Phenolic Compounds of Wheat. Their Content, Antioxidant Capacity and Bioaccessibility

Abstract

The importance of wheat has mainly been attributed to its ability to be ground into flour and semolina that form the basic ingredients of bread and pasta, respectively, while bran is mainly used for animal feeding. However, many epidemiological studies have demonstrated that health beneficial effects of whole wheat were attributed to the bioactive factors in bran and aleurone, such as non-digestible carbohydrates and phytochemicals. Among health-promoting phytochemicals residing in whole grains of wheat, phenolic compounds have gained much attention in many scientific research areas, as they have strong antioxidant properties. Phenolic acids and flavonoids represent the most common form of phenolic compounds found in whole wheat grains, and they are among the major and most complex groups of phytochemicals with a number of types that exist as soluble free compounds, soluble conjugates that are esterified to sugars and other low molecular mass compounds, and insoluble bound forms. Due to its strong antioxidant activity, possess anti-inflammatory, anti-carcinogenic activity and diabetes alleviation properties and could be associated with cardiovascular disease prevention, obesity and aging control.

Keywords: Wheta; Phenolic compounds; Phenolic acid; Flavonoids; Coumarins; Proanthocyanidins; Stilbenes; Lignans; Antioxidant capacity

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Introduction

Whole grain of cereals, primarily their aleurone layer, germ and bran, are rich sources of phytochemicals including phenolic compounds, carotenoids, vitamin E, lignans, β-glucans, inulin, resistant starch, sterols and phytates. These bioactive compounds are responsible for the health benefits of whole grains, as well as food rich in this.

Among health-promoting phytochemicals represented in cereals, phenolic compounds have gained much attention in many scientific research areas due to their strong antioxidant properties. Their concentrations in cereals are influenced by types, varieties and the part of the grain [1,2]. Generally, phenolic compounds are categorized as phenolic acids, flavonoids, stilbenes, coumarines, lignans and tannins [3]. However, the most abundant phenolic compounds found in cereals are phenolic acids and flavonoids [1]. These compounds mainly exist as glycosides in whole grain linked to various sugar moieties or as other complexes linked to organic acids, amines, lipids, carbohydrates and other phenols [4].

Among phenolic acids, predominant phenolics in cereals are benzoic acid derivatives such as gallic, vanillic and syringic acid and cinnamic acid derivatives such as ferulic, p-coumaric and caffeic acid, of which, ferulic acid is the most potent in all cereals and makes from 70 to 90% of total [5,6]. On the other hand, ferulic acid and diferulates, as the most abundant phenolics and major contributors to the in vitro antioxidant capacity of cereals grain, are not present in significant quantities in some fruits and vegetables. Antioxidant properties of phenolic acids stem from the reactivity of their phenol moiety, i.e. from the reactivity of hydroxyl substituent on the aromatic ring. The hydroxylation and methoxylation in the aromatic ring affect the radical-quenching ability and hence different antioxidant activities of phenolic acids. It is considered that radical scavenging via the hydrogen atom donation can be the predominant mode of the phenolic acids antioxidant activity [7]. In cereals grains, phenolic acids are mainly present in the insoluble bound form, linked to cell wall structural components such as cellulose, lignin, and proteins through ester bonds and the results showed their low bioaccessibility (<1 to 2%) [6,8,9]. Most often, phenolic acids are esterified to the arabinose side groups of arabinoxylans. The ratio of free, soluble conjugated, and bound ferulic acid in corn and wheat was about 0.1 : 1 : 100 [10]. Epidemiological evidence has supported the role of free phenolic acids in the prevention of several chronic diseases including cardiovascular disease, cancer, diabetes, as well as aging and other disorders, possibly due to its high antioxidant capacity [11]. However, given to the insoluble bound form of phenolic acids represented in the whole grain of cereals, data on the bioavailability are needed in order to elucidate their potential role in health benefits. In general, the bioavailability of ferulic acid from the whole grain of cereals is low. Thus, bioavailability of the acid from consumed wheat and corn bran was 2.5-5% and even lower 0.4-0.5% in rat, respectively [12]. This practically means that the majority of the cereals phenolic acids are those that can be released in the colon by intestinal microflora in order to exert their healthful benefits locally, such as colon cancer prevention.

Similar to phenolic acids, biochemical activities of flavonoids depend on their chemical structures and the relative orientation of various moieties in the molecules. Flavan nucleus, consisting of two benzene rings combined by an oxygen-containing pyran ring, makes basic structure of flavonoids [13]. The ability of flavonoids to be effective antioxidants depends on their metal-chelating potential, the presence of hydrogen-/electron-donating substituents in the molecules and the ability of the flavonoid to delocalize the unpaired electron [14]. Generally, flavonoids form the largest group of naturally occurring polyphenols. Approximately 9000 different flavonoids, grouped into families including flavones, flavonol, isoflavones, flavanones, flavanonol, flavanol and anthocyanidin, have been identified. Flavonoids normally accumulate in the vacuoles of plant tissues as their respective O-linked glycosidic conjugates. However, C-linked glycosides are major secondary metabolites in cereals. C-glycosides are generated with conjugation by different sugars occurring singly or doubly at the C-8 and/or C-6 position [15]. In addition to a soluble conjugated form, flavonoids are present in cereals in the free form, as the respective aglycines, and the insoluble bound form. The results of Lee et al. [16] showed that flavonoide aglycones had greater antioxidant activities than their glucosides when the potency was assessed using the LDL oxidation assay. Cereals grain mainly contains flavones. Further, colored whole grain of cereals contains a larger number of anthocyanins that are identified mostly as derivatives of cyanidin, pelargonidin, delfinidin and peonidin [6]. Delphinidin is known to be responsible for the bluish color, whereas cyaniding and pelargonidin are responsible for purple and red colors. Some cereals can also contain polymeric forms of flavonols, tannins and proanthocyanidins [14,1], whose antioxidant capacity is higher than that of monomers. Shi et al. [17] reported that antioxidant capacity of flavan-3-ols was 20-fold higher than of vitamin C and 50 times than of vitamin E. Flavonoids are of particular importance in the human diet. Summing up the results of many studies, He & Giusti [18] reported that flavonoids acted as antioxidants, possessed anti-inflammatory, anti-carcinogenic activity and diabetes alleviation properties and could be associated with cardiovascular disease prevention and obesity control.

Phenolic compounds in wheat grain

The aleurone layer is a wheat fraction with the highest antioxidant activity, followed by the bran [19]. According to results of Žilić et al. [2], debranned flour from bread and durum wheat had 3.7 and 2.4-fold lower antioxidant capacity than the respective bran fractions. The significant correlations between total phenolic contents and ABTS radical scavenging activities, point to their high contribution to antioxidant capacity. However, the variation in the antioxidant capacity, as well as in the content of phenolic compounds in bran and flour, highly depend on milling processes. In the study of Žilić et al. [2], the total phenolic contents in bread and durum wheat bran ranged from 7505.27 to 10823.29 mg GAE/kg and 7746.54 to 12384.55 mg GAE/kg, respectively. In the alkali hydrolyzates of hard and soft wheat bran, the total phenolic contents of 13514 and 14599 mg FAE/kg, respectively, were found [19]. The contribution of insoluble bound phenolics was 83% in total. Moore et al. [5] reported the content of acetone/water extractable phenolic compounds ranging from 2700 to 3500 mg GAE/kg in the bran of 20 wheat genotypes. The results of Žilić et al. [8] indicate that wheat grain phenolic compounds especially in the bound form, as dominant, exert a lower antioxidant capacity in comparison with its hydrolyzed and isolated free forms in analyzed extracts. The total antioxidant capacity of the whole wheat grain was 19.44 mmol Trolox Eq/kg, while the sum of antioxidant capacity of phenolic fractions in the sample amounted to 47.12 mmol Trolox Eq/kg. When the degree of phenolic compounds polymerisation exceeds a critical value in grains, the reduced availability of its hydroxyl groups and the increased molecular complexity promotes a decrease in antioxidant capacity [20]. A total of 70 phenolic compounds, including coumarins, phenolic acids, anthocyanins, flavones, isoflavones, proanthocyanidins, stilbenes and lignans, were identified in old and modern wheat genotypes using HPLC–TOF-MS analyses [15]. Figure 1 shows the structure of the main phenolic compounds of wheat grain.

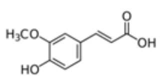
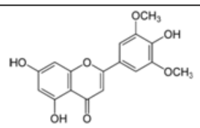
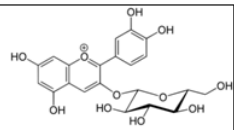
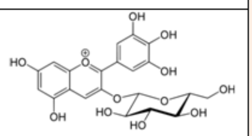
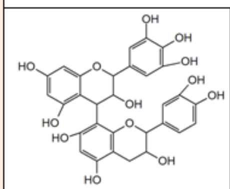
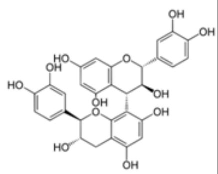
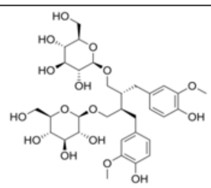
Cinnamic acid derivatives	Flavones	Anthocyanins	
			
Ferulic acid	Tricin	Cyanidin 3-glucoside	Delphinidin 3-glucoside
Proanthocyanidins		**Lignans**	
			
Prodelphinidin B-3	Procyanidin B-3	Secoisolariciresinol diglycoside	

Figure 1: The structure of the main phenolic compounds of wheat grain.

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Phenolic acid

Wheat grains mainly contain ferulic and p-coumaric acid. A mean content of these acids in the bran of 10 bread and durum wheat genotypes was 6997 and 163 µg/g, respectively, while their content in the flour ranged from 6.95 to 374.51 and 0.41 to 7.34 µg/g, respectively [2]. However, a number of phenolic acids such as isoferulic, o-coumaric, caffeic, sinapic, vanillic, p-OH-benzoic, protocatechuric and chlorogenic acid were also detected [21,22,2,8]. As primary phenolic acid, total ferulic acid ranged from 75 to 93% and 64 to 84% of total phenolic acid in the whole wheat grains and wheat brain, respectively [8,23,22]. Depending on wheat species and genotypes, free and soluble conjugated ferulic acid made small contributions (<0.5% and <27%, respectively) [23] (<2% and <6%, respectively) [8], while bound ferulic acid was the prevalent form of ferulic acid present in the grains. According to results of Adom et al. [24], free ferulic acid content for wheat varieties ranged from 0.10 to 0.74 µmol of ferulic acid/100 g of grain, while the soluble-conjugated ferulic acid content varied from 0.94 to 4.17 µmol/100 g. The bound ferulic acid content contributed >97% of the total ferulic acid content in all varieties. On the other hand, o-coumaric and p-OH-benzoic acids were mainly in the soluble conjugated form for cultivated bread wheat [23]. Further, p-OH-benzoic, o-coumaric and caffeic acids were not detected in the free form [5,23,8]. Ferulic acid, bound to arabinoxylans and other cell wall polysaccharides of wheat, was able to resist digestion in the upper gastrointestinal tract, while free ferulic acid was highly bioaccessible [25]. Zhao et al. [25] also showed how differences in the molecular size of ferulic sugar esters influence the degree of absorption and absorption site of FA within the gut. About 60 to 70% of ferulic acid esterified to just one arabinose or to several arabinoses and xyloses was not absorbed in the small intestine, leading to the conclusion that the major cleavage of ferulic acid esters takes place in the large intestine. It could be concluded that the improvement of processing methods in order to achieve a higher bioavailability of ferulic acid naturally present in wheat may be the most promising approach to expect health benefits of these phenolic compounds.

Flavonoids

Like the phenolic acids, flavonoids occur mainly in the bound form, attached to the cell wall of wheat. The content of total soluble flavonoids (free + conjugated fractions) was low, being 56.37 mg CE/kg in wheat grains [8]. The obtained content was slightly lower than the total soluble flavonoids contents of 10 durum wheat varieties evaluated by Dinelli et al. [26]. According to results of Žilić et al. [2], the flavonoids were not detected in debranned flour of bread and durum wheat. Total flavonoids recorded in bread and durum wheat bran amounted to 213.04 and 259.31 mg CE/kg, respectively. A total of 32 flavones detected in the investigated old and modern wheat varieties, comprised of 21 C-glycosidic forms, 7 O-glycosidic derivatives and 4 aglycone compounds [26]. Although a large number of conjugates could be identified, these were based on a restricted range of flavones, namely 5,7,4'-trihydroxyflavone (apigenin) and 5,7,3'4',-tetrahydroxyflavone (luteolin). Three additional flavones, all derivatives of the flavonol quercetin, were also detected. According to Naczk & Shahidi [27], tricin (5,7,4-trihydroxy 3,5-dimethoxyflavone) was found to be the dominant flavone pigment in wheat. Isoflavones were also present in the free phenolic fractions of some of the investigated wheat varieties [26]. Glycosylated cyanidins, delphinidins, malvinidins, pelargonidins, petunidins, and peonidins located in the aleurone layer or pericarp are responsible for a purple, blue, or red color of wheat grain. Other than white grain color is not natural for common hexaploid wheat. The blue color was introduced into wheat from blue colored diploid wild einkorn wheats and purple color from tetraploid emmer (Triticum dicoccum). According to results of Hosseinian et al. [28], the total anthocyanin profile content of purple wheat was 500.6 mg/kg. Thirteen major anthocyanins were isolated and cyanidin 3-glucoside (Cy-3-Glu) was the predominant followed by malvidin 3-glucoside (Mv-3-Glu) and cyanidin 3-galactoside (Cy-3-gal). These authors state that all anthocyanins were present as their glycoside forms and no aglycones were observed in the wheat samples. The concentration of anthocyanins in a large population of blue wheat lines was found to range from 35 to 507 mg/kg [29]. The anthocyanin composition of blue wheat differed from that of purple wheat. The study of Abdel-Aal et al. [29] is the first to identify the main anthocyanin in blue wheat as delphinidin 3-glucoside (Dp-3Glu), being ≈37% of the total anthocyanins. The second dominant anthocyanin at 32% of the total anthocyanins was delphinidin 3-rutinoside (Pp-3-Rut). As a consequence thereof, delphinidin was the main aglycone in blue wheat, making ≈69% of the total anthocyanidins.

Coumarins, proanthocyanidins, stilbenes and lignans

Research of Lachman et al. [30] indicated that grains of some wheat varieties contain various coumarins, among which the compound coumarin and its hydroxylated derivatives. Coumarins as the benzopyrones compounds may have a variety of bioactivities including anticoagulant, estrogenic, dermal photosensitising, antimicrobial, vasodilator, molluscacidal, antithelmintic, sedative and hypnotic, analgesic and hypothermic activity. However, Dinelli et al. [26] tentatively identified only one compound as coumarin. This compound was detected only in the free phenolic fraction of one old durum wheat genotype. The same authors detected two proanthocyanidins in the free phenolic form, prodelphinidin B-3 and procyanidin B-3, in four and two durum wheat varieties, respectively. The dimeric proanthocyanidins may also contain some propelargonidin units. Proanthocyanidins are colorless phenolic oligomers or polymers based on flavan-3-ol units, such as catechin, epicatechin, gallocatechin and epigallocatechin, who possess extremely high antioxidant capacity. In the aqueous phase antioxidant action of proanthocyanidins increases from monomer to trimer and then decreased from trimer to tetramer. On the basis of the observed yields and chromatographic evidence, it is estimated that the concentration of extractable soluble catechin plus proanthocyanidins was in the order of 20 to 40 µg/g fresh weight wheat bran [31]. The analysis of various small grain cereals has shown a high content of total proanthocyanidins in grain of hull-less barley, but using colorimetric method proanthocyanidins could not be detected in grain of 10 bread and durum modern lines [1]. As noted by McCallum & Walker [31], extraction of proanthocyanidins from wheat grain is complicated by the presence of the various carbohydrate and protein fractions that readily complex with these compounds. Commonly only a small proportion of proanthocyanidins can be extracted from plant tissue, and this may be as low as 10% in the case of polymeric prodelphinidins. Stilbenes are of small molecular weight and can possess anticancer, anti-inflammatory and anti-oxidant activities. Up to now, there have been very few the reports about stilbenes in cereals. Dinelli et al. [26] detected two stilbenes, double glycosylated pinosylvin and glycosylated pinosylvin, in some investigated durum wheat genotypes, as

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well as two isomers of glycosylated pinosylvin that occurred in the bound phenolics of all investigated old genotypes. Lignans in wheat grain are little bit more studied polyphenols. It is a group of diphenolic compounds that are present in the outer layers of wheat grain. The total lignans content, determined by CE-ESI-MS, was 2.60 and 5.00 µg/g for modern and old bread wheat varieties, respectively [32]. A large variation in the grain total lignan content was observed in the 73 wheat varieties (3.4 to 22.7 µg/g). However, large variations in the lignan composition cannot be observed, although relative amounts of individual lignans may vary over varieties [33]. Secoisolariciresinol and pinoresinol were detected in ten investigated modern and old bread wheat varieties, whereas arctigenin, hinokinin, and syringaresinol were exclusively detected in old genotypes. Secoisolariciresinol and pinoresinol were present in amounts ranging from 1.30 to 1.64 mg/g and from 0.98 to 1.22 mg/g, respectively [32]. According to Qu et al. [34], the concentration of a major lignin in wheat brain, secoisolariciresinol diglycoside, ranged from 42.7 to 82.9 µg/g. By intestinal microflora, when consumed, secoisolariciresinol diglycoside is converted to two lignan metabolites, enterodiol and enterolactone, that may contribute to the inhibition of cancer cell growth in mice [34]. These metabolites have a similar structure to the human hormone estrogen and so may have estrogenic/anti-estrogenic effects. In addition to above mentioned lignans, the study of Peñalvo et al. [35] showed the presence of aglycone lignans such as matairesinol, lariciresinol, syringaresinol and medioresinol in bread wheat.

Conclusion

Wheat grains are reach source of phenolic compounds with potential health benefits, but the nutritional properties will only be fully exploited if whole-wheat products are available. The most abundant phenolic compounds found in wheat are phenolic acids and flavonoids located in the outer layer of grain. Most of the phenolic compounds in bran are bound to carbohydrates, and can survive gastrointestinal digestion reaching the colon intact, where they provide an antioxidant environment. Relatively high variations among genotypes within species concerning the total phenolic content, phenolic acids, flavonoids, anthocyanins and lignans, provide a foundation for those interested in utilizing or improving modern wheat varieties for their health benefits.

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