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Effect of sand roasting and microwave cooking on antioxidant activity of barley

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ABSTRACT

Eight different barley cultivars were roasted in sand and cooked in a microwave oven and evaluated for antioxidant activity (AOA), total phenolic content (TPC), non-enzymatic browning, polyphenol oxidase activity (PPO), total flavonoid content (TFC), reducing power and metal chelating activity. Sand roasting resulted in higher puffing index. Greater increase in yellowness of the roasted flour was brought about by microwave cooking. A significant decrease in TPC (8.5 to 49.6%) and TFC (24.5 to 53.2%) was observed after roasting. The AOA significantly increased (16.8 to 108.2%) after roasting with sand roasted barley exhibiting higher AOA. The reducing power and metal chelating activity significantly increased by up to 77.5% and 78.9%, respectively after roasting with sand roasted samples showing greater effect. The non-enzymatic browning index increased (315 to 774%) and was higher for sand roasted barley. The microwave cooking brought about a greater reduction (45.1 to 76.8%) in PPO activity.

1. Introduction

Barley (*Hordeum vulgare*) is an important cereal crop ranking fifth in world production and it plays an important role in human nutrition (Madhujith, Izydorczyk & Shahidi, 2006; Sharma & Gujral, 2010a). It is utilized around the world mainly as a malting and brewing grain, however some of the barley is also used in baked products. Barley is a rich source of β-glucan and contains high levels of phenolic compounds (Goupy, Hugues, Boivin & Amiot, 1999; Madhujith & Shahidi, 2009; Sharma & Gujral, 2010b).

Minimal processed foods have more health benefits as compared to processed foods (Shahidi, 2009). Roasting is a rapid processing method that uses dry heat for short periods of time. The roasted grain exhibits improved texture, enhanced crispiness and volume due to puffing (Hoke, Houska, Pruchova, Gabrovska, Vaculova, & Paulickova, 2007). Roasting also improves the digestibility, colour, flavor, shelf life and reduces the antinutrient factors of cereals and legumes (Gahalawat & Sehagal, 1992).

In India, barley is widely consumed in the roasted form called *Sattu*. The whole barley is roasted in hot sand at 250–300 °C for a short time which causes the grain to puff and expand splitting the husk. The husk is removed and the roasted grain is ground to flour which is mixed with water and sugar to make a popular drink. Microwave cooking is becoming a common heating method as the time taken is short. The shortcomings of sand roasting like lack of temperature control and contamination with sand can be eliminated by switching over to microwave cooking. The objective of the present investigation was to study the effect of traditional sand roasting and microwave cooking on antioxidant properties of different hulled barley cultivars grown in India.

2. Materials and methods

2.1. Barley samples

Eight common hulled barley cultivars (PL-172, PL-426, RD-2503, RD-2508, RD-2035, RD-2052, RD-2552 (six rowed) and DWR-28 (two rowed)) grown in different locations in the states of Punjab, Haryana, Uttar Pradesh and Rajasthan were collected from Central State Seed farm, Sriganganagar, Rajasthan, India. The grain was cleaned and stored for further evaluation.

2.2. Reagents

Standard ferulic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine, protease (from *Streptomyces griseus*) and catechin were procured from Sigma-Aldrich (Steinheim, Germany). L-3-(3,4-Dihydroxyphenyl) alanine (L-DOPA), 3-(N-Morpholino) propane sulfonic acid (MOPS) and polysorbate-20 were procured from Johnson Matthey, U.K. L-ascorbic acid, potassium ferricyanide, ferric chloride, ferrous chloride, trichloroacetic acid, sodium carbonate and Folin–Ciocalteu's reagent were procured from Loba Chemie, Mumbai, India. All chemicals were of analytical grade. Each test was performed in triplicates on dry weight basis. The Milli Q water (Millipore, France) was used in all analytical tests.

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2.3. Sand roasting and microwave cooking of barley and flour preparation

Hulled barley (400 g) conditioned to a moisture content of 10% so as to eliminate the effect of differences in moisture content on roasting behavior was roasted at 280 ± 5 °C for 20 s in a traditional sand roaster. The roaster consisted of an iron pan having a diameter of 915 mm and depth of 610 mm and contained 2 kg of sand. The roaster was fired with a diesel burner. The grain was vigorously stirred with the sand to ensure uniform heating (Sharma, Gujral and Rosell, 2010). It was immediately removed from the hot sand by sieving and spread on a marble slab for cooling.

Barley (50 g) was taken in a glass beaker (250 ml) and placed at the center of the microwave oven (LG, Intellocook, 2450 MHz, 900 W) and roasted for 120 s at 900 W. Both the sand roasting and microwave cooking treatments were carefully optimized in such a way that it resulted in grain with maximum expansion and no burning.

Barley after cooling was dehusked in a rice polisher McGill Rice miller No. 2 (Rapsco Brookshire TX, USA) as described by Sharma and Gujral (2010a, b) and ground in the Newport Super Mill (Newport, Australia) to pass through 60 BSS (250 μm) sieve to obtain barley flour. Any fraction retained on the sieve was again ground in the Super mill so that all of it passed through the 60 BSS sieve, the process was repeated till all the flour passed through the sieve. The flour was stored in airtight container at −20 °C for further analysis.

2.4. Physical properties of barley samples

The bulk density was calculated by measuring the weight of known volume of control, sand roasted and microwave cooked barley. Sample was poured in a graduated cylinder (250 ml) and then weighed and the result expressed as g/l. The puffing index was calculated by dividing the bulk density of control barley by the bulk density of sand roasted or microwave cooked barley.

2.5. Colour characteristics of flour

The colour measurement of flour from control, sand roasted and microwave cooked barley was carried out using a Hunter Colorimeter fitted with optical sensor (Hunter Associates Laboratory Inc. Restan VA., USA) on the basis of CIE L*, a*, b* colour system. The colour difference (ΔE) was calculated by applying the following equation

$$\Delta E = [(\Delta L^*) + (\Delta a^*) + (\Delta b^*)]^{1/2}$$

2.6. Total phenolic content (TPC)

The total phenolic content was determined according the Folin–Ciocalteu specterophotometric method (Sharma and Gujral 2010b). Barley flour samples (200 mg) were extracted with 4 ml acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) at room temperature (25 °C) for 2 h. Aliquot of extract (200 μl) was added to 1.5 ml freshly diluted (10 fold) Folin–Ciocalteu reagent. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 ml of sodium carbonate solution (60 g/l). After incubation at room temperature (25 °C) for 90 min, the absorbance of the mixture was read at 725 nm (Shimadzu, UV-1800, Japan). Acidified methanol was used as a blank. The results were expressed as μg of ferulic acid equivalents per gram of flour.

2.7. Antioxidant activity (AOA)

Antioxidant activity was measured using a modified version of the method described by Brand-Williams, Cuvelier, and Berset (1995). Ground barley samples (100 mg) were extracted with 1 ml methanol for 2 h and centrifuged at 3000 *g* for 10 min. The supernatant (100 μl) was reacted with 3.9 ml of a 6×10^{-5} mol/l of DPPH solution. Absorbance (A) at 515 nm was read at 0 and 30 min using a methanol blank. Antioxidant activity was calculated as % discolouration.

$$\%\ \text{Antioxidant activity} = (1-(\text{A of sample}_{t=30} / \text{A of control}_{t=0})) \times 100$$

2.8. Reducing power

The reducing power was measured as described by Zhao et al. (2008). Barley flour (0.5 g) was extracted with 80% methanol (0.5 ml) on wrist action shaker for 2 h. The extract (1 ml) was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and 2.5 ml potassium ferricyanide (1%) was added followed by incubation at 50 °C. Trichloroacetic acid solution (10%) was added to mixture, which was then centrifuged at 10,000 *g* for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml deionized water and 0.5 ml ferric chloride (0.1%). The absorbance of the mixture was measured at 700 nm. Increased absorbance of the mixture indicated increased reducing power. A standard curve was prepared using various concentration of ascorbic acid and the results were reported as μmol ascorbic acid equivalents/g of flour.

2.9. Metal chelating (Fe^{+2}) activity

The metal chelating activity of barley extract was measured as reported by Dinis, Madeira, and Almeidam (1994). The extract (0.5 ml) was mixed with 50 μl of ferrous chloride (2 mM/l) and 1.6 ml of 80% methanol was added. After 5 min, the reaction was initiated by the addition of 5 mM/l ferrozine (100 μl) and the mixture was shaken on vortex. The mixture was incubated at room temperature (25 °C) for 10 min. Absorbance of solution was measured at 562 nm on a spectrophotometer. The chelating activity of the extract for Fe^{+2} was calculated as follows:

$$\text{Iron}\left(Fe^{+2}\right)\text{ chelating activity }(\%) = \{1-(\text{Absorbance of sample at 562 nm} / \text{Absorbance of control at 562 nm})\} \times 100$$

2.10. Total flavonoids content (TFC)

The total flavonoids content was determined as previously described by Jia, Tang, and Wu (1998). Barley extract (250 μl) was diluted with 1.25 ml distilled water. Sodium nitrite (75 μl of 5% solution) was added and the mixture was allowed to stand for 6 min. Further, 150 μl of a 10% aluminium chloride was added and the mixture was allowed to stand for 5 min. After that, 0.5 ml of 1 M sodium hydroxide was added and solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Catechin was used as standard and the results were reported as microgram CE/g of flour.

2.11. Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity was determined using the standard method 22–85 of the American Association of Cereal Chemists (2000) by measuring the absorbance at 475 (A475) nm using a spectrophotometer. Control barley samples were assayed without L-3-(3,4-dihydroxyphenyl) alanine substrate. The PPO activity was calculated as difference in the absorbance of sample and control and expressed as Δ475/min g flour.

2.12. Non-enzymatic browning (NEB) index

Non-enzymatic browning index of barley samples was carried out by the method of Palombo, Gerter, and Saguy (1984). Barley flour