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ORIGINAL RESEARCH PAPER

POLYPHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF BLACK RICE FLOUR

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Black rice is a very rich source of anthocyanins, being very popular among health conscious food consumers. Black rice presents a great importance in the food industry due to the high content of polyphenols. In order to evaluate the phytochemical profile and antioxidant activities, seven black rice milling fractions obtained at laboratory scale were investigated. For each fraction the antioxidant activity, anthocyanins, flavonoids and total polyphenolic content were determined. The highest content of bioactive compounds was recorded in the black rice milling fraction with particles ranging from 125 to 180 µm. The highest amount of anthocyanins was 0.119 mg/g fresh weight, whereas the total content of polyphenols was 483 mg/g fresh weight.

Keywords: black rice, phenolic content, anthocyanins, antioxidant activity

Introduction

Black rice represents one of the most important grain in Asia, with a high content of proteins, vitamins (vitamin B, riboflavin and niacin), minerals, being a rich source of antioxidants (Yawadio *et al*., 2007). Black rice, known as long life rice (Kong *et al*., 2008), is a whole grain that belongs to the *Oryza sativa* L. species, with more than 200 varieties worldwide. The black colour of the rice kernel appears due to a high content of anthocyanins. Black rice, according to Kushwaha (2016), is known under many names such as: purple rice, forbidden rice, heaven rice, imperial rice, king's rice and prized rice. This type of rice is considered a good panacea for many digestive diseases thanks to its curative effect.

According to Chaudhary (2003), black rice, besides the fact that it has so many health benefits, is also used in the food industry as an organic dye. In recent years, pigmented rice has been classified as one of the most consumed products among functional foods, due to the fact that it contains a high amount of phenolic compounds that have antioxidant properties (Mira *et al*., 2009; Shen *et al*., 2009). The anthocyanins are compounds that occur naturally in black rice, being a gluten-free, cholesterol-free, low in sugar, salt and fat type of cereal, in addition to being responsible for lowering the cholesterol in the human body (Lee *et al*., 2008).

Several studies showed that the supplementation with black rice pigment reduced considerably the oxidative stress, improved the lipid profile and modulated the atherosclerotic lesion (Guo *et al*., 2007).

Several research papers concluded that the black rice powder represents one of the nature's superfoods, with lots of remarkable abilities. Black rice is used mainly in Asia in the food industry in order to decorate several other products and to obtain noodles, sushi, and puddings. Besides the health benefits, Chaudhary (2003) observed that the demand for black rice has increased, being used as a coloring agent of organic products. Nonetheless, black rice is a good source of natural dyes which can afterwards be successfully extracted and used in the food industry (Sompong *et al*., 2011). For a long time, black rice anthocyanins (BRACs) were considered to be a significant part of different health-promoting functional foods because of their antioxidant activity (Nam *et al*., 2006, Philpott *et al*., 2006), hypoglycemic (Tsuda *et al*., 2003), anticancer (Hyun & Chang, 2004) and anti-inflammatory effects (Tsuda *et al*., 2002). These compounds are found in the pericarp (Shen *et al*., 2009), seed coat and the aleurone layer of black rice (Sompong *et al*., 2011), and they can be isolated as fractions to use them as functional colorants or as food ingredients (Ling *et al*., 2002). Kong and Lee (2010) studied the phenolic and anthocyanin contents as well as their antioxidant activity in different milled fractions, these compounds being considered one of the most efficient antioxidants in the plant kingdom (Velioglu *et al*., 1998).

Chutipaijit *et al*. (2011) showed that black rice may be an alternative treatment as a healthy product for some diseases. American Health Association, the American Cancer Society and the 2005 Dietary Guidelines for Americans recommended the increased consumption of black rice to prevent heart disease and various types of cancers (USA Rice Federation 2008). A recent research study of Qin *et al*. (2009) revealed that anthocyanin supplementation improves LDL and HDL levels. Thomasset *et al*. (2009) concluded that anthocyanin consumption can delay the cancer development in rodents and Guo *et al*. (2007) showed that black rice may have antiatherogenic activity. Other healthy compounds may fight against: the development of chronic inflammatory proliferative diseases (CIPDs) (Ishihara and Hirano, 2002), arteriosclerosis and cancer (Namsh *et al*., 2006; Chen *et al*., 2006), free radicals (Tananuwong and Tewaruth 2010), inflammation throughout the body. Furthermore, they promote blood circulation, slow the damage and aging of tissues, reduce cholesterol and blood sugar levels (Hirunpanich *et al*., 2005; Rechner and Kroner 2005), affect the pituitary gland function, inhibit the gastric acid secretion and the platelet aggregation (Butelli *et al*., 2008). Likewise, Kayahara and Tsukahara (2000) showed that after eating black rice based products, the ingestion of some microorganisms takes place that can help to prevent headaches, constipation, colon cancer, prevent heart disease, lower the blood pressure and prevent the Alzheimer's disease.

From the research point of view, this study is very important taking into account the serious lack of studies concerning the polyphenolic compounds found in the milled fractions of black rice. For a better understanding of the composition of black rice regarding these bioactive compounds and which of them is responsible for the antioxidant activity, the objectives of this study were to determine the total anthocyanin content, total polyphenolic content, total flavonoidic content, and the antioxidant activity in the different fractions of milled black rice flour.

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, sodium carbonate, sodium hydroxide, sodium acetate, sodium nitrite, potassium chloride, aluminum chloride, ethanol (HPLC grade) were purchased from Sigma Aldrich (Germany).

Black rice sample

Black rice (*Oryza sativa L*) was purchased from a local supermarket (Galaţi) in the period June-July 2014. Black rice was milled with a laboratory mill (Mlynek Laboratory JNY Tip WZ/2) in order to obtain whole cereal. The product resulting was sifted through sieves with 630, 550, 315, 180, 125 and 90 µm, resulting seven streams (from F1 to F7).

F1 - F7 fractions were extracted using the anthocyanins extraction protocol detailed below. Preliminary analysis was performed to establish the phytochemicals content in different black rice flour fractions.

Biological active compounds extraction

Bioactive compounds such as anthocyanins, poliphenols, flavoniods from black rice were extracted using 1 g of flour and a volume of 8 mL ethanol (70%), for 24 hours. The mixture was centrifuged at 5000 rpm, at 4°C for 30 minutes. The supernatant was collected and concentrated at 40°C till dryness (Rotavapor R-124, Buchi, Switzerland).

Determination of total monomeric anthocyanins content

The total monomeric antocyanins content (TAC) was determined as using the AOAC Official Method 2005.02 and expressed as mg cyanidin-3-glucoside equivalents (C3G) per g flour with 14 % moisture.

Total phenolic content

The total phenolic content (TPC) was determined using a modified colorimetric method (Gutfinger, 1981), where a volume of 200 µL of extract was mixed with 1 mL of Folin – Ciocalteu reagent and 15.8 mL deionized water. The mixture was allowed to react for 10 minutes in the dark, and then 3 mL of Na_2CO_3 (20%) were added. After 60 minutes, the absorbance was measured at 765 nm and the results were expressed as mg gallic acid (Sigma-Aldrich, Steinheim, Germany) equivalents per g flour (mg GA/g flour).

Total Flavonoid Content

For the black rice samples, the total flavonoid content (TFC) was assessed using a modified colorimetric method as described by Dewanto *et al*. (2002). The results were expressed as mg catehinic equivalents (EC) per g flour.

Determination of 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH-RSA)

The DPPH-RSA was determined following the colorimetric procedure described by Almela et al. (2006), a volume of 3.9 ml of diluted DPPH (1:10) was added to the samples (0.1 ml). The DPPH solution was added to the black rice extracts and stirred, and afterwards each mixture was maintained in the dark for 30 minutes. The decrease in the absorbance (A) was measured spectrophotometrically at 515 nm. The antioxidant activity was expressed as percentage of DPPH-RSA according to the following equation (1) and the obtained values were compared to the antioxidant activity of the black rice flour extracts.

$$\%DPPH\ RSA=((A_{control} - A_{sample})/A_{control})*100 \qquad (1)$$

Statistical analysis

The experiments were performed in triplicate and the results were expressed as average values together with standard deviations.

Results and discussion

Little is known about the bioactive compounds content and antioxidant activities of black rice fractions. According to Zhang *et al.* (2010) there are significant differences regarding the phytochemical content and antioxidant activity that may vary among black rice varieties.

Total Anthocyanin Content

The anthocyanins are responsible for the purple, red or blue pigmentation of various plants. The most important differences between the anthocyanins are closely correlated to the nature and number of sugars that are attached to the molecule, the main position of the attachment, the number of hydroxyl groups, and the aromatic or aliphatic acids number. In Figure 1 can be seen the results of the total anthocyanin content of black rice fractions.

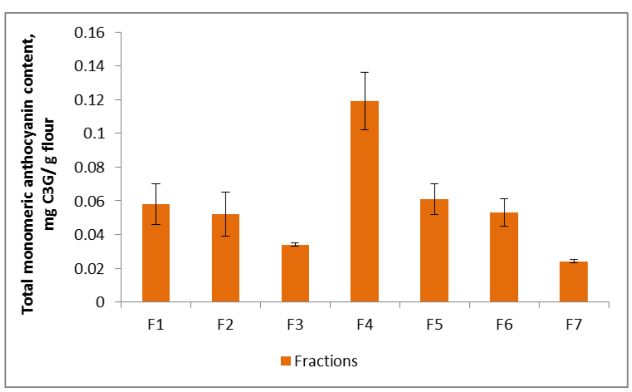

Figure 1. Total monomeric anthocyanin content of black rice fractions

Among the seven fractions of black rice flour, F4 presented the highest amount of TAC 0.119±0.017 mg C3G/g flour. All the other fractions presented close values such as 0.052±0.013 mg C3G/g flour in the case of F2, 0.053±0.008 mg C3G/g flour for F6, 0.058±0.012 mg C3G/g flour for F1 and 0.061±0.009 mg C3G/g flour for F5, whereas the lowest values were obtained in the case of F3 and F7 (0.034±0.001 and 0.024±0.001 mg C3G/g flour, respectively. Sompong *et al*. (2011) obtained TAC values ranging between 0.003 – 0.014 mg C3G/g in red rice varieties and 1.095 – 2.566 mg C3G/g in black rice varieties. Abdel-Aal *et al*. (2006) obtained 3.276 mg C3G/g in black rice, value that was 35 times higher than those for red rice. Zhang *et al*. (2010) showed, in the case of Heinuo 9933 rice bran variety, a content of TAC of 50.96 mg C3G/g DW. The value for monomeric anthocyanins reported by Asem *et al*. (2015) in scented black rice was found to be 0.74 mg C3G/g in *Chakhao Poireton* variety and 0.692 mg C3G/g in *Chakhao Amubi*. Similar values were obtained by Hosseinian *et al*. (2008) in the case of purple wheat, respectively, 0.526 and 0.5 mg C3G/g. Park *et al*. (2008) observed for black rice a TAC content of 1.214 mg C3G/g. Murdifin *et al*. (2015) analyzed the anthocyanins from black rice and presented a value of 0.66±3.61 mg C3G/g. Yao *et al*. (2009) studied different types of cereals and obtained different TAC contents such as: 3.83±0.04 mg C3G/g in the case of black rice, 0.05±0.01 mg C3G/g for the red rice, 1.22±0.08 mg C3G/g for the purple rice, 0.31±0.01 mg C3G/g for purple corn, 0.27±0.05 mg C3G/g for black barley, 0.19±0.02 mg C3G/g in the case of black soybean, and 1.63±0.03 mg C3G/g for black soybean coat.

Total Phenolic Content

The phenolic compounds are found in different food varieties, including fruits, vegetables and cereals. The types of compounds and their concentration may vary among different food products, which may be due to genetic and environmental factors and the processing conditions. Rice is one of the main ingredients of the population diet and has very important antioxidant properties. Phenols are compounds that contribute to the total antioxidant activity of products. Figure 2 shows the total phenolic content of black rice fractions.

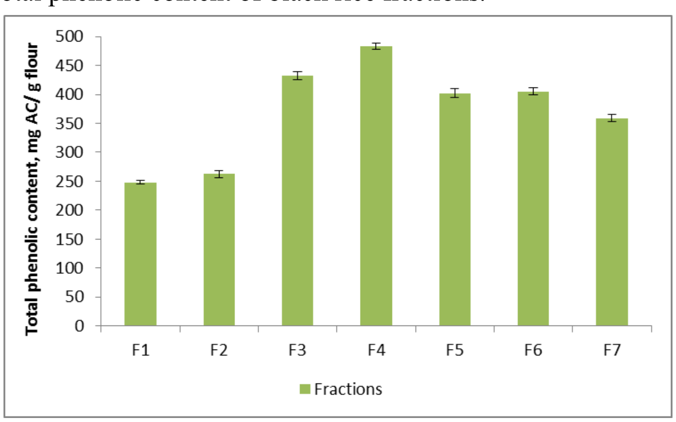

Figure 2. Total phenolic content of black rice fractions

In our study, it can be seen that F4 had the highest polyphenolic content of 483±5.32 mg GA/g flour, closely followed by F3 with a content of 432.13±7.32 mg GA/g flour, whereas F5 and F6 had almost the same content (402.26±8.01 and 405.32±6.32 mg GA/g flour, respectively). Bordiga *et al*. (2014) reported a phenolic content for six varieties of pigmented rice ranging from 1.404±0.322 (Selenio variety) to 0.011±1.161 (Artemide variety) mg GA/g. The phenolic content values of red rice varieties reported by Sompong *et al*. (2011) were between 0.14 and 0.34 mg GA/g, whereas the black rice varieties displayed 4-fold higher values (0.74 – 0.105 mg GA/g). The total polyphenolic content measured by Asem *et al*. (2015) had the following values: 5.77 mg GA/g for *Chakhao Poireiton* cultivar and 5 mg GA/g for *Chakhao Amubi* cultivar, respectively. The analysis of polyphenols from black rice assessed by Murdifin *et al*. (2015) presented a value of 1.197±9.00 mg GAE/g. Yao *et al*. (2009) obtained different TPC contents such as: 8.58±0.56 mg GA/g in the case of black rice, 0.10±0.01 mg GA/g for the red rice, 4.62±0.18 mg GA/g for the purple rice, 1.11±0.09 mg GA/g for purple corn, 0.46±0.04 mg GA/g for black barley, 0.75±0.06 mg GA/g in the case of black soybean, and 5.26±0.42 mg GA/g for the black soybean coat.

Total Flavonoid Content

In Figure 3 can be seen the results of the total flavonoids content of black rice fractions.

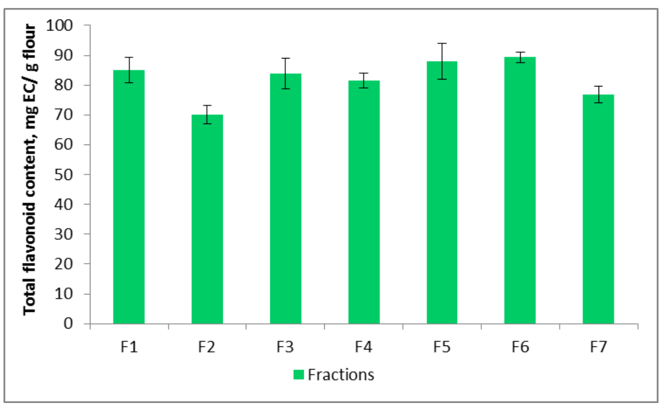

Figure 3. Total flavonoid content of black rice fractions

It can be seen in Figure 3 that the flavonoidic content for F1, F2-6, has similar values that range between 81.54±2.41 mg EC/g flour (F4) and 89.31±1.69 mg EC/g flour (F6), while F2 and F7 present smaller values of 70.10±2.94 and 76.82±2.91 mg EC/g flour. According to the studies of Asaduzzaman *et al*. (2013), the flavonoidic content from six aromatic rice varieties ranged from 6.60 to 12.80 mg RE/g flour. Similar results were also obtained for aromatic rice varieties that were analyzed by Saikia *et al*. (2012) who assessed a value of 6.80 mg RE/g in the case of BRRI dhan 37 variety, and 12.80 mg RE/g for Philipine Katari rice variety, respectively.

Antioxidant Activity

As it can be seen below, Figure 4 shows the antioxidant capacity of the black rice fractions. The antioxidant activity for each of the seven fractions ranged between 60±2.3 (F1) - 68.23±4.01% (F5). It can be stated that after performing the experiments, the high DPPH activity is due to the flavonoidic content and to the total polyphenolic content. Bordiga *et al*. (2014) obtained a DPPH radical scavenging capacity ranging from 55.1 to 7.30 mmol TE/kg rice. The studies on several red rice samples of Sompong *et al.* (2011) reported FRAP values ranging between 13 to 76%, whereas amongst the black rice samples FRAP ranged between 16 - 30.3%. According to the studies of Suttiarporn *et al*. (2016), the antioxidant activity in the case of thai black rice bran cv. Riceberry was 94.83%. Moreover, Zhang *et al*. (2015) obtained antioxidant activity values ranging between 6.73±0.16 µmol TE/g rice in the case of YF64 black rice variety, and 9.11±0.43 µmol TE/g rice in the case of YF53 variety, respectively. According to the results obtained by Asem *et al*. (2015), the antioxidant activity for *Chakhao Poireiton* and *Chakhao Amubi* varieties were 70.28% and 60.84%, respectively. These results were similar with those obtained by Park *et al*. (2008) on Korean black rice (Heugjunjubyeo), the values being 40.39 and 55.20%. A study of Saenkod *et al*. (2013) on Chinese black rice (Brown Himi variety) showed a high antioxidant activity of 70.82%. Moko *et al.* (2014) reported an 88.29% DPPH activity for red rice from Indonesia. Furthermore, the analysis of DPPH activity in the case of black rice, which was assessed by Murdifin et al. (2015), presented a very small inhibition value of 22.22±1.05%.

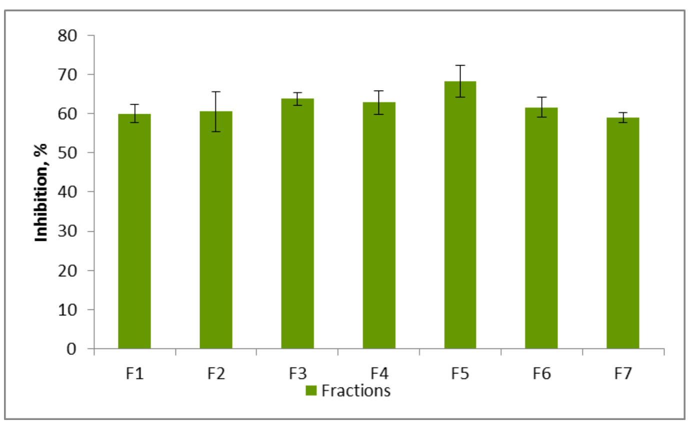

Figure 4. Antioxidant activity of black rice fractions

Based on the obtained results, it was observed that the total anthocyanin content decreased from F1 to F3 and also from F4 to F7, the highest value being assessed for F4. In the case of polyphenolic compounds, their content increased from F1 to F3 and decreased from F4 to F7, with the maximum value for F4. For all the fractions, the total flavonoid content and the antioxidant activity do not vary significantly. Throughout the sifting procedure, the biologically active compounds are mostly concentrated in F4 (the fraction that resulted after the sifting with a sieve mesh of 180 μm).

Conclusions

This study investigated different fractions obtained by grinding and sieving the black rice at laboratory scale, in order to assess the biologically active compounds content.

The results suggest that the high antioxidant potential is given mainly by anthocyanins, phenols and flavonoids, and hence the black rice is considered to be a very valuable natural product. The black rice flour fraction with the highest concentration of biologically active compounds has particle size of 180 μm (F4). The results could provide food manufacturers with useful insight so as to promote the consumption of rice products. In this regard to optimize the functional food processing, more research will be further addressed.

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