Food Chemistry 208 (2016) 116–123

Contents lists available at ScienceDirect

Food Chemistry

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

The revisited levels of free and bound phenolics in rice: Effects of the extraction procedure

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 23 March 2016
Accepted 28 March 2016
Available online 30 March 2016

Keywords:
Pigmented rice
Free phenolics
Bound phenolics
Extraction of phenolics
Phenolic acids

ABSTRACT

The effects of the type of solvolytic solution and number of extraction steps on the recovery of free phenolics, anthocyanins and proanthocyanidins from different rice samples were evaluated. Moreover, bound phenolic acids were determined as a function of enzymatic and/or alkaline hydrolysis treatment of the rice residue obtained after the extraction of free phenolics. The Acetone/Water (70:30 v/v) was the most effective solvolytic solution for extracting free phenolics from pigmented rice, as well as anthocyanins from black and wild rice, and proanthocyanidins from red rice. The application of three extraction steps increased the recovery of free phenolics up to 10%. The adoption of an enzymatic treatment, with α-amylase in order to reduce the paste viscosity of the residue, increased the extractability of bound phenolics. α-Amylase at 37 °C during 15 min followed by an alkaline hydrolysis at 37 °C was the best treatment for the recovery of bound phenolics.

1. Introduction

Rice is considered an important source of energy for populations from developed and developing countries. More than just energy, rice is a source of proteins, minerals, vitamins, and bioactive compounds (Monks et al., 2013). The most consumed rice is polished rice, which is industrially prepared by removing the pericarp and aleurone layers of rice caryopsis and the germ. Recently, studies have been performed in order to evaluate the amount and profile of bioactive compounds in pigmented and non-pigmented rice (Min, Gu, McClung, Bergman, & Chen, 2012; Paiva et al., 2014; Shen, Jin, Xiao, Lu, & Bao, 2009; Walter et al., 2013; Zaupa, Calani, Rio, Brighenti, & Pellegrini, 2015; Zhang, Shao, Bao, & Beta, 2015). These bioactive compounds included phenolic acids, anthocyanins, proanthocyanidins, tocopherols, and oryzanol. Phenolic compounds have been identified as compounds that present antioxidant, anti-inflammatory, anticarcinogenic and hypoglicaemic health benefits (Deng et al., 2013; Kim, Do, & Lee, 2006; Ling, Cheng, Ma, & Wang, 2001; Liu, 2007; Yawadio, Tanimori, & Morita, 2007).

The pigmented black, red and wild rice grains are rich sources of phenolic compounds while just a small amount of phenolic compounds are found in brown rice and polished rice (Paiva et al., 2014; Walter et al., 2013). Black and wild rice are important sources of anthocyanins and phenolic acids, while red rice is an important source of proanthocyanidins and phenolic acids. According to the results presented by Zaupa et al. (2015) and Zhang et al. (2015), the main phenolic acids found in both pigmented and non-pigmented rice are protocatechuic acid, synaptic acid, vanillic acid, *p*-coumaric acid, and ferulic acid.

Phenolic acids can be classified as free phenolic acids or bound phenolic acids (Renger & Steinhart, 2000). Free phenolic acids are extractable by a solvolytic solution, such as water, methanol, ethanol and acetone. The 80% methanol solution is the most used solvent for extracting free phenolics from rice samples; and two extraction steps are generally performed. On the other hand, bound phenolic acids are those present in the insoluble forms, which are covalently bound to structural components from the cell wall such as cellulose, hemicellulose (e.g. arabinoxylans), lignin, pectin, and rod-shaped structural proteins (Acosta-Estrada, Gutierrez-Uribe, & Serna-Saldivar, 2013). Bound phenolics are commonly extracted by adding a strong alkali to the residue obtained after the extraction of free phenolics. The alkaline hydrolysis promotes the increase in the viscosity of the residue, and the solubilization of the phenolics that are linked to the cell walls of rice caryopsis. Solubilized phenolics are then generally extracted from the viscous matrix by using ethyl acetate in a phase of

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http://dx.doi.org/10.1016/j.foodchem.2016.03.107
0308-8146/

separation procedure. The free and bound phenolic content is dependent on the rice genotype and processing (de Mira, Massaretto, Pascoal, & Marquez, 2009; Shen et al., 2009).

The extraction procedure used for the extraction of free and bound phenolics may be insufficient for a complete recovery of the phenolics distributed in rice. The solvolytic solution and the number of extraction steps adopted for the extraction of free phenolics are not standardized, in available literature. Moreover, literature data is limited about the effects of the solvolytic solvent and number of extraction steps for the recovery of free phenolics. Regarding bound phenolics, a great trouble arises from the alkaline hydrolysis treatment. The increase in matrix viscosity promoted by the alkali addition may hinder the extraction of the phenolics by an ethyl acetate solvent in the final step of the process. Zhou, Robards, Helliwell, and Blanchard (2004) reported that α-amylase pre-treatment of the residue from the extraction of free phenolics prior to an alkaline treatment facilitated the recovery of bound phenolics since α-amylase degrades α-1,4 linkages of starch polymers, reducing their viscosity. Literature data is also limited about the effects of enzymatic pre-treatment on the recovery of bound phenolics from rice. It is hypothesized that the content of free and bound phenolics from literature is underestimated.

The aim of this study was to evaluate the effects of the type of solvolytic solution and the number of extraction steps on the recovery of free phenolics, anthocyanins and proanthocyanidins from different rice samples. Moreover, the amount and profile of bound phenolic acids were determined as a function of enzymatic and/or alkaline hydrolysis treatment of the rice residue obtained after the extraction of free phenolics.

2. Materials and methods

2.1. Materials

Seven rice samples named black rice, red rice, wild rice, brown rice, long-grain polished rice, glutinous rice and short-grain polished rice were purchased from a local market from the city of Pelotas, State of Rio Grande do Sul, Brazil. The rice grains were immediately transported to the *Laboratório de Pós-Colheita, Industrialização e Qualidade de Grãos* of the *Universidade Federal de Pelotas* and stored at 16 °C under darkness until used for analyses. Rice samples were subjected to milling prior to analyses in the laboratory mill (Perten 3100, Perten Instruments, Sweden) equipped with a 35-Mesh sieve. The approximate composition of the rice samples was determined in accordance to the methods 46–13, 30–20 and 08–01 of the American Association of Cereal Chemists (AACC American Association of Cereal Chemists, 1995) for protein, fat, and ash content, respectively; and the results are presented in Table 1. All chemicals used in this study were of an analytical grade or better.

2.2. Free phenolic content as a function of the extraction solvent

The extraction of free phenolics was performed according to the method described by Qiu, Liu, and Beta (2010), with some modifications. Rice flour (2 g) was extracted twice with each of the following solvents at a ratio of 1:10 (w/v): (1) Acetone/Water/Acetic acid (70:29.5:0.5 v/v); (2) Acetone/Water (70:30 v/v); (3) Distilled water; (4) Ethanol; (5) Methanol; and (6) 80% Methanol. For each extraction the mixture was kept on a mechanical shaker (Certomat Biotech International) for 1 h at 150 rpm at room temperature. After centrifuging it (Eppendorf 5430-R) at 4000 rpm (1430g) for 5 min, the supernatants obtained from each extraction were combined and concentrated until dry by using a rotary evaporator at 35 °C. The dried extracts was redissolved in 20 mL of the tested solvent and used as crude extracts for total quantification of the free phenolics.

The content of the free phenolics of the mixture was evaluated by using the Folin–Ciocalteau method, with some modifications (Singleton & Rossi, 1965). Briefly, 100 μL of the properly diluted extracts were mixed with 400 μL of distilled water, 0.25 mL of 1 N Folin–Ciocalteau reagent, and then added to 7.5 g/100 mL of 1.25 mL of sodium carbonate. After reacting for 120 min, the absorbance of the mixture was measured at 725 nm (UV 17000 spectrophotometer, Shimadzu, Japan). Calibration curves were prepared for each one of the six solvents tested. Gallic acid was the standard compound used for calibration. Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of rice on a dry weight basis.

2.3. Free phenolic content as a function of the extraction steps

Free phenolics from black, red and wild rice samples were extracted with Acetone/Water (70:30 v/v) from 1 to 6 subsequent steps in order to evaluate the recovery of free phenolics as a function of the number of extraction steps. Acetone/Water (70:30 v/v) was chosen as a solvent since it was the most effective in extracting free phenolics. Black, red and wild rice samples were chosen because they were the most phenolic-rich samples. The extraction procedure and the quantification proceeded as previously described in paragraph 2.2., just varying the number of extraction steps.

2.4. Anthocyanin and proanthocyanidin content as a function of extraction solvent

The total anthocyanin and proanthocyanidin content in the rice samples were determined according to the spectrophotometric methods described by Abdel-Aal and Hucl (1999), and Porter, Hrstich, and Chan (1986), respectively. The same solvents were tested for both compounds: (1) Acetone/Water/Acetic acid (70:29.5:0.5 v/v); (2) Acetone/Water (70:30 v/v); (3) Distilled water; (4) Ethanol; (5) Methanol; and (6) 80% Methanol.

Anthocyanins were extracted from 0.5 g of rice flour using each of the reported solvents. 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. The absorbance was recorded at 535 nm (UV 17000 spectrophotometer, Shimadzu, Japan) and the total anthocyanin contents of the samples were calculated as mg of cyanidin-3-glucoside equivalents (Cy-3-G) per 100 g of the sample.

The proanthocyanidins (PAs) were quantified by adding 1 mL of each phenolic extract to 6 mL of butanol: water (95:5 v/v) containing 100 μL of 2% w/v solution of $NH_4Fe(SO)_2{\cdot}12H_2O$ in 2 M HCl. Glass tubes with screw caps were used. The material was mixed using a vortex and distributed in a water bath at 95 °C for 50 min. The absorbance was measured at 550 nm (UV 17000 spectrophotometer, Shimadzu, Japan). The quantification was performed based on a calibration curve of catechin. Results of a triplicate analysis were given as mg of catechin equivalents per 100 g of dry matter. The total PAs content was established as the sum of PA content in extracts.

2.5. Scanning electron microscopy

The pericarp, aleurone and endosperm layers of black, red and wild rice grains were analyzed by scanning electron microscopy in order to relate the grain structure to the differences in the phenolic content. Black, red and wild rice grains were cut into midsections and placed directly into vials containing 2%