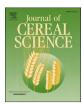
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# Evaluation of quality attributes, nutraceutical components and antioxidant potential of wheat bread substituted with rice bran



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#### ABSTRACT

In the present study, the effect of stabilized rice bran (RB) substitution on quality attributes, nutraceutical components and antioxidant potential of wheat bread was investigated. An increasing tendency was observed in protein, fat, ash, total dietary fiber content as well as in color and firmness of the breads as the level of substitution increased. Moreover, the RB substituted breads contained higher levels of vitamin E, phenolic content and antioxidant activity in comparison to the control. Baking resulted in a decrease in the vitamin E content and total flavonoids, whereas the amounts of phenolics as well as the antioxidant activities increased. Sensory evaluations revealed that breads substituted with 30% RB were overall acceptable, however, substitution level higher than 10% negatively affected the sensory properties in terms of color crumb and texture. Therefore, a significant improvement in the nutritional composition of the wheat bread with RB substitution was observed.

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# 1. Introduction

Nowadays, consumer's awareness of the need to consume superior quality and healthy foods known as functional foods is increasing. Bread is an ideal functional food, since it is an important part of our daily diet. Therefore, the tendency is to produce specialty breads made from whole grain flour and other functional ingredients known as functional foods (Dewettinck et al., 2008).

Rice bran (RB), is a by-product obtained by rice milling industry, is gaining commercial importance in the world as it has many beneficial nutritive and biological effects. It is rich source in fiber, protein, minerals, vitamins, phytochemicals such as  $\gamma$ -oryzanol, tocopherols, tocotrienols and polyphenols (Aguilar-Garcia et al., 2007), which help in preventing the oxidative damage of body tissues and DNA. Many studies reported that RB has cholesterol lowering properties, cardiovascular health benefits and anti-tumor activity (Nagendra Prasad et al., 2011).

In spite of its excellent nutrition value, its hypoallergenicity and

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recently claimed nutraceutical properties, a large amount of RB has been discarded and it is used as animal feed at considerably low prices. The limitation of using RB in food applications is largely due to its deterioration which are caused by enzymatic activities. It can be stabilized by a variety of methods like dry or wet heating, infrared radiation, ohmic heating etc (Nagendra Prasad et al., 2011).

Several researches have been undertaken in an attempt to increase utilization of RB as human foods. Stabilized RB or its components have been used in various food matrices such as bread (Hu et al., 2009; Tuncel et al., 2014a,b), cookies (Bhanger et al., 2008) and pizza (Delahaye et al., 2005) in order to introduce functional and nutritional properties.

Bakery products have proven to be good vehicles for incorporation of bran from other cereals. Although there are studies which have investigated the effect of RB on the physicochemical characteristics (Lima et al., 2002; Tuncel et al., 2014a), mineral and B vitamin contents (Tuncel et al., 2014b), a scarcity of information appears on the nutraceutical components, as well as the antioxidant properties of bread fortified with RB, and also on the fate of these beneficial health components during breadmaking.

Thus, the present study was designed to evaluate the effects of RB addition on quality attributes, nutraceutical components and antioxidant potential of substituted wheat breads. Furthermore, the effect of the baking process on their resultant breads have been studied.

Abbreviations: DPPH, 2, 2-diphenyl-1-picrylhydrazyl; FA, ferulic acid; FRAP, ferric reducing antioxidant power; pCA, p-coumaric acid; RB, rice bran; SA, sinapic acid; TDF, total dietary fiber; TFC, total flavonoid content; TPC, total phenolic content; WF, wheat flour.

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### 2. Materials and methods

### 2.1. Materials

Commercial WF (control) was purchased from the local market. Freshly milled raw RB was collected from a local milling industry (Bekas Agro SA, Thessaloniki, Greece). The RB was originated after the whitening milling progress of the grains of a Greek variety "Olympiada" (indica type). The grains were delivered to the industry by rice growers, while normal agro-practices were applied for local rice cultivation, including the application of herbicides until the tillering stage of the plants. After RB was provided by the industry, it was immediately stabilized in a laboratory oven at 120 °C for 10 min in order to inactivate the lipase. The stabilized RB was left to cool down at room temperature, then it was airtight sealed in aluminum bags using a vacuum sealer and stored at room temperature (25 °C) and at 4 °C for 4 months, in order to study the effect of time storing at different temperatures. Results showed that oxidative rancidity of RB could be prevented by heating, and storing at 4-5 °C for up to 4 months or at 25 °C for up to 2 months.

# 2.2. Chemical analysis

Moisture, protein, ash and fat content were determined following standard AACC methods (AACC, 2000). TDF content was determined using the procedure provided by Megazyme (Wicklow, Ireland) with their respective test kids. Carbohydrate composition was measured by the difference.

# 2.3. Baking tests

Basic dough formula on 100 g flour basis consisted of salt (2 g), compressed yeast (5 g), ascorbic acid (100 ppm), the amount of water required to reach 500 BU of consistency, and 10, 15, 20, 25 and 30% stabilized RB, respectively (when added). Two-step bulk fermentation and proofing was used. The doughs were mixed at farinograph bowl for 5 min at low speed, fermented for 30 min, then dough pieces (300 g) were hand-moulded and put into tin pans for proofing for other 60 min (30 °C and 60–70% relative humidity) and finally baked at 220 °C for 45 min. The bread quality attributes were evaluated after cooling for 1 h at room temperature. A small quantity of bread loaves were dried, ground to pass through 0.5 mm screen and stored at -25 °C for further analysis.

# 2.4. Bread quality evaluation

Bread quality parameters included specific volume, crumb color, acceptance and texture of crumb. Crumb color of the breads were measured using the HunterLab calorimeter model MiniScan XE Plus (Reston, USA). Lightness (L\*), redness  $(+a^*)$  or greenness  $(-a^*)$ , yellowness  $(+b^*)$  or blueness  $(-b^*)$  were measured four times for each loaf. The crumb firmness was determined on the central slices from each bread loaf using a texture analyzer TA-XT plus (Stable Micro Systems, Surrey, UK) according to Approved Method 74-09 (AACC, 2000). For each textural measurement, two slices from the center of each bread loaf were used. Measurements from at least three bread loaves were taken for each formulation.

Overall acceptability was carried out as follows: one slice of bread, identified by code numbers, was served to each un-trained panelist (n=20) under normal illumination. They evaluated each bread for quality attributes: crumb color, aroma, taste, texture and overall acceptability. Acceptability of each quality attribute was rated with a score 1 (lowest) to 9 (highest). Products were considered acceptable if their mean scores for overall acceptability were above 5 (neither like nor dislike).

# 2.5. Vitamin E analysis

HPLC analysis of vitamin E (tocopherols and tocotrienols) was accomplished using an Agilent Technologies 1200 with a fluorescence detector, and a Nucleosil 100  $C_{18}$  column (4.6 mm  $\times$  250 mm, 5  $\mu$ m), according to a previously described protocol (Irakli et al., 2011). The tocopherols and tocotrienols were separated using a linear gradient elution system starting with methanol and ending with a mixture of methanol—isopropanol—acetonitrile.

# 2.6. Determination of TPC

For free phenolic content estimation, samples were extracted twice with aqueous methanol (70:30, v/v), centrifuged and supernatant was stored in the freezer until analysis. The residue obtained was extracted with 4 M sodium hydroxide and used for bound polyphenol estimation and analyzed by Folin Ciocalteau's reagent at 725 nm using gallic acid as standard (Irakli et al., 2012). The results were expressed as milligrams of gallic acid equivalents per 100 g sample on a dry weight basis (mg GAE/100 g dw).

## 2.7. Determination of TFC

The TFC of phenolic extracts obtained as described above were analyzed by the AlCl<sub>3</sub> reagent method (Bao et al., 2005), using the standard catechin curve and expressed as milligrams catechin equivalent per 100 g of sample on a dry weight basis (mg CATE/ 100 g dw).

# 2.8. Phenolic acid composition

The HPLC-analysis was performed on an Agilent LC series 1200 HPLC system (Agilent Technologies, Urdorf, Switzerland) equipped with a quaternary gradient pump, a membrane degasser, a rheodyne injection valve with a 20  $\mu L$  loop and a diode array detector. Separations were conducted on a Nucleosil 100  $C_{18}$  column (250 mm  $\times$  4.6 mm, 5  $\mu m$ ). The mobile phase consisted of 1% aqueous acetic acid (eluent A) and methanol (eluent B), and was based in the method described by Irakli et al. (2012). The linear gradient started with 90% A, reaching 80% A at 10 min, 75% A at 20 min, 65% A at 30 min and finally 35% A at 40 min. Detection was carried out at 320 nm. Phenolic acids in the samples were identified by comparing their relative retention times and the respective UV spectra with those of authentic compounds.

# 2.9. DPPH radical scavenging capacity assay

Aliquots of aqueous methanol phenolic extract (free and bound) of the sample was mixed with DPPH<sup>+</sup> reagent and absorbance was read at 516 nm as outlined by Yen and Chen (1995). The percentage of scavenging effect was calculated by using the following equation: DPPH radical scavenging capacity (%) =  $[1 - (A_{516} \text{ of } sample/A_{516} \text{ of } blank)] \times 100$ . Results were expressed as mg Trolox equivalents per 100 g of sample on a dry weight basis (mg TE/100 g dw).

## 2.10. FRAP assay

The FRAP assay was carried out according to Benzie and Strain (1999). Briefly, the oxidant in the FRAP assay consisted of 20 mM ferric chloride solution, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 0.3 mM acetate buffer pH 3.6 in a proportion of 1:1:10, respectively, and was freshly prepared. 100  $\mu$ L aliquot of extract reacted with 3 mL of the FRAP solution at 37 °C for 4 min under dark conditions and the absorbance was recorded at 593 nm against blank. Results were expressed as mg Trolox

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