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Antioxidant activity of Iranian barley grain cultivars and their malts

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Barley (*Hordeum vulgare L.*) belongs to the grass family *Poaceae* and is an ancient and important cereal grain crop. Whole grain products are recommended for healthy diets as being recognized sources of dietary fiber and antioxidant substances such as polyphenols and vitamin E. The current study was conducted to evaluate the antioxidant activity for 19 cultivars (Bahman, MB-82-12, Nosrat, Kavir, Torkman, Makoeei, Karoun, Valfajr, Reihane, Dasht, MB-42-4, Nik, Rihane-03, Sahra, Yosef, DD-10, Nimrooz, Fajr-30, Gorgan-4) of barely (*Hordeum vulgare* L) grain and their malts. The Ferric reducing antioxidant power (FRAP) method was used to evaluate this activity. The Range of antioxidant activity in barely grain was ranged between 0.31-1.01 mg/kg and in malt was ranged between 0.64-3.34 mg/kg. The average antioxidant activity was significantly higher in malt (1.584±0.596 mg/kg) compared to barely grain (0.633 ±0.221 mg/kg) $p \leq 0.001$. Results of the current study show that Nosrat cultivar had significant difference from other cultivars of barley. But in malt products, MB-82-12 cultivar had significant difference with other malt products and had higher antioxidant activity. Finally, we recommend that, if the goal is to select the most antioxidant activity of barely and malt products, we preferred to use Nosrat cultivar of barley and MB-82-12 malt.

Key words: Antioxidant, barley cultivars, malt, ferric reducing antioxidant power (FRAP).

INTRODUCTION

Free radicals contribute to more than one hundred disorders in human body. Free radicals due to environmental pollutants chemicals, toxins, radiation, etc caves depletion of immune system antioxidants, change in gene expression and induce abnormal proteins (Pourmorad et al., 2006). Free radicals are controlled by enzymes such as medicinal plants, fruits, vegetables and seeds and can constitute an important source of antioxidants and they may be used to

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reduce oxidative damage and tissue injury (Amjad and Shafighi, 2013). Many plants have anti-oxidative and pharmacological activities. Bioactive phenols, especially bioflavonoids are very interesting as antioxidants because of the ability to act as free radical scavenging, inhibition of hydrolytic and oxidative enzymes (Frankel, 1995). Some researchers suggest that the biological activity of these compounds is related to their antioxidant action (Gryglewski et al., 1987). Antioxidants play an important role in inhibition and radical scavenging, thus providing protection against diseases. Antioxidants inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide radicals, proxy radicals, hydroxyl radicals and proxy nitrate (Karthikumar et al., 2007).

The antioxidant properties of phenolic compounds in grains have been associated with the health benefits attributed to these crops and the value-added products derived from them. Antioxidants may play an important role in the chronic disease prevention by arresting oxidative damage caused by reactive oxygen species (ROS) to vital biomolecules such as DNA, lipids, and proteins (Hollman, 2001). One of the richest sources of phenolics among the grains is barley. In beer, for example, 70 to 80% of the phenolic constituents originate from malted barley while the remaining 20 to 30% come from the hops (Gerhauser 2005). The scavenging activity of barley phenolics against DPPH and ABTS were comparable to a synthetic antioxidant, butylated hydroxyl-toluene (BHT) (Ragaee et al., 2006). Barley (*Hordeum vulgare* L.) is one of the ancient cereal crops that currently have received increasing demands worldwide. It is considered as one of the most important cereals worldwide. It is the major cereal in many dry areas of the world and is vital for the livelihoods of many farmers. In Iran, it is mainly grown for grain and straw for small ruminants during winter, with green fodder sometimes used for winter grazing. Barley assumes fourth position in total cereal production in the world after wheat, rice, and maize. Barley is more productive under adverse environments than other cereals. Barley has also been used as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods (Celus et al., 2006). It is used in soups and stews, and in barley bread of various cultures. Barley grains are commonly made into malt in a traditional and ancient method of preparation (Alazmani, 2015). Moreover, about two thirds of barley crop has been used for feed, one-third for malting and about 2% directly for food (Baik and Ullrich, 2008; Gupta et al., 2010). Barley grains were good source of phenols and contains very high amount of total phenolics. A wide range of phenolic antioxidant compounds has been found in barley such as benzoic and cinnamic acid derivatives, proanthocyanidins, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds (Hernanz et al., 2001). They are present in free and bound form in cereals. Bound phenolics are ester-linked to cell-wall polymers in the outer layers of kernel. Ferulic acid and its dehydrodimer derivatives is the major phenolic compound in cereals present mainly in bound form (Manach et al., 2004, Kim et al., 2007). Phenolics and other antioxidants found in cereals may act as free radical scavengers (Ragaee et al., 2006) and/or reducing agents, chelating pro-oxidant metals and singlet oxygen quenchers (Zielinski, 2002). Recently, no correlation was found between phenol and flavonoid contents and antioxidant activity in barley grain varieties (Sharifi et al., 2013). Malt and its products made from grains especially barley is considered as raw materials in industries with the highest conversion index and thus the greatest added value (Namaghi and Ghaboos, 2010). Over centuries, malting has been used for promoting enzymatic activity and decomposition of cell wall, softening the kernels, develop-ment of different aroma, flavor, and color, producing reduction sugars, and increasing the availability of vital nutrients of the grains. Malting is a biotechnologically complicated process including steeping, germination and drying germinated malt under temperature and humidity controlled conditions so that a friable nutrition product would be made (Gupta et al., 2010). During malting barley seeds are germinated to promote the mobilization of storage compounds process. Malting is influenced by various physicochemical factors including: barley variety, sulphur and nitrogen content, O_2 and CO_2 content, contents of carbohydrates, enzymes, antioxidants, proteins and lipids of barley and steeping and germination time (Eksiri et al., 2014). Today, malt has found a special application in food industry worldwide. Malt and its extract are used as sweeteners, flavorings, colorings a fermenting agent in malt vinegar and beer brewing, malt concentrate, malto-dextrin, maltose syrup, infant formula, coffee malt, and some bakery products. Malt also shows medicinal properties including lowering blood sugar level, functioning against intestinal diseases, stimulation of lactating glands, anti-diarrhea, strengthening hairs and preventing them to become gray (Namaghi and Ghaboos, 2010). Antioxidants are not equally distributed in barley grain. ρ-Coumaric acid exhibited the lowest amount in the kernel center and rapidly increased towards outer layers such as lignified huslc (Salomonsson et al.,1980). Phenolic acids are present mostly in the aleuronic layer and endosperm (Goupy et al., 1999). The highest amount of ferulic acid is found in cell walls of aleurone layer being rich in arabinoxylans. Natural antioxidants of cereals may act as free radical scavengers, reducing agents, potential complexes of pro-oxidant metals and singlet oxygen quenchers. Furthermore, many natural antioxidants present in barley exert wide – ranged biological effects including antibacterial antiviral anti-inflammatory, anti-allergic and anti – thrombotic effects and may also be involved in vasodilator actions (Cook and Sammon, 1996). Polyphones identified in barley include antho-cyanins, flavonols, phenolic acids catechins and proanthocyanidins. Antioxidants mostly play an important

role in malting and processing due to their ability to delay or prevent oxidation reactions and oxygen free radical reactions. Antioxidants such as sulfites, formaldehyde, or ascorbate can be added into the brewing process in order to improve beer flavor stability. Approximately 80% of phenolics of beer are derived from barley malt and the remaining comes from hops (Goupy et al., 1999). The phenolics in barley malting include polyphenols (benzoic and cinnamic acids derivatives), flavonoids, proantho-cyanidins, tannins and aminophenolic compounds. All these compounds identified as non-enzymatic inhibitors of lipid peroxidation have been also known as having important antioxidant and antiradical properties (Eksiri et al., 2014). Thus the presence of natural antioxidants in malting barley and screening of malting barley varieties with the highest level of radical scavengers seem important to produce beers with high levels of antioxidant activity (Gupta et al., 2010). Recently, barely malt extract was found to prevent the reduction of antioxidant enzymes activities, to decrease the levels of malondialdehyde and carbonyl in liver and brain, and to improve total antioxidant capability in the D-galactose induced mouse aging model (Qingming et al., 2010). Malt contains various compounds of barley (endogenous phenolic compounds) from the malting process (Maillard reaction products) which can play significant role in malting and brewing through their antioxidant properties (Goupy et al., 1999). Munich-style malts melanoidin rich atmosphere is known to have antioxidant properties that are beneficial in stabilizing the taste of beer (Briggs, 1998). Malt processing releases inherent bound phenolic compound and creates new antioxidants through the maillard reaction in barley leading to increased antioxidant activity (Baba et al., 2014). Germinated barley can contain more than 45 mg/g dry weight as fat and linoleic acid as the main component (50-60%). During malting, a significant reduction in fat content could be observed, indicating rapid degradation. Free fatty acids produced during lipolysis can be done by autoxidation and lipoxygenase yields highly reactive peroxide aqueous deoxygenated. This hydro peroxidase enzyme can produce carbonyl compounds such as trans-2-nonenal (Moll and Moll, 1986). Two ways may be used naturally for oxidative deterioration of beer and malting method of optimizing control: protecting antioxidants present in barely (mainly polyphones) and promotion of new products in antioxidants. Antioxidants are generally thought to play a significant role in malting and brewing due to their ability to delay or prevent oxidation reactions and oxygen free radical reactions (Zhao et al., 2008). Antioxidant compounds present in barley extracts are complex, and their activities and mechanisms would largely depend on the composition and conditions of the test systems. In order to better understand the antioxidant power of barley and malt, FRAP method (as a spectrophotometric method) was used to measure the antioxidant activity.

MATERIALS AND METHODS

Barley and malt samples

The barley cultivars were collected from the seed and plant improvement institute which included 19 Iranian barley cultivars: (Bahman, MB-82-12, Nosrat, Kavir, Torkman, Makoeei, Karoun, Valfajr, Rihane, Dasht, MB-42-4, Nik, Rihane-03, Sahra, Yosef, DD-10, Nimrooz, Fajr-30, Gorgan-4). These cultivars were collected from the growing area of Iran and corresponding malts were studied. All barley samples (with germination capacity above 95%) were malted in the same way using standard malting conditions. The following technology was used for malt production from the tested grains: washing and steeping of grains (H_2O T=17±2°C) until moisture content in grains reached 38–40%. Then the grains were placed for germination. The kilning procedure occurred in six successive steps of heating: 50°C for 12 h, 60°C for 1.5 h, 65°C for 1.5 h, 70°C for 1.5 h, 75°C for 1.5 h and 80°C for 4 h till constant moisture content was achieved in the grains (5±1%). Then the barley grains were held under warm and humid conditions for several days (germination) (Jones, 2005) and also to maintain embryo growth, enzymes synthesis and endosperm breakdown (Gupta et al., 2010). Finally they were dried under air current gradually increasing the temperature (kilning) for ensuring the product stability. This method is commonly used at the USDA Cereal Crops Research Unit described by Jones and Marinac (2000).

Extraction procedure

Barley (or malt) was finely ground in a laboratory mill. Fifty grams of ground sample was extracted with 150 mL of water at 45°C. After a 1 h extraction, the weight was filled up to 200 g. Then the extract was filtered with Whatman No.1 filter paper. The crude extract was stored at –20°C until used (MacGregor and Balance, 1980).

Ferric reducing antioxidant power (FRAP) assay

Briefly, 900 µL of the FRAP reagent (TPTZ 0.01 M: $FeCl_3$ 0.02 M: 0.1 M acetate buffer pH 3.6 (=1:1:10) was prepared daily, and 90 µL of distilled water and 10 µL of barley or malt extract were mixed and incubated at room temperature. Antioxidant activity was determined spectrophotometrically at 593 nm (UV visible spectrophotometer, GBC Cintra 40, Victoria, Australia) after 30 min. A series of concentrations of $FeSO_4$ including 1000, 750, 500, 250 and 125 µM were used for construction of calibration curve and were measured as described for sample solutions. All samples were performed in triplicate (Ozgen etal., 2006,Jannat et al.,2010).

Statistical analysis

The experimental results were expressed as means ± standard deviation (SD). SPSS version 18 was used to carry out the analysis of variance (ANOVA), $P < 0.05$ values were regarded as significant.

RESULTS AND DISCUSSION

Antioxidant activity of barley grain is not negligible. The results of the current study are shown in Table 1. The cultivar of barley had a significant influence on anti-oxidant efficiency. As shown in Table 1, malt of

Table 1. Antioxidant activity of different cultivars of barley grain and their malts.

Cultivar	Malt (Mean±SD) ($mgkg^{-1}$)	Barley (Mean±SD) ($mgkg^{-1}$)
Rihane-03	2.25±0.007	0.43±0.001
Makoeei	2.11±0.005	0.73±0.002
Mb-82-12	3.35±0.044	0.31±0.001
Gorgan-4	1.17±0.002	0.80±0.001
Mb-42-4	1.75±0.086	0.35±0.001
Karoun	0.65±0.001	0.71±0.004
Dasht	1.27±0.001	0.40±0.001
Nik	1.37±0.001	1.02±0.005
Fajr-30	1.48±0.017	0.56±0.001
Yosef	2.03±0.003	0.89±0.003
DD -10	0.98±0.001	0.32±0.001
Nosrat	1.12±0.002	1.02±0.002
Sahra	1.35±0.005	0.84±0.001
Kavir	1.65±0.002	0.59±0.001
Bahman	0.98±0.003	0.74±0.001
Torkman	1.89±0.003	0.53±0.001
Valfajr	1.31±0.001	0.47±0.001
Rihane	1.38±0.003	0.78±0.002
Nimrooz	2.01±0.001	0.55±0.001

Mb-82-12 cultivar had higher antioxidant activity than the others. On the other way, malt of Bahman and DD-10 cultivars varieties had lowest antioxidant activity and had significant difference with other cultivars. Most of the malts of the different barley cultivars had significant differences with each other, but Nik and Nosrat cultivars had no significant difference in antioxidant activities. In barley cultivars antioxidant activities, Nosrat cultivar had higher anti-oxidant activities and had a significant difference with other varieties. On the other hand, Mb-82-12 variety had lowest antioxidant activities. Differences in antioxidant activity between other barley varieties were significant. Majority of malts had higher antioxidant activities than their corresponding barleys. The increase of antioxidant activity could come from the development of such non-enzymatic browning products as Maillard products, which can also act as antioxidants, particularly melanoidins (Goupy et al., 1999; Yanagimoto et al., 2002; Yilmaz and Toledo, 2005; Osada and Shibamoto, 2006). In addition, hydrolytic enzymes can lead to the release of bound phenolic compounds, mainly the phenolic acids associated to lignin and arabinoxylans. Besides the synthesis of amylases, proteases and α-glucanases causing polymer degradation, other hydrolytic enzymes can lead to the release of bound phenolic compounds, mainly the phenolic acids associated to lignin and arabinoxylans (Maillard et al., 1996; Maillard and Berset, 1995). Moreover, kilning leads to more friable tissues and probably allows better extraction of phenolic acids, mostly present in the outer layers of the grain (Maillard et al., 1996 and 2007). At the later stage of the drying process, some observed increase in antioxidant activity could be attributable to the occurrence of antioxidant substances or phenolic compounds by thermal reactions such as non-enzymatic browning reactions (Yanagimoto et al., 2002; Yilmaz and Toledo, 2005; Osada and Shibamoto, 2006). Appearance of maillard reaction products during kilning has previously been proved. It has been shown that beers naturally contain products resulting from the thermal breakdown of carbohydrates or from the non-enzymatic browning reaction (Amadori compounds, enediols, enaminones, enediamines, reductones and melanoidins (Moll and Moll, 1986). Duenas et al. (2009) showed that germination produced significant changes in flavonoids and non-flavonoid phenolic compounds of *Lupinus angustifolius* seeds. The results obtained indicate that germination modifies the quantitative and qualitative polyphenolic composition of lupin (*Lupinus angustifolius* L.) seeds during the different days of the process, with a significant increase of flavonoids. An increase in the antioxidant activity was also observed as a consequence of the process (Duenas et al., 2009). In fact, germination releases reducing sugars and amino acids. During the first steps of kilning, the water at the surface layers of the grain is removed (the humidity falls from 0.45 to 0.12 g/g) and the first intermediates of maillard reaction can be produced. Then, high temperatures and low humidity contents achieve the extension of the maillard reaction. Caramelization of sugars can also occur during the last steps of kilning, catalyzed by the low concentration of organic acids (Karakus, 1975). Ferulic acid reacts with maillard reaction

intermediates derived from glucose and proline at kilning temperatures, leading to higher antioxidant activity and an increase of antioxidant properties of maillard reaction products with heating time (Samaras et al., 2005).

Conclusion

The present study demonstrated the influence of barley cultivar, and malting on antioxidant activity. The cultivar of barley has significant influence on antioxidant efficiency. Thus, this foundation can help the selection of barley cultivars, knowing their antioxidant efficiency and their phenolic composition. The choice of a cultivar must take into account two parameters: the antioxidant power of barley and its increase during malting. Antioxidant activity of barley seems to be somewhat related to the total phenolic content, particularly to the content of flavan-3-ols. Thus, it could be more judicious to choose a cultivar of barley showing a poly phenolic profile with the most antioxidative compounds. The influence of the kilning process was also demonstrated. During kilning, antioxidant activity increased, as well as the content of phenolic acids. However, more study is needed to demonstrate factors affecting the antioxidative capacity of barley and malt.

Conflict of interests

The author(s) did not declare any conflict of interest.

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