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Original Research Article

Physicochemical and nutritional properties of pigmented rice subjected to different degrees of milling

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ABSTRACT

Recent studies have reported the health benefits of pigmented rice cultivars due to the presence of bioactive compounds in the bran layer of caryopsis. This study evaluated the proximate composition, colour, total flavonoids, anthocyanins and proanthocyanidins contents, as well as the total phenolics and antioxidant activity of IAC-600 black rice cultivar and MPB-10 red rice lineage, as a function of degree of milling (DOM), at 0%, 4%, 7%, 10%, 12% and 15% of DOM. Black rice showed a thicker bran layer than red rice. Around 80% and 65% of the ash content of red and black rice, respectively, was distributed in the bran layer. 4% DOM reduced 47% of the fat content in red rice, while in order to reduce similar fat content in black rice, a 7% DOM was necessary. The total free phenolics were around 6- and 7-fold higher than bound phenolics for black and red rice, respectively. Although the non-milled black rice presented higher free and bound phenolics contents than non-milled red rice, the red rice showed higher DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ antioxidant activities.

1. Introduction

Rice (*Oryza sativa* L.) is considered a basic source of energy for more than half of the population worldwide (Monks et al., 2013). Consumer preference of rice depends on the cultural tradition of each region of the world and it is associated with the amylose content of the grains. Regardless of the amylose content, the most consumed rice is milled rice, which is industrially prepared by removing about 7–12% of the bran fraction by mechanical milling. The milling process removes most of the fibre and fat from the grain, improving its sensory properties and storage stability, respectively. Recently, pigmented rice, such as red rice, black rice and wild rice, have received the attention of consumers looking for healthier foods. The health benefits of pigmented rice are attributed to the presence of phenolic compounds that possess antioxidant, anticarcinogenic, antiallergic, anti-inflammatory, antiatherosclerosis and hypoglycaemic activities (Deng et al., 2013).

The phenolic constituents of rice are mainly distributed in rice pericarp, which accounts for 2–3% of rice caryopsis, and can be separated in three different groups: phenolic acids, flavonoids and proanthocyanidins. The simple phenolic acids and flavonoids are the most common phenolic compounds and they generally occur as soluble conjugated (glycosides) and insoluble forms (bound) (Nardini and Ghiselli, 2004). Free and soluble conjugated phenolic forms are absorbed in the stomach and small intestine; the dietary intake of bound phenolics presents a chemopreventive activity against colon cancer (Acosta-Estrada et al., 2014).

The phenolic acids are found in black-, red- and white-pericarp rice grains. Differently from fruits and vegetables, the rice grains present high amount of ferulic acid, which is considered a potential anti-inflammatory compound (Adom et al., 2003). The main flavonoid compounds found in black-rice are anthocyanins. Min et al. (2012) and Pereira-Caro et al. (2013) studied the presence of anthocyanins in black rice cultivars and reported cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside as the main individual anthocyanins present in black rice pericarp. According to Chen et al. (2006), both cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside are potential molecules for blocking tumour initiation, promotion and metastasis. Red-pericarp rice, on the other hand, contains proanthocyanidins, which are also known as condensed tannins. The proanthocyanidins dimers and trimers have been

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reported as the dominant phenolic antioxidant in red rice (Gunaratne et al., 2013).

Several studies have been conducted in order to quantify the phenolics indicated above in pigmented rice from different cultivars. However, little is known regarding their presence or loss as a function of mechanical bran removal. The milling of rice grains is an essential process carried out by all rice manufacturers and commercial farmers to remove the fat-rich aleurone layer that would otherwise promote rancidity of the rice grain, during long storage periods (Sellapan et al., 2009). Although pigmented rice cultivars clearly present higher nutritional benefits than milled white rice, which is the most consumed all over the world, the colour appearance and the difficulty to preserve the quality during supply, due to the presence of the fat-rich embryo, are some challenges for improving its production and consumption.

The objective of this study was to improve the knowledge about the presence of total phenolics, flavonoids, anthocyanins and proanthocyanidins in black rice and red rice grains subjected to different degrees of milling (0%, 4%, 7%, 10%, 12% and 15%). Moreover, the proximate composition, colour and antioxidant activity were also recorded as a function of degree of milling.

2. Materials and methods

2.1. Materials

One black rice cultivar (IAC-600) and one red rice lineage (MPB-10) were cultivated under an irrigation system in Pelotas, State of Rio Grande do Sul, Brazil. The rice grains were harvested when the moisture content was approximately 20%, transported to the *Laboratório de Pós-Colheita, Industrialização e Qualidade de Grãos* of the *Universidade Federal de Pelotas* and submitted to cleaning and drying processes, until 13% moisture content was achieved. All chemicals used in this study were analytical grade or better.

2.2. Rice milling

The rice grains (100 g) were dehusked and polished using a Zaccaria rice machine (Type PAZ-1-DTA, Zaccaria, Brazil). Brown rice samples, after cleaning and grading, were polished until five different degrees of milling were achieved: 4, 7, 10, 12 and 15%. The degree of milling was determined using the following equation: DOM = [1 − (weight of the milled rice/weight of the rice in hull)] × 100. Samples that resulted in DOMs of 4, 7, 10, 12 and 15% after the milling process, as well as the unpolished rice, were analysed.

Experiments were conducted in triplicate, and the average values were used for analysis. Broken grains were removed using the laboratory grader of the same Zaccaria rice machine (Type PAZ-1-DTA, Zaccaria, Brazil). The unpolished rice grains and the polished grains with the four different degrees of milling (4, 7, 10, 12 and 15%) were ground to 70 mesh size powder using a laboratory mill (Perten 3100, Perten Instruments, Sweden).

2.3. Proximate analysis

The moisture content of the rice samples was determined using a drying oven set at 105 ± 3 °C, with natural air circulation for 24 h, following the recommendations of the American Society of Agricultural Engineers (ASAE, 2000). Moisture content was expressed as percentage (%). Fat content was determined following method 30–20 of the American Association of Cereal Chemists (AACC, 1995). Nitrogen content was determined according to AACC method 46–13 (AACC, 1995), and the protein content was obtained using a conversion factor of nitrogen to protein of 5.95. Ash content was determined according to the AACC method 08-01 (AACC, 1995).

2.4. Colour

To illustrate the colour alteration in black- and red-pericarp rice grains as affected by the different degrees of milling, the grains were photographed by a professional photographer using a Nikon-310 digital camera (Nikon D100 6Mp, Nikon, Japan). The whiteness (L^* value) of black and red rice grains was also measured with a Minolta CR-310 chroma metre (Minolta CR-310, Osaka, Japan), as this parameter was the most usable to evaluate the effect of milling on the colour of rice grains.

2.5. Total flavonoids and proanthocyanidins

The extract for quantification of total flavonoids and total proanthocyanidins was prepared by adding 40 mL of 70% acetone in water (v/v) to 2 g of rice flour. The material was vortexed and placed in an ultrasonic water-bath for 20 min. The slurry was centrifuged at 3000 × *g* and 4 °C for 10 min. The supernatant was stored at −20 °C until used as extract for total flavonoids and proanthocyanidins analysis.

The total flavonoids content was measured according to the method described by Dewanto et al. (2002) with slight modifications. Briefly, 2.25 mL of distilled water and 0.15 mL of 5% sodium nitrite solution were added to 0.5 mL of the extract in a test tube, mixed and kept for 6 min. Afterwards, 0.3 mL of a 10% aluminium trichloride hexahydrate solution were added. The material was vortexed and allowed to stand for 5 min, followed by addition of 1 mL of 1 M sodium hydroxide. The mixture was vortexed again and absorbance was measured immediately at 510 nm (UV 17000 spectrophotometer; Shimadzu, Kyoto, Japan). Results were expressed as mg of quercetin equivalents per 100 g of sample.

Proanthocyanidins (PAs) were quantified according to the method described by Porter et al. (1986). Absorbance was measured at 550 nm. The quantification was performed based on a calibration curve of catechin. Results of triplicate analysis are given as mg of catechin equivalents per 100 g of dry matter. Total PAs content was established as the sum of PA content in the acetone extracts.

2.6. Total anthocyanins

Total anthocyanin content in rice was determined according to the spectrophotometric method described by Abdel-Aal and Hucl (1999), with modifications. Anthocyanins were extracted from 0.5 g of rice flour using acidified methanol (methanol and 1.0 N HCl, 85:15, v/v). Extracts were centrifuged at 27,200 × *g* for 15 min. This process was repeated three more times and the supernatants combined. The extracts were stored at −20 °C overnight, recentrifuged and then filtered through a 0.45-μm filter. Absorbance was recorded at 535 nm and the total anthocyanin contents of the samples were calculated as mg of cyanidin-3-glucoside equivalent (Cy-3-G) per 100 g of sample.

2.7. Total phenolics content

2.7.1. Extraction of phenolics

The extraction of free and bound phenolics was performed according to the method described by Qiu et al. (2010), with some modification. Rice flour (2 g) was extracted twice with 80% methanol at a ratio of 1:10 (w/v). For each extraction the mixture was kept on a mechanical shaker (Certomat Biotech Internacional) for 1 h at 150 rpm at room temperature. After centrifuging (Eppendorf 5430-R) at 4000 rpm (1430 × *g*) for 5 min, the supernatants obtained from each extraction were combined and concentrated to dryness by using a rotary evaporator at 35 °C. The dried methanol extract was redissolved in 20 mL of 50%