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Phenolic acid profiles of Chinese wheat cultivars

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ABSTRACT

Phenolic acid concentrations were determined in 37 Chinese commercial winter wheat cultivars grown at a single site over two seasons, and fractions comprising free and bound types were analyzed using HPLC with measurements of individual phenolic acids in each fraction. Most of the parameters were significantly influenced by cultivar, season, and their interaction effects, with cultivar variance being predominant. Wide ranges of concentration among the 37 cultivars were observed. The average concentration of bound type was 661 $\mu g\ g^{-1}$ of dm, making up 97.5% of the phenolic acid determined with ferulic accounting for 70.7% of it, while free type made up only 2.5% of the phenolic acid determined with syringic accounting for 44.7% of it. Bound type was the predominant source to the grain phenolic acid concentrations determined. There were highly significant and positive correlations between bound ferulic concentration and total bound phenolic acid concentration, and between free syringic concentration and total free phenolic acid concentration. Cultivars Liangxing 66 and Zhongmai 895 were stable in concentration of components of phenolic acids across seasons, with high values of free and bound phenolic acids indicating they could be selected as parents in wheat breeding for health beneficial phenolic acid.

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1. Introduction

Common wheat (*Triticum aestivum* L.) is a major crop and an important component of the human diet, accounting for approximately 30% of the total grain consumption with an annual production of over 660 million tonnes globally (FAO, 2010). It is used in the production of various food products including bread, noodles, steamed bread, and cakes (He et al., 2004), supplying protein, starch, micronutrients such as Fe and Zn (Bouis et al., 2000), together with dietary fiber, vitamins, and phytochemicals thought to contribute protective effects to health (Ward et al., 2008).

Current interest in the health benefits provided by wheat and other grains has led to an increased focus on variation of phytochemical concentrations among cultivars (Adom et al., 2003, 2005; Ward et al., 2008). Epidemiological studies have increasingly shown protective roles of wheat grain against the risk of many chronic diseases, especially those related to metabolic syndrome (Meyer et al., 2000; Ward et al., 2008). Grain consumption helps to lower the incidence of cardiovascular disease (Jacobs et al., 1998; Thompson, 1994) and cancer-related deaths (Jacobs et al., 1998; Nicodemus et al., 2001), partly due to unique phytochemical concentrations of a number of chemicals including derivatives of benzoic and cinnamic acids, flavonols, phenolic compounds, tocotrienols, tocopherols, and carotenoids (Adom et al., 2003, 2005; Ward et al., 2008). Grain phytochemicals exert their health benefits through multifactorial physiologic mechanisms including antioxidant activity, mediation of hormones, enhancement of the immune system, and facilitation of substance transit through the digestive tract (Lupton et al., 1995), butyric acid production in the colon, and absorption or dilution of substances in the gut (Gazzaniga and Lupton, 1987). Among the health-promoting phytochemicals residing in cereal grains, phenolic compounds as the most diverse and complex group have gained much attention in scientific research (Dykes and Rooney, 2007; Thompson, 1994). Phenolic acids are the most abundant form of phenolic compounds in cereal grain (Mattila et al., 2005), and play an important role in combating oxidative stress in the human body by maintaining a balance between oxidants and antioxidants (Temple, 2000). Phenolic acids can be divided into two groups (Kim et al., 2006; Li et al., 2008; Ward et al., 2008), with hydroxybenzoic acid derivatives including

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p-hydroxybenzoic, protocatechuic, vannilic, syringic, and gallic acids, and hydroxycinnamic acid derivatives including p-coumaric, caffeic, ferulic, and sinapic acids (Verma et al., 2009). These acids are present mainly in bound forms, linked to cell wall structural components such as cellulose, lignin, and proteins through ester bonds (Parker et al., 2005). They were reported to possess good antioxidant activities (Miller and Rice-Evans, 1997). The presence of phenolic acids both in free and bound form attached to various polysaccharides is of significant interest in preventing oxidative stress induced diseases. The free phenolic acids are easily absorbed into the circulation, whereas the bound type are released by the intestinal enzymes as well as by the colonic microflora and can be absorbed into the circulatory system (Andersson et al., 2010). Thus a strategy of breeding cultivars with enhanced abilities to load more phenolic acids into the grains offers sustainable, cost-effective health benefits and has the advantage of requiring no changes in consumer behavior (Adom et al., 2003; Irmak et al., 2008). Exploiting genetic variation in cultivars for increased phenolic acids is likely to be an effective method and is the first step toward enhancement in wheat based foods. Highly significant genotypic differences in phenolic acids among wheat genotypes from Europe, Canada and the United States of American have been reported (Irmak et al., 2008; Li et al., 2008; Verma et al., 2009; Ward et al., 2008).

China is the largest wheat producer and consumer in the world, with an annual production of more than 110 million tonnes from about 24 million hectares, supplying the cheapest source of calories and protein for the local inhabitants. Research has traditionally focused on improving yield, and disease and pest resistance, but more recently also on industrial quality characteristics for food products (He et al., 2001). To date, little attention was given to improving nutritional value, and no information is available on phenolic acid profiles in the leading wheat cultivars. Studies have indicated that the cardiovascular incidence in urban China is about 18% and has almost doubled in the last ten years (Chen, 2004). This has received recent national attention as metabolic syndrome. Therefore, full characterization of the phenolic acid profiles of the leading wheat cultivars could lead to new opportunities for breeding and eventual commercial production of value-added cultivars rich in beneficial components for making nutraceuticals and other functional foods. The objectives of this study were to determine the phenolic acid profiles and concentrations in grain of leading Chinese wheat cultivars and promising advanced lines. The information generated from the study could be important for Chinese wheat breeding programs, and may have potential application in other wheat producing countries.

2. Materials and methods

2.1. Wheat samples and sample preparation

Thirty-seven high-yielding commercial wheat cultivars were used in this study. All of them are from the Yellow and Huai River Valleys Winter Wheat Region which contributes 70% of Chinese wheat production (He et al., 2001), except for the superior pan bread quality cultivar Zhongyou 9507 which is from the North China Plain Winter Wheat Region. Yannong 19, Jimai 19, and Han 6172 have been the leading cultivars since 2001, each with cumulative areas of cultivation of more than four million ha. The cultivation area of Jimai 22 in the 2010–2011 season exceeded 2 million ha, whereas Zhongmai 155, Zhongmai 875, and Zhongmai 895 were the most promising lines. Xiaoyan 6 was a leading cultivar from 1981 to 1995 and widely used as a quality donor parent. Jishi 02-1, Yumai 34, and Zhengmai 366 displayed superior pan bread quality (contact for detailed information). The tested cultivars were sown in the 2008–2009 and 2009–2010 cropping seasons at the wheat breeding station of the Institute of Crop Science, Chinese Academy of Agricultural Sciences, at Anyang (lat. 36°06′N, long. 114°21′E, 61 m above sea level, with a loam soil type) in Henan province, located in the Yellow and Huai River Valleys Winter Wheat Region. The heading dates of the cultivars ranged from April 19 to 26 in the 2008–2009 season and from April 27 to May 3 in the 2009–2010 season, and the harvest dates varied from June 4 to 7 and from June 7 to 10 in the two seasons, respectively. The total precipitation and sunshine hour, and mean temperature between heading and harvest varied from 113.9 to 19.3 mm, from 326.7 to 257.0 h, and from 19.6 to 21.1 °C in the two seasons, respectively. When the total period of 3 months before heading to harvest was considered, total precipitation and sunshine hours varied from 154.1 to 68.3 mm and from 822.7 to 753.9 h, and mean temperature between 11.4 and 10.0 °C in the two seasons. Fertilization was similar in the two seasons, both with a total of about 300 kg ha^{-1} nitrogen applied. A completely randomized block design with two replications was used, and each plot consisted of 2 × 2 m rows, 0.2 m apart. Seeding rate was about 180 kg ha^{-1} and field management was according to local practices. Grain samples harvested from each replication were cleaned by hand. Thousand kernel weight was obtained as an average of three samples, with each containing 200 seeds. Grain diameter was determined on 300-kernel samples with a Perten 4100 Single Kernel Characterization System (SKCS, Perten Instruments North America Inc., Reno, NV). Grain protein content was obtained with a near-infrared (NIR) analyzer (Instalab 600, Newport Scientific Sales and Services Ltd., Australia) following AACC approved method 39-10 (AACC, 2000). Each sample was milled into fine powder with a 60-mesh screen and thoroughly mixed, and cooled immediately and stored at −20 °C until analyzed.

2.2. Analytical methods

2.2.1. Extraction of free phenolic acids

Free phenolic acids in wheat flour were extracted essentially according to a previously reported method (Adom et al., 2003, 2005). A 1 g sample of flour was extracted with 20 mL of 80% chilled ethanol. Eppendorf tubes containing samples were shaken on a shaker at room temperature for 10 min. After centrifugation at 2500 g for 10 min, the supernatant was transferred into a new tube, and extraction was repeated once more for the residue. Supernatants were pooled together, evaporated at 45 °C to less than 5 mL, and reconstituted into 10 mL with distilled water. The extracts were stored at −20 °C until further analysis within a three-month period.

2.2.2. Extraction of bound phenolic acids

Bound phenolic acids were extracted according to the published method of Mattila et al. (2005) with minor modifications; 15 mL of distilled water and 5 mL of 6 M NaOH were added to test tubes with the residue after the extraction of free phenolic compounds, and sealed and stirred overnight (about 16 h) at room temperature (20 °C) using a magnetic stirrer. The solution was then adjusted to pH 2, and liberated phenolic acids were extracted three times with 15 mL of a mixture of cold diethyl ether (DE) and ethyl acetate (EA, 1:1 v/v). DE/EA layers were combined, evaporated to dryness, and the residue was dissolved in 1.5 mL of methanol. Acid hydrolysis was then performed by adding 2.5 mL of concentrated 12 M HCl into the test tube and incubating in a water bath at 85 °C for 30 min after completion of the above alkaline hydrolysis. The sample was then cooled, and adjusted to pH 2, with DE/EA extraction performed in the same manner as that for alkaline hydrolysis. The results of the alkaline and acid hydrolyzes were calculated to represent the bound phenolic acid concentrations.

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