Food Chemistry 196 (2016) 638–645

Contents lists available at ScienceDirect

Food Chemistry

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

Antioxidant and antiproliferative activities in immature and mature wheat kernels

Mi Jeong Kim, Sang Sook Kim *

Division of Functional Food Research, Korea Food Research Institute, Seongnamsi 463-746, Republic of Korea

ARTICLE INFO

Article history:
Received 7 May 2015
Received in revised form 20 September 2015
Accepted 28 September 2015
Available online 30 September 2015

Keywords:
Immature wheat
Antioxidant capacity
Cell proliferation
Phenolic content
Flavonoid content
Vitamin E

ABSTRACT

The purpose of this study was to investigate health benefit-related parameters such as antioxidant contents and antiproliferative activities in immature wheat compared to mature wheat. Immature and mature wheats were harvested 35 and 45 days after the heading date, respectively; steamed immature wheat was also tested. The phenolic, flavonoid and vitamin E contents of immature and steamed immature wheat were compared with those of mature wheat. Additionally, antiproliferative activities against colon cancer cells (HT-29 and Caco-2) and cervical cancer cells (HeLa) were evaluated in three samples. The immature wheat contained higher phenolic and flavonoid contents but lower vitamin E contents than mature wheat. The antioxidant capacity, as measured by oxygen radical absorbance capacity (ORAC), was higher in immature wheat than in other samples. In the antiproliferation assays, immature wheat had the lowest EC_{50} values in HT-29 (39.3 mg/mL) and HeLa (31.4 mg/mL) cells, indicating stronger antiproliferative activity.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major food grains consumed by humans (Van Hung, Maeda, Miyatake, & Morita, 2009), and global wheat production in 2014 was estimated at 715 million tons (FAO, 2014). Although wheat is used primarily as a source of energy, whole wheat grain is an excellent source of dietary fiber, minerals, vitamins, and bioactive phytochemicals, such as antioxidant compounds (Cai, Choi, Lee, Park, & Baik, 2014). For this reason, numerous studies have been conducted to investigate the nutritional quality and health benefits of whole wheat.

In particular, the phenolic contents and antioxidant capacities in wheat have been studied to investigate its health benefits (Adom & Liu, 2002; Okarter, Liu, Sorrells, & Liu, 2010; Verma, Hucl, & Chibbar, 2008). Adom and Liu (2002) reported that the antioxidant capacity of wheat was higher than that of rice or oats. Verma et al. (2008) found that the antioxidant activity in wheat bran was highly correlated with its free, bound, and total phenolic contents. The phytochemicals found in wheat are ferulic acid, *p*-coumaric acid, syringic acid, vanillic acid and caffeic acid (Okarter et al., 2010). These phytochemicals exhibit strong antioxidant properties in that they scavenge or neutralize free radicals by donating electrons and thereby reduce or minimize oxidative damage to proteins, DNA, and lipids (Verma et al., 2008). Decreases in oxidative damage to cells or cell components may explain the inhibition of diseases such as cancer and cardiovascular diseases, which may be caused by oxidative stress (Willcox, Ash, & Catignani, 2004).

In addition to overall antioxidant capacity, wheat has several specific components that contribute to a reduced risk of colon cancer. Okarter (2011) found that phenolic extract from the insoluble-bound fraction of whole wheat inhibited the proliferation of human colon cancer cells in vitro. These researchers also reported a higher antiproliferative activity of bound phenolics in the cell wall. Recently, Whent et al. (2012) reported that antiproliferative activity against HT-29 cells was higher in the wheat cultivar WestBred 936 than in other wheat cultivars. Additionally, 5-alk(en)ylresorcinols isolated from wheat bran oil had strong antiproliferative activity (Zhu, Conklin, Chen, Wang, & Sang, 2011). However, neither immature wheat nor the bran of immature wheat has been characterized.

In Korea, wheat is harvested in June, which is considered the rainfall season. The quality of wheat is negatively affected by rain during the harvest season, which causes pre-harvest sprouting (PHS) of wheat kernel (Edwards, Ross, Mares, Ellison, & Tomlinson, 1989). The PHS reduces the functional quality of wheat flour and thus affects the economic value of the grain. The problem by PHS affects many wheat producing regions including Canada, Australia, South Africa, USA, Central Asia and Europe

* Corresponding author.
E-mail address: sskim@kfri.re.kr (S.S. Kim).

http://dx.doi.org/10.1016/j.foodchem.2015.09.095
0308-8146/© 2015 Elsevier Ltd. All rights reserved.

(Biddulph, Plummer, Setter, & Mares, 2007). As reported by Biddulph et al. (2007) and Clarke et al. (2005), farmers in Australia lost about 22% of their wheat revenue in the 2004/2005 and the Canadian wheat crop value loss was annually estimated around $100 million due to PHS damage. If immature wheat could be used as food material, the likelihood of wheat damage by PHS would be reduced. Immature wheat is typically obtained by early harvest when the wheat culms are still green in color (Musselman & Al-Mouslem, 2001). Immature or steamed immature grains have been used as food during times of famine in Korea (Park, Choi, Kum, & Lee, 2007). The identification of health benefits or advantages of consuming immature or steamed immature wheat could promote the utilization of immature wheat as food material. However, only few studies have reported characteristics of immature wheat compared to those of mature wheat, and those studies have typically focused on nutritional aspects (Kim et al., 2007; Yang et al., 2012). Yang et al. (2012) reported lower protein and higher dietary fiber contents in immature wheat than in mature wheat. Kim et al. (2007) also reported that immature wheat had higher essential amino acid contents than mature wheat and the immature wheat with more than 33 days after the heading date was similar to matured wheat in protein content, implying as a potential food material. Although previous studies reported nutritional value of immature wheat, information on health benefits such as antioxidant or anticancer activity in immature wheat is limited.

The purpose of this study was to investigate the health benefits of immature wheat by evaluating its antioxidant capacity as measured by oxygen radical absorbance capacity (ORAC) and determining contents of related compounds (e.g., total phenolic contents, flavonoids and vitamin E) and comparing their values to those in mature wheat. Additionally, the antiproliferative effect of immature and mature wheat against two colon cancer cell lines (HT-29 and Caco-2) and a cervical cancer cell line (HeLa) were compared.

2. Materials and methods

2.1. Grain samples and sample preparation

The wheat samples used in this study were obtained from a hard wheat cultivar (*T. aestivum* L. cv. Keumkang) grown in Iksan (Jeollabuk-Do, Korea, geographic coordinates: 35°56′ N, 126°53′ E) and harvested in 2014. Immature wheat (IW) was harvested 35 days after the heading date, and mature wheat (MW) was harvested 45 days after the heading date. In addition to MW and IW, steamed immature wheat (SIW) was prepared by steaming 1 kg of immature wheat kernels in covered stainless container (41 × 41 × 17 cm) for 30 s with 0.5 Mpa at 100 °C. Steaming has been used to prevent spoilage due to the enzymatic activity in immature grain with high moisture content. Three samples (MW, IW and SIW) were dried overnight to 14% moisture using a dry oven (HK-D0100F, Hankuk General Equipment Plant, Hwaseong-si, Korea). The ash, protein and fat contents of mature, immature and steamed immature wheat kernels based on 14% moisture were as follows: mature wheat (ash: 1.39%; protein: 10.5%; fat: 1.33%), immature wheat (ash: 1.46%; protein: 10.2%; fat: 2.03%) and steamed immature wheat (ash: 1.5%; protein: 11.1%; fat: 1.89%). Dried kernels were packaged in vacuum-sealed bags at 0 °C until used. Wheat kernel samples were milled into flour using a Cyclotec™ 1093 sample mill (Foss, Hillerod, Denmark) and stored at 4 °C for further analysis.

2.2. Methods

2.2.1. Extraction of free and bound phenolic contents in wheat kernels

The phenolic contents were extracted as previously described by Verma, Hucl, and Chibbar (2009), with slight modifications. In this study, free and bound phenolic contents were extracted as soluble and insoluble phenolic fractions.

Free phenolic contents in wheat were extracted by mixing 1 g of whole wheat flour with 20 mL of 80% chilled ethanol for 10 min. After centrifugation at 2500×*g* for 10 min, the supernatant was removed, and extraction was repeated. Supernatants were pooled, evaporated to dryness at 45 °C, and reconstituted with acidified methanol (methanol/hydrochloric acid, 80:20, v/v) to a final volume of 5 mL.

After extraction of the free phenolic contents, residues were subjected to extraction of bound phenolic compounds. First, the residues were digested with 6 M sodium hydroxide at room temperature for 1 h with shaking (SK-600, Lab Companion, Seoul, Korea). Then, the mixture was neutralized with an appropriate amount of hydrochloric acid and extracted with hexane to remove lipids. After washing with hexane, the mixture was hydrolyzed with hydrochloric acid at 85 °C for 30 min and then extracted five times with ethyl acetate. The ethyl acetate fraction was evaporated to dryness at 45 °C and reconstituted with acidified methanol (methanol/hydrochloric acid, 80:20, v/v) to a final volume of 10 mL. The extracts were stored at −20 °C for less than 3 days and used to measure total phenolic content, total flavonoid content and ORAC.

2.2.2. Determination of total phenolic content (TPC)

The total phenolic content (TPC) of free and bound extracts in three samples was determined using methods previously described by Lv et al. (2012), with slight modifications. Values of total phenolic contents were calculated by combining free and bound phenolic contents. Briefly, 100 μL of whole wheat sample extract was oxidized with Folin–Ciocalteu (Sigma–Aldrich, St. Louis, MO, USA) reagent; then, the reaction was neutralized with 20% sodium carbonate (Junsei Chemicals, Tokyo, Japan). After 2 h of reaction at ambient temperature in the dark, the absorbance was measured at 765 nm using a spectrophotometer (U-2900 double beam spectrophotometer, Hitachi High-Tech, Tokyo, Japan). Gallic acid (Sigma–Aldrich, St. Louis, MO, USA) was used as standard, and TPC was expressed as milligrams of gallic acid equivalents per gram of whole wheat.

2.2.3. Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) of free and bound extracts in three samples was determined as previously described by Gujral, Sharma, Gill, and Kaur (2013). Total flavonoid contents were calculated by summing free and bound flavonoid contents. The extract (250 μL) was diluted with 1.25 mL distilled water. Then, 70 μL of 5% sodium nitrite (Wako Chemical, Richmond, VA, USA) was added, and the mixture was allowed to stand for 6 min. Next, 150 μL of 10% aluminum chloride (Junsei Chemical, Tokyo, Japan) was added, and the mixture was allowed to stand for 5 min. Then, 0.5 mL of 1 N sodium hydroxide was added, and the solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer (U-2900 double beam spectrophotometer, Hitachi High-Tech, Tokyo, Japan). As standard, (+)-catechin hydrate (Sigma–Aldrich, St. Louis, MO, USA) was used; the results were reported as milligrams of catechin equivalents per gram of whole wheat.

2.2.4. Determination of vitamin E

For determination of vitamin E, the oil was extracted from wheat flour samples (5 g) with *n*-hexane for 6 h. The oil was then recovered by evaporation of the solvent. The tocopherol and tocotrienol contents were analyzed as previously described by Ko et al. (2012). In brief, extracted oil from wheat samples were diluted with *n*-hexane and then filtered through a Millipore 0.45 mm FH membrane for a liquid chromatograph analysis. A liquid

Download English Version:

https://daneshyari.com/en/article/1184188

Download Persian Version:

https://daneshyari.com/article/1184188

Daneshyari.com