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FULL LENGTH ARTICLE

Effect of microwave roasting on antioxidant and anticancerous activities of barley flour

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Abstract The antioxidant and anticancerous activities of native and microwave roasted barley flour were compared in three different solvents (methanol, ethanol and water) using various antioxidant assays. The extracting solvents significantly affected the antioxidant potential and inhibition of DNA damage capabilities of barley flour extracts. Among the different solvents methanol was found to be the most suitable for extraction, as the extracts showed highest antioxidant as well as anticancerous activities and also prevented DNA damage to the maximum extent. Microwave roasting resulted in a mixed response toward the antioxidant potential of barley, as % inhibition of DPPH and reducing power increased while all other parameters such as OH radical scavenging activity, H_2O_2 radical scavenging activity and total phenolic content showed a decrease. An increase of 13.24% in methanol, 8.14% in ethanol, and 6% in water was reported in case of DPPH & an increase of 21.37% in methanol, 21.43% in ethanol, and 15.23% in water was seen in case of reducing power as a result of roasting. A decrease of 34.39%, 121.477%, and 50% in TPC values was reported as a result of microwave roasting for Methanolic, Ethanolic and Aqueous extracts, respectively. However, all the tests showed an overall increase in a dose dependent manner as the concentration of extracts increased. Roasting also resulted in a decrease in the anticancerous potential and the inhibition of DNA damage by the barley extracts irrespective of the solvents used. The anti-proliferative activities of the native and microwave roasted barley flour extracts were tested on Colo-205, T47D and MCF7 cell lines. Barley flour extracts inhibited cancer cell growth which was more for native barley flour (39.81%) than roasted barley flour (22.91%).

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1. Introduction

Barley (*Hordeum vulgare*) is an important cereal crop ranking fifth in the world production and it plays an important role in human nutrition (Madhujith et al., 2006; Sharma and Gujral, 2010). Barley is a widely consumed cereal among the most ancient cereal crops. Almost 80–90% of barley production is used as animal feeds and malt. However barley is now gaining renewed interest as an ingredient for the production of

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functional foods due to high concentrations of bioactive compounds such as β-glucans and polyphenols (Jadhav et al., 1998). Barley is also reported to contain B-complex vitamins, tocotrienols, and tocopherols (Madhujith et al., 2006; Sharma and Gujral, 2011) which are known to inhibit non enzymatic lipid peroxidation and are widely recognized for antioxidant and antiradical properties. The abundant contents of phenolic compounds in barley reveal that barley may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion.

Loss of nutritional components, generation of health deteriorating compounds, non-ecofriendly and economic considerations are major setbacks for the processing industry. Due to these considerations minimally processed foods are gaining importance in day to day life. It is well documented that the minimally processed foods have more health benefits (Shahidi, 2009; Sharma and Gujral, 2011). Roasted foods are one of the minimally processed foods that have been used all over the world from the ancient times. In India, barley is widely consumed in the roasted form called *sattu*. Microwave cooking is becoming a common heating method because it is time saving and the short-comings of sand roasting e.g., lack of temperature control and contamination with sand can be eliminated by switching over to microwave cooking.

2. Materials and methods

2.1. Materials

The indigenous hull-less variety of barley (*H. vulgare*) was procured from Regional Research Station, Ladakh. All chemicals used were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and calf thymus DNA were procured from sigma Aldrich, whereas, Folin–Ciocalteu reagent, gallic acid, sodium phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid, H_2O_2, KH_2PO_4, thiobarbituric acid, phosphate buffer, hydrogen peroxide, BHT, $FeSO_4$, phosphate buffer, 1.5% agarose gel Ethidium bromide and Tris, Tris/boric/EDTA gel buffer were procured from Himedia laboratories

2.2. Microwave cooking of barley

Hulled barley (400 g) was conditioned to a moisture content of 10% so as to eliminate the effect of differences in moisture content on microwave roasting behavior. Barley was kept in microwave oven (LG, Intellocook, 2450 MHz, 900 W)and roasted for 120 s at 900 W. Microwave roasting was carefully optimized in such a way that it resulted in grain with maximum expansion and no burning.

2.3. Extraction of barley flour for antioxidant assays

0.3 g each of native as well as roasted barley was dissolved separately in 10 ml of methanol, ethanol and water, and then stirred for 2 h on a magnetic stirrer followed by the centrifugation for 10 min at 3500 rpm. The supernatants obtained from native and roasted barley were labeled as N^m, N^e, N^a & R^m, R^e, R^a, respectively and superscripts 'm', 'e' and 'a' indicate methanol ethanol and aqueous solvents, respectively. 300 µl each of N^m, N^e, N^a, R^m, R^e & R^a was kept for TPC determination in triplicates. Rest of the quantity was then evaporated at 40 °C. The dried samples were redissolved in their respective solvents to make the stock sample of varied concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml & 50 µg/ml).

2.4. Antioxidant activity of microwave roasted barley flour

2.4.1. DPPH radical scavenging activity

DPPH radical scavenging activity of the extract solutions (viz; methanolic, ethanolic and aqueous) was determined according to the method of Gaulejac et al. (1998) with some minor changes. 100 µl each of native as well as roasted barley sample extracts in different solvents of varied concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml & 50 µg/ml) was added to 2.9 ml of 6×10^{-5} mol/l methanolic solution of DPPH. The absorbance at 517 nm was measured with a spectrophotometer (Hitachi U-2900) after the solution was allowed to stand in the dark for 60 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Percentage inhibition was calculated by using the formula:

$$\% \text{ inhibition} = A_{control517} - A_{sample517}/A_{control517} \times 100$$

where $A_{control517}$ is the absorbance of the control and $A_{sample517}$ is the absorbance of the extract.

2.4.2. Determination of total phenolic content

The TPC of the barley extract was determined according to the Folin–Ciocalteu spectrophotometric method (Singleton and Rossi, 1965) with some modifications. 100 µl each of N^m, N^e, N^a, R^m, R^e & R^a was mixed with 2.5 ml of 10-fold diluted Folin–Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 2 ml of 7.5% Na_2CO_3 solution was added to each of the samples, and the final volume was made up to 10 ml with deionized water. After 1 h of reaction at room temperature, the absorbance at 760 nm was determined. The measurement was compared to a standard curve of gallic acid (GA) solution, and the total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry barley (mg GAE/g db).

2.4.3. Reducing power

The reducing power was determined by the method of Oyaizu (1986) with minor changes. 100 µl of each extract with varying concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml) was mixed with sodium phosphate buffer and potassium ferricyanide. The mixture was incubated at 50 °C for 20 min followed by the addition of trichloroacetic acid and then centrifuged at 3000 rpm for 10 min. The upper layer was mixed with deionized water and $FeCl_3$, and then the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.4.4. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of extracts was assayed by the method of Wu et al. (2007) with certain modification. The reaction mixture contained 25 mM calf thymus DNA (1 ml), 10 mM ferric chloride (200 µl), 100 mM ascorbic acid (200 µl), 2.8 mMH_2O_2 (200 µl) in 10 mM KH_2PO_4 (pH 7.4) and various concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml,