Innovative Food Science and Emerging Technologies 25 (2014) 19–27

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Effect of bioprocessing and particle size on the nutritional properties of wheat bran fractions

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ARTICLE INFO

Article history:
Received 22 July 2013
Accepted 27 November 2013
Available online 6 December 2013

Editor Proof Receive Date 27 December 2013

Keywords:
Lactic acid bacteria
Yeast
Fermentation
Bran
Bioprocessing
Micronization
Fiber
Antioxidant

ABSTRACT

In this work, the influence of bioprocessing on the nutritional quality and health effects of wheat bran of different particle sizes (750, 400, 160, 50 μm) was evaluated. Bioprocessing was carried out by a 24 h-fermentation using *Lactobacillus brevis* E95612 and *Kazachstania exigua* C81116 as starters, with or without the addition of an enzyme mixture with specific carbohydrase activities. Bioprocessing clearly affected the microstructure and chemical and nutritional features of wheat bran depending on the particle size. Bioprocessing significantly improved the antioxidant and phytase activities (up to 3.7 fold, respectively), peptides and total free amino acids and content (up to 40%) and the *in vitro* digestibility of proteins. The antioxidant power and nutritional indexes were higher for the bioprocessed brans compared to the native, mainly in bran having smaller particle size. In every case, the addition of the enzymes further improved the positive effect of the microbial fermentation.
Industrial relevance: Wheat bran is a source of nutritionally important compounds such as dietary fibers, minerals, vitamins and phenolic acids. Commonly, processing of bran has mostly been performed for technological purposes, to facilitate its use as a DF rich ingredient in foods improving its structural and organoleptic features. The bioprocessing technology here applied offers a tool to enhance also the nutritional value of wheat bran, especially of finer particle size. As a result, bioprocessed wheat bran showed higher potential compared to the native bran, and qualified as a suitable ingredient for food preparations.

1. Introduction

Worldwide, food and health authorities recommend an increase of the consumption of whole grain cereals due to the evidence that more whole grains in the diet lead to a reduction of the risk of several chronic diseases (Frølich, Åman, & Tetens, 2013). Besides the nutrient content and the presence of compounds such as dietary fibers, minerals, and phenolic acids, also the structure and texture of cereal foods have long been recognized as parameters involved in the health benefits of whole grain foods (Frølich et al., 2013). Therefore, different novel technologies were developed for transformation processes in order to better exploit the cereal nutritional potential. Among these, micro- and nanotechnologies are showing great potential in nutraceuticals and functional food manufacture for human health improvement (Chen, Weiss, & Shahidi, 2006). Recently, the application of micronization in food research has shown that the reduction of the particle size of various fiber-rich plant materials alters the structure, surface area and functional properties of the particles (Hemery et al., 2011). *In vitro* digestion studies of bran-enriched breads have shown that the bioaccessibility of phenolic acids and minerals was improved with the decreasing of bran particle size and with the increasing concentration of micronized aleurone material (Hemery, Mabille, Martelli, & Rouau, 2010). From the industry point of view the possibility to exploit bran fractions with a different granulometry is a useful opportunity to obtain new functional ingredients for the preparation of several foods (Esposito et al., 2005). This is important considering that, besides the nutritional value mainly related to dietary fibers, phenols and minerals, bran has negative effects on sensory and technological properties which limit its use as a food ingredient in general and in bread making in particular. One possibility to overcome this effect is to pre-treat bran with bioprocessing techniques such as fermentation, using specific yeast and lactic acid bacteria starter cultures and/or enzymes, such as cell wall degrading enzymes (Delcour, Rouau, Courtin, Poutanen, & Ranieri, 2012).

The use of bioprocessing techniques has been shown to be also a good approach to improve the bioaccessibility of health-promoting compounds in bran (Coda et al., 2014; Katina et al., 2007; Mateo Anson, van den Berg, Havenaar, Bast, & Haenen, 2009). Enzymatic and

Abbreviations: as, Ash; BHT, butylated hydroxytoluene; BV, Biological Value; DF, dietary fiber; EAA, Essential Amino Acid; EAAI, Essential Amino Acid Index; FAA, free amino acid; FID, flame ionization detector; GC, gas chromatography; DFi, insoluble dietary fiber; NI, Nutritional Index; PA, phytase activity; PCA, Principal Component Analysis; PER, Protein Efficiency Ratio; Pr, protein; RP, reducing power; DFs, soluble dietary fiber; ST, starch; DFt, total dietary fiber; TFAA, total free amino acid; TP, total phenols; TFA, trifluoroacetic acid; Wx, water extractable arabinoxylans.

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http://dx.doi.org/10.1016/j.ifset.2013.11.012

fermentation technologies offer an array of tools to modify the grain matrix. During fermentation, the grain constituents are modified by the action of both endogenous and bacterial enzymes, thereby affecting their structure, bioactivity, and bioavailability (Hole et al., 2012). Yeast-started fermentation improved the bioactivity and baking properties of wheat bran prepared from peeled kernels, resulting in solubilization of arabinoxylans (Katina et al., 2012). Fermentation can exert an impact on the nutritional quality and health effects of whole grain foods. For instance, fermentation with lactic acid bacteria has a well-known role in enhancing the nutritional properties as well as the texture and palatability of whole grain and fiber rich products (Poutanen, Flander, & Katina, 2009). It increases the level of bioactive peptides, dietary fiber solubility and mineral bioavailability, and decreases glycemic index (Rizzello, Cassone, Di Cagno, & Gobbetti, 2008). Lactic acid bacteria fermentation is a useful tool to obtain specific results, but selection of proper strains is an important pre-requisite for successful achievements. The capacity of lactic acid bacteria to release bioactive compounds during cereal fermentation is very well known but few studies are available on bran fermentation. Bran seems to have a great potential to improve the technological performances and/or integrate foods with healthy compounds, if a proper processing technology is applied. In this study, the impact of the combination of milling procedure and bioprocessing on wheat bran having different particle sizes was evaluated. Chemical and nutritional properties of fermented bran were investigated.

2. Materials and methods

2.1. Raw materials

Commercial wheat bran (Fazer Mills, Lahti, Finland) was ground by TurboRotor technology (Mahltechnik Görgens GmbH, Dormagen, Germany) to three different levels of fineness. The median particle sizes of the four brans obtained, and analyzed by sieving, were: 750 (unground), 400, 160 and 50 μm, as provided by the supplier. All the four brans were used in bioprocessing. Total DF contents of the brans were 48.0 ± 1.3% (750 μm), 48.9 ± 1.9% (400 μm), 47.9 ± 1.9% (160 μm) and 48.4 ± 1.7% (50 μm), respectively, as determined according to the method AOAC 9852.

2.2. Bran bioprocessing

Lactobacillus brevis E95612 and *Kazachstania exigua* C81116 belonging to VTT Culture Collection (VTT, Technical Research Centre of Finland) were used as starters for fermentation. *L. brevis* E95612 was cultivated for 24 h at 30 °C on MRS (Oxoid LTD, Basingstoke, Hampshire, United Kingdom) in anaerobic conditions, while the yeast was cultivated for 24 h at 25 °C in YM (3 g/L malt extract, 3 g/L peptone, 10 g/L dextrose). After the late exponential phase of growth was reached, cells were recovered by centrifugation (10,000 ×*g* for 10 min), successively washed twice in 0.05 M phosphate buffer, pH 7.0, and re-suspended in tap water (ca. 15% of the initial volume of the culture). Wheat bran and water (ratio 20/80) were mixed using a Bamix blender (Bamix, Switzerland). Lactic acid bacteria and yeast were both inoculated at cell density of ca. 10^6 cfu/g. Enzyme preparations Depol 740L (Biocatalyst Ltd., Great Britain) and Grindamyl 1000 (Danisco, Denmark) were added at the beginning of fermentation. In particular, Depol 740L (liquid preparation) was dissolved in water, while Grindamyl 1000 (powder) was added to bran. The enzymes used contained a variety of hydrolytic enzymes, mainly xylanase, endoglucanase and β-glucanase in Depol 740L (Mateo Anson et al., 2009), and alpha-amylase in Grindamyl. Enzyme dosages were: 161 nkat xylanase/g of bran for Depol 740L (xylanase activity according to Bailey, Biely, and Poutanen (1992)), and 75 nkat α-amylase/g of bran for Grindamyl 1000 (n α-amylase activity according to Megazyme Ceralpha method). Bioprocessing of bran was carried out inoculating the two starters without and with the addition of enzymes, as described above. Fermentations were carried out using Termarks incubators, KBP6151, Norway at 20 °C for 24 h. After fermentation, bran was freeze-dried and characterized.

2.3. Microbiological analyses and pH determination

Bran samples (10 g) were homogenized with 90 mL of sterile saline in a Stomacher 400 lab blender (Seward Medical, London). Serial dilutions were made and enumeration of lactic acid bacteria and yeasts was carried out by plating on MRS and YM agar after incubation for 48 h at 30 °C or 25 °C respectively. The pH value was measured by a TitroLine autotitrator (Alpha 471217, Schott, Mainz, Germany) suspending an aliquot of 10 g of fermented bran in 100 mL of distilled water. All samples were analyzed in duplicate.

2.4. Microscopic analysis

Aliquots of the different bran fractions were collected before and after bioprocessing, embedded in 2% agar and fixed in 1% glutharaldehyde dissolved in 0.1 M phosphate buffer (pH 7.0), dehydrated in a graded ethanol series, and embedded in hydroxyethyl methylacrylate, as recommended by the embedding kit manufacturer (Leica Historesin, Heidelberg, Germany). Polymerized samples were sectioned (2 mm sections) in a rotary microtome HM 355 (MicromLaborgeräte GmbH, Walldorf, Germany) using a steel knife. Protein was stained with aqueous 0.1% (w/v) Acid Fuchsin (Gurr, BDH Ltd., Poole, U.K.) in 1.0% acetic acid for 1 min, and β-glucan was stained with aqueous 0.01% (w/v) Calcofluor White (fluorescent brightener 28, Aldrich, Germany) for 1 min. The sections were also stained with Light Green (BDH Chemicals Ltd, Poole, Dorset, UK)/Lugol's iodine solution. When imaged in bright field, Light Green stains protein green/yellow, whereas Lugol's iodine solution stains the amylose component of starch blue and amylopectin brown. Most starch appears dark blue because amylose masks the amylopectin. The samples were examined with an Olympus BX-50 microscope (Tokyo, Japan). In exciting light (epifluorescence, 400 and 410 nm; fluorescence, >455 nm) intact cell walls were stained with Calcofluor that appear in blue and proteins were stained with Acid Fuchsin that appear in red. Starch is unstained and appears black. Micrographs were obtained using a SensiCam PCO CCD camera (Kelheim, Germany) and the Cell^P imaging software (Olympus).

2.5. Dietary fiber and arabinoxylan analysis

Total dietary fiber (DF) content was analyzed. Soluble and insoluble DF of the bran and bread samples was determined by the enzymatic gravimetric AOAC Method 2009 with an assay kit (Total DF K-TDFR, Megazyme, Ireland), following manufacturer's instructions.

The water-extractable arabinoxylan fraction was obtained by extracting 1 g of the cereal sample with 7 mL of cold water (4 °C) (Santala, Nordlund, & Poutanen, 2013). Successively, the water-soluble fraction was hydrolyzed with 1.2 mL of 7.5 N H_2SO_4 in a boiling water bath for 2 h (Santala et al., 2013). To measure the total amount of arabinoxylans, 50 mg of cereal sample was pre-hydrolyzed with 1.56 mL of 72% (w/w) H_2SO_4 at room temperature (25 °C) for 30 min (Blakeney, Harris, Henry, & Stone, 1983). Samples were then diluted with 15.6 mL of Milli-Q water (Millipore, Billerica, MA) and hydrolyzed in a boiling water bath for 2 h. After cooling, the solutions were neutralized by adding appropriate volume of 4 M NaOH. The sugars obtained from the hydrolysis steps and the monosaccharide standards (50 mg/mL; glucose, arabinose, xylose, galactose, and mannose) were analyzed as their alditol acetates, as described by Blakeney et al. (1983). Standard curves were obtained analyzing dilutions obtained by these monosaccharide solutions. Myo-inositol was used as the internal standard (0.5 mg/mL sample). The acetylated monosaccharides were analyzed with gas chromatography (GC) using an Agilent 6890