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Antioxidant potential of wheat flour chapattis as affected by incorporating barley flour

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ABSTRACT

Whole wheat flour was replaced with whole barley flour at levels of 28, 56 and 84 g/100 g to prepare chapattis and their antioxidant properties were evaluated before and after baking. The total phenolic content (TPC) in wheat flour was 2062 μg ferulic acid equivalents/g, total flavonoid content (TFC) was 966 μg catechin equivalents/g and antioxidant activity was 12.3% and they increased by 57.1, 101 and 22.6%, respectively upon incorporating barley flour at the highest level. Baking significantly decreased the TPC by up to 17.0% and TFC by up to 30.7% however the AOA increased by 13%. Barley flour incorporation significantly increased the reducing power which upon baking showed an insignificant decrease. Polyphenol oxidase (PPO) activity significantly increased by up to 109% upon incorporating barley flour however baking lowered PPO activity by 67.9%. Chapattis containing barley flour can be used to deliver the bioactive components of barley.

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1. Introduction

Processed foods these days require the incorporation of bioactive ingredients to satisfy the demands of health conscious consumers. Barley (*Hordeum vulgare* L.) is considered as a functional grain because it contains β-glucan, B-complex vitamins, tocotrienols, tocopherols and bioactive compounds (Madhujith, Izydorczyk, & Shahidi, 2006). Intake of dietary phenolics can reduce the risk imposed by free radicals and oxidation products that cause various forms of cancer and cardiovascular disease (Shahidi, Chandrasekara, & Zhong, 2011). Barley has greater antioxidant activity than wheat and rice and contains many phenolic compounds that are concentrated in the outer layer of barley grain (Madhujith et al., 2006). Beside the antioxidants barley also contains higher amount of (up to 6 g/100 g flour) of β-glucan, which is widely reported to lower blood cholesterol levels and glycemic index (Brennan & Cleary, 2005). The Food and Drug Administration has allowed whole grain barley and barley-containing products to carry a health and recommends that 3 g daily intake of β-glucan reduces the risk of coronary heart diseases by lowering blood cholesterol. Considering the health benefits of barley β-glucan, the human consumption of barley should be encouraged.

Chapattis are a good option for utilizing barley in baked foods because they have been a staple food of the Indian subcontinent and parts of the Middle East for hundreds of years (Gujral, Singh, & Rosell, 2008). A healthy person consumes 5–6 chapattis in a day and this quantity can deliver the required (3 g) daily requirement of β-glucan and antioxidants if part of the wheat flour is replaced with barley flour. In India most of the wheat is (~90%) consumed in the form of chapatti (Gujral, 2010) and chapattis would be a better way to supply the bioactive compound of barley in the human diet. The antioxidant properties of bread containing barley flour and fiber have been carried out (Holtekjolen, Baevere, Rodbotten, Berg, & Knutsen, 2008) however there is no literature available on antioxidant properties of chapatti containing barley flour. Antioxidant properties of chapattis containing germinated brown rice and oat flours have been reported by Gujral, Sharma, Bajaj, and Solah (2012) and Gujral, Sharma, Gill, and Kaur (2013). The objective of the present investigation was to utilize barley flour in chapatti making and to study the effect of incorporating barley flour on the antioxidant properties of chapatti and changes in the antioxidant properties upon baking.

2. Materials and methods

2.1. Milling of barley and wheat samples

Barley cultivar PL-172 is a widely grown cultivar of our region and it was selected for chapatti making because it contained high levels of β-glucan (5.3 g ± 0.1/100 g) which was determined using β-glucan assay kit provided by Megazyme International, Ireland (Sharma, Gujral, & Rosell, 2011). Barley sample was dehusked as previously described by Sharma and Gujral (2010). The commonly cultivated wheat cultivar PBW-343 was selected for blending with

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barley flour. Wheat was milled in a stone mill (Amar Industries, Amritsar, India) to produce whole wheat flour with 100% extraction rate. Whole barley flour was produced by milling dehusked barley in the same mill to get 100% extraction rate.

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-Ascorbic acid, potassium ferricyanide, ferric chloride, ferrous chloride, trichloroacetic acid, sodium carbonate and Folin–Ciocalteu's reagent were procured from Loba Chemie, Mumbai, India. L-3-(3,4-Dihydroxyphenyl) alanine (L-DOPA), 3-(N-Morpholino) propane sulfonic acid (MOPS) and polysorbate-20 (tween twenty) were procured from Johnson Matthey, U.K. All chemicals were of analytical grade. Each test was performed in triplicates on dry weight basis. The Milli Q water (Millipore, France) was used for all analytical tests.

2.3. Preparation of wheat–barley flour blends

Food and Drug Administration (FDA, 2005) has recommended a minimum 3 g intake of β-glucan per day therefore wheat flour was replaced with barley flour in such a way so as to give 1.5, 3.0 and 4.5 g/100 g β-glucan (equivalent to 28, 56 and 84 g/100 g barley flour incorporation) in the wheat barley flour blends, the blends containing barley flour were hereafter defined as BF-28%, BF-56% and BF-84%, respectively. The wheat flour (100%) was considered as control. The chapatti making behavior of the wheat barley flour blends and β-glucan incorporated wheat flour was evaluated.

2.4. Color characteristics of flour

The color measurement of chapattis 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^* color system. The color difference (ΔE) was calculated by applying the following equation

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

2.5. Chapatti making

Preliminary trials were carried out to determine the amount of water to be added to the flour (200 g) to develop a non sticky viscoelastic dough that could be easily rolled and sheeted to make a chapatti (Gujral et al., 2012). The flour was mixed with optimum water for 3 min in a laboratory pin mixer (National Manufacturing Company, Lincoln, NE). The water required to form a non sticky viscoelastic dough was reported as water absorption (g water/100 g flour). Water absorption of wheat flour was 68.7 g/100 g while blends containing barley flour at levels of 28, 56 and 84 g/100 g wheat flour had water absorption of 71.3, 73.0 and 76.7 g/100 g, respectively.

The dough was left to rest for half an hour. Dough ball (50 g) was rounded and then placed on a rolling board and sheeted with a rolling pin. The dough was rolled in one direction, inverted, and then rolled in a perpendicular direction.

The raw chapatti was immediately placed on an electric hot plate at 280 °C ± 3.0 and baked on one side and then inverted and baked on the other side followed by final baking on the first side. The baking time for the control chapatti was 77 s however the chapattis containing barley flour at level of 28, 56 and 84 g/100 g wheat flour, exhibited a baking time of 99, 120 and 165 s, respectively due to differences in dough water absorption. The chapatti was allowed to cool for 10 min at 25 °C (80% relative humidity) and then frozen at −20 °C and freeze dried in a freeze drier (Heto, LL 3000, Denmark) and the freeze dried chapattis were ground in Newport Super Mill (Newport, Australia) and stored at −20 °C for further analysis.

2.6. Sensory evaluation of chapattis

A semi-trained panel of 8 male and 7 female members comprising of staff and students from the department, evaluated the sensory properties of the chapattis. The age of the members ranged from 22 to 46 and the members are regular consumers of chapatti and are aware of its quality attributes. The samples were coded with specific numbers to eliminate bias. Panelists were instructed to evaluate color, taste, aroma, breakability, chewability, stickiness and overall acceptability. A nine-point hedonic scale with 1, dislike extremely; 5, neither like nor dislike and 9, like extremely was used. Water was provided to rinse the mouth between evaluations. Three batches were prepared for each treatment and three chapattis were evaluated from each batch.

2.7. Total phenolic content (TPC)

The total phenolic content (TPC) was determined according the Folin–Ciocalteu spectrophotometric method (Gao, Wang, Oomah, & Mazza, 2002). Sample (200 mg) was 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 (FAE) per gram of sample.

2.8. Antioxidant activity (DPPH radical scavenging activity)

Antioxidant activity (AOA) was measured using a modified version of the method described by Brand-Williams, Cuvelier, and Berset (1995). Sample (100 mg) was 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 % discoloration.

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

2.9. Reducing power

The reducing power was measured as described by Zhao et al. (2008). The sample (500 mg, dry weight basis) was weighed in polypropylene tubes and 0.5 mL of 80% methanol was added and shaken on shaker for 2 h. After that, the tubes were centrifuged at 3000*g* for 10 min and supernatant (extract) was collected. The extract (1 mL) was mixed with phosphate buffer (2.5 mL) and 2.5 mL potassium ferricyanide was added followed by incubation at 50 °C. Trichloroacetic acid solution (10 g/100 mL) 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. The absorbance of the mixture was measured at 700 nm. A standard curve was prepared using various

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