

## PHENOLIC ACIDS AND ANTIOXIDANT ACTIVITY OF WHEAT SPECIES: A REVIEW

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Wheat (genus *Triticum*) is considered to be an important source of polyphenols, plant secondary metabolites with numerous health-promoting effects. Many phytochemicals are responsible for the high antioxidant activity of whole grain products. However, there is a lack of information about composition of phenolic acids and their concentrations in different *Triticum* species. Despite the fact that the increased consumption of whole grain cereals and whole grain-based products has been closely related to reduced risk of chronic diseases, bioactive compounds found in whole grain cereals have not achieved as much attention as the bioactive compounds in vegetables and fruits. Recent studies have revealed that the content of bioactive compounds and antioxidant capacity of whole grain cereals have been regularly undervalued in the literature, because they contain more polyphenols and other phytochemicals than was reported in the past. Phenolic acids represent a large group of bioactive compounds in cereals. These compounds play a significant role in the possible positive effects of the human diet rich in whole grain cereals, especially in wheat and provide health benefits associated with demonstrably diminished risk of chronic disease development. Ferulic acid, the primary and the most abundant phenolic acid contained in wheat grain, is mainly responsible for the antioxidant activity of wheat, particularly bran fraction. In this paper, selected phenolic compounds in wheat, their antioxidant activity and health benefits related to consumption of whole grain cereals are reviewed.

Key words: antioxidant activity, health benefits, phenolics, phytochemicals, wheat

Wheat is an important agricultural commodity and a main food all around the world. It is one of the major food grains consumed by people (Van Hung *et al.* 2009) with global wheat production forecast in 2015 at 735 million tonnes (FAO 2015). It contains important beneficial components for human nutrition. Due to the high content of natural antioxidants, wheat and wheat-based products can perfectly serve as a basis for the functional foods development designed to improve the health of millions of consumers (Vaher *et al.* 2010). Although wheat is used mainly as a source of energy, whole wheat grains are an excellent source of dietary fiber, vitamins, minerals and other bioactive phytochemicals such as antioxidant compounds (Cai *et al.* 2014). For this

reason, numerous studies have been conducted to investigate the nutritional quality and health benefits of whole wheat grains.

The phenolic compounds, one of the most widely occurring groups of phytochemicals, are secondary metabolites synthesized during the plant development and in response to stress conditions (Brandolini *et al.* 2013). These compounds, most of which are present in wheat bran fractions, play a significant role in plant growth and reproduction because they provide protection against pests and pathogens and contribute to the colour and sensory characteristics of plant species. In addition to their role in plants, in human diet phenolic compounds provide many health benefits associated with diminished risk of

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chronic diseases caused by reactive oxygen species (Harborne & Williams 2000; Gani *et al.* 2012).

According to Heim *et al.* (2002), the beneficial effects of phenolic compounds have been attributed to their antioxidant activity. It depends on the structure, particularly the number and position of the hydroxyl groups and the type of substitutions on the aromatic rings (Balasundram *et al.* 2006). Phenolics have lower electron reduction potential than the oxygen radicals and they are excellent oxygen radical scavengers. Additionally, the phenoxyl radicals are less reactive than the oxygen radicals and they scavenge reactive oxygen intermediates without promoting further harmful oxidative reactions (Ainsworth & Gillespie 2007). This strong antioxidant activity of phenolic compounds leads to anti-inflammatory, anti-thrombotic, anti-atherogenic, cardioprotective and vasodilatory effects on human health (Quiñones *et al.* 2013).

The phenolic compounds have the structure that varies from simple molecules to complex polymers. They contain one or more aromatic rings with one or more hydroxyl groups. This crucial structural diversity has an impact on their differences in bioavailability. Simple phenolic acids cross the intestinal barrier more easily, while complex molecules are hardly absorbed (Scalbert *et al.* 2002). Cereals represent staple food of humankind and are an important source of phenolic compounds. One of the most common types of phenolic compounds in cereals are phenolic acids (Li *et al.* 2008), which are present in three forms: soluble free, soluble conjugated with mono- and polysaccharides and insoluble bound (Naczki & Shahidi 2004). According to Li *et al.* (2008), the most abundant fraction in wheat grains is the insoluble bound (77%), followed by the soluble conjugated (22%) and the soluble free (<0.5 – 1%). In the past, the phenolic content of whole grain cereals had been undervalued in the literature, because most of researches only studied the free phenolic content and not the content of bound phenolics (Adom & Liu 2002). Phytochemicals in the bound form cannot be digested by human enzymes. They could survive stomach and small intestine digestion and therefore can reach the colon. The colonic microflora may release the bound phytochemicals by fermentation processes and after absorption provide health benefits in colon or other body tissues. This

can partly explain the mechanism of consumption of whole grain cereals in the prevention of colon cancer, other gastrointestinal cancers, breast cancer, prostate cancer and other chronic diseases, which is supported by many epidemiological studies (Liu 2007).

#### *Antioxidant activity of phytochemicals in wheat*

The important group of phytochemicals with small molecular weight present in wheat grains are antioxidants. Antioxidants are defined as molecules that can delay or prevent oxidative stress at low concentration and specific assay conditions. Oxidative stress has been related to cardiovascular diseases, cancer and other chronic diseases that account for a major part of deaths today (Willcox *et al.* 2004; Vaher 2010).

Wheat contains a diverse range of biologically active compounds that may contribute to its antioxidant capacity. Wheat antioxidants are mainly concentrated in bran fraction and the amount of these antioxidants depends on the grain variety (Kim *et al.* 2006). First of all, wheat bran represents a good source of phenolic acids, which significantly contribute to the total antioxidant activity of wheat (Baublis *et al.* 2000; Yu *et al.* 2003; Kosík *et al.* 2014c). Extract of wheat bran with a high concentration of phenolic acids was shown to have stronger antioxidant activity than other wheat fractions (Onyeneho & Hettiarachchy 1992). In addition, wheat bran is able to inhibit lipid oxidation catalysed by either peroxy or iron radicals (Baublis *et al.* 2000). Most recently, Zhou *et al.* (2004) reported that wheat grain, bran and other fractions had different antioxidant activities and total phenolic contents. Their research also showed that ferulic acid, with a concentration range of 99–231 µg/kg, was the main contributor to the antioxidant activity of wheat. It has been suggested that ferulic acid could be used as a marker of wheat antioxidants. According to Lacko-Bartošová *et al.* (2013), antioxidant activity of whole grain flour was significantly higher than the white flour. Antioxidant activity of white flour (23.26%) was two times lower than antioxidant activity of whole grain flour (49.57%). The highest antioxidant activity (76.47%) was determined in wheat bran.

Phytochemicals in wheat exhibit strong antioxidant properties. They scavenge or neutralize free radicals and reduce oxidative damage to DNA, proteins and membrane lipids. Decreases in oxidative damage to cells or cell components may explain the reduction of chronic diseases, which may be caused by oxidative stress (Willcox *et al.* 2004; Kim & Kim 2016). To investigate health benefits of wheat, total phenolic contents and antioxidant capacities in wheat have been studied (Verma *et al.* 2008; Okarter *et al.* 2010). 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. Most of the antioxidant compounds in wheat are bound and may survive digestion to reach the colon intact, where they induce an antioxidant environment (Pérez-Jiménez & Saura-Calixto 2005). Okarter (2011) found that phenolic extract from the insoluble-bound fraction of whole wheat inhibited the proliferation of human colon cancer cells *in vitro*. A major action in the protection of colon from cancer probably exert ferulic acid – the typical whole wheat phenolic acid with only 0.5–5% of absorption within the small intestine. Thus, phenolic acids in the bound form can act along the whole length of the digestive tract by trapping oxidative compounds (Fardet 2010).

#### Phenolic acids

Phenolic acids are derivatives of benzoic and cinnamic acids and are present in all cereals. They can be subdivided into two major groups, hydroxybenzoic acid and hydroxycinnamic acid derivatives (Figure 1). Hydroxybenzoic acid derivatives include *p*-hydroxybenzoic, protocatechuic, vanillic, syringic and gallic acids. Hydroxycinnamic acid derivatives include *p*-coumaric, caffeic, ferulic and sinapic acids (Figure 1) (Mattila *et al.* 2005; Gani *et al.* 2012). The common phenolic acids found in whole grain cereals include ferulic acid, vanillic acid, caffeic acid, syringic acid and *p*-coumaric acid (Sosulski *et al.* 1982; Liu 2007). Ferulic acid is the primary and the most abundant phenolic acid in wheat grains. Smaller concentrations of *p*-hydroxybenzoic, *o*-coumaric, *p*-coumaric, vanillic, syringic, salicylic and sinapic acids are also present in wheat (Moore *et al.* 2005; Liyana-Pathirana *et al.* 2006).

#### Composition, content and effects of food processing

The phenolic acids reported in cereals occur in both free and bound form. Free phenolic acids are found in outer layers of the pericarp. Bound phenolic acids are esterified to cell walls and released from the cell matrix by acid or base hydrolysis (Mattila *et al.* 2005; Gani *et al.* 2012). Phenolic acids in wheat grains are present mostly in the bound form with other grain components such as saccharides – starch, cellulose,  $\beta$ -glucan and pentosane (Yu *et al.* 2001; Vaher *et al.* 2010). Also, food processing, such as fermentation, thermal processing, pasteurisation and freezing, contributes to the release of these bound phenolic acids (Dewanto *et al.* 2002). They can be degraded at high temperature and high pH, particularly caffeic acid (Dimberg *et al.* 2001).

Phenolic acids are predominantly present in the bran, i.e. the aleurone layer and the outermost pericarp, which are usually eliminated during milling (Bondia-Pons *et al.* 2009; Belobrajdic & Bird, 2013). As a result, whole meal flours contain higher amounts of phenolic acids than commercial wheat flours. In whole meal flours, the number of polyphenols is highly variable and is closely related to species and variety of cereals (Adom *et al.* 2005; Andersson *et al.* 2014; Kosík *et al.* 2014b). Different extraction conditions also influence the content reported in different studies (Yu *et al.* 2001).

Einkorn is a high-nutritional-value cereal with the high content of proteins, carotenoids and tocopherols (Hidalgo *et al.* 2006; Brandolini *et al.* 2008). However, a significant gap remains in its phenolic acid composition. Li *et al.* (2008) recorded a total phenolic acid content similar to winter, spring and durum wheats, slightly higher than spelt, but marginally lower than emmer. Serpen *et al.* (2008) measured higher values of bound ferulic acid in emmer than in einkorn. However, Abdel-Aal and Rabalski (2008) reported more phenolic acids in einkorn than in several primitive and modern wheat species. According to HEALTHGRAIN diversity screen, the large EU project, the content of phenolic acids in wheat species varied between 326 and 1171  $\mu\text{g/g}$  dry matter (DM) (Li *et al.* 2008). Other studies have revealed that the total concentrations of phenolic acids are typically in the range of 200–900  $\mu\text{g/g}$  DM in whole grain wheat (Belobrajdic & Bird 2013). The content of phenolic acids in spelt also showed differences

between varieties and ranged from 507 to 1257  $\mu\text{g/g}$  DM (Gawlik-Dziki *et al.* 2012). Some studies have concluded that the content of phenolic acids is related to genotype (Hernández *et al.* 2011; Ragaei *et al.* 2012). However, others have found that location (i.e. environmental factors) is more important (Vaher *et al.* 2010).

The conjugated and bound phenolic acids recorded in selected wheat species are summarised in the Table 1. In the study of Brandolini *et al.* (2013), 39 different *Triticum monococcum*, *T. turgidum* and *T. aestivum* accessions were evaluated. The total conjugated phenolic acids in whole meal flour ranged between 36.0 and

52.6 mg/kg DM. The total bound phenolic acids varied between 441 and 715 mg/kg DM that correspond with the results (208–964 mg/kg DM) reported by Li *et al.* (2008). Volkan *et al.* (2015) analysed 15 wheat accessions from different *Triticum* species. The phenolic acid contents in the conjugated and bound extracts varied between 28.2–70.8 mg/kg DM and 482–766 mg/kg DM, respectively. Hidalgo and Brandolini (2017) tested conjugated and bound phenolic acids content of three einkorn accessions. The total conjugated phenolic acids ranged between 33.9 and 54.9 mg/kg DM. The concentrations of total bound phenolic acids varied between 484.2 and 579.5 mg/kg DM.

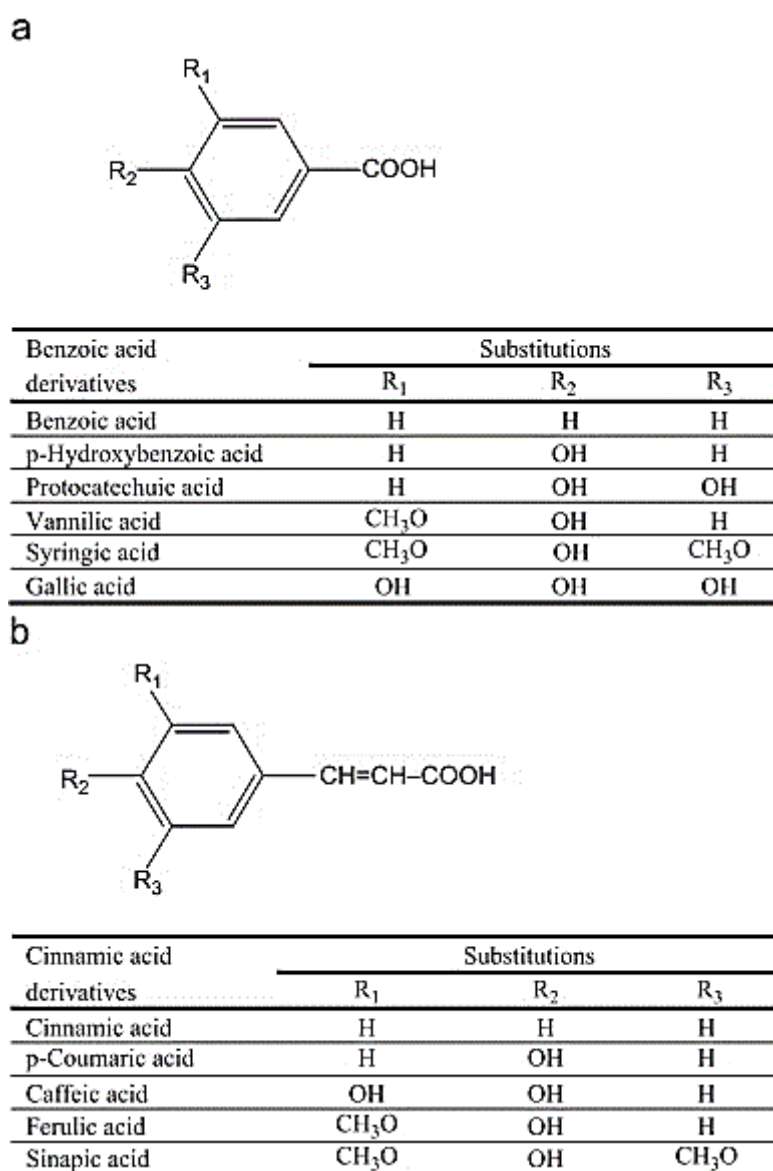


Figure 1. Structure of common phenolic acids: (a) benzoic acid derivatives and (b) cinnamic acid derivatives (Liu 2007)

Although the content of phenolic acids depends on variety and various agricultural practices, it seems that bound phenolic acids variation is lower in different growing conditions than free and conjugated forms (Fernandez-Orozco *et al.* 2010; Belobrajdic & Bird 2013; Kosík *et al.* 2014a). Organic cultivation systems have been found to lead to a small increase in the total amount of phenolic acids, especially ferulic and *p*-coumaric acid, in spring and winter wheats, compared with conventionally grown wheat (Zuchowski *et al.* 2011). These differences were attributed to a concentration effect due to differences in the size of the grain, which was smaller in organically grown crops. It is also important to note that the study was performed on plants grown within the two cropping systems only in one year (Andersson *et al.* 2014).

Both free and bound phenolic acids are affected by processing, e.g. baking. A study performed by El-Sayed and Rabalski (2013) found that baked products contained higher amounts of free phenolic acids and lower amounts of bound ones than the whole grain wheat flour used. This is probably the result of release of bound compounds. Most of the changes occurred during baking, but no change could be seen in the dough. In other studies, baking has resulted in a reduced amount of free phenolic acids, although in those studies a fermentation process preceded the baking process, which might explain the differences in the results (Menga *et al.* 2010). The free phenolic acids also increased and actually seemed to be more stable in biscuits and muffins than in bread products. El-Sayed and Ra-

balski (2013) explained this by the fact that phenolic acids are less accessible to oxidation in fat products than in aqueous food systems. However, different mechanisms may occur simultaneously in the cereal products during processing, influencing the content of various phenols. Concurrently to release of bound compounds from the food matrix, phenols may be oxidised, polymerised or thermally degraded.

#### *Bioactivity and implications for human health*

Some studies have been conducted to evaluate the bioavailability of hydroxycinnamates from cereals and it has been found that esterification of ferulic acids impairs their uptake in the intestine (Belobrajdic & Bird 2013). However, colonic fermentation in the digestive tract releases phenolic compounds, which in turn may be taken up in the large intestine (Maki *et al.* 2012). Zhao and Moghadasian (2010) suggested that the bioavailability of hydroxycinnamic acids follow the order *p*-coumaric acid > ferulic acid > caffeic acid, and that the free compounds have higher bioavailability than their corresponding bound forms. The metabolism of these compounds in the colon is not fully understood, but it is supposed that they undergo reduction, dihydroxylation, demethylation and  $\beta$ -oxidation.

Phenolic acids have many functions in cereals. They act as stabilisers of the cell wall structure, but they may also be involved in the physical and chemical defence against various microorganisms, pests and insects. Phenolic acids have also been reported to inhibit the biosynthesis of trichothecenes of *Fusarium* fungi, which are potent human toxins (Boutigny *et al.* 2009). They are mentioned as bio-

T a b l e 1

Soluble conjugated, insoluble bound and total phenolic acids in selected wheat species [mg/kg DM]

Species	Phenolic acids		
	Soluble conjugated	Insoluble bound	Total
Einkorn <sup>a,b,c</sup>	34.4–63.9	482–766	516–831
	43.8–54.9	534.1–579.5	578–634
	52.6	584	637
Emmer <sup>a,c</sup>	44.3	506	551
	49.9	546	596
Spelt <sup>a,c</sup>	39.7	538	578
	42.8	594	637

<sup>a</sup>Volkan *et al.* (2015); <sup>b</sup>Hidalgo & Brandolini (2017); <sup>c</sup>Brandolini *et al.* (2013)

DM – dry matter

active phytochemicals promoting human health in a multidisciplinary way. They may provide health benefits because of their antioxidant properties (Thompson 1994; Bondia-Pons *et al.* 2009). Ragae *et al.* (2012) found a high correlation between the concentration of bound phenolic acids in whole grain wheat and DPPH (diphenylpicrylhydrazyl) scavenging capacity. Phenolic acids may also inhibit LDL-cholesterol oxidation (low density lipoproteins) and oxidative damage to DNA and lipid membranes, which might otherwise lead to several pathological conditions, including cardiovascular diseases, type 2 diabetes and cancer. *In vitro* studies on phenolic acids have shown anti-inflammatory effects and anti-proliferative activities on cancer cells. Several phenolic acids have also been shown to be responsible for inhibiting cataracts of the eye lens (Chethan *et al.* 2008).

#### *Ferulic and p-coumaric acid in cereals*

Ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid) is the most abundant hydroxycinnamic acid found in cereal grains. It occurs primarily in the plant seeds and leaves, mainly covalently conjugated to mono- and disaccharides, plant cell wall polysaccharides, glycoproteins, polyamines and lignin. Wheat bran is considered to be a good source of ferulic acid, which is esterified to hemicellulose of the cell walls (Dewanto *et al.* 2002). The content of ferulic acid in wheat grains is near to 0.8–2 g/kg dry weight basis that represents up to 90% of total phenolic compounds (Sosulski *et al.* 1982; Lempereur *et al.* 1997; Gani *et al.* 2012).

Ferulic acid in cereals can be found in free, soluble-conjugated and bound forms. About 90–95% of the total amount of ferulic acid represent the insoluble bound ferulic acid (Lv *et al.* 2012). The content of bound ferulic acid was significantly higher than free and soluble-conjugated ferulic acid in wheat, corn, oats and rice. The ratio of free, soluble-conjugated and bound ferulic acid in corn and wheat was 0.1:1:100. The order of total ferulic acid content among the tested grains was corn > wheat > oats > rice (Adom & Liu 2002; Boz 2015). In wheat, the insoluble dietary fraction contains 3544 µg/g of phenols, whereas the soluble fraction contains 95 µg/g. This may in-

dicate that ferulic acid is bound to arabinoxylan but not to β-glucan, which is a more soluble polysaccharide (Caprita *et al.* 2011). The content of ferulic acid seems to be higher in soft wheat than in hard wheat (Ragae *et al.* 2012).

Ferulic acid and other phenolic acids protect wheat kernels by providing both physical and chemical barriers through cross-linking saccharides, antioxidant activities against destructive radicals and astringency that deters insects and animals from consumption (Arnason *et al.* 1992; Liu 2007). Like several other phenolic compounds, ferulic acid exhibits antioxidant activity in response to free radicals *via* donating one hydrogen atom from its phenolic hydroxyl group (Kumar & Pruthi 2014). Beneficial effects on human health such as anti-inflammation and free radical scavenging have been demonstrated. It has been suggested that ferulic acid can treat diverse disorders including Alzheimer's disease, cardiovascular diseases, *diabetes mellitus*, cancer and skin diseases (Mancuso & Santangelo 2014; Karunaratne & Zhu 2016). Suzuki *et al.* (2007) found that ferulic acid may have positive effects on blood pressure through stimulation of nitric acid production, which induces arterial vasodilation and increases the blood flow.

*p*-coumaric acid (4-hydroxycinnamic acid) is a hydroxyl derivative of cinnamic acid (Garrait *et al.* 2006). It is present in the lowest amount in the centre of the kernels and in increasing amount towards the outer layers (Awika & Rooney 2004; Madhujith *et al.* 2006). This acid is another phenolic acid of great interest due to its chemoprotectant and antioxidant properties (Torres y Torres & Rosazza 2001). It has been suggested to have anti-tumour activity against human malignant tumours. There is a report revealing that *p*-coumaric acid reduces the risk of stomach cancer (Ferguson *et al.* 2005). It has also potentially protective effect against heart diseases because of its ability to decrease the resistance of LDL lipoproteins, cholesterol oxidation and lipid peroxidation (Garrait *et al.* 2006). It shows anti-mutagenesis, anti-genotoxicity and anti-microbial activities, inhibits cellular melanogenesis and plays a role in immune regulation in humans (Kiliça & Yeşiloğlu 2013).

## CONCLUSIONS

Whole grain cereals may represent a significant share in intake of bioactive compounds in human diet. They are mainly present in bran fraction of cereals, which is usually eliminated by milling. Growing evidence indicates that the consumption of whole grains at regular intervals can be related to many health benefits, for example the reduced risk of cardiovascular diseases, obesity, type 2 diabetes and certain types of cancer. Bioactive compounds play an important role in plant growth, protection against pathogens and pests, affect the colour and sensory properties of plants. Despite this fact, antioxidant properties of cereals reported in the literature have been undervalued, since only unbound forms have been studied. Most of the bioactive compounds in wheat are in the insoluble bound fraction and may survive gastrointestinal digestion. They can be released from cell matrix by acid or base hydrolysis, usually by colonic microflora through fermentation. The antioxidant potential of whole grains is closely related to their phenolic content, primarily ferulic acid as the main source of antioxidant activity. Einkorn, emmer and spelt wheat belong to the non-traditional wheat species with different valuable traits and properties. Significant gap remains in their antioxidant activities, phenolic acid composition and the possible role in human diet. More knowledge is needed to prepare strong arguments for an increased consumption of whole grain cereals and whole grain-based products and to provide better information about their health benefits. For this reason, the study of bioactive compounds reviewed in this paper is constantly important issue.

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