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Review

Maximising the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions

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ABSTRACT

Germinated brown rice (GBR) is considered a healthy alternative to white rice in the fight against chronic diseases. As the functional quality of GBR depends on genotype and germination conditions, the objectives were to identify suitable Ecuadorian brown rice cultivars and optimal germination time and temperature to maximise γ-aminobutyric acid (GABA), total phenolics compounds (TPC) and antioxidant activity of GBR. Regression models for the prediction of phytochemical composition and antioxidant activity in GBR were also obtained. Germination improved GABA, TPC and antioxidant activity in all cultivars. Maximum GABA and antioxidant activity were attained at 34 °C for 96 h, while the highest TPC was found at 28 °C for 96 h in all cultivars. GBR cv. GO displayed the highest antioxidant activity and cv. 15 was the most effective at accumulating GABA and TPC in the optimal germination conditions. Therefore, Ecuadorian GBR could be used for the preparation of functional foods serving as preventative strategies in combating chronic diseases.

Contents

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1. Introduction

Rice is the most widely consumed cereal grain for a large part of the world's human population. It is the second most produced cereal in the world after maize, (FAOSTAT, 2013). Rice is also the largest crop in Ecuador where long-grain varieties with greater resistance to diseases and pests, high yields and resistance to postharvest are mostly grown. Ecuadorian rice production is increasing gradually and, although rice is the staple food in this region, there has been an overproduction in 2010, and this overproduction could occur again in the future. Therefore, alternatives that diversify the application of rice in human nutrition and improve its nutritional value are required.

Brown rice (BR) is composed of external thin layers (bran) that enclose the embryo and endosperm. The nutritional components in BR mainly exit in the germ and bran layers which are mostly removed as a consequence of milling or polishing (Monks et al., 2013). For this reason, BR has a higher nutritional quality than polished rice. Recently, human and animal studies have shown that consumption of BR reduces the risk of type-2 diabetes, cardiovascular disease (CVD) and cancer, and these protective health effects have been linked to the presence of bioactive compounds such as polyphenols, GABA, acylated steryl β-glucoside and γ-oryzanol (Zhang et al., 2010; Kim, Kang, Nam, & Friedman, 2012; Goffman & Bergman, 2004).

Germination is a low-cost technology which starts with seed water uptake and ends at the protrusion of radicle from the seed. Reactivation of metabolism occurs during the seed germination process which results in the hydrolysis of storage proteins and carbohydrates and the synthesis/accumulation of metabolites with health-promoting properties. Germination of BR increases the content of γ-aminobutyric acid (GABA) and antioxidants, such as phenolic compounds, γ-oryzanol and vitamin E among other bioactive compounds (Kim, Hwang et al., 2012). GABA exerts a series of health-promoting effects, such as regulation of blood pressure and heart rate, alleviation of pain, anxiety and sleeplessness (Ito, 2004). In addition, GBR extract with enhanced levels of GABA stimulates immune cells (Oh & Oh, 2003) and it inhibits cancer cell proliferation (Oh & Oh, 2004). More recently, studies show that GABA is also a strong secretagogue of insulin in the pancreas and effectively prevents diabetes (Imam, Azmi, Bhanger, Ismail, & Ismail, 2012). Polyphenols have a wide range of biological activities which are linked to their protective effects on oxidative stress-induced diseases as shown in several epidemiological studies (Arts & Hollman, 2005). Recently, Esa, Abdul-Kadir, Amon, and Azlan (2013) have demonstrated that attenuation of oxidative stress by germinated brown rice (GBR) consumption is reached through increases in antioxidant levels in plasma and antioxidant enzyme activity in the liver, thereby, preventing the formation of atherosclerotic plaques in hypercholesterolemic rabbits.

Accumulation of bioactive compounds during BR germination was shown to vary greatly depending on the cultivar, pH, presence of additives and aeration of the soaking solution temperature and time during the phase of water uptake (also known as soaking or steeping), germination and post-germination seedling growth (Watcharatparpaiboon, Laohakunjit, & Kerdchoechuen, 2010). These facts clearly indicate the relevance of cultivar selection and optimisation of germination conditions before planning strategies of designing a functional food for improving consumer's health. Previous studies have focused on optimisation of the germination process to maximise the nutritional quality of GBR (Rusydi, Noraliza, Azrina, & Zulkhairi, 2011). So far, little has been reported about the optimisation of soaking and germination conditions to produce GBR with improved phytochemical content and antioxidant activity. Thus, we have focused this work on the optimisation of the phytochemical load (GABA and phenolic compounds) and antioxidant activity of sprouts from different commercial Ecuadorian BR cultivars.

The objectives of the present study were to evaluate the effect of germination time and temperature of BR on potential health-promoting phytochemicals (GABA and TPC), to evaluate the antioxidant activity to assess suitable rice cultivars and to optimise germination time and temperature in relation to concentrations of these bioactives and antioxidant activity in BR sprouts. Moreover, this study shows model equations that predict the phytochemical composition and antioxidant activity of BR sprouts based on germination time and temperature.

2. Material and methods

2.1. Plant materials

Commercial certified BR cultivars INIAP 14, INIAP 15 and INIAP 17 (coded cv. 14, cv. 15 and cv. 17) and experimental cultivar GO39839 (coded cv. GO) were provided by the National Autonomous Institute of Agricultural Research, Ecuador (Instituto Autónomo de Investigaciones Agropecuarias, INIAP). All varieties had similar harvest yields and seed appearance was translucent, white centre with extra-long grains.

2.2. Chemicals and reagents

Liquid chromatography (LC)-grade acetonitrile and methanol were purchased from Lab-Scan (Gliwice, Poland). Analytical grade methanol was provided by Scharlau (Barcelona, Spain). Other chemical reagents and standards used were purchased from Sigma–Aldrich (Steinheim, Germany). Water was purified using a Milli-Q system (Millipore Billerica, MA, USA).

2.3. Seed germination

BR seeds of each cultivar (50 g) were rinsed in distilled water and surface sterilised by 0.1% sodium hypochlorite (seed:NaOCl ratio, 1:5 w/v) for 30 min and drained. Afterwards, hygienised grains were rinsed with sterile distilled water to neutral pH. Seeds were then placed in deionised water (seed:water ratio, 1:5 w/v) and soaked at 28 °C for 24 h. Soaking water was drained and the seeds were placed on a drilled grille over moist filter. Seed were covered by moist filter paper and the grille was placed in plastic germination trays containing distilled water. Germination trays containing hydrated rice seeds were introduced in a germination cabinet (model EC00-065, Snijders Scientific, Netherlands) provided with a water circulating system to keep 90% air humidity. Germination was carried out at 28 and 34 °C in darkness for 48 and 96 h. Germination percentage was calculated as an estimation of seed viability and the germinated percentage was calculated from the following equation: $GP = (\text{GBR seeds}/\text{total BR seeds}) \times 100$. GBR seeds were those with the radical projected from the embryo. Finally, GBR samples were freeze-dried (Virtis Company, INC. Gardiner, NY, USA), and homogenised by using a ball mill (Glen Creston Ltd., Stanmore, UK). Powdered samples were stored in plastic bags, under vacuum, in darkness at 4 °C until further analysis. Each cultivar had three replications for each germination condition.

2.4. Determination of γ-aminobutyric acid

The content of γ-aminobutyric acid (GABA) was determined using reversed-phase high performance liquid chromatography as described previously (Torino et al., 2013). Briefly, 0.5 g of sample was suspended in 12 ml distilled water. The suspension was stirred