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# Dephytinization of wheat and rice brans by hydrothermal autoclaving process and the evaluation of consequences for dietary fiber content, antioxidant activity and phenolics



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#### ABSTRACT

Autoclaving process applied to wheat and rice bran samples to decrease the phytic acid content and to enhance the functional and nutritional properties (dietary fiber and phenolic content, antioxidant activity) of bran samples. All hydrothermal treatments caused significant decreases in phytic acid contents of both wheat (95.2%) and rice bran (95.6%) samples. The most effective process conditions on enhancing the total dietary fiber content for both bran samples were pH 4.0 level and 1.5 h holding time. Autoclaving treatment resulted in a decrease in total phenolic contents after holding for 90 min and at 121 °C at their native pH levels. Autoclaving for 90 min caused the greatest degree of increment in the total antioxidant activity of wheat (12%, pH 4.0) and rice bran samples (2%, pH 3.5). Autoclaving treatment was found as quite effective method for both dephytinization and enrichment of wheat and rice brans as a functional food ingredient.

*Industrial relevance*: Authors believe that the study presents important new information in terms of both enhancing functional properties of wheat and rice brans by hydrothermally dephytinization treatment and revealing the correlation between hydrothermal treatment and functional ingredients of brans. In this way, proposed method transforms inexpensive and easily accessible sources into important food ingredients and gives them added value. Hydrothermal treatment also enables food industry to use cereal brans as functional ingredients in the applications of both designing and enriching new and healthy food formulations.

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## 1. Introduction

Scientific studies conducted by different research teams combining nutritionists, oncologists and biochemists have demonstrated that the human health is inextricably linked with eating habits. Despite this fact, there has been an increasing unhealthy food trend which leads to many health hazards such as obesity, type 2 diabetes, cardiovascular complications, gastrointestinal disorders and certain types of cancer (Patel, 2015). Thus, much of the research in this area has focused on specific foods or components such as cereal brans depending on their functional properties related with nutritional and health benefits.

Cereal brans obtained as milling by-products have been characterized with their rich composition in terms of dietary fiber, vitamins, minerals, antioxidants, phenolic lipids, phytosterols and other phytochemicals (Patel, 2015). Among all cereal brans, wheat and rice brans are the most studied and popular dietary supplements. Specific studies are aimed to describe the detailed phytochemical composition of wheat and rice brans showed that the rice bran is rich for phenolic compounds,

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vitamins (vit. E), dietary fiber, oils, steroid derivatives, polysaccharides, naturally occurred antioxidants (tocopherols, tocotrienols, oryzanol), and proteins (essential amino acids, especially lysine) whereas fibers, lignins, oligosaccharides, polyphenols, lignans, phytic acid, minerals, alkylresorcinols, glutathione, sulphur compounds,  $\alpha$ -linoleic acid, carotenoids, vitamin B and E are identified as the major components in wheat bran (Friedman, 2013; Sharif, Butt, Anjum, & Khan, 2014). Consequently, those brans have been considered as novel functional ingredients due to their unique nutritional compositions and positive health benefits on the battle against metabolic syndrome, type 2 diabetes, chronic diseases (in particular cardiovascular diseases), certain cancers and associated ailments (Stevenson, Phillips, O'Sullivan, & Walton, 2012).

Apart from their functional and health benefits mentioned above, bran of wheat and rice contains a considerable amount of phytic acid (myo-inositol hexaphosphate) which acts as an antinutrient substance and limits the nutritional effect and usage in the food formulations (Akhter, Saeed, Irfan, & Malik, 2012). Antinutrient effect of phytic acid has been originated from its strong binding capacity of mineral cations (iron, magnesium, zinc and calcium) and consequently changing their solubility, functionality, absorption and digestibility (García-Estepa, Guerra-Hernandez, & García-Villanova, 1999; Reddy & Sathe, 2001; Sharif et al., 2014; Stevenson et al., 2012). Binding effect of phytic acid

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has been considered as a potential public health problem for the people with diets mainly based on cereals or having critical deficiency of essential minerals (Servi, Özkaya, & Colakoglu, 2008; Stevenson et al., 2012).

Various methods for reducing the phytic acid content have been of great importance in the applications of fiber-enrichment of foods. In previous studies (Akhter et al., 2012; Jayarajah, Tang, Robertson, & Selvendran, 1997; Mosharraf, Kadivar, & Shahedi, 2009; Sandberg, 2002; Sanz Penella, Collar, & Haros, 2008; Türk, Carlsson, & Sandberg, 1996), different processes such as soaking, malting, fermenting, heat treatments (i.e. baking, autoclaving, frying) or the addition of enzymes were made to reduce the phytate content during food processing. However the fact remains that none of these processing methods achieved a complete elimination of phytic acid. In the last decades, hydrothermal process, also known as autohydrolysis, appears as a quite useful alternative method with several advantages such as being simple, economical, environmentally friendly because of not requiring any other chemical and not causing corrosion problems. During the process, high temperature and pressure are applied with water (Ruiz, Rodríguez-Jasso, Fernandes, Vicente, & Teixeira, 2013). According to the study of Aguedo, Ruiz, and Richel (2015), browning in the media due to the formation of some small compounds caused by uncontrolled depolymerization, debranching and de-esterification of the solubilized fragments is seen as one of the drawbacks of the process. Despite the several advantages of the hydrothermal process, there are limited number of studies related with the effects of hydrothermal dephytinization treatments on the bioactive contents of cereal brans, especially of wheat and rice. However, it has been reported that dephytinization should be made before enrichment of food to provide both the preservation of food quality and an effective phytate reduction in the end product (Servi et al., 2008).

On the basis of these considerations, this study has aimed to achieve two important objectives. The main goal of this work is to investigate the effects of hydrothermal dephytinization process on the bioactive compounds and dietary fiber contents of wheat and rice brans. Besides, it is also aimed to reduce the phytic acid content of wheat and rice bran by hydrothermal process at maximum rate before they were incorporated into the food formulation.

## 2. Materials and methods

## 2.1. Sample preparation

## 2.1.1. Sample preparation for hydrothermal treatments

Wheat bran samples were obtained from a commercial flour mill in Ankara, Turkey while rice bran samples were provided by a rice processing plant located in Edirne, Turkey. Moisture, ash and protein contents of bran samples were determined using AACC Approved Methods 44/1,08/1 and 46/12, respectively (AACC, 1999). Moisture, ash and protein contents of wheat bran were found as 10.7%, 5.6% and 14.2% whereas those of rice bran were found as 8.8%, 9.7% and 16.5%, respectively. Both bran samples passed through laboratory type bran finisher (Buhler Type MLU-302) and later bran slurries for each sample were prepared by mixing with deionized, distilled water at the ratio of 1:15 (w/v). Bran slurries were subjected to the hydrothermal treatments.

## 2.1.2. Extraction of free phenolic compounds

Free phenolic compounds of bran samples were extracted by blending 0.5 g of sample with 5 mL of acethone:water mixture (1:1, v/v) for 1 h in stirring shaker. After centrifugation at 2500g for 10 min, the supernatant was filtered through Whatman No. 42 filter paper and extraction was repeated two times. Supernatants were pooled and evaporated at 40 °C using a rotary evaporator (Buchi, Rotavapor R-210, Switzerland). The resulting solutions were kept at  $-20\,^{\circ}\text{C}$  under nitrogen gas in dark containers until further analysis, after the extracts were dissolved in 2 mL DMSO (dimethyl sulfoxide solution).

## 2.1.3. Extraction of bound phenolic compounds

The residues remained after extraction of free phenolics were digested with 2 N sodium hydroxide (1:40, w/v) at room temperature for 4 h by stirring. Acidity of samples was adjusted to pH 2.0 with 6 M HCL solution. After neutralization, the mixture was extracted with 20 mL of hexane to remove lipids. After centrifugation at 2500g for 10 min, hexane was removed from the samples. This procedure was repeated one more time. The final solution was extracted five times with an appropriate amount of diethylether:ethyl acetate mixture (1:1, v/v). After centrifugation at 2500g for 10 min the diethylether:ethyl acetate fraction was pooled and evaporated at 40 °C using a rotary evaporator (Buchi, Rotavapor R-210, Switzerland). Samples were dissolved in 2 mL DMSO and were kept at  $-20\,^{\circ}\text{C}$  under nitrogen gas in dark containers until further analysis.

## 2.2. Hydrothermal process (autoclaving)

The pH values of both slurries were adjusted to 4.5, 4.0 or 3.5 with acetic acid. The slurries were then held at 121 °C for 0.5, 1.0 or 1.5 h in an autoclave. The bran slurries of wheat and rice without pH adjustment were used as control samples and they left resting for the same periods as the related treatment. After hydrothermal application, the slurries were sieved (opening 250  $\mu$ ) and the sieved samples were rinsed five times with 500 mL of water each time, and dried at 60 °C to moisture content of maximum 12%. Analyses of the bran samples were carried out in triplicate, and mean values on a dry basis were reported in the tables.

#### 2.3. Proximate analyses

The analyses of moisture, ash and protein contents of bran samples were carried out according to the AACC approved methods 44-01, 08-01 and 46-12, respectively (AACC, 1999). pH of the bran slurries was measured by a digital pH meter. Total dietary fiber content was determined according to the AOAC method 991.43 (AOAC, 2012). Total phosphorus content was measured spectrophotometrically using phosphovanadomolybdate method as described by Rickey and Evans (1955), after the bran samples were prepared by the wet ash method (Garcia, Blessin, & Inglett, 1972). Phytic acid and total phosphorus content was measured by using the colorimetric procedure of Haug and Lantzsch (1983), and phytic acid was calculated accordingly. Phenolic content and antioxidant capacity of each sample were determined according to the methods described by Yu et al. (2002), after the extraction process mentioned by Adom and Liu (2002).

The phenolic contents of each extracts were determined using Folin-Ciocalteau reagent. Briefly,  $100~\mu l$  of extracts was oxidized with Folin-Ciocalteau reagent, and the reaction was neutralized with sodium carbonate. The final volume was made up to 10~m l with pure water. After 2~h of incubation, the absorbance at 765~n m was measured and used to calculate the phenolic contents using gallic acid as standard. Results were expressed as milligram of gallic acid equivalent per kilogram of sample.

The antioxidant activity of samples was determined using 2.2-diphenyl-2-picryl-hydrazyl (DPPH) reagent. Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid) was used as an antioxidant standard. DMSO was used to prepare the solution of Trolox. The absorbance at 515 nm was measured, and the results were expressed as Trolox equivalent.

## 2.4. Statistical analyses

For each treatment, sampling was conducted according to a completely randomized experimental design with a factorial arrangement. All statistical analyses were performed using SPSS software (V.11.0 for Windows, SPSS Inc., Chicago, IL). Results were analyzed by three-way analysis of variance (ANOVA) with the general linear

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