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POSSIBILITIES OF ENRICHING FOOD PRODUCTS WITH ANTHOCYANINS BY USING NEW FORMS OF CEREALS

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Abstract: At present, there are a number of high-tech trends in the development of food raw materials in the world market, the future nutritional value and composition of which can be laid even at the stage of breeding a variety, as a result of production of which the raw materials will be obtained containing the specified components – vitamins, minerals and biologically active compounds. It is possible to refer to such kinds of food raw materials common wheat of a purple color which has a high content of anthocyanins. Despite the antioxidant capacity and the related health benefits, the studies on the use of raw materials with a high level of anthocyanins as an ingredient for food products are extremely rare. This article presents the results of the use of food products of new forms of cereals in production, namely, the isogenic wheat lines specially created for comparative studies at the Institute of Cytology and Genetics of the SB RAS with the help of molecular genetic methods, which only differ in a small segment of the genome containing the regulatory anthocyanin biosynthesis gene *Pp3/TaMyc1*. Flour and wheat bran with anthocyanins (PG) and the control (RG) group were used for the production of flour confectionery products in order to obtain products with a high level of anthocyanins beneficial for human health. The share of anthocyanins in the products obtained from PG wheat was 2.5–2.6 times higher than in the similar products obtained on the basis of the control line. The revealed differences between PG and RG in the end-use products testify to the resistance of anthocyanins to technological processing. It has been estimated that when eating 100 grams of biscuit made from flour with the addition of bran of purple wheat grain, the consumption of anthocyanins will be up to 0.83 mg. Thus, a high content of anthocyanins in PG allows to produce the enriched confectionery products with a high nutritional value.

Keywords: Enriched food products, bioflavonoids, anthocyanins, food market, cereals, flour confectionery, nutritional value

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INTRODUCTION

The main trends in the development of the industry in the field of healthy nutrition in Russia include the development of technologies for the production of qualitatively new common and special food products with the specified properties based on the new types of raw materials obtained using new technological methods. Scientific researches of A.A. Pokrovsky, V.A. Tutelyan, M.M. Gaparov, V.B. Spirichev and V.M. Poznyakovsky and other scientists in our country made a great contribution to solving the problems of the development and manufacturing of nutrient-rich food products.

The basis of the modern food production strategy is to find the new resources of essential food components, to use the non-traditional types of raw materials and create the new advanced technologies that increase the nutritional and biological value of a product and give it the desired properties and extend its shelf life. Food enrichment is an effective way to eliminate the deficit of essential nutrients in human nutrition (Pokrovsky, 2002) [1].

The creation of consumer good products with a high food and biological value requires the expansion and improvement of the raw materials base of the food industry. The modern molecular genetics offers some approaches that, when combined with traditional breeding methods, make it possible to accelerate the creation of new varieties. At present, there are a number of high-tech trends in the development of food raw materials in the world market, the future nutritional value and composition of which can be laid even at the stage of breeding a variety, as a result of production of which the raw materials will be obtained containing the specified components – vitamins, minerals and biologically active compounds. For example, it has been shown that it is possible to regulate the content of amylose in bread (to increase the proportion of resistant starch for a diet for celiac disease) or biologically active polyphenolic compounds (anthocyanins) by creating plants with the specified characteristics even at the stage of wheat selection (Schönhofen et al., 2017; Khlestkina et al., 2017) [2, 3].

Plants with the specified content of protein, carbohydrates, microelements, vitamins and the secondary metabolites useful for human health (for example, the polyphenolic compounds which are plenty in red grapes, tea, wine, pomegranate, blueberry and cranberry, but which can also be synthesized by some varieties of cereals) can be created by using the natural variability of wheat and other cereals.

This article describes the possibility of increasing the content of anthocyanins in flour confectionery production using a new type of raw materials – common wheat of a purple color, development of which can be essentially accelerated under control of diagnostic molecular genetic markers. The choice of such a format as flour confectionery products for the creation of fortified food products with a high content of anthocyanins was determined, first of all, by the global production.

Flour confectionery products are the largest segment of the confectionery market in terms of sales, and the biscuit market, in turn, constitutes a significant part of the group of flour confectionery products. In 2010–2015, the production of biscuit and other flour confectionery products for long-term storage in the Russian Federation, according to Rosstat [Russian Statistics Committee], showed a steady positive trend, its growth was 126.6% over the period (from 1,097 thousand tons to 1389 thousand tons). At the same time, it should be noted that there is a significant proportion of the products in this group that do not meet the principles of healthy nutrition and, therefore, the developments of such product formulations are needed that preserve the usual taste properties and have a high nutritional value.

STUDY OBJECTS AND METHODS

Plant material. Spring bread wheat near-isogenic lines (red-grained, RG (i:S29*Pp-A1Pp-D1pp3*^{P} with an uncoloured pericarp) and a purple-grained, PG (i:S29*Pp-A1Pp-D1Pp3*^{P})), differing in a small segment of 2A chromosome (between the markers *Xgwm339* and *Xgwm817*) containing the regulatory anthocyanin biosynthesis gene *Pp3/TaMyc1* (Tereshchenko et al., 2012; Shoeva et al., 2014; Gordeeva et al., 2015) [4, 5, 6] were used for accurate comparative estimation.

The RG and PG wheats were grown in the Novosibirsk region at the experimental site of the selection and genetic complex of the Institute of Cytology and Genetics of SB RAS (55^{o}02'N, 82^{o}56'E) in the summer of 2016.

Estimation of the technological properties of flour, bran and the quality of the finished products. In the development of products from wheat that synthesizes bioflavonoid pigments anthocyanins, the flour was used from the grain of the RG and PG lines, as well as the bran obtained by milling these samples of grain. For the purposes of a comparative analysis, wheat flour of the premium grade was used (GOST R 52189-2003).

To estimate the quality of raw materials, standard methods were used (GOST 27676-88; GOST 27839-2013): the humidity was determined using an accelerated method for drying in a drying cabinet, acidity – using the method for the acid-base titration of water-flour dough; whiteness – using a R3-TBMS-M whiteness meter; the falling number – using ChP-1; the mass fraction of crude gluten was obtained by washing with water followed by weighing; the quality of crude gluten was characterized by color, elastic properties (using IDK-3M) and its hydration ability; the gas-forming ability of flour – using the volumetric method using Yago-Ostrovsky's device.

To estimate the quality of the end-use products, their humidity and absorption ability were determined using standard methods (GOST 5900-14, GOST 10114-80). To obtain the objective estimation of organoleptic indicators, closed tasting estimation was performed, expressed in points according to a 30-point scale. Table 1 provides product variants.

The studied products were enriched by modifying the regulatory trade formulation of Ovsyanochka biscuit, namely by replacing and / or supplementing separate components with experimental raw materials with a high content of anthocyanins.

In the first two variants (1 and 2), the formulation replaced oatmeal with the bran from RG and PG flour. The third and fourth variant envisaged not only the addition of bran instead of oatmeal, but also the replacement of wheat bread flour with PG and RG flour. Thus, the fourth variant provided for the maximum use of raw materials with a high content of anthocyanins.

Table 1. Variants of products made and studied in the present paper

No.	Used flour and bran				
	Flour as per GOST R 52189–2003	Flour from the grain of the red grain strain	Flour from the grain of the purple grain strain	Bran from the grain of the red grain strain	Bran from the grain of the purple grain strain
1	+	–	–	+	–
2	+	–	–	–	+
3	–	+	–	+	–
4	–	–	+	–	+
5	–	–	–	+	–
6	–	–	–	–	+

Analysis of the content of anthocyanins and antioxidants in bran and finished products. The extracts were obtained by adding 10 ml of a 1% aqueous solution of HCl to 1 g of the ground sample, mixing and incubating for 1 hour at 37°C. The supernatant obtained by centrifugation for 15 min at 4°C at 5000 rpm was used for the analysis.

The anthocyanin content was estimated using a SmartSpec^{TM} Plus spectrophotometer (BioRad, www.bio-rad.com). The recalculation from OD530-700 into the mass concentration was carried out using the method described above (Abdel-Aal, Hucl, 1999) [7] using cyanidin-3-glucoside as a standard. The antioxidant activity was estimated using a Blizar antioxidant activity analyzer (Interlab, Russia) according to the manufacturer's instructions. Gallic acid (mg/l) was used as a reference substance.

The average of the three successive measurements was used for the analysis. The significance of differences between the samples, in terms of the studied parameters, was estimated using the Mann-Whitney test.

RESULTS AND DISCUSSION

Tables 2 and 3 present the results of the comprehensive analysis of the used flour and bran, respectively. The mass fraction of the crude gluten of flour from the grain of the red and purple grain strains was practically the same and very high, much higher than that of wheat flour of the premium grade. At the same time, the gluten was poor in quality and long in elasticity, but had a good hydration ability. On the basis of the "falling number" indicator, the low autolytic activity of flour from the grain of RG and PG strains was detected, which may be due to the low activity of its own enzymes and/or a starch state.

Four biscuit variants were made and analyzed in the course of trial baking: two variants with the replacement of oatmeal with bran from RG and PG flour, two variants with the addition of bran instead of oatmeal and the replacement of wheat bread flour with PG and RG flour (see Table 1).

The following parameters were used to estimate the quality of products: shape, surface, color, structure, a shape in the fracture, taste and water absorption (Table 4).

To obtain the objective estimation of organoleptic indicators, closed tasting estimation was performed, expressed in points according to a 30-point scale.

The wheat flour products with the addition of PG bran had the highest scores, according to the organoleptic estimation, due to the high estimate of taste and aroma, texture and consistency (Table 5). Biscuit from PG flour and bran was looser with a distinct porous structure. Water absorption is higher in the samples with the addition of PG bran.

Table 2. Quality parameters of bran

Name of the parameter, units	RG bran	VG bran
Humidity, %	15.7	15.6
Acidity, deg.	10.4	15.4

Table 3. Quality parameters of flour

Name of the parameter, units	Wheat flour, premium grade	RG flour	VG flour
Humidity, %	13.0	15.4	14.9
Acidity, deg.	4.2	5.0	5.6
Whiteness, conventional units	54	33	27
Falling-number, s	180	402	386
Mass fraction of crude gluten, %	33	48	47
Quality of crude gluten:			
Color	light	light	light-beige
Elasticity, cm	15	21	25
GDI, conventional units	85	109	102
Group	2 satisfacto-rily weak	3 unsatisfacto-rily weak	2 satisfacto-rily weak
Hydratation capacity, %	179	186	171

Note. GDI is a gluten deformation index.

Table 4. Quality parameters of products (numbers 1–4 of products are according Table 1)

Parameter	1	2	3	4
Shape	Round			
Surface	Rough with interspersions			
Color	Gold brown	Brown	Gold brown	Brown
Structure	Loose	Loose with a porous structure	Loose	Loose with a porous structure
Shape in the fracture	Loosened	More loosened	Loosened	More loosened
Taste	Natural, without a foreign taste	Distinct slightly nutty	Distinct	Distinct, slightly nutty
Smell	Natural, without a foreign taste	Natural, without a foreign taste	Distinct	Distinct
Water absorption, %	192.0	209.0	180.0	211.0

Table 5. Quality parameters of organoleptic indices (numbers 1–4 of products are according Table 1)

Parameter	1	2	3	4
Average score	22.5	23.2	20.2	21.0

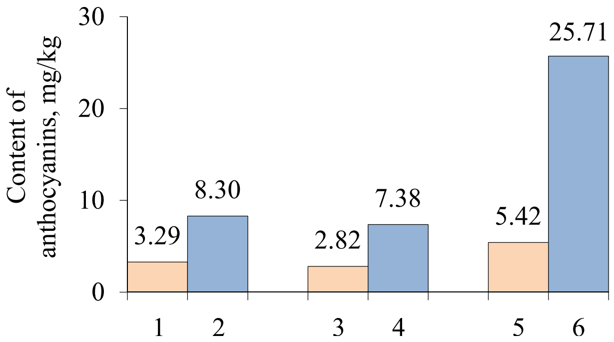

Fig. 1. Content of anthocyanins (mg/kg) in bran and the finished products in terms of cyanidin-3-glucoside (numbers 1–6 of products are according Table 1).

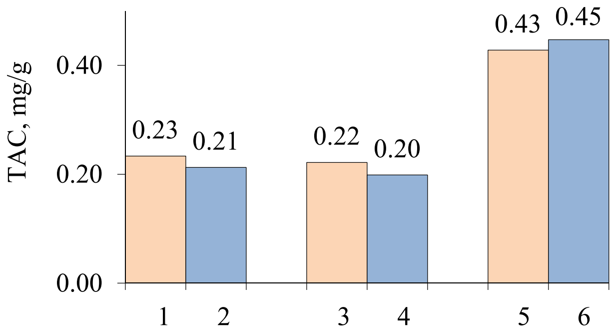

Fig. 2. Content of antioxidants (mg/g) in biscuit made with the addition of the bran of the control strain and the strain of wheat with purple grain and the content of anthocyanins in bran (numbers 1–6 of products are according Table 1). Calculated equivalent to gallic acid.

The unusual color of products with the addition of bran and/or flour from PG was not a negative factor in estimating the quality of products. The addition of bran was estimated well thanks to a "crispy taste" – a smoothly pronounced crispy taste.

Fig. 1 presents the results of anthocyanin content in bran and bran containing products.

The bran and confectionery products made with the addition of bran differed in a significantly higher anthocyanin content if the bran was from the PG strain ($p < 0.05$). The origin of flour (GOST, RG or PG) did not have a significant effect on these indicators.

The content of anthocyanins in the bran of the PG strain was about 4.5 times more than that in the bran of the RG strain. The share of anthocyanins in the products obtained from flour and bran of the PG strain was 2.6 times higher than in the case of a similar product obtained on the basis of RG.

In the case of using GOST flour, the addition of PG bran resulted in an anthocyanin content of 2.5 times higher than when using RG bran. The revealed differences between PG and RG not only in bran, but also in the finished products testify to the resistance of anthocyanins to technological processing. The calculations show that when eating 100 grams of biscuit made from flour with the addition of bran of purple wheat grain, the consumption of available anthocyanins can reach up to 0.83 mg.

Fig. 2 presents the results of the estimation of the content of antioxidants in bran and the finished products. Bran has a high mass fraction of antioxidant compounds. This indicator does not depend on the origin of bran (RG or PG).

The comparison with the earlier obtained data shows that the amount of the antioxidants consumed with biscuit, which is made with the addition of bran (Figure 2), is not lower than that when consuming bread (Khlestkina et al., 2017) [3].

There are continuous changes in the structure of a variety of confectionery products, since innovations are immanently inherent in the food industry and are the strategic parameter of its development. At present, product developments are aimed not only at providing an attractive appearance and taste variety, but also at taking into account the interests of the consumers who evaluate the useful properties of new products closely.

The new properties of products that provide an increase in their nutritional and biological value, meet the modern world trends in the field of healthy nutrition and allow us to form new niches in the field of various specialized functional products. At the same time, the characteristic feature of modern food products is the complexity of their formulations, and the manufacturing of a food product of a certain nutritional value is provided by the content of a large number of food ingredients of a different chemical nature (Usenko, 2016) [8].

The enrichment of food products with natural ingredients has an advantage over chemical preparations and premixes, and is as an alternative to the processes of chemicalization in the food industry. The composition of these products, in addition to vitamins and minerals, includes protein substances, dietary fiber and other valuable food components, which are in the form of natural compounds and in a form that is better absorbed by the body.

In terms of the orientation to manufacture natural products, researchers are increasingly focusing on the creation of new types of raw materials, the future nutritional value and composition of which can be laid even at the stage of creating a variety. A special place in these studies is held by the products with a high content of flavonoids. Polyphenols are mainly represented by flavonoid compounds, which include both a number of colorless compounds, for example, rutin, and colored molecules (for example, anthocyanins, which give a pinkish, blue and red color to plant tissues). It is not only customary berries that can be brightly colored, but also cereal grain. In this case, anthocyanins are only synthesized in the aleurone layer and the pericarp giving the grain a bluish or purple color, respectively (Adzhieva et al., 2015) [9].

Since anthocyanins perform protective functions in plants, in particular, protect their tissues from excessive UV radiation, donor plants of an anthocyanin color of grain were found in the areas with a high level of insolation, for example, in Ethiopia. The "collectors" of plant resources, such as N.I. Vavilov, have long been interested in the dark-colored southern forms of cereals, and some breeders have begun to "transfer" the color from donors to the cultivated "northern" varieties. However, in the northern latitudes, with insufficient insolation, the anthocyanin color of a stem and other parts of a plant that provides a color for grain (due to pleiotropy) is non-uniform, which can become an obstacle to the registration of the variety, so the breeders tried to get rid of the signs of the anthocyanin color.

At the same time, by the end of the 20th century, there was already quite a lot of evidence about the benefits of anthocyanins for human health, in particular, the use of anthocyanins prevents cardiovascular diseases, vision pathologies, diabetes, arthritis and has an anticancer and anti-inflammatory effect (Howard and Kritchevsky 1997; Wang et al., 1999; Tsuda et al., 2003; Lila 2004; de Pascual-Teresa et al., 2010; Hui et al., 2010; Cassidy et al., 2011; Sancho and Pastore, 2012) [10, 11, 12, 13, 14, 15, 16, 17].

Therefore, since the beginning of the 21st century, the creation and production of varieties of cereals (rice, corn, wheat) with colored (purple) grain (Garg et al., 2016) [18] is becoming increasingly popular [18], as well as the unconventional forms of vegetable crops (for example, the tomatoes and peppers colored with anthocyanins) and potatoes with a purple flesh (Strygina, Khlestkina, 2017) [19]. The value of such products, although the number of anthocyanins contained therein is lower than, for example, in blueberry or red grapes, is that the products from cereals and potatoes are included in the daily diet. In addition, these products are a more effective way of storing nutrients than in such perishable food products as berries and fruits.

Since 2000s, the breeding and production of cereals (rice, wheat, maize) enriched with anthocyanins became broadly adopted. The grain that produces these compounds has a purple or black color. Among cereals, rice as an important crop and also as a model species, has left ahead other crops in genetic studies, breeding and the production of purple (black)-grained rice. There are more than 200 types of black rice varieties in the world. Of this number more than 54 varieties of modern black rice with high yield characteristics and multiple resistances were developed in China.

China is the leading rice producer in the world responsible for 62 % of the global production of black rice. Other major rice producers in Asia are Sri Lanka, Indonesia, India, Philippines, etc. Thailand occupies the ninth place in black rice cultivation (Chaudhary, 2003; Sompong et al., 2011; Ujjawal, 2016) [20, 21, 22]. In 2001, Thailand announced its debut of purple rice Hom Nil, a cross between the indigenous black seed and fragrant jasmine varieties, to the world market (http://www.asiabiotech.com/05/0524/0673_0680.pdf). In 2008, glutinous rice (the colored rice included in this category) accounted for only 4.9% of the total exported volume, while non-fragrant white rice and fragrant rice accounted for over 80%. Although the trend of colored rice consumption in Thailand and worldwide has been increasing due to its high nutritive and medicinal value, only 0.1% of the total rice cultivation area was allocated to colored and organic rice (Rice Department, Ministry of Agriculture and Cooperatives, Thailand, 2016).

In addition, Chaudhary (2003) reported an upcoming demand of black rice as an organic food coloring agent in the EU and the USA. In 2015, the size of the global market of natural food color was estimated as 1.32 billion USD and continued to grow. The demand for natural food color in the Asia Pacific region expected to gain the highest growth rate over 7% by revenue from 2016 to 2025 due to the rapid growth of the food industry and health awareness related to synthetic color (Vayupharp and Laksanalamai, 2015; Grand View Research, 2017) [24, 25]. Anthocyanins are the water-soluble pigments found in black rice grain and bran. The shade of anythocyanis can be varied from orange to red to blue depending on the pH of a food matrix (Vayupharp and Laksanalamai, 2015) [24]. The main concern of food industry toward the stability of natural colour and extraction yield drove a lot of researchers to determine appropriate pretreatment and extraction methods (Ngamwonglumlert et al., 2015) [26].

The conventional extraction methods including soxhlet extraction, maceration and hydrodistillation are commonly used but they require a long extraction time and a large amount of a solvent. The recent development of the advanced extraction methods such as supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed-electric field extraction and enzyme-assisted extraction have emerged as an alternative choice since they consume less of a solvent, need a shorter extraction time and are more environmentally friendly (Cheok et al., 2014) [27].

Table 6. Rice Varieties in Thailand

Market Classification	Approximate Varieties	Areas or Province
Fragrant rice		
1. Jasmine or Kao Horm Mali	Kao Hom Doc Mali 105 (KDML105 and Gor-Kor)	Thung Kula plain (2 million hectares), Surin, Buri Rum
2. Jungwad	The same variety as Hom Mali 105 Khao' Jow Hawm Suphan Buri Khao’ Jow Hawm Khlong Luang 1	A province other than in (1) Non-photosensitive, irrigated Non-photosensitive, irrigated, mostly planted in the Central Plain
3. Pathumthani	Pathumthani 1	Non-photosensitive, irrigated
Non-fragrant white rice	A lot of varieties, photosensitive and non-photosensitive	Most provinces
Glutinous rice	Niaw Ubon, Niaw San-pah-tawng	Northeastern and Northern

Source: Titapiwatanakun, 2012 [23].

The natural question was whether these compounds can be destroyed during processing. A number of studies have shown that potato and wheat anthocyanins are fairly resistant to processing (Mulinacci et al., 2008; Lemos et al., 2015; Khlestkina et al., 2017 [28, 29, 3]). Another problem is to "teach" plants to produce anthocyanins not only in the hulls of grain, but also in the endosperm. So far, geneticists have managed to achieve this only with the help of methods of genetic engineering (Zhu et al., 2017) [30], but GMO-plants, as is known, cannot be grown in many countries, including ours. However, the potential for using the anthocyanins contained in grain hulls remained underestimated.

Taking into account the growing interest in the products that contain natural antioxidants, at the initiative of the Research Center for Food Safety of Novosibirsk State University, the possibilities of using the developments of Russian geneticists for the purpose of creating products from the new types of wheat grains were analyzed for the first time within the framework of this interdisciplinary study. The Center was created at Novosibirsk State University (NSU) within the framework of Project 5–100 in August 2015 as a result of the competition of the joint laboratories of NSU and the Siberian Branch of the Russian Academy of Sciences. The priority areas of the Center are the scientific studies in the field of the modern technologies in the food market that affect the consumer and technological characteristics of food raw materials and food products and joint interdisciplinary studies in the development of innovative food products as part of the implementation of interdisciplinary scientific projects to ensure global food security. At a time when the food market is dominated by the technological solutions that break food chains, the fundamentally new approaches are needed based on the interdisciplinary and systemic interaction of scientists from different fields of science, taking into account state and public interests. Thus, the problem of ensuring food security requires the fundamentally new, nonstandard solutions based on the interdisciplinary and systematic interaction of scientists from different fields of science in order to improve the quality of life of the population and the rational use of natural resources.

One of the developments of the Center was the study of the antioxidant activity of anthocyanins of purple wheat in flour confectionery products. Using the isogenic lines of wheat specially designed for comparative studies, which differ only in a small segment of the genome that has the regulatory gene of biosynthesis of anthocyanins *Pp3/TaMyc1*, it was shown that in terms of the baking and organoleptic properties of the product, purple wheat is not inferior to and in some cases manifests itself better than the control strain (Khlestkina et al., 2017) [3]. In addition, the presence of anthocyanins increases the shelf life of bakery products and their stability in the provocative conditions that promote the development of mold fungi. It was found that when eating 100 grams of bread made from flour with the addition of bran of purple wheat grain, more than 1 mg of anthocyanins will get into the body (Khlestkina et al., 2017) [3]. It is worth noting that, regardless of pigmentation, the addition of bran increases the antioxidant potential of the product by itself, which can also be of value to human health.

In this paper, we used the flour and bran from the same isogenic wheat lines for confectionery production. Note that the conditions were simulated for the extraction of anthocyanins closest to those in digestion in the digestive tract in order to estimate the amount of anthocyanins available for consuming. It has been shown that when eating 100 grams of biscuit made from flour with the addition of bran of purple wheat grain, the consumption of anthocyanins will be up to 0.83 mg.

Thus, our interdisciplinary study demonstrates a way to manufacture enriched confectionery products with a high antioxidant potential. The further developments of confectionery products based on the use of raw materials (wheat and bran) with a high content of anthocyanins can form a whole family of products with health-promoting properties. The uniqueness of these products is evidenced by the fact that at the present time none of bread wheat varieties have been registered in the State Register, from the grain of which the products containing anthocyanins can be produced.

Although a lot of researchers have reported that purple (or black) rice contains a high amount of an antioxidant agent, especially anthocyanins in pericarp (Yawadio et al., 2007; Chotimarkorn et al., 2008; Leardkamolkarn et al., 2012) [31, 32, 33] and contains a higher amount of proteins, vitamins and minerals than common white rice (Suzuki et al., 2004) [34], it is not well accepted among main rice consumers since it is difficult to cook. Moreover, purple (or black) rice contains some undesirable characteristics, such as, a distinct taste, a dark appearance and a hard cooked rice texture (Ujjawal, 2016) [22]. Thus, an alternative to the use of black rice have been studied, such as, wheat replacement in bakery products (Jung et al., 2002; Joo and Choi, 2012) [35, 36], noodles (Sirichokworrakit et al., 2015) [37] and ice cream (Chuaykarn et al., 2013) [38]. In addition, the results of the studies testify to the fact that the potential consumer who has received information about the health benefits of anthocyanins is interested in purchasing such cereals at a price higher than that of similar products (Bruschi et al., 2015) [39].

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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