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# Effects of pre-fermented wheat bran on dough and bread characteristics

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### ABSTRACT

This study was aimed at investigating the influence of wheat bran arabinoxylans on the dough development and on the qualitative characteristics of the final bread. Furthermore, the impact of microbial fermentation and enzymatic treatment (xylanase, amylase, cellulase) on the solubility of wheat bran arabinoxylans was studied. For this purpose different wheat breads were produced: Control Bread (CB): soft wheat flour 100%; Bran Bread (BB): soft wheat flour 80%, wheat bran 20%; Bran Sourdough Bread (BSB): soft wheat flour 80%, pre-fermented bran 20%; Bran Enzyme Bread (BEB): soft wheat flour 80%, wheat bran 20%, enzyme mixture (0.02%); Bran Enzyme Sourdough Bread (BESB): soft wheat flour 80%, pre-fermented bran 20%, enzyme mixture (0.02%). Flours and doughs were characterized for their rheological properties by alveographic, farinographic and rheofermentometer tests and for dietary fiber and total and soluble arabinoxylan content. Final breads were characterised for dietary fiber, arabinoxylans, moisture content, bread volume, and for miscroscopic evaluation of the bread crumb by scanning electron microscopy. Results highlighted that the use of pre-fermented bran (starter culture fermentation in combination with enzyme treatment) influenced the arabinoxylans solubility, having positive effects on the bread making quality, the water balance and the overall characteristics of the final breads.

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# 1. Introduction

Nowadays, there is a growing interest in supplementing food products for human consumption with dietary fiber (DF), since this ingredient is highly recommended for its well-known health benefits (Verardo et al., 2011; Ktenioudaki and Gallagher, 2012). The most commonly recognized source of fiber comes from nondigestible carbohydrates which naturally occur in different food, such as whole grains (oat, wheat, barley, rice, etc.), beans, fruits and vegetables. Many fibers may be extracted from these sources and used as independent food ingredients to supplement the fiber content in foods.

In particular, wheat bran cereal, a source rich in healthy ingredients such as dietary fiber and phytochemicals, is becoming increasingly valuable for food processing, since the consumers prefer foods naturally fortified. The dietary fiber is concentrated in the outer layers of the wheat grain (the bran fraction) and it

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The quantification and the assessment of the overall AX content is relevant because the arabinoxylans have health benefits and influence the cereal processing and the breadmaking quality,

the Ara/Xyl ratio (Izydorczyk and Biliaderis, 1995).

consists of cellulose, lignin and highly substituted (insoluble) arabinoxylans (AX), whereas the cell walls of aleurone layer consist of

Arabinoxylans have been identified in different cereals: wheat,

rye, barley, oat, rice, sorghum. Although AX are minor components

of the whole caryopsis in cereals, they still account the main frac-

tion of soluble and insoluble dietary fiber of wheat flours (Skendi

et al., 2011). Arabinoxylans consist mainly of a xylan chain with

 $\beta$ -1,4-linked D-xylopyranosyl residues (Xyl) to which mostly single

 $\alpha$ -L-arabinofuranose units (Ara) are linked at the O-2 and/or O-3

positions of the xylose units as side residues (Izydorczyk and Biliaderis, 1995). In wheat AX, approximately 66% of the xylose

residues, that form the backbone chain, are unsubstituted Xyl

(Saulnier et al., 2007). Moreover, some Ara units carry ferulic acid

residues esterified to O-5 of Ara linked to O-3 of the xylose residues.

Water-extractable and water-unextractable AX structures are

similar, showing only slight differences in molecular weight and in

mostly soluble AX and  $\beta$ -glucans (Nordlund et al., 2013).









including, performance of the milling, starch and gluten separation, workability of the mixtures, volume and qualitative characteristics of the bread (Courtin and Delcour, 2002; Frederix et al., 2004; Goesaert et al., 2005). Several studies (Biliaderis et al., 1995; Courtin and Delcour, 2002) showed that the effects of AX are more evident during the main stages of baking. The waterabsorbing capacity of AX also influences the distribution of moisture among dough constituents, resulting in modified rheological properties and prolonged mixing time. The redistribution of moisture among gluten and other molecules allows AX to react directly with gluten molecules, generating a more complex network containing both gluten and AX (Sivam et al., 2010). It was ascertained, in fact, that the soluble fraction of AX may fix the amount of water from 3.5 to 6.3 times their weight, while the insoluble fraction from 6.6 to 9.9 times their weight (Courtin and Delcour, 2002).

The adverse effects of fiber on the quality of baked products have been well documented and many approaches have been employed to improve the quality of the fiber-rich products consisting mainly through the use of enzymes and/or through the use of bioprocessing techniques such as fermentation (Salmenkallio-Martilla et al., 2001; Rizzello et al., 2010; Katina et al., 2012; Nordlund et al., 2013).

In detail, properties of wheat AX can be improved by modifying its structure by enzymes (Katina et al., 2006). Endoxylanases, for instance, are hydrolytic enzymes that cleave the backbone of AX, thereby decreasing the degree of polymerization, increasing the solubility of the polymer and releasing xylo-oligosaccharides (Santala et al., 2011). Endoxylanases are nowadays frequently applied as cereal processing aids (Ingelbrecht et al., 2000; Courtin and Delcour, 2002; Goesaert et al., 2005).

Moreover, sourdough fermentation, especially in rye breadmaking, in addition to enhance the starch and proteins hydrolysis and to improve the texture, the flavor and the shelf-life of breads (Salmenkallio-Martila et al., 2001; Poutanen et al., 2009; Rizzello et al., 2010), also increases the solubility of arabinoxylans (Lappi et al., 2010).

In this context, the present study aimed to investigate the effects of lactic acid bacteria fermentation and enzymatic treatment (xylanase, amylase, cellulase) on AX solubility and technological characteristics of dough and bread.

# 2. Materials and methods

## 2.1. Samples

Five different wheat breads were produced: Control Bread (CB): Soft wheat flour 100%; Bran Bread (BB): Soft wheat flour 80%, wheat bran 20%; Bran Sourdough Bread (BSB): Soft wheat flour 80%, prefermented bran 20%; Bran Enzyme Bread (BEB): Soft wheat flour 80%, wheat bran 20%, enzyme mixture (0.02%); Bran Enzyme Sourdough Bread (BESB): Soft wheat flour 80%, pre-fermented bran 20%, enzyme mixture (0.02%).

Soft wheat flour (WF) and soft wheat bran (WB) were obtained from a local mill sited in Campobasso (Italy). The basic ingredients required for bread dough preparation were: commercial dehydrated yeast powder (1.5%), salt (1.5%), sugar (1.5%), and water (60%). For the production of bran-enriched breads, the proper amount of water required to bring the mixture to an optimal consistency was determined by farinograph measurements (AACC Method 54-21).

For BEB and BESB doughs the enzyme mixture (0.02%) composed by xylanase, amylase, cellulase was used (Funfamyl Super AX and Celluclast BG, Novozymes, Denmark).

BSB and BESB samples were produced by adding 20% wet pre-

fermented bran, obtained as subsequently described.

Bread samples were produced by an Automatic Bread Machine (Backmeister mod. 8650). All the ingredients were put in the machine with a mixing time of 25 min, a fermentation time of 60 min and a cooking time of 55 min.

### 2.2. Pre-fermented bran

Pre-fermented bran was obtained by use of *Lactobacillus brevis* R5 strain (Reale et al., 2011), a lactic acid bacteria previously isolated from Italian traditional sourdough and belonging to microbial culture collection of the Department of Agricultural, Environmental and Food Sciences, University of Molise. The strain was selected for its technological properties such as sugar fermentation activity, acidification and leavening ability. As reported by Prückler et al. (2015) the acidification by lactic acid bacteria, independently from the homo- and heterofermentative species, partially masks or reduces the unpleasant flavor of the bran without inducing severe changes to the bran matrix.

Before use, microbial strains were revitalised in fresh media, MRS broth (Oxoid, Milan) and YPD (10 g/L yeast extract, 20 g/L bacteriological peptone, 20 g/L glucose), for lactic acid bacteria and yeasts, respectively. The fresh microbial cultures were centrifuged (13 000 rpm for 15 min at 4 °C; Centrifuge 5415 R; Eppendorf, Hamburg, Germany) and washed twice in NaCl solution 0.9% (w/v) and used for the inoculum. Sterilized bran (100 g) was mixed with 350 g of sterilised water and inoculated with 8 log cfu/g of *Lb. brevis* R5. The inoculated bran was incubated at 28 °C for 16 h. Immediately after fermentation the wet pre-fermented bran was used in the production of BSB and BESB samples.

#### 2.3. Reagents

NaOH 50% (p/v) was purchased from Baker (Mallinckrodt Baker B.V., Deventer, Holland), high-purity laboratory water was produced by means of a MilliQ-Plus apparatus (Millipore S.p.A., Milano, Italy); glucose, xylose, arabinose, fructose, standards and all other chemicals and reagents of HPLC grade were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

#### 2.4. AX analysis by HPAEC-PAD

Determination of arabinoxylans was realised as reported by Messia et al. (2009). Briefly, for water soluble AX: 100 mg of a flour sample was shaken in 10 mL of water at 30 °C for 2 h and centrifuged. Aliquots (1 mL) of the supernatant were hydrolyzed with 1 mL of 4N HCl for 2 h. For total AX: flour sample (10 mg) was weighed into a glass tube, 2 mL of 2N HCl was added, and the mixture was hydrolyzed at 100 °C for 2.5 h. After cooling, neutralization was carried out by the addition of 2N sodium carbonate.

Fermentable sugars were removed according to the following procedure: After hydrolysis, the sample was neutralized with sodium carbonate, added to a suspension of commercial bakery yeast cells (25 mg/mL) in 0.2 M sodium phosphate buffer (pH 7) and incubated for 15 h at 30 °C. The mixture was centrifuged and the supernatant obtained was diluted and injected in the chromatographic system.

For the sample injection, a Rheodyne injector (Cotati, CA, USA) with a 25  $\mu$ L loop, was used. The chromatographic separation was carried out with a Carbopac PA1 (250  $\times$  2 mm) (Dionex Corporation, Sunnyvale, CA, USA) analytical column. The chromatographic run (22 min) and the quantitative determination were conducted with a 0.25 mL/min flow rate, using a mobile phase of water and 200 mM sodium hydroxide (90%–10%). The control of the

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