluated according to GOST 18078–722. The optical characteristics of the extracts were obtained with the help of KFK-3–01–30MZ photometer (Russia, OAO ZOMZ).

The experimental data were obtained in triplicate and statistically analyzed with MS Excel (Microsoft, USA) software. Results were presented as mean-root-square (S), mean-root-square (standard) deviation (±SD). Differences between control and test samples were recognized as significant at a probability level not higher than 0.05.

During the experiment, to find the optimal parameters of microwave heating during the extraction of blueberry leaves, the Mathcad 15 software (RTS, USA) was used. To

[1] GOST R51487–99 "Vegetable oils and animal fats. Method for determination of peroxide value". Moscow: Standartinform, 2003. Retrieved from https://docs.cntd.ru/document/1200028330 Accessed November 10, 2022 (In Russian)

[2] GOST 18078–72 "Fruit and berry extracts. Specifications" Moscow: Publishing House of Standards, 1998. Retrieved from https://docs.cntd.ru/document/1200022569 Accessed November 10, 2022 (In Russian)

begin with, two main variable factors affecting efficiency were determined — the duration of heating and the power of the microwave field, then the "desirability" function — the total content of antioxidants in the obtained extract — was established. The matrix of a two-factor experiment was composed. There was a condition that the level of the first factor crosses once with each level of the second factor. In result of solving the problem, the maximum values of the variables function were obtained.

Results and discussion

To extract the biologically active substances from the leaves of blueberry that grows in the Trans-Baikal region, the possibility of their extraction with a microwave field in order to increase the degree of extraction was studied. To extract useful components from the raw materials, including the antioxidant substances, various extractants were used. The most suitable among them was water-alcohol solution, as it is a food component and has a high extracting capacity.

To define the modes of technology for extracting, it is necessary to optimize the parameters of microwave extraction of plant components from the blueberry leaves. For this, a factorial experiment was used with involvement of mathematical methods.

The main extraction parameters to be optimized are the following:

- the duration of microwave extraction;
- the power of the microwave field.

Based on the planned parameters (Table 1), a matrix of a two-factorial experiment was compiled. This matrix served as the basis for a number of tests. The limitations of the studied parameters were chosen on the basis of the literature data of the authors, who recommend microwave processing for the plant substances extraction. The total value of antioxidants content was taken as the function of "desirability", which characterizes the efficiency of the extraction of biologically active antioxidant substances from the plant raw materials.

Table 1. Levels of factors studied

Factor	Level						
	1	2	3	4	5	6	7
X_1, Duration, minutes	0	2	4	6	8	10	—
X_2, Microwave power, W	0	450	550	650	750	850	950

The figure 1 below shows the contour of the "desirability" and its function of the microwave processing duration and microwave power on the content of polyphenols in the resulting extract.

In order to find the maximum value of the response function and the values of the corresponding factor, a standard procedure was performed to find the maximum of a function of two variables in a bounded domain of definition. When solving the extremum problem, the following solution was obtained (Table 2).

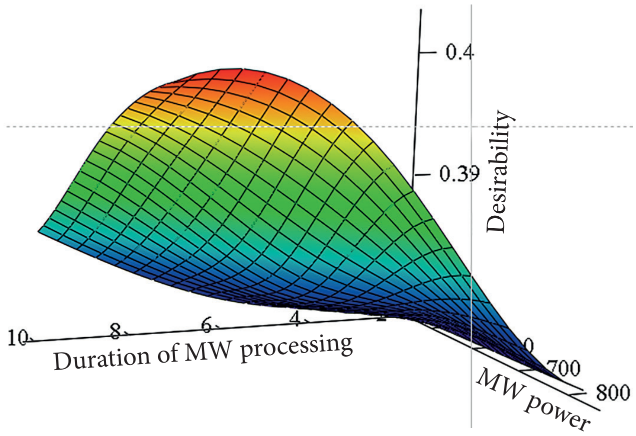

Figure 1. Effect of power and duration of microwave treatment on the content of antioxidants in the extract

Table 2. Results of solving the extremum problem

Duration at the peak value	Power at the peak value	Response function at the peak value
X_1 = 9.2 min	X_2 = 850 W	Y = 14.5%

The microwave field, due to its influence on the solvent dipoles orientation, converts microwave energy into thermal energy, thus heating up the entire bulk of the material. The heating induces destruction of plant tissue. This contributes to a more efficient extraction of biologically active substances from the plant materials into the solvent medium.

As a result of the experiments, the following parameters for obtaining an extract from blueberry leaves were chosen: extraction with a water-alcohol solution of 40% concentration, the ratio of raw materials to the extractant was 1:5 (the ratio recommended for plant raw materials ranges from 1:1 to 1:10), the microwave heating power was 850 W, duration of heating was 9–10 minutes in order to ensure the efficiency of the process.

Further, comparative studies of the characteristics of the control sample (without microwave heating) and test sample (exposed to the microwave heating) of blueberry leaves extracts were run (Table 3).

Table 3. Characteristics of blueberry leaves extract

Parameters	Blueberry leaves extract	
	Control	Test sample (with MW)
Sediment	Liquid without any sediment and foreign inclusions	Liquid without any sediment and foreign inclusions
Taste and smell	Weakly tart, without bitterness	Tart, slightly herbal, without bitterness
Color	Light brown	Rich brown
pH	4.22 ± 0.21*	4.17 ± 0.20
Sugar, %	14.50 ± 0.61*	15.40 ± 0.52
Organic acids, %	7.20 ± 0.42**	8.40 ± 0.53
Ascorbic acid, mg%	0.25 ± 0.03*	0.29 ± 0.02
Total content of antioxidants, %	9.23 ± 0.25**	14.42 ± 0.16
Total content of polyphenols, %	2.92 ± 0.22**	9.20 ± 0.34

* $p > 0.05$; ** $p < 0.05$

It was noted that blueberry leaves extracts obtained by maceration (control sample) and obtained by the microwave heating (test sample) were a liquid without sediment with a different shade of color.

The saturation of the extracts color, which depends on the amount of the extracted coloring pigments of anthocyanins of the polyphenolic group, was further determined by the spectrophotometric method.

When extracting the substances from the crushed plant raw materials, biologically active substances of blueberry leaves, including polyphenols, sugars, organic acids, ascorbic acid, etc., passed from the leaves into a water-alcohol solution. The test data presented in the Table 3 and their statistical processing showed that in terms of pH, sugar content and ascorbic acid content, no significant differences were found in the control sample and the test sample of the extract ($p > 0.05$).

Biologically active substances of the polyphenolic group showed the greatest antioxidant activity in plant raw materials. The data of the Table 3 indicate that the content of polyphenols significantly increased in the test sample of blueberry leaves extract — it increased three times ($p < 0.05$).

This fact proved the increase in the biologically active substances concentration, and hence it increased the efficiency of the extracting components from the blueberry leaves with the help of a microwave field.

Figure 2 shows the scheme for preparing an extract from the blueberry leaves.

To confirm the differences in saturated color of the extract obtained with the microwave heating, which proves a higher concentration of biologically active substances in the solution, the values of the optical density of the analyzed extract samples at the absorption maximum (340–400 nm) were studied (Table 4).

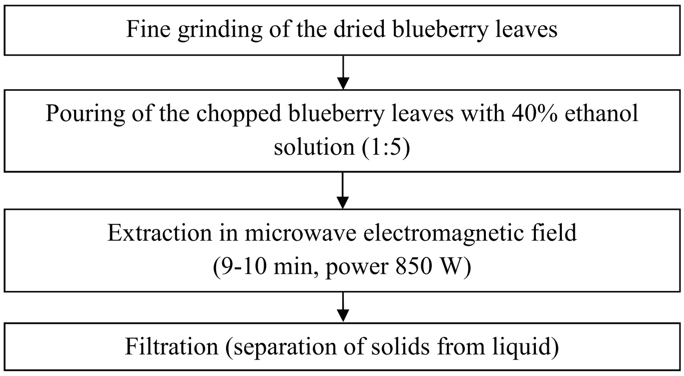

Figure 2. Scheme for preparing an extract from blueberry leaves

Table 4. The value of the optical density of the extract obtained from blueberry leaves

Sample	The value of optical density D, units	Total content of antioxidants (TCA), %
Control	1.75	9.23 ± 0.25
Test	2.72	14.42 ± 0.16

The obtained data confirmed the darker, more saturated color of the test sample. The value of the optical density of the test extract is higher by 55.4% compared to the control sample ($p < 0.05$). A higher value of the total content of antioxidants in the test sample of the extract obtained by microwave heating was noted in comparison with the control sample by 6.19%, which is 56.2 rel.% higher.

At the next stage an experiment was carried out to study the influence of the extract from blueberry leaves obtained with a microwave heating on the dynamics of oxidative process in animal fat. Samples without blueberry leaves extract were used as the control samples (Figures 3 and 4).

The data presented in the Figures 3 and 4 prove that blueberry leaves extract is able to inhibit oxidative processes in raw pork fat and raw horse fat. These processes are

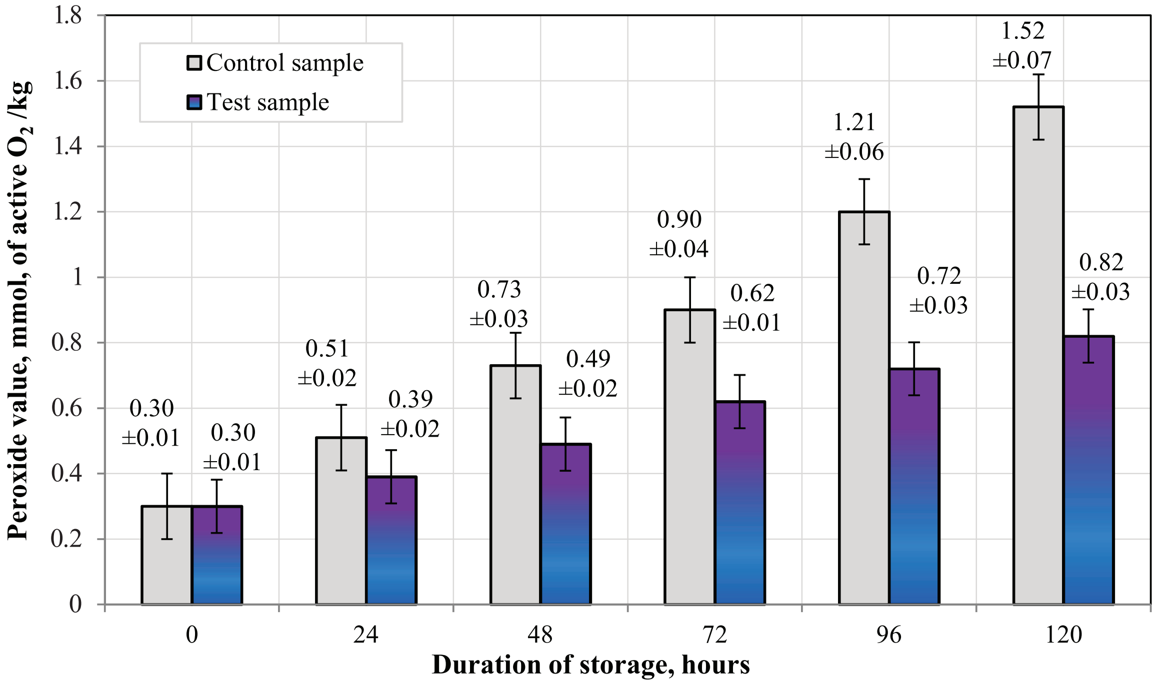

Figura 3. Dynamics of peroxide value change in the pork fat with blueberry leaves extract added

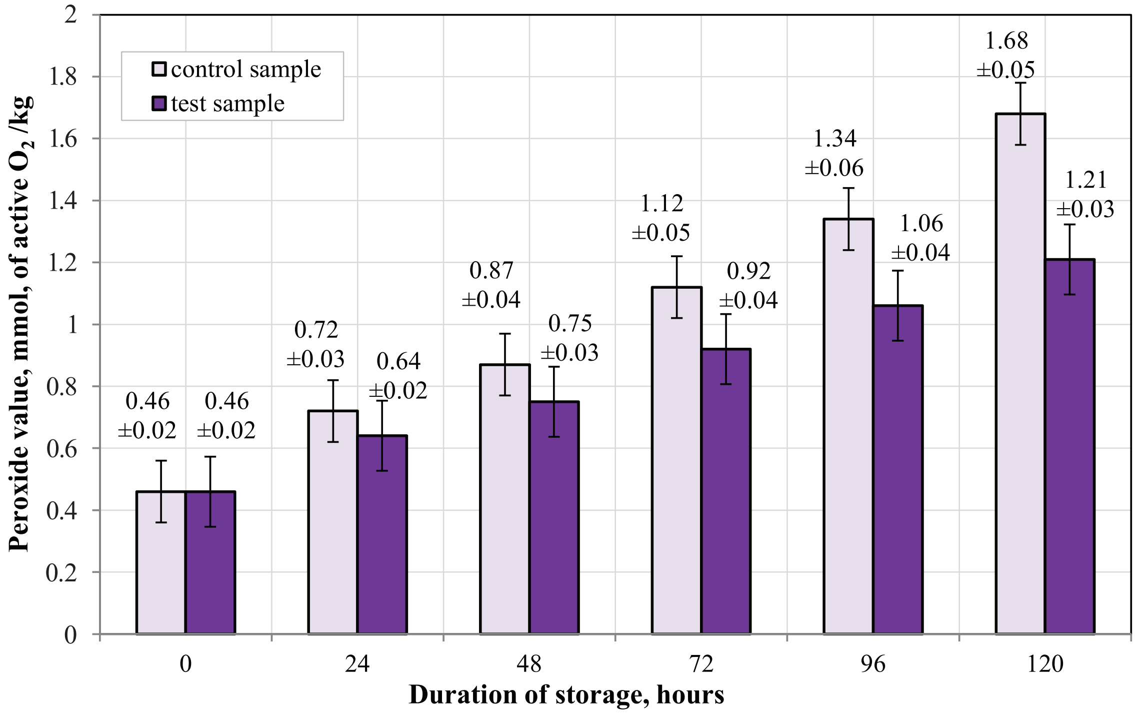

Figure 4. Dynamics of peroxide value of horse fat with blueberry leaves extract added

the continuous chain reaction, which can be inhibited by certain inhibitors with high antioxidant properties. The data of the Figure 3 showed that after 120 hours of storage of pork fat, inhibition of its peroxidation was observed, since the value of the peroxide value in the test sample is 46% lower in comparison with the same value in the control sample ($p<0.05$). In horse fat, the difference between the peroxide value in the control samples and test samples after 120 hours of storage reached 67% ($p<0.05$). In horse fat the oxidation process was more intense, since horse fat has more unsaturated fatty acids than pork fat. The influence of the biologically active substances in the extract is effective for both types of animal fat, therefore, the addition of the blueberry leaves extract as a renewable plant raw material into meat food can help inhibit oxidative processes, thereby extending the shelf life of finished food products.

The obtained test results are consistent with research data obtained by scientists in the food industry both in the Russian Federation and in foreign countries.

The researches of the Nanjing Agricultural University obtained data on the effect of fermented blueberry, added in amounts of 2, 4 and 6% to the meat food products, on the degree of oxidation of the fat fraction in the boiled sausage during its storage (4 °C) for 28 days. They found that adding of blueberry slows down the oxidative deterioration of fat, which was confirmed by a significant decrease in peroxide and thiobarbituric values [30].

The article [31] presents data on the effect of aromatic herbs (coriander, basil, parsley, rosemary) on preservation and food safety of sausages. It was found that the finished food product can be stored for up to five days without chemical additives due to the presence of substances with antioxidant properties among the ingredients.

Fat peroxidation value decreased during storage of the protein-fat composition with added thistle extract. That is explained by the interaction of flavonoids from the extract and free radicals, which interaction increases the storage duration of the fat-containing food [32].

The positive effect of aloe vera extract on slowing down the oxidative spoilage of fermented sausages has been established. After 30 days of storage in sausages containing only sodium nitrite and sausages containing only aloe vera extract, a decrease in the content of the thiobarbituric value was recorded in comparison with the control sample by 48% and 45%, respectively. The least TBV (decreased by 68%) was recorded in the sausages with added aloe vera extract and sodium nitrite ($p < 0.05$) [33].

The presented publications provide data on the positive effect of the plant raw materials on oxidative processes during the storage of animal fat.

Conclusions

The influence of electromagnetic field power and duration of extraction on the efficiency of extraction of substances of a polyphenolic group was studied. The following extraction parameters were obtained: extraction with a water-alcohol solution with a concentration of 40%; the ratio of raw materials to the extractant was 1:5; the solution was exposed to the microwave heating with a power of 850W for 9–10 minutes long. Experiments have shown that blueberry leaves extract is capable to inhibit oxidative processes in animal fat due to the action of polyphenols with antioxidant properties, thus extending the shelf life of fat-containing finished food products. The antioxidant properties of blueberry leaves extract are proven by the high total content of antioxidants (14.42%), which are able to neutralize the free radicals and prevent oxidation processes.

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