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Effect of particle size of rice bran on gamma-oryzanol content and compounds

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The reduction of particle size can facilitate the extraction of phytochemical compounds. In this study was evaluated the effect of rice bran particles size in the yield and characteristics of γ-oryzanol compound. The γ-oryzanol extraction was realized with hexane and isopropanol solvents and quantification by spectrophotometric method. The γ-oryzanol extracts were characterized in relation of theirs majority components in HPLC-UV and the antioxidant capacity verified by the free radical DPPH● consumption. The γ-oryzanol yield varied of 0.10–1.54 mg/g of bran, and the highest yield was obtained in particles smaller than 0.39 mm. The γ-oryzanol majority components presence (cycloartenyl ferulate, 2,4-methylenecycloartanyl ferulate, campesteryl ferulate e β-sitosteryl ferulate) in the extracts was confirmed and verified differences in the profile of this components in function of different particles sizes. The γ-oryzanol extract obtained from particle sizes between 0.73 and 1.67 mm demonstrated most specific inhibition of DPPH radical (6.7%) and IC_{50} 6.63 μg/mL. When the particle size is reduced, the access surface to the extraction solvent is increased resulting in more γ-oryzanol extraction, however the extract from larger particles was more efficient as antioxidant.

1. Introduction

The large amount of co-products, generated in agricultural industry, arouse interest in exploiting them to obtain phytochemicals and antioxidants, it is extremely important to use the agro-industrial co-products in efficient form, adding value to them and reducing the environmental impact. The interest in natural foods has required research to identify natural antioxidants focusing on its extraction.

In the productive chain of rice (*Oryza sativa*), one of the most produced and consumed grain, there is a high amount of by-products. Each 100 kg of rice in husk generate about 5–10 kg of bran, which has conventional utilization to animal feed and oil extraction (Burlando and Cornara, 2014).

However, rice bran is a rich source of oil that due to its unsaponifiable fraction with antioxidant as γ-oryzanol, gives to rice oil more resistance in oxidation and in deterioration γ-oryzanol. The γ-oryzanol, an example of natural compound and antioxidant, it can be extracted from rice bran, it is a mixture of ferulic acid esters and exercises functions in cholesterol reduction (Cicero and Gaddi, 2001), being anticancer (Yasukawa et al., 1998), anti-inflammatory (Islam et al., 2008), antidiabetic (Son et al., 2011) and antioxidant (Winkler-Moser et al., 2012). This mixture of compounds has shown to be promising in the development of functional foods and industrial application to stabilize oils and fats (Lerma-Garcia et al., 2009).

The γ-oryzanol antioxidant potential is due to ability hydrogen donation of ferulic acid phenolic group (Nystrom et al., 2005). Ferulic acid esterified plant sterols, such as the γ-oryzanol, increases the antioxidant potential promoting molecular access to hydrophobic components that are more susceptible to oxidative cellular destruction, because the antioxidant potential increases with the extent of hydroxylation of aromatic rings (Graf, 1992).

The efficiency of solid/liquid extraction processes is affected by critical processing parameters, such as temperature, solvent nature, the solid matrix structure (especially size particle) and extraction time (Franco et al., 2007).

Antioxidants are responsible for inhibition and free radicals

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reduction in cell (Liochev, 2013), and for this it is necessary same characteristics to be considered a good antioxidant, as have substituents hydrogen or electrons donor to the radical, due to its reduction potential; having displacement capability of radical formed in its structure and to chelate transition metals involved in the oxidative process; and have access to the site of action, depending on their hydrophilic or lipophilic (Manach et al., 2004).

This study had the objective to evaluate for the first time the extraction yield of γ-oryzanol in different particle sizes of rice bran and characterize the extracts in relation to the majority components profile and their antioxidant properties.

2. Material and methods

The rice bran (RB) was provided by industries that benefit rice from Rio Grande do Sul, Brazil. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) standard was purchased from Sigma Aldrich, USA and the γ-oryzanol standard from Wako Chemicals, USA (purity>98%).

To obtain the different particle sizes of rice bran, it was classified in sieves with opening sizes 0.73 mm, 0.50 mm, 0.39 mm and lower than 0.39 mm, respectively. One sieves with opening of 1.67 mm was coupled above the rest sieves to retain particle larger than 1.67 mm. The particle morphology was visualized by scanning electron microscopy (SEM), where the samples were covered with a driver, in this case gold, by sputtering and then analyzed in the SEM. The analysis of the samples surfaces were realized under vacuum with a 10 kV accelerating voltage.

2.1. γ-oryzanol extraction

The extraction of γ-oryzanol was realized, in rice bran in different granulometry fractions, according Heidtmann-Bemvenuti et al. (2012). For γ-oryzanol quantification, was realized extract dilution in isopropanol and reading in a spectrophotometer at a wavelength of 326 nm, employing calibration curve with standard concentrations of γ-oryzanol between 3 and 20 μg/mL, which was diluted in isopropanol.

2.1.1. Major components profile of γ-oryzanol

The confirmation of the major components in the γ-oryzanol was performed on high performance liquid chromatograph with ultraviolet detector (HPLC-UV) using the γ-oryzanol standard (30 μg/mL) and γ-oryzanol extracts from different granulometry fractions (30 μg/mL) under the same conditions of Paucar-Menacho et al. (2007). The elution of components of the mixture was performed with acetonitrile:methanol: isopropanol 50:45:5 (v/v/v) at flow rate of 1.0 mL/min, using as stationary phase C18 column (10 μm) 250 × 4.6 mm and detection was performed at a wavelength of 315 nm. The majority four peaks correspond, respectively to cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and β-sitosteryl ferulate.

2.2. Antioxidant activity

The antioxidant capacity of γ-oryzanol extracts obtained from rice bran in different particle sizes (3 size fractions) were checked by methods of capture free radical DPPH•, which the consumption of the free radical DPPH• by γ-oryzanol extracts was determined from the decrease in absorbance units. The measurements were performed in spectrophotometer UV–Vis wavelength of 515 nm. For the γ-oryzanol concentrations used in the assays antioxidant, the extracts and standard were diluted in isopropanol. In tubes containing 3.0 mL of methanolic DPPH solution was added 0.5 mL of methanol (control) and 0.5 mL of antioxidants: γ-oryzanol extracts (5, 10 and 25 μg/mL) and standard γ-oryzanol (5, 10 and 25 μg/mL). The reactive mixture was left at room temperature without the incidence of light and the change of violet to yellow color was measured after 0, 15, 30, 45, 60, 90, 120, 150 and 180 min reaction (Herrero et al., 2005). The ability to scavenge free radical was expressed as percentage specific inhibition (SI) (% inhibition/$\mu g_{oryzanol}$) and it was calculated by Equation (2), by the ratio between the percentage of radical oxidation inhibition (I) calculated by Equation (1) and the concentration of γ-oryzanol.

$$I = \left[\frac{(\text{AbsDPPH} - \text{AbsExt})}{\text{AbsDPPH}}\right] * 100 \quad (1)$$

where Abs_{DPPH} is the absorbance of DPPH solution (control) and Abs_{Extr} is the absorbance of the sample solution.

$$SI = \frac{I}{\gamma - \text{oryzanol concentration}} \quad (2)$$

The absorbances were plotted against the solutions concentration to obtain the amount of antioxidant needed to decrease the initial concentration of DPPH by 50% (IC_{50}).

2.3. Statistical analyses

For the purpose to evaluate the significance of differences of the dependent variables (γ-oryzanol, γ-oryzanol major components and antioxidant activities) from different particle sizes rice bran and reaction time (independent variable) was used analysis of variance (ANOVA), followed by the means differences Tukey test, using Statistica 6.0 software. Differences with probability value of $p < 0.05$ were considered significant, and the data were presented as mean ± standard deviation. Multivariate analysis was performed using the software PAST (folk.uio.no/ohammer/past), where the principal component analysis (PCA) was applied to the data set after normalization by Pearson correlation matrix. The PCA were performed to establish the correlation of γ-oryzanol major components with the antioxidant activity.

3. Results and discussion

3.1. γ-oryzanol

The γ-oryzanol yield from rice bran in different particle sizes varied of 0.10–1.54 mg/g of bran (Fig. 1).

The best yield of γ-oryzanol extraction was obtained in fraction of rice bran with particles sizes lower than 0.39 mm and fractions with particles sizes between 0.73 and 1.67 mm obtained lower amounts. The increase of γ-oryzanol yield with reduction of granulometry is according Pinelo et al. (2007) and Wanyo et al. (2014), who showed that reducing the particle size can increase the extraction of certain bioactive compounds. The yield of 1.54 $mg_{oryzanol}/g_{bran}$ verified in the bottom fraction (<0.39 mm) was similar the yield obtained in Xu and Godber (2000) study, which was evaluated the extraction efficiency of γ-oryzanol from rice bran and the best yield result was 1.68 $mg_{oryzanol}/g_{bran}$ and concentration 11.8 $mg_{oryzanol}/g_{extract}$.

Microscopic analysis (Fig. 2) showed the particles had an almost planar shape, with low sphericity. Particles sizes lower than 0.39 mm showed increase in surface particle deposits, this may be due to the bran milling process which damages cell wall, leading to the displacement of microparticles from the internal part of the vegetable matrix to its surface (Santos et al., 2015), which facilitate the removal the cell content.

The images analysis obtained in SEM show evidences that the increase of γ-oryzanol extraction yields can be explained by the