Food Chemistry 173 (2015) 298–304

Contents lists available at ScienceDirect

Food Chemistry

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

Effects of germination on the nutritive value and bioactive compounds of brown rice breads

Fabiola Cornejo [a,b,1], Patricio J. Caceres [b,c,1], Cristina Martínez-Villaluenga [c], Cristina M. Rosell [a,*], Juana Frias [c]

[a] Institute of Agrochemistry and Food Technology (IATA-CSIC), Avenida Agustín Escardino, 7. Paterna 46980, Valencia, Spain
[b] Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ingeniería Mecánica y Ciencias de la Producción, Campus Gustavo Galindo Km 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador
[c] Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form 28 July 2014
Accepted 7 October 2014
Available online 14 October 2014

Keywords:
Brown rice
Germination
Nutritive value
Gluten free

ABSTRACT

The effect of germination conditions on the nutritional benefits of germinated brown rice flour (GBR) bread has been determined. The proximate composition, phytic acid, *in vitro* protein digestibility and *in vitro* enzymatic hydrolysis of starch, glucose and starch content, as well as the most relevant bioactive compounds (GABA, γ-oryzanol and total phenolic compounds) and antioxidant activity of breads prepared with GBR at different germination conditions was determined. When comparing different germination times (0 h, 12 h, 24 h and 48 h), germination for 48 h provides GBR bread with nutritionally superior quality on the basis of its higher content of protein, lipids and bioactive compounds (GABA and polyphenols), increased antioxidant activity and reduced phytic acid content and glycaemic index, although a slight decrease in *in vitro* protein digestibility was detected. Overall, germination seems to be a natural and sustainable way to improving the nutritional quality of gluten-free rice breads.

1. Introduction

In the last decade, the use of brown rice (BR) has increased in both standard diets and in those diets catered to people with celiac disease or allergies to other cereals. In addition, the germination of BR grains provides higher nutritional and functional values since they are associated with the quality and quantity of their nutrients, biologically active compounds and antioxidant potential. Currently consumers demand natural foods, and sprout products have become increasingly popular among people interested in improving and maintaining their health by changing dietary habits. In this scenario, sprouted BR grains are excellent examples of functional food, because besides their nutritive value they lower the risk of various diseases and/or exert health promoting effects.

Germinated brown rice (GBR) is considered as a gluten-free grain characterised by an excellent nutrient profile and germination greatly enhances the content of bioactive compounds, such as GABA (γ-aminobutyric acid), phenolic compounds, γ-oryzanol and the antioxidant activity (Caceres et al., 2014). For instance, while the consumption of rice is associated with diabetes mellitus due to its high glycaemic index, GBR takes a leading role against diabetics and at the same time, a reduction on phytic acid is achieved enhancing mineral availability (Kim et al., 2012).

Scientific research supports the beneficial effects of these bioactive compounds, which includes regulation of blood pressure and heart rate, alleviation of pain and anxiety, improves sleeplessness and autonomic disorders associated with menopausal or presenile periods, suppresses liver damage, inhibits cancer cell proliferation and protects against oxidative stress (Oh & Oh, 2004). In Japan, GBR was launched to the market in 1995. Since then, the popularity of GBR is increasing within the Japanese population and, simultaneously, numerous derived food products have increased. Consequently, the use of GBR as a functional ingredient has caught the attention of researchers studying changes in nutritional composition and bioactivity. Thus, there is an increasing trend focusing on the use of GBR in the formulation of high quality health products. In this scenario, GBR is used as a raw material for obtaining different food products, like GBR balls, soup, bread, doughnuts, cookies and rice burgers (Ito S, 2004).

Bread is a staple food in many parts of the world providing most calories in the diet. Bread is mostly prepared from wheat flour, which makes it unsuitable for people suffering from celiac disease patients, which is a lifelong disorder with a prevalence of 1% of the

* Corresponding author. Tel.: +34 963900022; fax: +34 963636301.
E-mail address: crosell@iata.csic.es (C.M. Rosell).
[1] These authors have made equal contribution to the work.

http://dx.doi.org/10.1016/j.foodchem.2014.10.037
0308-8146/

world population. The only acceptable treatment is the restriction of gluten from the diet and, therefore, GBR bread is an attractive healthy alternative for this group of patients. The availability of palatable BR-containing gluten-free products would represent a significant advance towards ensuring an adequate intake of nutrients and bioactive compounds, mostly for subjects with celiac disease, but also for general consumers. Accordingly, developing bread based on GBR with desirable nutritional quality and providing bioactive compounds is worthy of investigation.

To date, experimental GBR breads have been characterised with adequate instrumental and sensory attributes (Cornejo & Rosell C.M., 2014). However, to our knowledge, investigations on the effect of germination conditions on the nutritive composition of BR-bread are very limited. Therefore, the aim of the present study was to assess the proximate composition, phytic acid content, *in vitro* protein digestibility and *in vitro* enzymatic hydrolysis of starch, glucose and starch, as well as the most relevant bioactive compounds (GABA, γ-oryzanol and total phenolic compounds) and antioxidant activity of breads prepared with GBR at different germination conditions.

2. Materials and methods

2.1. Materials

Commercial certified BR cultivar INIAP 15 was provided by the National Institute of Agricultural Research from Ecuador (INIAP). Seeds were harvest between May and December 2011. The gluten-free bread formulations also contained compressed yeast (LEVAPAN, Lessaffre, Valladolid, Spain) and hydroxypropylmethylcellulose (Methocel K4M) obtained from Dow Chemical Company (Michigan, USA).

2.2. Germination and flour preparation

Brown rice was sterilized with 0.1% sodium hypochlorite solution (1:5 w/v) for 30 min, and then rinsed with distilled water. Afterwards, rice was soaked in distilled water (seed water ratio, 1:5, w/v) for 24 h at 28 ± 1 °C. Soaking water was drained and the rinsed seeds were placed into plastic trays containing moist filter paper and were also covered with paper. The filter papers were kept wet by capillarity. Germination was carried out at 28 ± 1 °C and 100% relative humidity under darkness for 12, 24 and 48 h. Germination period was selected on the basis of preliminary assays where nutritional pattern was followed in parallel to technological functionality of flours. After germination, seeds were dried at 50 ± 1 °C for 24 h. Once dried, seeds were ground with a diameter inferior to 1 mm with a cyclone mill (UDY Corporation, USA). Brown rice flour was also obtained for comparison purposes, besides flour from soaked rice without germination. Two sets of samples were prepared for each treatment.

2.3. Bread preparation

The dough was prepared using the recipe of Marco and Rosell (2008). Half of the rice flour was mixed with boiling water (half of the water) and mixed for five minutes. The dough was left to rest until the temperature decreased to 30 °C. Then, the remaining flour, the other ingredients and water were added and mixed for 5 min. The dough was put into pans and fermented for 40 min at 35 °C and 85% RH. Finally, the fermented dough was baked for 35 min at 175 °C. The bread was analysed after 24 h of baking. Bread samples were coded BR for breads made with unprocessed BR flour, Pre-GBR for breads made with soaked brown rice and GBR preceded with germination time for those germinated brown rice flour (e.g. 12 h GBR for GBR germinated for 12 h).

2.4. Nutritional composition

Chemical composition of gluten-free breads was determined following AOAC (2005) methods, which included: moisture (method 925.10), ash (method 923.03), fat (method 922.06) and protein (method 920.87). The carbohydrate content of the samples was calculated by subtracting the sum of grams of moisture, protein, fat and ash from 100 g of bread. The components were converted to food energy using conversion factors (4.0 kcal g^{-1} for proteins and carbohydrates and 9.0 kcal g^{-1} for fats) (FAO, 2003).

2.5. Determination of phytic acid

An accurate photometrical Haug and Lantzsch's determination of phytic acid phosphorus was used (Reichwald & Hatzack, 2008) with some modifications. One ml of 1 M HCl was added to 50 mg of a sample in an airtight stoppered vial and heated for 1 h in a glycerol bath at 80 °C under constant agitation at 10×*g*. The mixture was then cooled to room temperature and centrifuged at 10,621×*g* for 5 min and 0.250 ml of the supernatant was diluted with 1 ml of distilled water. An aliquot of 0.4 ml of sample, standard (phytic acid solution in 0.2 M HCl) or blank (0.2 M HCl) were added to 0.8 ml of ferric solution (0.05 g of FeCl3 in 500 ml of 0.2 M HCl) in an airtight stoppered vial and was heated for 1 h in a glycerol bath at 80 °C with agitation at 10×*g*. The mixture was cooled in an ice bath for 15 min and centrifuged at 10,621×*g* for 5 min at room temperature. Aliquot of 0.6 ml of the supernatant was added to 0.8 ml of the complexing reagent (0.5 g of 2,2′-bipiridine and 65 μl of thioglycolic acid dissolved in 50 ml of 0.2 M HCl) and absorbance was read at 540 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA) controlled by the Gene 5TM software version 1.1. (BioTek Instruments).

2.6. In vitro protein digestibility

The *in vitro* protein digestibility of the samples was determined by the modified method of Hsu, Vavak, Satterlee, and Miller (1977). Briefly, 50 ml of aqueous protein suspension containing 6.25 mg protein/ml was prepared. Then, samples were placed in a 37 °C water bath and the pH was adjusted to 8.00 using 0.1 M NaOH and/or 0.1 M HCl, while stirring. Trypsin at a concentration of 1.6 mg/ml was maintained in an ice bath and the pH was adjusted to 8.00 with 0.1 M NaOH and/or 0.1 M HCl. Five millilitres of enzyme solution were then added to the protein suspension, which was kept stirred at 37 °C. The trypsin had an activity of 13,766 BAEE units/mg protein. The pH drop was recorded at 15 s after enzyme addition and at one minute intervals for 10 min. The enzyme solution was always freshly prepared before each series of experiments. The percent protein digestibility (Y) was calculated by using Eq. (1) (Hsu et al., 1977):

$$Y = 210.464 - 18.1x, \quad (1)$$

where x is the change in pH after 10 min.

2.7. In vitro starch digestibility and expected glycaemic index

Starch digestibility of bread was determined from dried samples, following the method previously described (Dura, Blaszczak, & Rosell, 2014) with minor modifications. Briefly, for free sugars removal, powdered sample (0.1 g) suspended in 2 ml of 80% ethanol was kept in a shaking water bath at 85 °C for 5 min, and then centrifuged for 10 min at 1000×*g*. The remaining pellet was incubated with porcine pancreatic α-amylase (6 U/ml) (Type VI-B, ⩾10 units/mg solid, Sigma Chemical, St. Louis, USA) in 10 ml of 0.1 M sodium maleate buffer (pH 6.9) in a shaking water bath at 37 °C. Aliquots of 200 μl were withdrawn during the incubation