Food Chemistry 242 (2018) 427–434

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Cooking quality properties and free and bound phenolics content of brown, black, and red rice grains stored at different temperatures for six months

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ARTICLE INFO

Keywords:
Oryza sativa
Postharvest quality
Flavonoids
Phenolic acids

ABSTRACT

The changes in cooking quality and phenolic composition of whole black and red rice grains stored during six months at different temperatures were evaluated. Brown rice with known cooking quality properties and low phenolic levels was used for purposes comparison. All rice genotypes were stored at 13% moisture content at temperatures of 16, 24, 32, and 40 °C. Cooking time, hardness, free and bound phenolics, anthocyanins, proanthocyanidins, and free radical scavenging capacity were analysed. The traditional rice with brown pericarp exhibited an increase in cooking time and free phenolics content, while rice with black pericarp exhibited a reduction in cooking time after six months of storage at the highest studied temperature of 40 °C. There as increases in ferulic acid levels occurred as a function of storage temperature. Red pericarp rice grains showed decreased antioxidant capacity against ABTS radical for the soluble phenolic fraction with increased time and storage temperature.

1. Introduction

Rice (*Oryza sativa* L.) is consumed by over half of the world's population, mainly as a source of carbohydrate and protein. Rice, especially with a pigmented pericarp, is also a source of bioactive compounds, such as phenolics. Several studies have shown that pigmented rice genotypes, such as black and red rice varieties, exhibit greater phenolics content than brown rice genotypes (Finocchiaro, Ferrari, & Gianinetti, 2010; Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011). Phenolic compounds provide numerous human health benefits, including antioxidant, anticarcinogenic, antiallergenic, anti-inflammatory, hypoglycemic, and antiatherosclerotic properties (Deng et al., 2013).

Phenolic compounds are found in nature in both free and bound forms (Nardini & Ghiselli, 2004). Bound phenolics are linked to one or more sugar units through an hydroxyl group (O-glucosides) or through carbon-carbon linkages (C-glucosides). Free phenolics are those easily extractable with solvents, which may be easily absorbed in the small intestine. Bound phenolics may resist enzymatic hydrolysis and absorption, reaching the colon, where they are released (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). In rice, bound phenolics may account for up to 88% of the total phenolic content (Zhou, Robards, Helliwell, & Blanchard, 2004).

Anthocyanins and proanthocyanidins are the main classes of flavonoids found in black and red rice, respectively (Cai, Sun, Xing, Luo, & Corke, 2006; Gunaratne et al., 2013). Rice grains with a black pericarp have been shown to possess 35 times greater anthocyanin content than red rice grains (Abdel-Aal, Young, & Rabalski, 2006). The main anthocyanins found in black rice genotypes are cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside. Proanthocyanidins found in red rice consist of single or polymerized units of flavan-3-ol, such as (+)-catechin and (−)-epicatechin. The degree of polymerization (DP) affects the absorption of proanthocyanidins in the small intestine and, thus, their bioactivity (Ou & Gu, 2014). In red rice, 51% of total proanthocyanidins exhibit a DP between 1 and 10, while 49% exhibit a DP greater than 10 (Chen, McClung, & Bergman, 2016).

Pigmented rice is consumed as whole rice. The grains are simply subjected to dehusking before packaging. Changes in cooking quality properties of black and red rice grains over the course of their shelf-life are not fully understood. In addition, limited information regarding changes that occur in phenolic compounds' availability and profile is available. Long-term storage at 30 and 40 °C is detrimental to the physicochemical, structural, texturometric, viscoamylographic, and sensory properties of brown rice grains (Chen et al., 2015; Park, Kim, Park, & Kim, 2012). Zhou, Chen, Zhang, and Blanchard (2014) found a decrease in the total content of phenolic compounds, flavonoids,

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http://dx.doi.org/10.1016/j.foodchem.2017.09.077
Received 2 March 2017; Received in revised form 4 September 2017; Accepted 14 September 2017
Available online 18 September 2017
0308-8146/

proanthocyanidins, and antioxidant activity of both light- and dark-colored pericarp rice, in both free and bound fractions, after storage at 37 °C for six months. In a previous study with soybeans stored for 12 months, Ziegler et al. (2016) observed an increase in the free phenolic content and antioxidant activity of grains maintained at 12, 15, and 18% moisture and 11, 18, 25, and 32 °C.

Although some studies have previously evaluated the storage of rice grains, a more in-depth study to understand cooking behavior and individual phenolic compounds during the shelf-life period is necessary. In this context, the aim of this study was to evaluate cooking quality properties and the phenolic composition of brown-, black-, and red-pericarp rice during six months of storage at 16, 24, 32, and 40 °C.

2. Materials and methods

2.1. Plant material and storage conditions

Brown, black, and red rice from IRGA 417, IAC-600, and MPB-10 genotypes, respectively, were used. All genotypes were cultivated under a flooded production system in Jaguarão (32° 33′ 37″ S, 53° 22′ 52″ W, 23 m), Rio Grande do Sul state, Brazil. Grains were harvested with approximately 20% moisture content and immediately transported to *Laboratório de Pós-Colheita, Industrialização e Qualidade de Grãos* (Labgrãos), from the Federal University of Pelotas, where they were subjected to cleaning and drying processes until 13% moisture content be achieved. Then, rice was subjected to pest control using aluminum phosphide. Rice grains were then dehusked using a Zaccaria rice-milling machine (Type 1-PAZ-DTA, Zaccaria, Brazil). Afterwards, dehusked rice was packaged into low-density polyethylene bags (0.2 mm-thick, with capacity for 0.9 kg of rice) and stored under dark at 16, 24, 32, and 40 °C, for six months, in triplicate. Analyses were performed on the first day of storage (initial) and at the 2nd, 4th, and 6th month of storage. Some analyses required dehusked rice samples to be milled into flour using a laboratory mill (Perten 3100, Perten Instruments, Huddinge, Sweden) set with a 35-mesh sieve.

2.2. Cooking time

Optimum cooking time was determined for whole rice by the Ranghino test (Juliano & Bechtel, 1985). In a 250-mL beaker, approximately 200 mL of distilled water was boiled, and 10 g of rice sample was immersed into the boiling water. Cooking time measurement started immediately after immersion. After 10 min and at every minute thereafter, ten rice grains were removed and pressed between two clean glass plates and observed under polarized light. Cooking time was recorded when at least 90% of the grains no longer had an opaque core or an uncooked center.

2.3. Hardness of cooked rice

Texture profile analysis (TPA) was performed using a texture analyzer (TA-XT2, Texture Technologies Corp., UK) with a 5-kg load cell using a two-cycle compression method. The texture analyzer was coupled to a computer that recorded the data via the XT.RA Dimension software program (v. 8, Texture Technologies Corp., USA). Rice samples were prepared by cooking samples of 10 g of rice in a 250-mL beaker with 200 mL of boiling distilled water until the white color of the core disappeared. Cooked rice was completely drained of the water using a strainer. A 20-mm diameter probe was used to compress 3 grains, with pre-test and post-test speeds of 1 mm s^{-1} and a test speed of 5 mm s^{-1}. A two-cycle compression force versus time program was used to compress the samples to 90% of their original thickness, after which the probe returned to its original position before performing the second compression cycle. Hardness was determined from the first test curve. Results were expressed in Newton (N).

2.4. Total phenolic content

2.4.1. Extraction process

The extraction of free and bound phenolics was performed according to the method described by Qiu, Liu, and Beta (2010). Rice flour (2 g) was extracted twice with methanol (80% v/v) at a ratio of 1:10 (m/v). For each extraction, the mixture was kept on an orbital shaker (Certomat Biotech International) for 1 h at 150*g* at room temperature. After centrifuging (Eppendorf 5430-R) at 4000 × *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 methanol (50% v/v) and used as crude extract for the quantification of total free phenolic content and the quantification of individual phenolics, anthocyanins, and proanthocyanidins. The residue obtained from the crude extraction was washed with 40 mL of distilled water to eliminate any remaining organic solvent, and then filtered through a Whatman No. 1 filter paper. After drying in a hood at room temperature, the dried residue was kept in a sealed container at 4 °C prior to alkaline hydrolysis.

2.4.2. Alkaline hydrolysis

The residue remaining from the extraction process was separately hydrolyzed with 40 mL of NaOH (4 M) on a shaker for 4 h at 150*g*. After digestion, the solution was adjusted to pH 1.8 with HCl (6 M) and then extracted three times with 70 mL aliquots of ethyl acetate. The combined ethyl acetate fractions were dried with anhydrous Na_2SO_4, evaporated to dryness, and reconstituted in 5 mL of methanol (50% v/v), comprising the bound phenolics fraction.

2.4.3. Determination of the total phenolic content

The total phenolic content of both free and bound phenolics fractions was evaluated using the Folin-Ciocalteau method (Singleton & Rossi, 1965). Results were expressed as mg of gallic acid equivalents (GAE) (calibration curve: 7–250 μg/mL, equation: $Y = 0.0065x + 0.0286$, $R^2 = 0.9993$) per 1 g of rice on a dry weight basis (dw).

2.5. Anthocyanins

Total anthocyanin content was determined according to the method described by Abdel-Aal and Hucl (2003). Rice flour (500 mg) was put into a 15 mL Falcon™ tubes. Extraction was performed by adding 10 mL of acidified methanol (85% methanol: 15% 1 N HCl) to the tubes. The material was first homogenized by vortexing for 30 s, followed by stirring for 30 min at 200 × *g* on an orbital shaker. Afterwards, the samples were centrifuged at 7600 × *g* for 15 min. The supernatant was collected and the absorbance recorded at 535 nm (Jenway 6705 UV/Vis Spectrophotometer). Total anthocyanin content was expressed as mg equivalent of cyanidin-3-*O*-glucoside (CGE) per 1 kg of rice, using the following formula:

$$C = (A/\varepsilon) \times (\text{vol}/1000) \times \text{MW} \times (1/\text{sample wt}) \times 10^6$$

where *C* is concentration of total anthocyanin (mg/kg), *A* is absorbance reading, Ɛ is molar absorptivity (cyanidin-3-glucoside = 25,965 cm^{-1} M^{-1}), vol is total volume of anthocyanin extract, and MW is molecular weight of cyanidin-3-glucoside (449).

2.6. Identification and quantification of phenolics, flavonoids, and anthocyanins

The same extract used for total phenolic content determination was used for LC-ESI-QToF-MS analysis. Samples were filtered through a 0.45 μm nylon membrane filter (Merck Millipore Corporation, Darmstadt, Hesse, Germany). The LC-ESI-QTof-MS analysis was performed on a Prominence UFLC system (Shimadzu, Japan) coupled to a quadrupole time-of-flight mass spectrometer (Impact HD, Bruker