Stabilization of rice bran and its effect on bioactive compounds content, antioxidant activity and storage stability during infrared radiation heating

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ABSTRACT

Rice bran (RB), a by-product of rice milling industry has limited uses despite its nutritional value. Thus, the aim of the current study was to improve the process of RB stabilization and it was conducted in three stages a) optimization of the infrared radiation (IR) conditions in order to inactivate lipase activity b) investigation of the effect of IR treatments on γ-oryzanol, vitamin E, total phenolic contents and antioxidant activity of stabilized RB as well as on color and fatty acid composition c) stability tests of free fatty acid (FFA) content and lipophilic compounds. Results indicated that IR heating at 140 °C for 15 min is an effective method for RB stabilization without any loss of γ-oryzanol and fatty acid composition, but with a significant decrease in vitamin E content. Contrary, phenolic content and antioxidant activity of bound extracts of stabilized RB increased as the IR power increased, whereas an insignificant increase was observed on free extracts. Storage stability tests showed that γ-oryzanol of stabilized RB was relatively less stable after 6-months storage than vitamin E. In conclusion, the selection of optimum IR conditions is important to guarantee the quality RB in terms of health beneficial components and undesirable components.

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1. Introduction

Rice bran (RB), a by-product of rice milling with highest nutritional value, corresponds to approximately 10% of the weight of brown rice. It is source of proteins, dietetic fibers and functional compounds such as γ-oryzanol, tocopherols and tocotrienols. γ-oryzanol, often identified as the active molecule of RB oil, is mainly composed of the ferulic acid esters of triterpene alcohols and plant sterols. It has shown to have a cholesterol reducing effect in both humans and experimental animals (Perez-Ternero et al., 2017).

Despite its excellent health benefits, the great majority of RB is just used as animal feed. Human diet utilization of RB is restricted due to rapid rancidity issues that starts with the milling operation. RB is sensitive to rancidity during storage due to its large amount of lipids as well as the presence of hydrolytic and oxidative enzymes mainly lipases, which are primarily responsible for the hydrolysis of triglycerides into glycerol and free fatty acids (FFA). Hence, it is necessary to stabilize the RB by suitable methods improving its

shelf life at ambient conditions, without damaging its nutritional profile.

Many approaches on the RB stabilization have been developed namely hydrothermal treatment (Thanonkaew et al., 2012; Pradeep et al., 2014), toasting (Silva et al., 2006), extrusion (Sharma et al., 2004), microwave treatment (Patil et al., 2016), ohmic heating (Dhingra et al., 2012) and infrared radiations (Yilmaz et al., 2014; Wang et al., 2017). Infrared radiation (IR) has been considered as a potential technique for food processing, since the heat energy of IR can be absorbed by food materials directly. It has many advantages compared to conventional heating technology, including uniform heating, versatility, simple required equipment, short heating time, low quality losses and energy consumption (Kathiravan et al., 2007).

Few studies are focused on the effect of IR on nutraceutical profile of RB during stabilization and storage. Yilmaz et al. (2014) reported that lipases in RB inactivated with short wave IR for 6 months storage without loss of γ-oryzanol content or fatty acid composition, however, degradation of tocopherols are reported. Similarly, Yilmaz and Tuncel (2015) found insignificant change on chemical composition and phenolic content of IR stabilized RB

compared to an untreated. On the other hand, Rodchuajeen, et al. (2016), studied the stability of γ-oryzanol and phenolic compounds of RB after exposure to IR and found an insignificant effect on γ-oryzanol after 90-days storage. However, we could not find literature reports concerning the stability of tocopherols and tocotrienols during storage of RB treated with IR.

Hence the objective of our study mainly focused on optimization of IR heating parameters (temperature and time of exposure) for RB stabilization in respect to minimize the FFA content and to investigate the effect of IR stabilization on γ-oryzanol and vitamin E content during 6 months of storage period. The fatty acids composition, the color values and phenolic content as well as antioxidant activity of IR treated RB samples were evaluated and compared to rough RB.

2. Materials and methods

2.1. Sample preparation

RB was obtained in lab scale after dehulling and polishing progresses of paddy rice (*Oryzae sativa* L., cultivar: Axios-Long A type). Subsequently, freshly RB was stabilized immediately with 12 different treatments, packed in aluminum foil vacuum-sealed bags and stored in a laboratory chamber at 20 °C.

2.2. Optimization of IR heating

For the optimization of IR stabilization conditions for RB, a custom device with an aluminum body was developed and constructed at DEMETER's engineering department (Supplementary Fig. 1). It was consisted of radiator with two infrared lambs (single tube 1200 W) connected to a series with a maximum power of 2400 W adjusting the temperature with a connected thermostat and an aluminum tray adapted on a compact shaker. The RB is loaded on the shaking aluminum tray and the distance was set to 15 cm above the lambs. The temperature and exposure time levels were selected on the basis of preliminary trials. The IR treatments were namely; T1 (100 °C, 5 min), T2 (100 °C, 10 min), T3 (100 °C, 15 min), T4 (100 °C, 20 min), T5 (120 °C, 5 min), T6 (120 °C, 5 min), T7 (120 °C, 15 min), T8 (120 °C, 20 min), T9 (140 °C, 5 min), T10 (140 °C, 10 min), T11 (140 °C, 15 min) and T12 (140 °C, 20 min). Untreated raw RB was used as a control. All experiments were performed twice.

2.3. FFA content

RB samples were taken at 0, 1, 2, 3, 4, 5 and 6 months of storage, and the FFA content was determined using a standard titration method (AACC, 2000). The FFA content was calculated as oleic acid equivalent, expressed as percentage of total lipids.

2.4. Peroxide value

Peroxide value was determined following AOAC official method and expressed in meq/kg oil. (AOAC, 2000).

2.5. Storage studies

The storage studies were performed as follows: five samples (T0, T6, T8, T10 and T12) were chosen according to the best results of free FFA content. Each of them was stored in a laboratory chamber at 20 °C for up to 6 months. The RB samples were analyzed for tocotrienols, tocopherols and γ-oryzanol contents at 1-month intervals.

2.6. Color measurements

Color values were measured using a colorimeter (HunterLab, model MiniScan XE Plus, Virginia, USA). The CIE color values were recorded as L* (lightness), a* (redness) and b* (yellowness). The total color differences (ΔE) between the control and treated samples were calculated using the equation: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ (Yueh et al., 2002).

2.7. Analysis of vitamin E

The tocopherols and tocotrienols contents (vitamin E) in the RB samples were determined according to Irakli et al. (2016) with some modifications: 0.5 g sample was extracted with acetonitrile (10 mL) by sonication and the extract was collected after centrifugation at $1500 \times g$ for 10 min. The same procedure repeated twice. The collected supernatants were evaporated, the remaining residue was redissolved in 1 mL acetonitrile/methanol (85:15, v/v) and finally a 20 μL aliquot was injected into the HPLC system (Agilent technologies, 1200 series, Urdorf, Switzerland). A YMC C_{30} column (250×4.6 mm id, 3 μm, MZ Analysentechnik, Mainz, Germany) was used and the mobile phase was consisted of acetonitrile (A), methanol (B) and dichloromethane (C). The linear gradient elution was 0 min, 85% A and 15% B; 5 min, 65% A and 35% B; 10 min, 10% A, 85% B and 5% C; 15 min, 30% A, 40% B and 30% C. Tocopherols and tocotrienols isomers were detected by fluorescence detector with excitation and emission wavelengths at 290 and 320 nm, respectively.

2.8. Analysis of γ-oryzanol

The same RB extract was used for the determination of γ-oryzanol content as vitamin E analysis. The separation was carried out using Target C_{18} (4.6×150 mm, 5 μm, MZ Analysentechnik, Mainz, Germany) column at 30 °C with mobile phase consisted of acetonitrile/methanol/dichloromethane (40:45:15, v/v/v) under isocratic conditions at a flow rate of 1.5 mL/min γ-oryzanol was detected at 330 nm. Quantitation was based on a linear calibration curve of the sum of the areas of four curves of γ-oryzanol standard namely cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and sitosteryl ferulate as were analysed by HPLC and confirmed by nuclear magnetic resonance (NMR) and MS by others researchers (Fang et al., 2003).

2.9. Analysis of fatty acids composition

Fatty acid composition of the RB oils extracted with ether was determined by gas chromatography (GC) (Model Varian CP-3800, equipped with flame ionization detector) according to AOAC 996.06. Individual fatty acids were identified by comparison of their retention times with those of external standard (Supelco 37 Component FAME Mix). The amounts of fatty acids identified were expressed in % of the total fatty acid areas of chromatograms identified.

2.10. Extraction and estimation of soluble and bound polyphenols

For soluble polyphenol estimation, RB samples were extracted with 60% methanol and centrifuged, supernatant was filtered through filter paper and the filtrate was stored in the freezer until analysis. The residue obtained was extracted with 4N NaOH and used for bound polyphenol estimation and analysed by Folin Ciocalteau's reagent at 760 nm using gallic acid as standard (Irakli et al., 2015). The results were expressed as mg of GA equivalents (GAE) per 100 g of RB (mg GAE/100 g).