CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF CORN HYBRIDS GRAIN OF DIFFERENT PIGMENTATION

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Received September 13, 2016; Accepted in revised form November 10, 2016; Published December 30, 2016

Abstract: The article presents the results of studies of the chemical composition and antioxidant activity of corn hybrids grain of different pigmentation, created in All Russian Research Scientific Institute of Corn (Pyatigorsk). We included kernels of yellow corn as control samples (Uralskiy 150 hybrid). Test kernel samples were differently pigmented: kernels of white color (White), of orange color with yellow tops (Orange), of brown color with yellow tops (Brown), with grey tops and light yellow sides (Grey), of purplish-red color (Rubin). Botanical studies of differently-colored corn kernels revealed that the kernel color depends on the outer layer (pericarp) pigmentation. It was shown that corn kernels Orange, Rubin, and Grey contained three groups of biologically active compounds: flavonoids: 80, 70, 73 mg/%, carotenoids: 2.40, 1.70, 1.60 mg/%, and anthocyanins: 30, 120, 30 mg/%, respectively. Corn kernel samples Orange and Rubin demonstrated high antioxidant activity (1.0 and 0.76 mg/l in gallic acid equivalents, respectively). Antioxidant activity of common yellow corn (Uralskiy 150 hybrid) amounted to 0.09 mg/l in gallic acid equivalent, which is about ten times less than in the test samples. Thus, presence of biologically active substances – carotenoids, flavonoids, and anthocyanins – in differently-pigmented corn kernels is correlated with high antioxidant activity. Our results suggest that corn kernels Orange and Rubin might be recommended for further use in food industry producing products with high content of biologically active compounds.

Keywords: grain, corn, chemical composition, anthocyanins, flavonoids, antioxidant activity

DOI: 10.21179/2308-4057-2016-2-85-91 *Foods and Raw Materials*, 2016, vol. 4, no. 2, pp. 85–91.

INTRODUCTION

Search for new types of plant-based raw materials containing biologically active substances contributes to diversification of feedstock used for healthy foods production, which corresponds to the basic provisions of the Strategy for Improving Food Products Quality in the Russian Federation until 2030. In order to achieve the Strategy goals, we should prioritize research on dietary profiles among the population, including prophylaxis of most common non infectious diseases, and development of food processing technologies that target food products quality improvement and promotion of healthy dietary habits [1].

In recent years, research in antioxidant activity of different plant-based raw materials and processed products seems to be particularly relevant. Findings show that antioxidant and antiradical properties of the products under investigations result from bioflavonoids these contain: flavonoids, isoflavonoids, anthocyanins, catechins, and other phenolic compounds. Low dietary antioxidants intake leads to free radical pathology, which is a concomitant condition of various diseases. Bioflavonoids, when included into the diet in physiological concentrations, demonstrate anti-allergic and adaptogenic properties, which enhances body's resistance to common illnesses [2, 3, 4].

Along with main nutrient elements synthesized during the corn growth, we can distinguish a separate class of biologically active substances that impart certain color to corn kernels. Xanthophylls (lutein and zeaxanthin) are yellow pigments that, when accumulated, make the corn kernels yellow, and anthocyanins provide different coloring, from red to purple.

Anthocyanins have exceptional antioxidant properties: they can scavenge free radicals and protect cells against membrane damage. They are also known to strengthen capillary walls and to reduce edema naturally. Anthocyanins inhibit lipid peroxidation, thus protecting them against oxidative stress, which, in turn, contributes to cardiovascular prophylaxis. Health-promoting properties of anthocyanins are used in pharmaceutical industry, for producing various biologically active food supplements mostly used in ophthalmology. Anthocyanins are easily accumulated in retinal tissues, at the same time, they strengthen blood vessels and reduce capillary permeability and fragility within the retina. Anthocyanins improve fibers and cells structure in connective tissue, restore outflow of intraocular fluid and reduce intraocular pressure, which facilitates glaucoma treatment [2, 5].

A number of studies have been performed on specificity of anthocyanins accumulation in different parts of corn plant [6, 7], and on effects of anthocyanin corn extracts onto physiological functions of body systems [8].

However, complex research of locally-bred corn hybrids with differently pigmented kernels has not received sufficient attention in Russia.

Thus, a study of chemical composition and antioxidant activity of biologically active substances in differently-pigmented corn kernels shall determine types of corn of major biological value.

OBJECTS AND METHODS OF STUDY

Studies were conducted in the Corn Quality and Processing laboratory of the All-Union Scientific Research Institute of Corn, and in the scientific research laboratory for Nanobiotechnologies and Biophysics at the public access center within the North-Caucasus Federal University.

Sampling materials were differently-pigmented hybrid corn kernels developed in the All-Union Scientific Research Institute of Corn. A sample of yellow corn (hybrid Uralskiy 150) was used as control. Test kernel samples were differently pigmented: kernels of white color (White), of orange color with yellow tops (Orange), of brown color with yellow tops (Brown), with grey tops and light yellow sides (Grey), of purplish-red color (Rubin).

Feedstock materials were planted on testing fields of the All-Union Scientific Research Institute of Corn, in Predgorny district of Stavropol Krai in 2014–2015. Corn hybrids were seeded on 2-row-plots ($S=7.8\ m^2$, with planting density of 4–5 plants per $1m^2$), within the optimal seeding time, from April, 26 to May, 6.

Soils of the testing field were represented by common chernozem, thick, with heavy loam texture. Topsoil layer contained 55.96% of physical clay, with prevailing slit fraction (31.00 %), fine sand (21.69%), coarse slit (21.32%). As for humus content, these soils are low-humic.

Agricultural technology included plowing (to the depth of 23–25 cm), spring-tooth harrowing (BZT-1.0), tilling (KPS-4), marking (SUPN-8), two inter-row cultivations (KRN-5,6). Soil-applied herbicide Merline (150 g/ha) was introduced under preplanting tilling. Plant nutrition was administered at the phase of 8–10 leaves: Humistim (2.0 l/ha) + Nibid (0.4 g/ha) + Megamix (0.2 l/ha) (spraying device: MTZ-X2 – OP-200). Harvesting was performed manually, at firm ripe stage.

Kernels were soaked and sectioned manually with blades, then colored with an alcoholic phloroglucinol solution and sulphuric acid solution (50%). Temporary slides were mounted in glycerine solution and observed under a Biolam microscope (zoom x4; x10; x40). Sections were photographed with a digital camera SONY CS 5.1.

In hybrid corn kernels, protein, starch, and fats content was determined by infrared spectroscopy with InfraLUM FT-12 analyzer.

Elemental analysis of minerals was performed by MPU 4 – C method: semi-quantitative spectroscopy for minerals derived from carbon electrode crater of AC plasma arc (DG-2). Mineral spectrum was obtained with a spectrophotograph ISP-28.

In order to determine flavonoids content in hybrid corn kernels, we used ethanol (95%) as extracting agent. For quantitative analysis, we used differential spectrophotometry method based on flavonoid-aluminum chloride complexation, which allows to exclude any influence coming from related compounds.

In order to determine carotenoids content in hybrid corn kernels, we used ethanol (95%) as extracting agent. Optical density of the solution was measured by a spectrophotometer at 440 ± 5 nm wavelength, in a 10mm cuvette cell. Total carotenoids content (mg%) was evaluated in the amount of β–carotene.

In order to determine anthocyanins content in hybrid corn kernels, we used ethanol (50%) with hydrochloric acid (1%). Optical density of the permeate was measured by a spectrophotometer at 510 ± 5 nm wavelength, in a 10 mm cuvette cell. Total anthocyanins content was evaluated in the amount of cyanidin-3,5-diglucoside.

Antioxidants content was quantified in the "Cvet Jauza-01-AA" measuring instrument plotting the output to a gallic acid calibration curve.

An amperometric method for total antioxidants content estimation in plant-based raw materials was developed by Khimavtomatika SPA JSC and certified by the All-Russian Research Institute of Metrological Service. A stock solution of gallic acid (100 mg/l) was prepared. 1 ml of stock solution was poured into a volumetric flask, making up the volume to 10 ml with distilled water. The diluted solution was further mixed. The solution was prepared immediately prior to calibration. Preparing the calibrating solutions of gallic acid (0.2, 0.5, 1.0, 2.0, 4.0 mg/l). 20, 50, 100, 200, 400 μl of gallic acid solution (100 mg/l) were introduced into 10 ml volumetric flasks, making up the volume with distilled water, and further mixed.

Mobile phase (eluent) used was orthophosphoric acid solution (0.0022 mol/l). 150 µl of concentrated orthophosphoric acid were added to approximately 700 ml of distilled water in a 1 l volumetric flask. The volume was made up with distilled water and stirred.

Tested solution was introduced into the instrument, and electrical currents produced on the electrode were transduced into digital output displayed as peaks on the screen.

RESULTS AND DISCUSSION

Hybrid Uralskiy 150 is an early-season hybrid belonging to the normal corn variety (*indentata sturt.*). Kernels are small and flat, elongated in shape, with pronounced facets. There is an indentation on top of the kernel, the surface is smooth and shiny (Fig. 1a). Horny endosperm is only present on the lateral sides, while floury endosperm develops in the center and in the top of the kernel, beneath the indentation. Kernels are marigold yellow.

Seed coat consists of several layers of heavily deformed cells elongated in tangential direction. Aleurone layer is located just beneath the seed coat and consists of one layer of thick-walled cells, rounded-square in shape. The main part of the seed, endosperm, is located under the aleurone layer. Endosperm is made up of thin-walled cells with 4 to 6 straight facets; these cells are filled with starch. Endosperm cells nearest to the layer are smaller than other cells inside the seed (Fig. 2a).

White corn belongs to the normal corn variety (*indentata sturt.*). Kernels are large and flat, elongated in shape, with pronounced facets. There is an indentation on top of the kernel, the surface is smooth and lustrous (Fig. 1b). Kernels are white.

Other test samples (Orange, Broun, Grey, Rubin) belong to the flint corn variety (*Indurata sturt.*). Kernels are smallish, rounded and elongated in shape, compressed, both dorsal and ventral side concave, rounded tops, surface flat, lustrous (Fig. 1c–f). Horny endosperm is scarcely present only on the lateral sides of the kernel, while floury endosperm is present in the center and in the top. Seed coat consists of several layers of cells elongated in tangential direction, arranged in neat rows. Cross section reveals three distinct layers within the pericarp: these are exocarp, mesocarp, and endocarp. Exocarp is a single layer of cells elongated in tangential direction, with thickened walls. Mesocarp consists of 2–3 layers of parenchyma cells with thickened walls, elongated in tangential direction, and arranged in neat rows. Endocarp is made up of thick-walled cells elongated in tangential direction fused into the seed coat. Cell walls constituting the hull are heavily pigmented from dark orange to raspberry brown (Fig. 2 c–f).

Botanical studies of differently-pigmented corn seeds have revealed differences in internal organization of the seed and in the hull coloring. Color of a corn kernel depends on pigmentation of the outer layer (pericarp), while aleurone layer and endosperm are not relevant for this parameter [9]. Corn color might be modified by crossing with some differently-pigmented feedstock. However, pericarp being the maternal tissue of the seed, pollen of the paternal variety from the year of crossing does not participate in its formation. Dominance of paternal traits in kernel pigmentation depends on amount and composition of biochemical compounds introduced by the pollen.

In corn kernels, protein, starch, and fats content was determined by infrared spectroscopy with “InfraLUM FT-12” analyzer. Findings of the analysis are presented in Fig. 3.

Fig. 1. Differently-pigmented corn kernels: (a) Uralskiy 150; (b) White; (c) Orange; (d) Brown; (e) Grey; (f) Rubin.

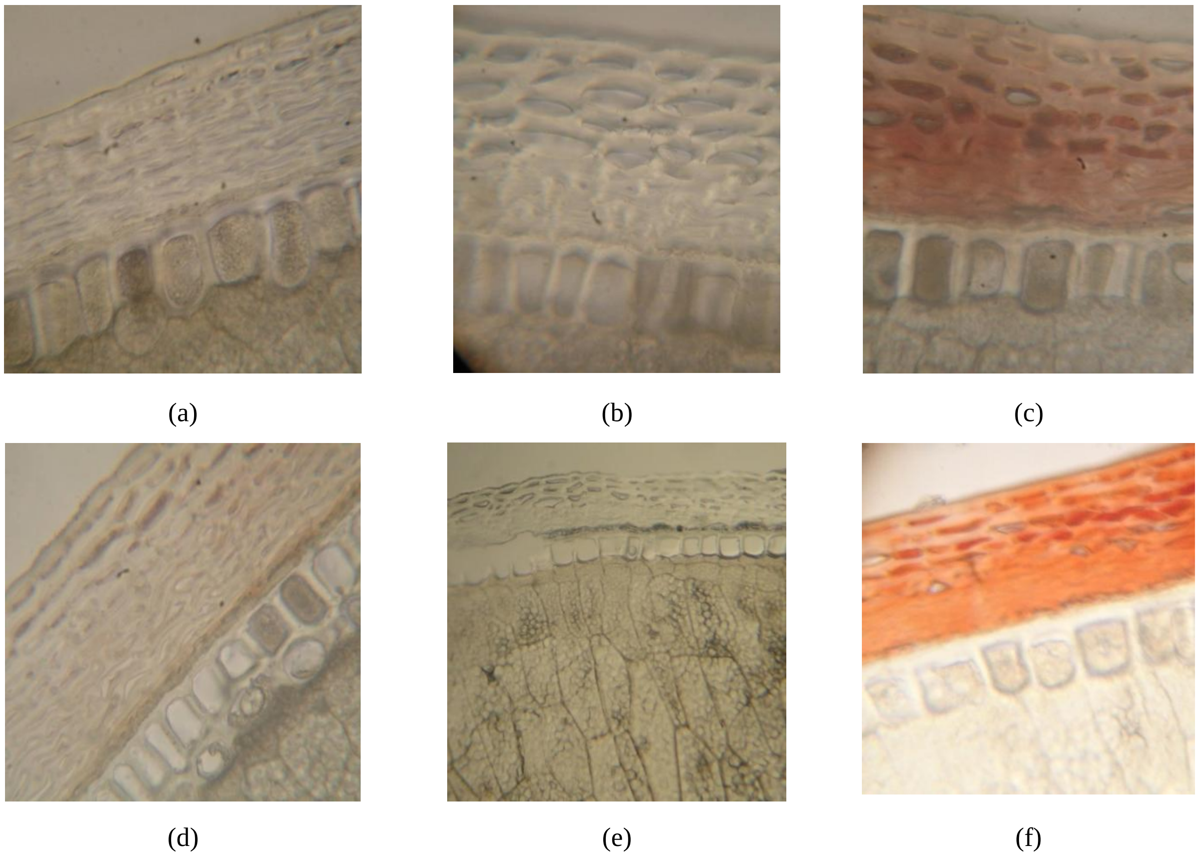

Fig. 2. Section view of differently-pigmented corn kernels: (a) Uralskiy; (b) 150 White; (c) Orange; (d) Brown; (e) Grey; (f) Rubin.

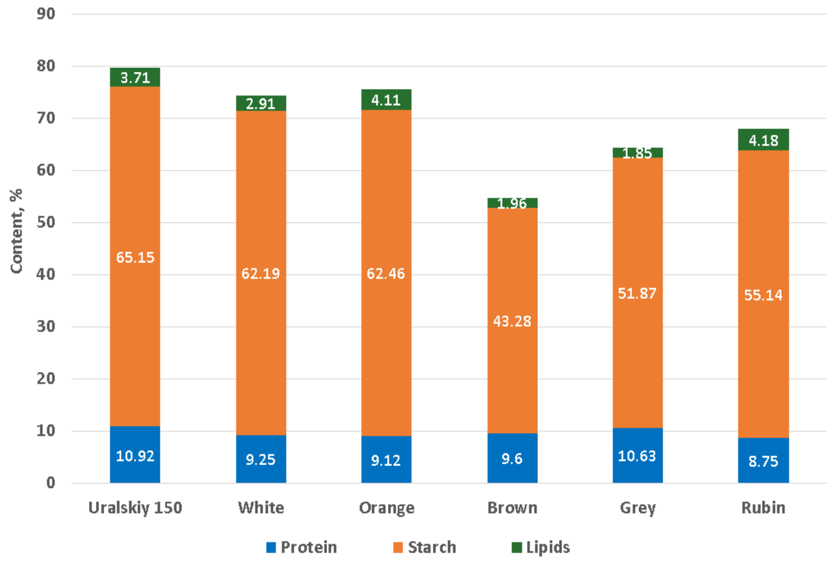

Fig. 3. Chemical composition of differently-pigmented corn kernels

The data provided in Fig. 3 indicate that protein content in corn kernels varied from 8.75% to 10.92%. Maximum protein content (10.92%) was found in Uralskiy 150 corn sample. High protein content (10.63%) was determined in Grey corn kernels.

In corn kernels, main substance is starch, its average content amounting up to 60–75%. Maximum starch content (65.15%) was found in Uralskiy 150 corn kernels. White and Orange corn samples contained approximately 62% of starch. Minimum starch content (43.28%) was found in Brown corn kernels.

Increased fat content in corn kernels is particularly relevant for corn oil extraction, and also for kernels used as fodder. Among the test samples, increased fat content was observed in Rubin and Orange corn kernels: 4.18% and 4.11%, respectively. Grey and Brown corn contained under 2% of fat.

Minerals amount to 2–5% of dry solids content within a kernel. These form ash particles during sample combustion. Table 1 presents minerals content in hybrid corn kernels.

Table 1. Minerals content in corn kernels, mg/100 g

Sample name	Copper	Zinc	Lead	Phosphorus	Manganese	Titanium	Iron	Potassium	Sodium	Calcium	Magnesium	Aluminum	Silicon
Uralskiy 150	2.45	7.8	0.52	32.4	9.5	2.60	3.90	73	54.2	59	186	11.2	47
White	1.22	9.2	0.48	52.3	12.3	3.20	1.90	92	38.9	63	162	9.4	63
Orange	0.84	8.4	0.54	42.0	8.4	4.20	4.20	84	50.4	84	168	25.2	84
Brown	0.85	8.5	0.51	33.6	8.5	4.25	2.55	85	42.5	85	170	8.5	51
Grey	3.15	15.7	0.63	52.5	31.5	5.25	5.25	128	53.5	105	210	10.5	105
Rubin	5.10	51.0	0.44	168.0	25.2	8.40	16.80	240	84.0	168	252	25.2	168

As per the data presented in Table 1, Rubin corn kernels contain maximum amount of: phosphorus – 168 mg/100 g; iron – 16.8 mg/100 g; potassium – 840 mg/100 g; sodium – 84 mg/100 g; calcium – 168 mg/100 r; magnesium – 252 mg/100 r; zinc – 51 mg/100 g; silicon – 168 mg/100 g.

Macro- and microelements found in Grey corn kernels were as follows: phosphorus – 52.5 mg/100 g; iron – 5.25 mg/100 g; potassium – 128 mg/100 g; sodium – 53.5 mg/100 g; calcium – 105 mg/100 g; magnesium – 210 mg/100 g; copper – 3.15 mg/100 g; zinc – 15.7 mg/100 g.

Other samples had lower minerals content, therefore, Grey and Rubin corn might be recommended as a source of macro- and microelements for enhancing mineral value of food products.

Flavonoids contain anthocyanins, a group of plant pigments responsible for hues of vegetative tissues. Anthocyanins are colored crystals readily soluble in water and other polars. Free anthocyanins and anthocyanidins bases are colored in purple. Red, blue, and purple hue of some vegetative tissues might come from the same anthocyanidin type involved in different cell sap reactions. In blue parts of a plant, it might be present as potassium or other alkali salt, in red ones, as oxonium salts of an organic acid, in purple ones, it is mostly a pigment base [10].

Quantities of biologically active substances in corn kernels were determined. Findings are presented in Table 3.

As per the data provided in Table 2, Orange, Rubin, and Grey corn kernels contain all three groups of biologically active compounds: flavonoids: 80, 70, 73 mg/%, carotenoids: 2.40, 1.70, 1.60 mg/%, and anthocyanins: 30, 120, 30 mg/%, respectively. Maximum concentration of flavonoids was registered in Orange corn sample: 80 mg/%, while Brown and Orange corn kernels were characterized by high content of carotenoids (2.40 mg/%), which is 2.2 times higher than the respective value in conventional yellow corn varieties. Maximum anthocyanins content was found in Rubin corn kernels: 120 mg/%.

Use of Orange and Rubin corn kernels in food industry seems promising for producing food products enriched with biologically active substances.

Recent years have witnessed a growing interest in natural antioxidants and their application in food industry. Apart from maximizing shelf life of foods, they also have antioxidant properties and boost human immune system. Considerable advances have been made in structural research of many complex natural compounds of plant origin [3, 6]. Phenolic compounds-flavonoids, anthocyanins, carotenoids, occurring widely in plants, and in corn kernels as well, have received much attention.

Express integral assessment of their quality should be based on antioxidant activity value reflecting content and impact of any organic reducing agent present within the test item.

Antioxidant activity of biologically active substances in corn kernels was quantified by amperometric assay in the "Cvet Jauza 01-AA" measuring instrument (Fig. 4).

Table 2. Biologically active substances content in corn kernels, mg/%

Sample name	Contained in a corn grain		
	flavonoids	carotenoids	anthocyanin
Uralskiy 150	76	0.90	–
White	67	–	–
Orange	80	2.40	30
Brown	–	2.40	–
Grey	73	1.60	30
Rubin	70	1.70	120

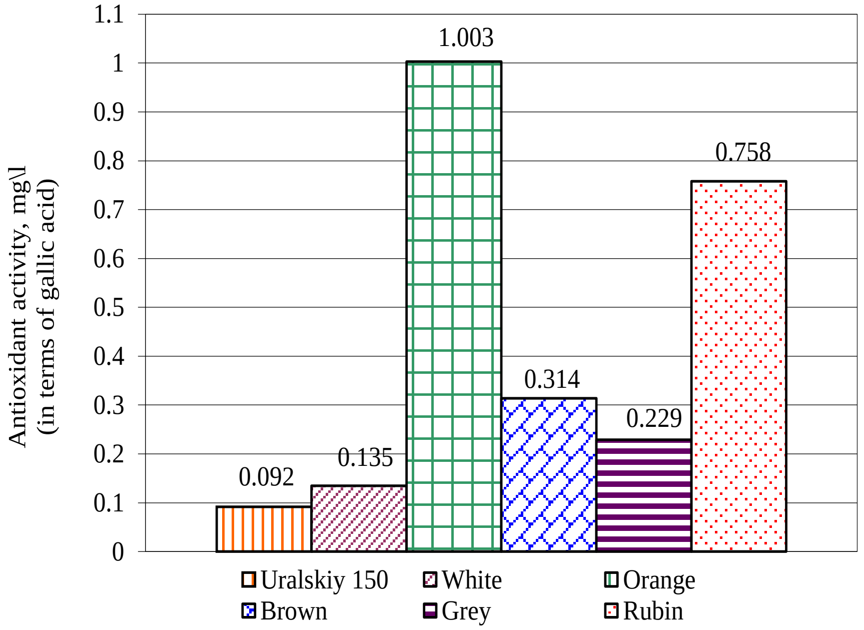

Fig. 4. Antioxidant activity of differently-pigmented corn kernels

According to the data presented in Fig. 4, high antioxidant activity (1.0 and 0.76 mg/l in gallic acid equivalents) was found in Orange and Rubin corn kernels, respectively. It might be explained by presence of all three groups of biologically active substances in these corn samples: carotenoids, flavonoids, and anthocyanins. Antioxidant activity of common yellow corn (Uralskiy 150 hybrid) amounted to 0.09 mg/l in gallic acid equivalent, which is about ten times less than in the test samples. Antioxidant activity of Grey and Brown corn kernels amounted to 0.23 and 0.31 mg/l in gallic acid equivalent, which exceeds control sample data over two and three times, respectively.

Thus, this complex study of chemical composition and antioxidant activity in differently-pigmented hybrid corn kernels suggested options for their differentiated targeted use in food industry. We showed that protein content in Grey hybrid corn kernels is similar to that of the hybrid yellow corn Uralskiy 150, conventionally used in food processing, which allows to diversify feedstock base for food processing industry. Starch content values deserve particular attention, as corn kernels represent a top priority feedstock in starchy foods products. Our research suggests that White and Orange hybrid corn kernels might be recommended for these specific technological purposes. Orange and Rubin hybrid corn kernels are particularly suitable for corn oil extraction. Grey and Rubin hybrid corn kernels can be used as a relevant source of macro- and microelements in developing food products with enhanced nutritional value. We observed increased antioxidant activity of tested hybrid corn samples in comparison to the control sample of hybrid corn Uralskiy 150. Findings showed certain groups of biologically active substances: flavonoids, anthocyanins, and carotenoids. Maximum flavonoids content was registered in Orange hybrid corn kernels (80 mg/%), maximum anthocyanins, in Rubin hybrid corn (120 mg/%). High antioxidant activity of the above hybrid corn kernels suggests their processed derivatives might be used in dietary prophylaxis as promising sources of biologically active substances. In conclusion, differently-pigmented hybrid corn cells are a valuable plant feedstock for food industry. Processed foods derived from differently-pigmented hybrid corn kernels shall amplify significantly the scope of grain feedstock application and shall contribute to the growth of domestic foods product assortment, which is particularly relevant under current economic circumstances.

ACKNOWLEDGEMENTS

This research and resulting article were prepared under grant MD – 4862.2016.11.

REFERENCES

1. *Postanovlenie Pravitel'stva RF № 1364-r «Strategiya povysheniya kachestva pishchevoy produktsii v Rossiyskoy Federatsii do 2030 goda» ot 29.06.2016* [RF Government Decree no. 1364-r Strategy for Improving Food Products Quality in the Russian Federation until 2030. dated 29.06.2016].
2. Makarova M.N., Makarov V.G., and Liflyandskiy V.G. Flavonoids and Cardiovascular Diseases. *Issues of Healthy and Diet Nutrition*, 2011, no. 2, pp. 53–60. (In Russian).
3. Hellström J.K., Shikov A.N., Makarova M.N., et al. Blood pressure-lowering properties of Chokeberry (*Aronia mitchurinii*, var. Viking). *Journal of Functional Foods*, 2010, vol. 2, iss. 2, pp. 163–169. DOI: http://dx.doi.org/10.1016/j.jff.2010.04.004.

4. Kurkin V.A., Kurkina A.V., and Avdeeva E.V. Flavonoids as Biologically Active Compounds in Medicinal Plants. *Fundamental Research*, 2013, no. 11-9, pp. 1897–1901. (In Russian).
5. Pisarev D.I., Novikov O.O., Selyutin O.A., and Pisareva N.A. Biological Activity of Polyphenols of Plant Origin. Prospect of Medical Practice of Anthocyanins. *Belgorod State University Scientific Bulletin. Medicine Pharmacy*, 2012, no. 10 (129), pp. 17–24. (In Russian).
6. Tretyakov M.Y., Khoroshilov S.A., Sidorov A.N., et al. Corn as a Source of Anthocyanin. *Achievements of Science and Technology of AIC*, 2012, no. 9, pp. 30–32. (In Russian).
7. Ji H.C., Lee H.B., and Yamakawa T. Major agricultural characteristics and antioxidants analysis of the new developed colored waxy corn hybrids. *Journal Faculty of Agriculture Kyushu University,* 2010, vol. 55, no. 1, pp. 55–59.
8. Navolokin N.A., Polukonova N.V., Maslyakova G.N., et al. Internal Organs Morphology and Tumors in Laboratory Rats with Transplanted Liver Cancer PC-1 by Intoduction Containing Extract of Gratiola (*Gratiola Officinalis* L.) and Anthocyan Maize (*Zea Mays* L.). *Saratov Journal of Medical Scientific Research*, 2013, vol. 9, no. 2, pp. 213–220. (In Russian).
9. Gorshkova T.A. *Rastitel'naya kletochnaya stenka kak dinamichnaya sistema* [Plant Cell Wall as a Dynamic System]. Moscow: Nauka Publ., 2007. 425 p.
10. Murav'eva D.A. and Yakovlev G.P. *Farmakognoziya* [Pharmacognosy]. Moscow: GEOTAR-MEDIA Publ., 2016. 976 p.

Please cite this article in press as: Zhirkova E.V., Skorokhodova M.V., Martirosyan V.V., Sotchenko E.F., Malkina V.D., and Shatalova T.A. Chemical composition and antioxidant activity of corn hybrids grain of different pigmentation. *Foods and Raw Materials*, 2016, vol. 4, no. 2, pp. 85–91. DOI: 10.21179/2308-4057-2016-2-85-91.