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ANTIOXIDANT, ANTIMICROBIAL ACTIVITY AND MINERAL COMPOSITION OF LOW-TEMPERATURE FRACTIONING PRODUCTS OF *MALUS DOMESTICA BORKH* (COMMON ANTONOVKA)

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ABSTRACT
The low-temperature fractionation of fruit *Malus domestica Borkh (Common Antonovka)* has been performed. We obtained by fractionation the biologically active products that are the dehydrated concentrate of juice and the powder of pomace fibers. Use of low temperature minimizes biological value losses during processing. These fractions of fruit *Malus domestica Borkh (Common Antonovka)* are experimentally studied. It is found that the fractions have high antioxidant activity and include bioflavonoids and organic and phenol carboxylic acids. Analysis of chromatograms showed availability of the identical compounds in the products of low-temperature fractionation. Sodium and potassium are part of the cells of biological systems as highly mobile ionic forms. Therefore, these elements prevail in the concentrated juice. Iron, manganese, copper, and zinc are biogenic trace elements or components of enzyme systems and are evenly distributed as in plant cell walls as well in protoplasm. It follows from the results of the study of the mineral composition that the products of the low-temperature fractioning can be used for a functional food as a result of its high content of magnesium and iron. The low-temperature fractionation of fruit *Malus domestica Borkh (Common Antonovka)* has antimicrobial activity against the standard strains of spoilage: *Bacillus subtilis VKM-B-501*, *Micrococcus luteus VKM-As-2230, Aspergillus flavus VKM-F-1024*, *Penicillium expansion VKM-F-275*, *Mucor mucedo VKM- F-1257*, *Rhizopus stolonifer VKM- F-2005*. Experimental data show that the products of low-temperature fractioning of *Malus domestica Borkh (Common Antonovka)* inhibit microorganism's growth. The detected composition of *Malus domestica Borkh (Common Antonovka)* fractions allows using these products as natural additives in food technology to maintain and increase period of storage and also for preventive nutrition.

Keywords: concentrated juice; pomace powder; *Malus domesti*ca Borkh. Common Antonovka fruit; antioxidant activity; bioflavonoids

INTRODUCTION
The synthetic food additives used in food technologies are suspected of toxicity and general public increases pressure on the food manufacturers with the view of application natural alternatives for maintaining or extending lifetime of products **(Seneviratne and Kotuwegedara, 2009)**.

Products of oxidation can lead to a deterioration of qualities and even endanger the food safety (**McClements and Decker, 2006**), so the choice of natural sources that have an antioxidant effect, shall guarantee safety of food additives.

Numerous plants contain biologically active compounds that can be considered as a good alternative to synthetic antioxidant food additives **(Nakatani, 2000; Yanishlieva et al., 2006)**. Antioxidant properties of biologically active compounds, mainly determined by their redox activity, allow to chelate heavy metals and to bind active oxygen **(Krishnaiah et al., 2011; Suja et al., 2016)**. Antioxidant activity into plant tissue is associated with presence of natural compounds, in particular bioflavonoids, hydroxyl acids, C and E vitamins, β-carotene and selenium. Antioxidants are used as compounds that can effectively interact with free radical, which determine biological membrane lipids oxidation protecting human body from diseases **(Rice-Evans, 1996; Van Acker, 1996; Du et al., 2016; Brzóska et. al., 2016)**.

Concentrated juices have high antioxidant potential to combat oxidative processes. They are also a source of calcium **(Chambi et al., 2016)**.

Apple pomace is rich in polyphenols, which are used as food additives due to their strong antioxidant and antimicrobial properties **(Vineetha et al., 2014; Zhang et al., 2016)**. Polyphenols of apple pomace are presented by chlorogenic acid, coffee acid, syrigin, epicatechin, cinnamic acid, kumarinovy acid, and quercetin. Determination of antioxidant activity showed that the extracts have a strong antioxidant activity DPPH radical to 90.96% ±10.23% **(Bai et al., 2013; Francini et.al., 2017)**. The high antioxidant activity of apple pomace and juice is determined **(Maragò et al., 2015)**. It is established that the pomace and biowaste generated in the process of industrial processing apples, possess both antioxidant and geroprotector activity. Markers of liver damage alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were reduced in the serum of the monkeys, which used the apple pomace and biowaste. **(Vineetha et al., 2014; Sharma et al., 2016)**.

Thermal processing is usually considered as a degrading factor for biologically active compounds because most of them are unstable during heating. In this connection, processed fruits are deemed to have much lower nutritional value and antioxidant activity than fresh **(Zhang and Hamauzu, 1995; Turkmen et al, 2005; Silva et al., 2015)**. Low-temperature fractioning of plants can be a possible alternative for food technology **(Emel'yanov, 2009)**. The fractioning leads to obtain biologically active products, which are the dehydrated juice and the dietary fiber. The result of pressing pulp is the juice containing main soluble solids and pomace mainly consisting of dietary fiber. The juice of direct extraction is evaporated under vacuum to produce a concentrated juice. Next, the concentrate and the pomace are dried in the convection oven at temperature up to 50 °C until the moisture that provides long shelf life under normal conditions at room temperature. Usage of low temperature minimizes loss of biological value during processing. As a result, the dehydrated fractions are biologically active one and can be used for manufacturing of functional purpose products **(Emel'yanov and Emel'yanov, 2009)**.

To obtain biologically active food additives and investigate their antioxidant and antimicrobial activity and mineral composition, we carried out the low-temperature fractioning of *Malus domestica Borkh (Common Antonovka)* fruits.

MATERIAL AND METHODOLOGY

Samples of *Malus domestica Borkh* (*Common Antonovka*) fruits were selected in Orel district, Orel Region of the Russian Federation in 2010 – 2012.

Preliminarily prepared raw materials, which are *Malus domestica Borkh (Common Antonovka)* fruits, are physically separated into juice of direct extraction and pomace of pulp. We evaporated juice under vacuum at temperature t = 30 – 50 °C to obtain a concentrate. Input power and speed of moisture removal, normalized to mass unit of evaporated fluid, respectively, accounted for $N/G_0 \approx 270$ W/kg and 60% h^{-1} **(Emel'yanov, 2009)**. The concentrate and pulp pomace are dried at atmospheric pressure and temperatures up to 50 °C to obtain dehydrated juice and dried pomace. After evaporation under vacuum, the concentrate took a paste form. As a result of convective drying concentrate, we have rised viscosity to a value that allows extruding product. We dried the granular juice and finally pulverized it to powder. Figure 1 presents photos of products of the low-temperature fractionation of *Malus domestica Borkh (Common Antonovka)* fruits.

To study the antimicrobial activity of products of the low-temperature fractioning of *Malus domestica Borkh (Common Antonovka)* fruits, we used the strains of microorganisms *Bacillus subtilis VKM B – 501*, *Micrococcus luteus VKM - As – 2230, Aspergillus flavus VKM F – 1024, Penicillium expansion BKM-F-275, Mucor mucedo BKM-F-1257*, and *Rhizopus stolonifer BKM-F-2005*. The sensitivity of test cultures to the action of the pomace powder and the juice concentrate is studied with assistance of diffusion in agar using wells in agar medium. Test organisms served as 18-20 hour cultures of microorganisms grown on stubble MPA. Suspension of microbes was added at the rate of 106 microbial bodies per 1 cm^3 of the nutrient medium. We used agaritine dense nutrient medium, which are MPA (for spore-forming bacteria and micrococci) and wort-agar (for fungi), as a nutrient media.

Antioxidant activity of processing products of *Malus domestica Borkh (Common Antonovka)* fruits is determined by spectrophotometric method in an alcoholic extract described in **(Silva et al., 2005)** based on percentage of inhibition of DPPH radical (2,2-diphenyl-1-picrylhydrazyl). We determined the optical density of solutions in the interaction DFPG with extractive substances of plants by spectrophotometer "Specord M40" at a wavelength of 515 nm.

Bioflavonoids amount is determined. by spectrophotometer analysis of complexes with aluminum chloride. Rutin **(Lobanov et al., 2004)** served as a standard.

Determination of trace is performed after dry digestion in a muffle furnace at 450 °C and dissolving the ash in the mixture of 10% hydrochloric acid and nitric acid by atomic absorption spectrophotometry and the air-acetylene

Figure 1 Concentrated juice from apple pulp as a plate form and pulp pomace powder.

flame device firm HITACHI 180-80 with deuterium background corrector. We used standard solutions of elements of the company «Merk» for calibration. Determination of the qualitative composition of extracts is performed by HPLC method on Milichrom UV-5 device equipped wit a computer processing system "Multichrom". Reversed-phase HPLC, chromatographic column Separon C-18.5, eluent - acetonitrile solution in an aqueous buffer at pH 3 – 7 with a volume ratio 0.02 and KH_2PO_4: acetonitrile solution – 85:15 were used for analysis. The detection is performed at wavelengths of 252 nm.

RESULTS AND DISCUSSION

It is known that fresh apples have high antioxidant and anticancer activity **(Eberhard et al., 2000).** It is found that the exact combination of biochemical substances in apple fruits is responsible for health benefits. Consumption of apples can play a significant role in reducing the risk of chronic diseases such as cancer. The main and most active natural antioxidants have a phenolic nature. This is about natural polyphenols, different types of flavonoids, phenolic hydroxyl acids, and vitamins. Table 1 shows the results of determination of antioxidant activity and total amount of bioflavonoids in extracts of processed products made of *Malus domestica Borkh* (*Common Antonovka*) fruits.

These data suggest that the processed products obtained from *Malus domestica Borkh (Common Antonovka)* fruits, which are the pomace powder and the juice concentrate, have a great value for food processing industry as a result of high antioxidant activity. The pomace powder has elevated bioflavonoids content, since this group of compounds is more abundant in the cell walls of plants. However, the concentrated juice can be used as additive in food technologies due to high percentage of DPPH inhibition. Sodium and potassium are presented by highly mobile ionic forms in the cells of biological systems. Therefore, these elements prevail in the concentrated juice. Iron, manganese, copper, and zinc that are biogenic trace elements or components of enzyme systems are evenly distributed in plant cell walls and protoplasm. Investigation of the mineral composition of the low-temperature fractioning products showed that they can be used for a functional food due to high content of magnesium and iron. The chromatographic analysis of the pomace powder and the concentrated juice extracts is performed.

To determine qualitative composition of processed products obtained from *Malus domestica Borkh (Common Antonovka)* fruits, we analyzed the differences between three groups of biologically active substances, which are organic acids, phenol carbonic acids, and flavonoids. Groups of biologically active agents are identified by their

Table 1 Antioxidant activity and content of bioflavonoids in the extracts of processed products made of *Malus domestica Borkh* (*Common Antonovka*) fruits.

Parameter	Pomace powder	Concentrated juice
% of DPPH inhibition	71.45 ±0.25	57.31 ±0.20
Bioflavonoids sum, %	2.19 ±0.05	1.24 ±0.08
	Mineral elements content, mg.100g^{-1}	
potassium	132.00 ±8.00	178.00 ±3.00
calcium	17.00 ±0.45	12.00 ±0.35
magnesium	114.00 ±3.00	67.00 ±2.00
sodium	11.00 ±0.30	19.00 ±0.20
phosphorus	13.00 ±0.10	7.00 ±0.05
ferrum	2.00 ±0.02	3.50 ±0.02
manganese	0.49 ±0.01	0.68 ±0.01
cuprum	0.14 ±0.01	0.30 ±0.01
zinc	0.24 ±0.01	0.35 ±0.01

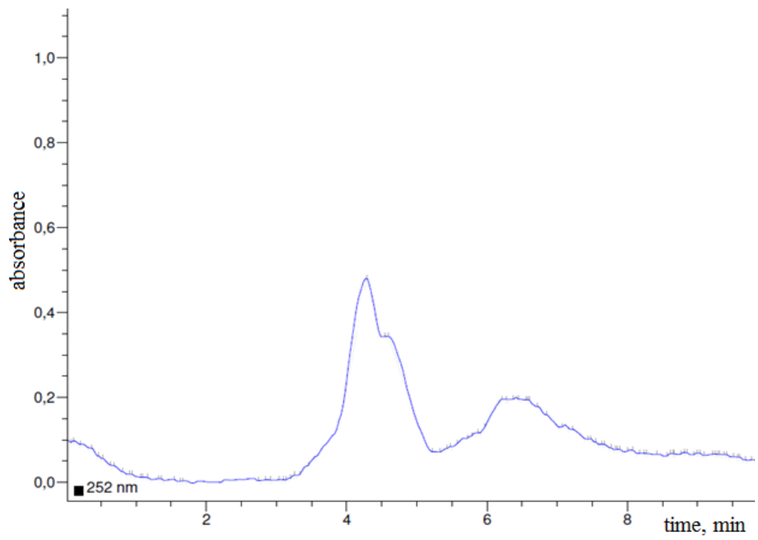

Figure 2 Chromatogram of the pomace powder of *Malus domestica Borkh* (*Common Antonovka*) fruits.

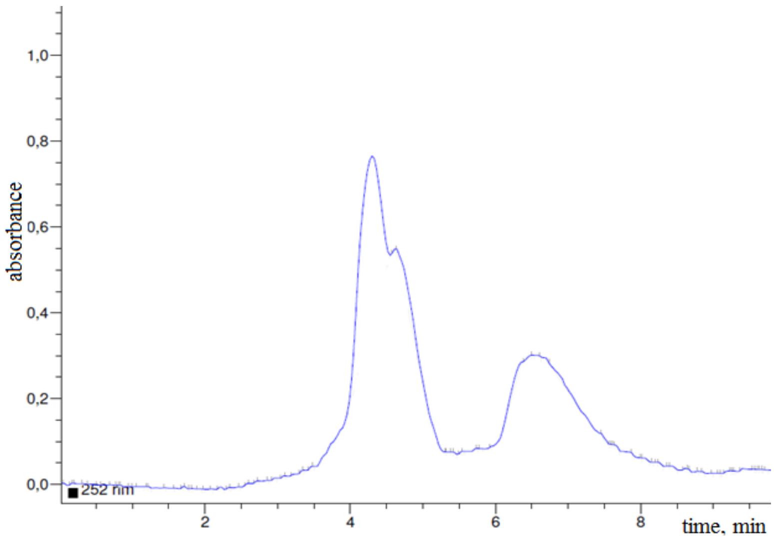

Figure 3 Chromatogram of the concentrated juice extract of *Malus domestica Borkh* (*Common Antonovka*) fruits.

Table 2 Antimicrobial activity of the products of the low-temperature fractioning of *Malus domestica Borkh* (*Common Antonovka*) fruits.

The type of microorganism	The diameters of zones of inhibition of growth of test cultures of microorganisms mm
Bacillus subtilis BKM-B-501	11.7 ±0.6
Micrococcus luteus BKM-As-2230	5.8 ±0.6
Aspergillus flavus BKM-F-1024	14.1 ±0.6
Penicillium expansion BKM-F-275	11.6 ±0.6
Mucor mucedo BKM-F-1257	9.8 ±0.6
Rhizopus stolonifer BKM- F-2005	8.2 ±0.6

retention time on the chromatograms presented in the form of peaks. The retention times from 2 to 5 minutes correspond to organic and phenol carbonic acids. The times from 5 to 8 minutes correspond to bioflavonoids.

Figures 2 and 3 show the chromatograms of the pomace powder and the concentrated juice extract obtained from *Malus domestica Borkh (Common Antonovka)* fruits. The chromatograms confirm the results of content determination of organic acids and bioflavonoids. Analysis of chromatograms shows the contents identical compounds in the products of the low-temperature fractionation. Both processed products contain organic and phenol carbonic acids in its composition. The compounds that are experimentally discovered have antiseptic and antioxidant activity.

The results of the antimicrobial activity study of the products obtained from *Malus domestica Borkh* (*Common Antonovka*) fruits with respect to the test cultures of microorganisms *Bacillus subtilis VKM-B-501, Micrococcus luteus VKM-As-2230, Aspergillus flavus VKM-F-1024, Penicillium expansion VKM-F-275, Mucor mucedo VKM-F-1257, Rhizopus stolonifer VKM- F-2005* are presented in Table 2.

Data show that the products of the low-temperature fractioning of *Malus domestica Borkh* (*Common Antonovka*) fruits inhibit the microorganism's growth. It is found experimentally that the zone diameters of growth inhibition of test cultures vary from 5.8 to 14.1 mm. The tested strains of microorganisms are the most common causative agents of food spoilage. The experimental results indicate that products of the low-temperature fractioning of *Malus domestica Borkh (Common Antonovka)* fruits have antiseptic properties. These points to the prospects of using the powder from pomace and the fruit juice concentrate of *Malus domestica Borkh (Common Antonovka)* in food technology as natural food additives.

CONCLUSION

During the experiment, it was found that the products of the low-temperature fractioning of *Malus domestica Borkh (Common Antonovka)* fruits have a high antioxidant activity. Determination of the amount of flavonoids and qualitative composition of the extracts from products of the low-temperature fractioning of *Malus domestica Borkh (Common Antonovka)* fruits showed the presence of identical compounds in both fractions. Study of the mineral composition of the obtained products showed that the majority of the chemical elements predominate in the concentrated juice. The pomace powder contains most calcium, magnesium and phosphorus. Processing products of apples have antimicrobial activity against the standard strains of food spoilage. Antioxidant and antimicrobial activity and magnesium and iron content are quite high in the highlighted fractions. That reflects the prospects of using the obtained natural additives in food technologies in order to maintain and increase the shelf life as well as for use in functional and preventive nutrition.

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