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Influence of non-starchy polysaccharides on barley milling behavior and evaluating bioactive composition of milled fractions

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ABSTRACT

Hulless barley cultivars grown at various altitudes were subjected to different conditioning treatments prior to roller milling. Amongst all treatments, conditioning grains to a moisture content of 14% for 30 min was found to be optimum. The bran fractions displayed greater levels of non-starchy polysaccharides and bioactive components as compared to refined flour fraction. The presence of greater levels of β-glucan in whole barley flour and bran of high altitude cultivars affected the refined flour yield inversely. Cultivars having higher total and insoluble arabinoxylans also resulted in lower flour yields ($R = -0.76$; $R = -0.73$). The damaged starch content of barley cultivars ranged between 5.1% and 8.7% which correlated positively with the content of β-glucans (up to $R = 0.77$) and arabinoxylans (up to $R = 0.80$) in bran and refined flour fractions. The anthocyanin and total phenolic contents of refined flours ranged between 3.9–7.6 μg/g and 1299–1607 μg FAE/g and was higher for high altitude cultivars.

1. Introduction

Nearly 1.75 million tonnes (FAOSTAT, 2013) of barley is cultivated in the Indian subcontinent. This grain plays a major role in sustainable agriculture due to its low water uptake and high drought tolerance. Barley has found limited application in human foods, in comparison to wheat which is used extensively for preparation of processed foods like bread, biscuits, cakes and chapattis. However, barley can serve as a healthier alternative to wheat due to its high content of bioactive compounds like, β-glucan, arabinoxylans and phenolic compounds (Fardet, Rock, & Remesy, 2008; Madhujith, Izydorczyk, & Shahidi, 2006; Madhujith & Shahidi, 2007; Sharma & Gujral, 2010). Worldwide it's hulled barley that is extensively used for malting and beer making, whereas, hulless barley has remained under-utilized. However, it is highly popular in some areas of the world like Leh-Ladakh in India and Lhasa in China (Dermience et al., 2014; Moza & Gujral, 2016).

The major non-starch polysaccharides of barley include mixed linked (1,3) (1,4) β-D-glucans and arabinoxylans (Newman & Newman, 2008). β-glucan has been reported to play a vital role in control of diabetes and cholesterol in humans (Jenkins et al., 2000; Madhujith & Shahidi, 2007). Uniqueness of barley lies in the fact that, its β-glucan is distributed throughout the aleurone and endosperm rather than being confined to outer branny layers (Bhatty, 1986; Zheng, Rossnagel, Tyler, & Bhatty, 2000).

Hulless barley can be subjected to roller milling to produce bran and refined flour which can be added to processed cereals to obtain a high fibre product without any undesirable change in the sensory characteristics (Bhatty, 1986; Kim & Lee, 1977). The morphological structure of hulless barley is similar to wheat (naked caryopsis) and hence, wheat milling machinery could be used to produce refined barley flour. The roller milling behavior of hulless barley has been explored (Bhatty, 1997, 1999b) and it has been found that the high β-glucan level in endosperm cell walls leads to sticking of endosperm to the bran fraction which eventually reduces the refined flour yield.

The objective of the following study was to optimally roller mill the hulless barley grains and then correlate the non-starchy polysaccharides (NSP), mainly β-glucan and arabinoxylans with the refined flour yield and damaged starch content.

2. Materials and methods

2.1. Materials

Hulless barley cultivars were procured from different locations in India: Geetanjali (CSAUAT, Kanpur) and Upasana (NDUAT, Faizabad) were the cultivars grown at low altitudes (below 126 m); Dolma and HBL-276 (CSKHPKV, Bajaura) grown at 1219 m, BHS-352 (IARI, Shimla) grown at 2200 m; Sindhu, Nurboo, SBL-8 and SBL-9 (HMAARI, Leh-Ladakh) were high altitude (3500 m) cultivars. The grain was manually cleaned and stored in PET jars at 4 °C in a refrigerator for evaluation.

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2.2. Chemicals

"Mixed Linkage β-Glucan kit (K-BGLU)" and "Starch Damage Assay Kit (K-SDAM)" were obtained from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland). Folin Ciocalteu's reagent was obtained from Loba Chemie (Mumbai, India). Phloroglucinol, D (+) xylose, D-glucose, sodium acetate and sodium carbonate were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Sulphuric acid, hydrochloric acid and acetic acid (glacial) were procured from Merck (India). All chemicals were of analytical grade. Milli-Q (Millipore, France) water was used for all the experiments.

2.3. Proximate composition of whole barley flours

The protein, carbohydrate, fat and ash content of the whole barley flours was determined according to the method of AOAC (1990).

2.4. Roller milling of hulless barley

Three conditioning treatments were given to the hulless barley grains prior to milling in Brabender Quadrumat® Junior (Brabender, Germany) as shown below

Treatment	Conditioning moisture (%)	Time of conditioning
1. Milling (M1)	14	24 h
2. Milling (M2)	14	30 min
3. Milling (M3)	10	24 h

Conditioning of grains was done using the formula

$$\text{Amount of water to be added} = \left(\frac{100 - \text{Actual moisture of grains}}{100 - \text{Desired moisture of grains}} - 1\right) \times \text{Weight of grains}$$

After the required amount of water was added (using the above formula) to the grains, they were mixed thoroughly till all the water got dispersed evenly. The grains were then rested for the required conditioning period and then were roller milled.

The grind obtained after milling was passed through 52 (300 μm) and 60 BSS (250 μm) sieves. The fraction retained on 52 BSS sieve was the bran fraction and the one passing through 60 BSS (250 μm) sieve was the refined flour fraction.

2.5. Colour characteristics

Colour measurement of refined flour samples was carried out using a Hunter Colorimeter fitted with optical sensor (Hunter Associates Laboratory Inc., Reston, VA, USA) on the basis of CIE L*, a*, b* colour system as described by Edney, Rossnagel, Endo, Ozawa, and Brophy (2002).

2.6. Quantification of total β-glucan

The total β-glucan was quantified according to the method reported by Mc Cleary and Glennie (1985) using a 'β-glucan assay kit'.

2.7. Quantification of total and water extractable arabinoxylan

A colorimetric method described by Finnie, Bettge, and Morris (2006) was used for estimating total and soluble arabinoxylan. Sample (125 mg) was weighed in polypropylene tubes. Water was added and the tubes were shaken vigorously. An aliquot (1 mL) was immediately pipetted out and transferred to a polypropylene tube. This aliquot was used to determine the total arabinoxylan content of the sample. The original suspension was then shaken in the orbital shaker at 25 °C for 30 min. After the extraction period was over, the sample was centrifuged at 2500*g* and the supernatant was collected. Aliquot (1 mL) was pipetted out from the supernatant and was used to determine the water extractable arabinoxylans.

Water (1 mL) was added to the tubes containing all the representative aliquots. Further, an extracting solution containing acetic acid, hydrochloric acid, Phloroglucinol (20% in ethanol) and glucose (1.75% aqueous solution) was added to the tubes, and tubes were allowed to boil in a boiling water bath (100 °C) (Narang Scientific Works, India) for 25 min. Tubes were then taken out, cooled immediately and absorbance of the samples was measured at 552 and 510 nm using a spectrophotometer (Douglas, 1981). D-(+)-xylose (X-1500, Sigma) was used to prepare the standard curve, which was used to determine the content of total and soluble arabinoxylans. The result was expressed in percentage (%). The tests were performed in quadruplets.

2.8. Starch damage

Starch damage was measured enzymatically using the 'Starch Damage Assay Kit'. Refined flour (100 mg, dry basis) was weighed in polypropylene tubes and equilibrated to 40 °C in orbital shaking incubator (Narang Scientific, New Delhi, India) for 5 min. Fungal alpha-amylase solution (50 U/ml) was prepared and was pre-equilibrated at 40 °C for 5 min. 1.0 ml of this solution was then added to sample tubes. The tubes were stirred on a vortex mixer for 10 s and incubated at 40 °C for exactly 10 min (from time of addition of the enzyme). Further, 8.0 ml of dilute sulphuric acid solution (0.2 % v/v) was added to each tube after 10 min from the time of addition of the fungal amylase and the tubes were stirred for 10 s for inactivation of enzymes and termination of the reaction. The tubes were centrifuged and an aliquot (100 μL) was taken. Amyloglucosidase (100 μL) was added to the tubes and after shaking vigorously on a vortex shaker, tubes were incubated at 40 °C for 10 min. Further, 4.0 ml of glucose oxidase/peroxidase reagent (GOPOD, glucose determination reagent) was added in each tube including glucose standard and all the tubes were incubated at 40 °C for 20 min. Along with the samples, a reagent blank was also prepared which contained 200 μL acetate buffer and GOPOD reagent (4.0 ml). The absorbance of the samples was measured at 510 nm on a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The damaged starch content was calculated as

$$\textit{Damaged Starch content}\ (\%) = \Delta E \times F \times 90 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

where

ΔE = (Absorbance of sample − Absorbance of reagent blank)
F = [150 (μg of glucose)/(Absorbance of 150 μg of glucose)]
W = Weight in milligrams of sample analysed

2.9. Total anthocyanin content

The determination of total anthocyanin content (TAC) was done using the spectrophotometric method reported by Siebenhandl et al. (2007). The samples were mixed with acidified methanol and the pH was set to 1 using 1 N HCl. Samples were then extracted for 2.5 h at 25 °C. After extraction, the samples were centrifuged and the absorbance of the supernatant was measured at 535 nm against a reagent blank. TAC was expressed as microgram cyanidin-3-glucoside per gram of sample and calculated using the equation shown below: