Food Chemistry 119 (2010) 903–906

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of processing on the antioxidant properties of extracts from Mexican barley (*Hordeum vulgare*) cultivar

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ARTICLE INFO

Article history:
Received 24 March 2009
Received in revised form 15 July 2009
Accepted 22 July 2009

Keywords:
Antioxidant capacity
Barley
Cooking
Germination
Roasting

ABSTRACT

The effect of traditional cooking, roasting and germination on the antioxidant capacity of a Mexican barley cultivar is presented. Barley dried grains were processed and phenolic extracts obtained by successive extractions with hexane, aqueous acetone (70%), aqueous methanol (50%) and water. Total phenolic content was evaluated by the Folin–Ciocalteu method and the radical scavenging capacity by the DPPH method. The inhibition of LDL oxidation was also determined. Results showed that cooking and roasting barley extracts increase, the total phenolic content in comparison with control (unprocessed) barley extracts, but the germination reduces it. Germinated seeds subjected to roasting, cooking and unprocessed barley grains showed a higher antioxidant activity (IC_{50}). Traditionally cooked barley grains showed the highest inhibition of LDL oxidation. These results indicate that processing affects significantly the antioxidant capacity of barley phenolic extracts.

1. Introduction

Cereals constitute the most important food component because they have high carbohydrate content, cereals such as wheat, corn and barley also provide vitamins, trace minerals, dietary fiber and bioactive compounds (Madhujith & Shahidi, 2007). Barley (*Hordeum vulgare*) is a widely consumed cereal. Barley was one of the first agricultural domesticates together with wheat, pea, lentils dating from about 10,000 years ago (Smith, 1998). Unfortunately, barley has not been perceived as such an important grain in human diet. Barley is more associated with the beer industry, malting, and animal feed; 80–90% of barley production is used for malting and animal feedstocks (Baik & Ullrich, 2008).

Barley is gaining renewed interest as an ingredient for production of functional foods due to its high contents of bioactive compounds such as β-glucans, tocopherols and tocotrienols. Moreover, there are many classes of phenolic compounds in barley, such as benzoic and cinnamic acid derivatives, proanthocyanidins, quinones, flavonols, chalcones, flavones, flavanones and amino phenolic compounds (Holtekjolen, Baevere, Rodbotten, Berg, & Knutsen, 2008).

Phenolic compounds have attracted the attention of food and medical scientists because of their strong *in vitro* and *in vivo* antioxidant activities and their ability to scavenge free radicals, break radical chain reactions and scavenging metals. The consumption of phenolics has been connected with a reduced risk of cardiovascular diseases and cancer (Zhao & Moghadasian, 2008). The abundant content of phenolic compounds in barley reveals that it may serve as an excellent dietary source of natural antioxidants with antiradical and antiproliferative potentials for disease prevention and health promotion (Madhujith & Shahidi, 2007; Zhao et al., 2008).

Thermal and food preparation methods applied to cereals improves their texture, palatability and nutritive value by gelatinization of starch, denaturation of proteins, increased nutrient availability, inactivation of heat labile toxic compounds and other enzyme inhibitors (Bakr & Gowish, 1991). However, information regarding the effect of thermal or germination effect on phenolic compounds and antioxidant capacity from barley is still limited, especially in cultivars commonly consumed in countries such as Mexico. As far as we know, there are not reports on the effect of processing in the antioxidant properties of Mexican barley cultivars.

2. Materials and methods

Barley (*H. vulgare*) cultivar *Esmeralda* was grown in Durango, Mexico in 2006 at the local Experimental Station of INIFAP (National Research Institute for Forestry Agriculture and Livestock) and gratefully donated.

2.1. Cooking experiments

Regular boiling (under atmospheric pressure) of barley was conducted according to a traditional Mexican recipe. Briefly, whole

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0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.07.044

barley grains (100 g) were immersed in a vessel with boiling water (500 mL) for 60 min. At the end, barley grains were drained and cooled down at room temperature for 1 h in covered plastic containers. Samples were ground (Micro-mill grinder, Bel Art, Pequannock, NJ, USA) to obtain a fine powder with a particle size of 0.425 mm and stored at 20 °C before analysis.

For roasting barley, whole barley grains were heated (25 g) along with sea sand (to prevent burning) in an open hot plate at 125 ± 2 °C for 30 min. The seeds were separated from sea sand by sieving and cleaning. Samples were ground (Micro-mill grinder, Bel Art, Pequannock, NJ, USA) to obtain a fine powder with a particle size of 0.425 mm and stored at −20 °C before analysis.

Germination experiments were conducted placing whole barley grains (20 g) in 1 L Erlenmeyer flask with distilled water (500 mL) and kept in a shaker for 24 h. The pre-soaked seeds were rinsed with distilled water and flat lined in moist cotton for germination. The flats were covered with aluminum foil and the seeds were allowed to germinate in the dark. Sprouts were excised when their size was 1.5 cm (approx. 48 h of germination) for the assays. Samples were ground (Micro-mill grinder, Bel Art, Pequannock, NJ, USA) to obtain a fine powder with a particle size of 0.425 mm and stored at −20 °C before analysis.

Control (unprocessed sample). About 25 g of whole barley grains were ground (Micro-mill grinder, Bel Art, Pequannock, NJ, USA) to obtain a fine powder with a particle size of 0.425 mm and stored at −20 °C before analysis.

2.2. Extraction

Grain samples (15 g) from each process were defatted with *n*-hexane for 24 h under continuous stirring in a shaker (Gallenkamp, England) at room temperature and stored at −20 °C until use. Defatted barley grains (10 g) were extracted with 200 mL of 70% acetone (v/v) into 500 mL flasks. After 4 h at 45 °C, the supernatants and sediments were separated by vacuum filtration. Extract solutions were concentrated to dryness in a vacuum-evaporator at 45 °C. Dried extracts were weighed and the yields calculated. Extracts were kept in the dark at −20 °C until further analyses.

2.3. Total phenolic content (TPC)

The TPC of extracts was determined using the Folin–Ciocalteu method. Briefly, 0.5 mL of diluted extract solution was shaken for 1 min with Folin–Ciocalteu reagent (100 μL) and distilled water (6 mL). Then, 15% Na_2CO_3 (2 mL) was added and the mixture shaken again for 30 s. Finally, the solution was diluted to 10 mL with distilled water and the absorbance measured at 750 nm after 1 h (Spectrophotometer UV–Vis Cary 50 Bio, Varian, Palo Alto, CA, USA). Results were expressed as catechin equivalents.

2.4. Free radical scavenging activity

Free radical scavenging capacity of processed barley grain extracts were evaluated according to the reported procedure (Manzocco et al., 1998) using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). A calibration curve at 515 nm (UV–Vis Spectrophotometer Varian, Cary 50, Varian, Palo Alto, CA, USA) was made to calculate the remaining DPPH concentration in the reaction medium at several concentrations (0–5000 μg/mL). The radical scavenging activities of the samples were expressed in terms of IC_{50} (concentration required for a 50% decrease in absorbance of DPPH radical) and calculated as % inhibition of DPPH. IC_{50} was obtained by plotting the percentage of residual DPPH at steady state as a function of the antioxidant concentration. A volume of 1.9 mL of DPPH in methanol was used. The reaction was started by the addition of 30 μL of experimental samples at IC_{50} for 60 min. Then the rate constant (k) was evaluated according to the method of Manzocco et al. (1998).

2.5. Inhibition of LDL oxidation

Human plasma samples (donated by Hospital General of Durango) were poured in plastic tubes with ethylenediaminetetraacetic acid (EDTA) (1 mg/mL) and treated with a kit for LDL precipitation (Biosystems, Zapopan, Mexico). Before assay, the amount of protein in isolated LDL was determinate by the Bradford method. Isolated LDL (0.2 mg/mL) was incubated at 37 °C with $CuSO_4$ (25 mM/L) and different concentrations of barley grain extracts for 3 h. $CuSO_4$ (25 mM/L) at the same conditions was taken as control. The amount of peroxides was evaluated by the thiobarbituric acid method following procedure proposed by Loy, Simon, and Delgado (2002).

3. Results and discussion

Results from the physical characterization of barley grains are shown in Table 1. Extract yields from barley under the different processes are shown in Fig. 1. The yields (1–2.2%) were lower than the ones reported by Liu and Yao (2007), who found yields of 3.96–4.92%. Differences may be attributed to the cultivar features and agro ecological conditions of *Esmeralda* barley.

3.1. Total phenolic content

Total phenolic content (TPC) results are shown in Fig. 2. In extracts from germinated barley grains, TPC was significantly ($p < 0.05$) lower than in the control (unprocessed), cooked and roasted seeds. Higher values of phenolic content were associated to roasted barley grains; several authors have observed similar behaviour in roasted common beans in comparison with other processing methods (Boateng, Verghese, Walker, & Ogutu, 2008), they explain that increase of phenolic compounds could be associated to release more bound phenolics from the breakdown of cellular constituents in function of thermal treatment. Similarly, Siddhuraju and Becker (2001) observed lower phenolics after germination but increased after cooking and roasting in common beans. It is important to note that the increase content in phenolic compounds depends on the type of grain, the seed preparation, and procedure used (Boateng et al., 2008).

Earlier research in cereals indicated that a major portion of phenolics is present as soluble conjugated or insoluble bound forms (Sosulski, Kryger, & Hogge, 1982). Dewanto, Wu, Adom, and Liu (2002) explained that thermal processing might release more bound phenolic acids from the breakdown of cellular constituents. Randhir, Kwon, and Shetty (2008) suggested that the dissociation of conjugated phenolic forms due to thermal processing

Table 1
Physical characteristics of *Esmeralda* barley grain cultivar.

Sample	Length (mm)	Width (mm)	Thick (mm)	Moisture (%)
Esmeralda barley grain	0.98 ± 0.0015	0.30 ± 0.0020	0.22 ± 0.0010	74.36 ± 0.65

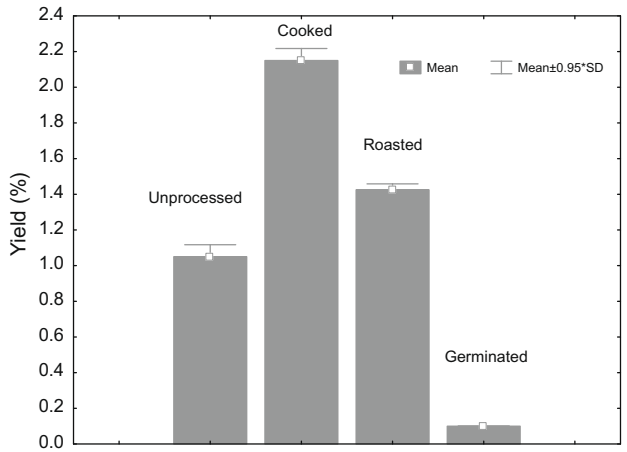

Fig. 1. Yield of extractables from processed and unprocessed barley grains.

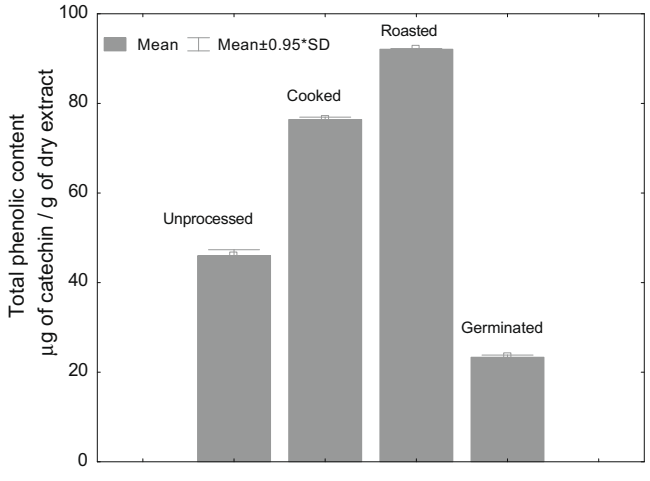

Fig. 2. Total phenolic content in extracts from processed and unprocessed barley grains.

followed by some polymerization/oxidation of phenolic constituents increased the amount of total phenolics.

The decrease in total phenolic content as observed in barley grains may be due to some degradation of phenolic compounds, unfortunately to support this affirmation is necessary more analytical research. These results are different from these for other barley and malt experiments (Maillard, Soum, Boivin, & Berset, 1996). According to Goupy, Dufour, Loonis, and Dangles (2003), an increase of 50% for phenolic compounds was observed during the germination of barley grains followed by kilning. Maillard et al. (1996) showed that the differences in phenolic contents in germinated barley grains might be explained by a poor extraction due to high moisture or bad milling of seeds. Increase in phenolic compounds in common beans in special in ferulic and *p*-coumaric acids was associated to a moderate reduction of hydroxycinnamic acid derivatives (or conjugated hydroxycinnamic acids) this result could indicate that thermal treatment induced the hydrolysis of conjugated phenolic compounds resulting in the release of free phenolic acids (Galvez Ranilla, Genovese, & Lajolo, 2009). Similar results were observed by Rakic et al. (2007) who indicating that thermal treatment of oak acorns from Serbia hydrolysable tannins were degraded resulting in increase of simple phenolics such as gallic acid. Clifford (2000) indicated that cinnamic acids such as caffeic, ferulic and *p*-coumaric acids are generally found in a conjugate form and they are released after a hydrolysis process such as that produced during thermal treatment. Free cinnamic acids can be further decarboxylated and degraded to several types of simple phenolics (Galvez Ranilla et al., 2009). According to Morello, Motilva, Tovar, and Romero (2004) phenolic acids in general exhibit better thermal stability than other phenolic compounds.

3.2. DPPH

Results for DPPH test expressed as IC_{50} are shown in Table 2. Lowest value of IC_{50} was obtained for catechin (110 µg/mL), highest value was shown for extracts from unprocessed barley grains (control) (4000 µg/mL).

Xu and Chang (2008) found after processing different grains that the DPPH radical scavenging was significantly reduced when compared to the original samples. However, Dewanto et al. (2002) found an increase in antioxidant activity after thermal treatment for sweet corn. Rocha-Guzman, Gonzalez-Laredo, Ibarra-Perez, Nava-Berumen, and Gallegos-Infante (2007) reported higher values of DPPH activity in cooked common beans (Bayo Victoria) than in uncooked beans, but this effect was related to the cultivar. Several authors claim that higher antioxidant properties of thermally processed foods could be due to the formation of Maillard products such as HMF (5-hydroxymethyl-2-furaldehyde), which render high antioxidant activity (Dueñas, Hernandez, & Estrella, 2006; Siddhuraju, 2006). Nicoli, Anese, Manzocco, and Lerici (1997) showed that medium dark roasted coffee brews had the highest antioxidant activity due to the formation of Maillard reaction products.

3.3. Kinetical analysis

The antioxidant kinetic analysis effect of different extracts of differently processed barley grains showed a decreasing *k* in the order of catechin > cooking > roasting > unprocessed = germination.

There were not differences ($p \geqslant 0.05$) between unprocessed barley and germinated seeds for the *k* parameter (Table 2), although in both cases, no thermal changes were induced. Several researchers claim that fast kinetics is associated with a vinyl-alcohol group and the fast and slow kinetics are associated with a free or monosubstituted catechol group. Thus, differences between processes could be associated with changes in the structure of the different chemical components in the extracts caused by thermal

Table 2
Chain-breaking activity and IC_{50} of extracts from processed and (control) unprocessed barley grains.

Sample	IC_{50}	Chain-breaking activity ($-O.D.^{-3}$/min/mg $_{d.m.}$)	R^2
Catechin	110 µg/mL[a]	4.2700[a]	0.991
Extract from (control) unprocessed barley grains	4000 µg/mL[b]	0.0076[d]	0.987
Extract from cooked barley grains	2600 µg/mL[c]	0.2342[b]	0.985
Extract from roasted barley grains	2000 µg/mL[d]	0.0980[c]	0.978
Extract from germinated barley grains	1650 µg/mL[e]	0.0064[d]	0.963

Different literal in the same column indicates statistical differences ($p < 0.05$).

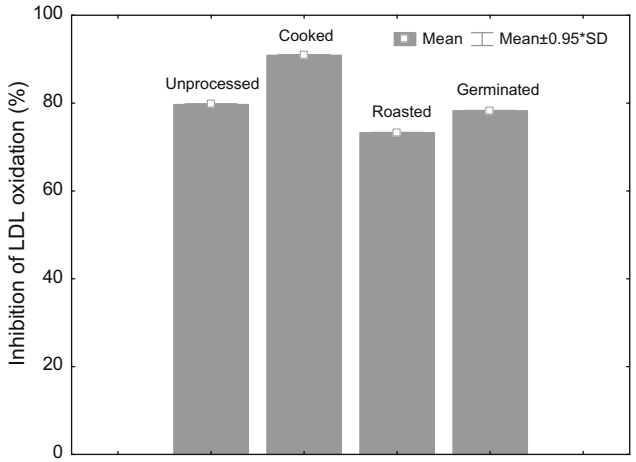

Fig. 3. Inhibition of LDL oxidation shown by extracts from barley grains.

processing, which can degrade vinyl-alcohols and increase the presence of free or monosubstituted catechol groups.

3.4. LDL

Oxidized LDL is processed by a scavenger receptor of macrophages, leading to cholesteryl ester accumulation. These lipid-laden macrophages become foam cells, which in time create fatty streaks leading to atherogenesis. Antioxidants are known to offer protective effects in controlling oxidative modification of LDL cholesterol, thus reducing the chances of developing atheromas in arteries.

Results from LDL assay are shown in Fig. 3 (experimental samples were evaluated at the IC_{50} for each treatment). The high level of protective effect against LDL cholesterol oxidation can be in part attributed to the chelation of Cu(II). Liyana-Pathirana and Shahidi (2006) showed that Cu(II)-mediated oxidation of LDL cholesterol can exhibit different kinetics depending on Cu(II) concentrations. Propagation can proceed when antioxidants are depleted at high Cu(II) concentrations, or when they are present at low concentrations. Madhujith and Shahidi (2007) found in aqueous methanolic extracts from six different whole barley cultivars 19.64–33.93% of LDL oxidation inhibition; these values were lower than found in the present work (78.1–94.3%). Differences could be associated to the use of different cultivar or the release of soluble conjugates by thermal treatment (Madhujith & Shahidi, 2009) (except for germinated and control). These results are interesting because in all cases higher levels of inhibition were found for processed and unprocessed barley grain extracts.

4. Conclusions

Processing of barley grains affects their total phenolic content, radical scavenging activity, antioxidant kinetics behaviour and inhibition of LDL oxidation. Particularly, barley grain extracts (processed or unprocessed) were very good for the inhibition of human LDL oxidation, thus the use of barley for human consumption in several processed forms has great potential for enhancing human health.

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