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Effect of rice parboiling on the functional properties of an enzymatic extract from rice bran

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ABSTRACT

We have studied the influence of the parboiling process on enzymatic extract obtained from rice bran. Two rice bran enzymatic extracts have been obtained; one from non-parboiled (RBEE), the other from parboiled rice (RBEEp), and their chemical composition and antioxidant capacities have been compared. These extracts differ in their main chemical composition; RBEEp has less carbohydrates and more fat content than RBEE. No differences in protein content were found.

With regard to bioactive compounds, both extracts are rich in phytosterols, tocopherols, tocotrienols and γ-oryzanol, being γ-oryzanol similar in both extracts. However RBEEp is richer in total tocols and phytosterols and RBEE has the highest content in hydrophilic phenols. Functional properties such as total antioxidant activity revealed that RBEE has higher capacity to scavenger peroxyl radicals than RBEEp. Accordingly, specific antioxidant studies showed that RBEE has greater protective capacity against lipid and protein oxidation than RBEEp. Therefore, we can conclude that RBEE has better bioactive properties than RBEEp.

In conclusion, these findings suggest that the parboiling pretreatment of rice modifies the bioactive composition of derived products such as rice bran enzymatic extract, that have been shown to exert bioactive properties with application in the nutraceutical and cosmetic fields.

1. Introduction

There is an emerging interest in the use of agroalimentary byproducts as potential source of functional ingredients for nutraceutical, medicinal or cosmetic purposes. Rice is the most important staple crop, feeding more than half of the world's population and generating tons of waste (Sharif et al., 2014). Thus, rice bran, the greatest abundant byproduct obtained in the milling process is generated in large quantities and is underutilized despite having singular products with a biological significance (Hagl et al., 2013; Okai and Higashi-Okai, 2006), such as naturally-occurring antioxidant compounds (Goufo and Trindade, 2014). These antioxidants principally include γ-oryzanol, tocopherols, tocotrienols and polyphenols. Rice bran also contains high amounts of fiber and vitamin B complex. Moreover, proteins from rice bran have a high nutritional value, so balanced amino acid profile and hypoallergenic nature of bran protein are suggestive for its application in infant foods (Sharif et al., 2014). Nevertheless, several factors, including the presence of antinutrients and the lipid deterioration that starts soon after bran removal (Sharif et al., 2014), limit the nutritional use of rice bran. It is therefore necessary to find a good method for preventing the rapid deterioration of the bran, thus ensuring a quality material for further processing. Our group has developed an enzymatic method that inactivates the lipase, preventing rancidity and thus turning it into waste products that are rich in soluble compounds with applications in different fields (Parrado et al., 2006).

The new products obtained from rice bran are water-soluble rice

Abbreviations: AAPH, 2-2′-azobis-(2-amidinopropane)-hydrochloride; CH, cumene hydroperoxide; DMACA, p-dimethylaminocinnamaldehyde; DNPH, dinitrophenylhydrazine; MDA, malondialdehyde; ORAC, Oxygen radical absorbance capacity assay; PBS, phosphate buffer; RB, untreated rice bran; RBp, parboiled rice bran; RBEE, rice bran enzymatic extract; RBEEp, parboiled rice bran enzymatic extract.

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bran enzymatic extracts (RBEE) which preserve functional properties and improve both the proteins solubility and the rice bran antioxidant components (Parrado et al., 2006). The enzymatic treatment also increases the concentration of minor functional components making the RBEE rich in bioactive compounds (Parrado et al., 2003). We have previously described that RBEE has antioxidant properties (Santa María et al., 2010) and hypocholesterolemic activity (Revilla et al., 2009), as well as antiproliferative and inmunoactivatory abilities (Revilla et al., 2013). Moreover, it has shown beneficial activities against hyperinsulinemia and hypertension, restoring endothelial function and vascular contractility in obese Zucker rats (Justo et al., 2013a, 2013b).

Traditionally, rice is consumed as polished white rice with the husk, bran, and germ fractions removed. However, there is another variation of white rice called parboiled (*par*tially *boiled)* rice, where the hulled rice is hydrated and steamed in order to retain the nutrition of the bran within the rice grain. Parboiled rice is widely used in cooking as the process alters the nature of the starch, resulting in transparent grains that will be less sticky and more separate when cooked. The aim of the current study was to evaluate how the parboiling process affects the chemical composition and antioxidant capacity of a rice bran extract obtained according to our enzymatic protocol.

2. Materials and methods

2.1. Preparation of rice bran

For this work it has been selected two types of rice bran (*Oryza sativa, var. indica*): untreated rice bran (RB) and parboiled rice bran (RBp), provided by Herba Ricemills, S.L.U (Sevilla, Spain). RB is obtained during the polishing/milling of raw rice grains once they have been stripped of the husk. It looks similar to flour, with a slightly granular texture, a light/beige brown color and it has a smell reminiscent of rice. Parboiled rice bran differs from untreated rice bran in one respect only; the grains have undergone a steaming process. This process consists of four consecutive steps: gross grain is soaked in water at 50–70 °C for 3–4 h and drained of the free water; steam and hot water are introduced into the cooking vessel and the rice is kept under pressure to gelatinize the starch in the rice grain and finally the grain is dried with hot air. Once dry, the grain undergoes the same process of husking and polishing/milling as the untreated white rice.

2.2. Preparation of enzymatic extracts

RBEE and RBEEp were prepared according to an enzymatic process previously described by our group (Parrado et al., 2006). Briefly, both types of rice brans were modified by enzymatic hydrolysis, using subtilisin (EC 3.4.21.62), a protease from *Bacillus licheniformis* as hydrolytic agent (Biocom, Spain) and a bioreactor at controlled temperature of 60 °C and pH 8. The processing of this product follows different steps, including centrifugation, filtration, and concentration. The final product is brown syrup that is completely water-soluble.

2.3. Chemical characterization

Rice bran and ezymatic extracts were chemically characterized using the AOAC standard protocols (1990). Total protein content was determined by method 954.01. Total fat content was determined by method 920.39. Fatty acids were determined by method 969.33 and total ash content was determined by method 942.05.

Carbohydrates were determined by HPLC as previously described (Zhang et al., 2003). Lipids containing γ-oryzanol were extracted using ethylacetate: hexane (1:1). The γ-oryzanol components were separated and quantified by analytical reversed-phase HPLC (Miller et al., 2003). Concentrations of tocopherols and tocotrienols isomers in RBEE were determined according to IUPAC standard method 2432 (IUPAC, 1987). Tocopherols and tocotrienols were purified using a silica column. The oil sample was diluted with hexane and an aliquot of the diluted sample was subjected to HPLC analysis using a fluorescence detector (Agilent Technologies, USA) with a silica column packing with LiChrosorb SI 60.

2.4. Gaseous chromatography analysis for phytosterols

The phytosterols concentrations were determined by gas chromatography using 5-cholestane as an internal standard. A gas chromatograph (Varian 3800; Varian Inc., Walnut Creek, CA, USA) was equipped with an SAC-5 fused-silica capillary column (30 m 60.32 mm, Supelco, Bellefonte, PA, USA) and a flame ionization detector. The column was held at 280 °C for 1 min and programmed to rise to 300 °C at a rate of 2°C/min. It was then held at 300 °C for 20 min. The carrier gas was helium and the total gas flow rate was 20 mL/min. Injector and detector temperatures were 310 °C and 320 °C, respectively. Comparing the retention times with standard times enabled the sterols to be identified.

2.5. Analyses of phenolics by spectrophotometry

The spectrophotometric determination of total flavonols, flavanols and phenolic content of RBEE and RBEEp were performed with a Hewlett-Packard UV–vis HP8453 spectrophotometer (Palo Alto, CA, USA), using 10-mm path length glass cells and distilled water as reference. For this purpose, 5 mg of samples (RBEE and RBEEp, respectively) were dissolved in 2 mL of ultrapure water and filtered through Millipore-AP 20 filters (Bedford, MA) prior to the spectrophotometric analysis. Total flavanols content was determined following a modification of the method described by Vivas et al. (1994). Ten microlitres of supernatant was mixed with 190 μL of methanol and 1 mL of p-dimethylaminocinnamaldehyde (DMACA) reagent. The absorbance was recorded at 640 nm after 10 min of reaction. A calibration curve of (+)-catechin was used for quantification. Results were expressed as mg of flavanols (expressed as catechin equivalents) per g of dry weight.

Total flavonols content was determined using a modification of the method originally described by Glories for wine phenols (Glories, 1979). The method consisted of placing 0.25 mL of sample or standard in a test tube and adding 0.25 mL of 0.1% HCl in 95% ethanol and 4.55 mL of 2% HCl. The solution was mixed and allowed to sit for approximately 15 min before reading the absorbance at 360 nm. Standard used was quercetin in 95% ethanol and the results were expressed as mg of flavonols (expressed as quercetin equivalents) per g of dry weight.

Total phenolics content was determined using a modification of the Folin-Ciocalteau method (Singleton and Rossi, 1965). Briefly, 0.25 mL of sample, 1.25 mL of Folin-Ciocalteu reagent, and 3.75 mL of a solution of sodium carbonate at 20% were mixed, and distilled water was added to make up a total volume of 25 mL. The solution was homogenized and left to stand for 120 min for the reaction to take place and stabilize. Absorbance was measured at 765 nm. Gallic acid was used as a calibration standard, and results were expressed as mg of polyphenol (expressed as gallic acid equivalents) perg of dry weight.