Grain quality of durum wheat varieties

Abugalieva A.I., Morgounov A.Y.

in

Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.).
Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110

2014
pages 549-553

Phenolic compounds and antioxidant activity in tetraploid wheat

Laura Nunzia Delvecchio, Francesca Taranto, Giacomo Mangini, Antonio Blanco, Antonella Pasqualone

Department of Soil, Plant, and Food Sciences, University Aldo Moro, Bari, Italy

Abstract. Phenolic compounds have been widely studied for their health value, as they exert a protective role against chronic degenerative diseases, mainly due to their antioxidant properties. Cereals are at the basis of the food pyramid and, even if they are not one of the main sources of phenolic compounds, they can effectively contribute to the dietary uptake of these secondary metabolites. After an overview of functions and mechanisms of bioavailability, the extraction methods and varietal variability of phenolic compounds in tetraploid wheat are reviewed, in comparison with bread wheat. The quantitative distr bution of the various fractions and classes of phenolic compounds in the caryopsis are discussed, with special attention to ferulic acid. The state of the art about the production of phenolic extracts from bran is reviewed, pointing out the most recent technologies adopted to recover the insoluble-bound phenolic fraction.

Keywords. Phenolic compounds – Tetraploid wheat – Milling by-products – Functional ingredients – Antioxidant activity.

Composés phénoliques et activité anti-oxydante chez le blé tétraploïde

Résumé. *Les composés phénoliques ont été largement étudiés pour leurs vertus sanitaires, car ils exercent un rôle protecteur contre les maladies dégénératives chroniques, principalement en raison de leurs propriétés anti-oxydantes. Les céréales sont à la base de la pyramide alimentaire et, même si elles ne sont pas l'une des principales sources de composés phénoliques, elles peuvent contribuer efficacement à l'apport alimentaire de ces métabolites secondaires. Après avoir donné un aperçu des fonctions et des mécanismes de la biodisponibilité, nous passerons en revue les méthodes d'extraction et la variabilité variétale des composés phénoliques chez le blé tétraploïde, en faisant une comparaison avec le blé tendre. La distribution quantitative des différentes fractions et classes de composés phénoliques dans le caryopse sera aussi discutée, en focalisant l'attention sur l'acide férulique. Enfin, l'état des lieux sur la production d'extraits phénoliques du son sera présenté, en examinant les technologies les plus récentes adoptées pour récupérer la fraction d'acide phénolique insoluble lié.*

Mots-clés. *Composés phénoliques – Blé tétraploïde – Sous-produits de mouture – Ingrédients fonctionnels – Activité anti-oxydante.*

I – Chemistry of phenolic compounds

Phenolic compounds constitute one of the most numerous and widely distributed groups of secondary metabolites in the plant kingdom, all characterized by the presence of at least an aromatic ring bearing one or more hydroxyl substituents. They can be divided into at least 10 different classes depending on their basic chemical structure (Table 1). The most abundant are phenolic acids, lignans, stilbenes, flavonoids and tannins (Bravo, 1998); phenolic acids and flavonoids represent 30 and 60%, respectively, of total phenolic compounds in the Mediterranean diet (King and Young, 1999).

Phenolic compounds range from low molecular weight compounds to highly polymerized compounds, such as melanins, suberin, tannins, and lignins. Flavonoids are derivatives of benzo-γ-pyrone; on the basis of the oxidation state of heterocycles, and aromatic rings position, are classified into: anthocyanidins, flavonols, flavans, flavanones, flavones, isoflavones and hydrolysable tannins.

The predominant phenolic compounds in cereals are phenolic acids. They are derivatives of either benzoic acid or cinnamic acid. The latter group includes ferulic acid (4-hydroxy-3-methoxycinnamic acid) that is the most abundant phenolic acid of wheat at all stages of development. Its concentration increases steadily during grain development, prior to a 50% decrease during grain ripening (Mc Keehen *et al.*, 1999). This acid arises from the methabolism of phenylalanine and tyrosine (Graf, 1992) and is ubiquitously present in plant cell walls (Bravo, 1998). The presence of the CH=CH–COOH group in its structure is considered to be the key for significantly higher antioxidative efficiency than that of hydroxybenzoic acids (White and Xing, 1997). The *trans* isomer of ferulic acid is predominant, as it represents 90% of the total phenolic acids of wheat (Lempereur *et al.*, 1997).

Table 1. Basic chemical structure of the main phenolic compounds.

Class	Basic carbonious skeleton
Hydroxybenzoic acids	C_6–C_1
Hydroxycinnamic acids	C_6–C_3
Stilbenes, anthraquinones	C_6–C_2–C_6
Flavonoids	C_6–C_3–C_6
Lignans	$(C_6$–$C_3)_2$
Melanins	$(C_6)_n$
Lignins	$(C_6$–$C_3)_n$
Condensed tannins (proanthocyanidins or flavolans)	$(C_6$–C_3–$C_6)_n$

II – Functions and distribution of phenolic compounds in plants

With regard to the subcellular distribution of phenolic compounds, sites of biosynthesis and accumulation are different, due to the reactivity of these compounds against protoplasmic constituents, that could render them toxic for the cell. This toxicity can be prevented by conjugation with monosaccharides and cellular compartmentalization in the synthesis and transport processes (Wink, 2010). The synthesized products, in glycosylated form, are seized in specific regions of the endoplasmic reticulum to form membranous vesicles. Subsequently, these vesicles can move to the vacuole, where different classes of phenolic compounds are stored. Alternatively, they can head to the plasma membrane for secretion within the cell wall, thus contributing to the process of lignification (Wink, 2010). Phenolic compounds, in fact, confer mechanical stability to cells, by forming polymeric constituents of support structures, such as lignin and other constituents of the cell wall (Renger and Steinhart, 2000).

The structural variety of phenolic compounds reflects in a large array of functions and explains their extensive diffusion. Due to their strong antioxidant activity, they protect plants from UV radiation and oxidative stress, and have phytoalexin functions (Hammerschmidt, 1999). In addition, phenolic compounds have antibiotic, antifungal, and antiviral properties (Dixon, 2001). The lignificating ability and antioxidant properties of ferulic and other phenolic acids constitute a physical and chemical barrier to insect attacks. In wheat, they play a role in midge resistance (Abdel-Aal *et al.*, 2001) and contribute to *Fusarium* resistance (Mc Keehen *et al*., 1999).

III – Health value of phenolic compounds

Phenolic compounds have been extensively studied for their health value, as they exert a protective role against chronic degenerative diseases, mainly due to their antioxidants properties. In fact, phenolic compounds are scavengers of free radicals, primarily responsible for the oxidative damage caused to DNA, lipids and proteins (Graf, 1992). In particular, flavonoids and phenolic acids, including ferulic acid, protect low density lipoproteins (LDL) from oxidation by reactive

oxygen species (ROS), associated with the initial steps of the atherosclerosis process (Yu *et al*., 2005).

Moreover, phenolic compounds play a preventive role in the various stages of carcinogenesis, with different mechanisms: (i) by scavenging the carcinogenic agents (especially free radicals); (ii) by altering the production of key proteins and stopping the cell cycle; (iii) by inducing apoptosis of tumor cells; (iv) by expounding an angiogenic action (Thomasset *et al.*, 2007; Ramos, 2008). Finally, phenolic compounds exert anti-inflammatory, anti-hypertensive, anti-microbial and photoprotective activities (Bravo, 1998).

IV – Bioavailability of phenolic compounds

Phenolic compounds may be classified as soluble or insoluble in the most common solvents. The bonds with other molecules influence the physical and chemical characteristics of these compounds, including their solubility, and determine their cell location, functions, absorption, and metabolism. In particular, water-soluble phenolic compounds include (i) free aglycones; (ii) glycosides obtained by conjugation with one or more mono- or di-saccharides; (iii) esters of organic acids (Bravo, 1998). Phenolic acids may form both ester and ether linkages owing to their bifunctional nature through reactions involving their carboxylic and hydroxyl groups, respectively. This allows phenolic acids to form cross-links with cell wall macromolecules. The insoluble fraction originates from bonds between phenolic compounds and cell wall polymeric constituents. Ferulic acid, for example, contributes to the formation of insoluble fiber by cross-linking arabinoxylans (Renger and Steinhart, 2000). Phenolic compounds can bind also lyposoluble molecules such as phytosterols, terpene alcohols or triterpenes, commonly associated with the cell membrane (Miller and Engel, 2006). While lyposoluble and insoluble-bound phenolic compounds mainly play a structural role in the cell wall, water-soluble phenolic compounds have generally antioxidant and antimicrobial functions (Smith and Hartley, 1983).

To be absorbed, glycosides must be hydrolyzed by glycosidase in the gastrointestinal tract; this enzyme can be endogenous, or produced by colonic microflora (Kim *et al.*, 1998). The product of this enzymatic reaction are hydrophilic aglycones that can be absorbed in the small intestine by diffusion throughout biological membranes (Bravo, 1998). Phenolic compounds present in foods in insoluble-bound form, especially high molecular weight polymers, such as condensed tannins, are not bioavailable and have antinutritional properties: these molecules complex and precipitate proteins and divalent cations, interfering with their digestion and absorption (Bravo, 1998).

V – Extraction and quantification of wheat phenolic compounds

Phenolic compounds are usually extracted from wheat grains, preliminarily milled, by procedures that involve the use of polar solvents. The most commonly used are methanol, ethanol, and acetone (Table 2). After the addition of solvent, the supernatant (corresponding to the soluble fraction composed of free and conjugated phenolic compounds), and the solid residue (insoluble-bound forms) are separated. The residue undergoes subsequent treatments, usually alkaline or acidic hydrolysis, to release the bound fraction of phenolic compounds (Adom *et al.*, 2003; Kim *et al*., 2006; Arrantz and Saura Calixto, 2010). The subsequent determination of the total amount of phenolic compounds in the recovered fractions is performed by VIS spectrophotometry after Folin-Ciocalteu reaction, while reversed-phase HPLC/MS is usually applied for identifying and quantifying the individual compounds. The elution proceeds by increasing the concentration of either acetonitrile or methanol in the mobile phase, under acidic conditions (Lempereur *et al.*, 1997; Kim *et al.*, 2006; Arrantz and Saura Calixto, 2010; Heimler *et al.*, 2010).

Table 2. Comparison of phenolic compound (PC) contents in wholemeal of durum and bread wheat.

Number of cultivars	Extracting conditions	Extracted fraction	Content of PC Min - max	Reference
Triticum turgidum L. ssp. *durum (Desf.) Husnot*				
5	Alkaline hydrolysis and subsequent ether extraction	Esterified ferulic acid	0.69–2.44 mg/g d.m. as ferulic acid	Lempereur *et al.*, 1997
9	Ethanol/water/formic acid (70:29.5:0.5)	Free and soluble-conjugated pPC	0.70-1.20 mg/g f.w. as gallic acid	Heimler *et al.*, 2010
30	Methanol-water (80:20 *v/v*) acidified with 1% HCl	Free and soluble-conjugated PC	0.78–0.95 mg/g d.w. as ferulic acid	Menga *et al.*, 2010
Triticum aestivum L. ssp. a*estivum*				
3	Ethanol	Free and soluble-conjugated PC	0.49–0.93 mg/g d.w. as gallic acid	Yu *et al.*, 2002
25	Methanol/water (80:20 *v/v*) acidified with 1% HCl	Free and soluble conjugated PC	0.78–1.07 mg/g d.w. as ferulic acid	Menga *et al.*, 2010
17	Ethanol/water/formic acid (70:29.5:0.5)	Free and soluble-conjugated PC	0.65-1.12 mg/g f.w. as gallic acid	Heimler *et al.*, 2010
11	Ethanol	Free PC	119.6-201.2 µmol gallic acid/100 g of grain	Adom *et al.*, 2003
11	Alkaline hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent extraction with hexane and ethyl acetate	Insoluble-bound PC	508-700 µmol gallic acid/100 g	Adom *et al.*, 2003
Commercial sample	Methanol/acetone (M/A)	Free and soluble-conjugated PC	1.12 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
Commercial sample	Acidic hydrolysis of residue of extraction of free and soluble-conjugated fraction and subsequent M/A extraction	Insoluble-bound PC	2.62 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
Commercial sample	Alkaline hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent M/A extraction	Insoluble-bound PC	0.002 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010

The primary phenolic compounds detected in wheat caryopsis are phenolic acids and derivatives (Mateo Anson et al., 2008). More than 70% of them are insoluble-bound forms (Adom *et al.*, 2003; Kim *et al.*, 2006). Among phenolic acids, the most abundant in both soft (Klepacka and Fornal, 2006) and durum wheat (Lempereur *et al.*, 1997) is ferulic acid, followed by *p*-coumaric, sinapic and caffeic acid (Lempereur *et al.*, 1997). Bound ferulic acid, esterified to arabinose units of cell-wall arabinoxylans, accounts for 97% of total ferulic acid content of wholemeal flour (Adom *et al.*, 2003).

Most of wheat phenolic compounds are concentrated in the outer layers of the kernel, as they play a key role in plant defense against pests and diseases (Abdel-Aal *et al.*, 2001). Hence, the removal of these fractions, during flour refining processes, causes a relevant decrease of these functional ingredients. In particular, phenolic compounds are concentrated in the aleurone layer (Lempereur *et al.*, 1997; Mateo Anson *et al.*, 2008). Ferulic acid occurs in high concentrations in aleurone, pericarp and embryo cell walls; only in trace amounts in the starchy endosperm. Lempereur *et al.* (1997) detected high concentrations of ferulic acid esterified to cell-wall arabinoxylans in the aleurone layer of 5 durum wheat cultivars (69% of total ferulic acid), while the remnant was found in germ and in seedcoat (26.6% of total ferulic acid). Only 1.4% of total ferulic acid was detected

in the starchy endosperm. Adom *et al.* (2005) observed that the content of phenolic compounds in wheat bran and germ is significantly higher than in the endosperm.

Table 2 shows a comparison between phenolic contents of durum and bread wheat. It can be observed that the majority of studies regarded bread wheat. Wide varietal variability was observed by various authors. Significant differences among wheat cultivars may depend on both intrinsic factors, related to genotype, and extrinsic ones, such as agronomic conditions. However, differences may be imputable to technical issues related to analytical protocols of phenolics extraction, and different instrumental specificity and sensitivity of the quantifying methods.

No reports regard the analysis of phenolic compounds in wild accessions of tetraploid grains. Table 3 shows the results of quantitative determination of soluble phenolic compounds (free and soluble-conjugated with mono- or di-saccharides) in a set of various tetraploid grains comprising *Triticum turgidum* L. ssp. *turanicum* (Jakubz.) Á.Löve & D.Löve, *Triticum turgidum* L. ssp. *turgidum*, *Triticum turgidum* L. ssp. *carthlicum* (Nevski in Kom.) Á.Löve & D.Löve and *Triticum turgidum* L. ssp. *dicoccum* (Schrank ex Schübler) Thell. On the whole, relevant levels of phenolic compounds were observed, ranging from 1.74 to 2.69 mg/g as ferulic acid. These values seem higher than those reported for cultivated varieties of durum and bread wheat, listed in Table 2.

Table 3. Soluble phenolic compounds (PC) (free and soluble-conjugated components of the total phenolic compounds) extracted with acetone:water 50:50 (v/v) from wholemeal of different tetraploid wheat accessions.

Type of wheat	Samples No.	Content of PC (mg/g as ferulic acid (min-max)
Triticum turgidum L. ssp. *turanicum*	5	1.74-2.18
Triticum turgidum L. ssp. *turgidum*	4	2.08-2.40
Triticum turgidum L. ssp. *carthlicum*	3	2.01-2.36
Triticum turgidum L. ssp. *dicoccum*	5	2.48-2.69

VI – Effect of polyphenol oxidase on tetraploid wheat phenolic compounds

Phenolic compounds act as terminators of free radicals and chelators of metal ions that catalyze lipid peroxidation. They exert the antioxidant activity by donation of a hydrogen atom to radicals (Bravo, 1998). Moreover, the phenoxy radical intermediates are resonance stabilized; therefore, a new chain reaction is not easily inititated. Oxidation of phenolic compounds lead to quinones, that are characterized by brown colour (Nicolas *et al.*, 1993).

Many vegetable foods contain polyphenoloxidases (PPO) (E.C. 1.14.18.1), a class of enzymes catalizing the oxidation of phenolics to quinones in presence of oxygen. In bread wheat, PPO causes discoloration of oriental noodles, at an extent related to the enzymatic activity (Fuerst *et al.*, 2006). The same enzyme is responsible for dough browning also in tetraploid wheat (Feillet *et al.*, 2000; Taranto *et al.*, 2012). The assessment of PPO activity in a set of 113 wild tetraploid wheat accessions and durum cultivars evidenced significantly lower levels of enzyme activity in the latter (Pasqualone *et al.*, 2004; Taranto *et al.*, 2012).

VII –Phenolic content of bran: from waste to source of antioxidants

Table 4 reports the content of phenolic compounds of bran. It is well established that the majority of bran phenolic acids occur in insoluble-bound form (Kim *et al.*, 2006). To release them, increasing the extraction rates, either chemical (Adom *et al.,* 2003; Kim *et al.*, 2006; Arranz and Saura Calixto, 2010) or enzymatic hydrolysis (Bartolome *et al*., 1999) have been proposed. The reported results

about the efficiency of chemical hydrolysis in alkaline or acidic conditions are controversial. Kim *et al.* (2006) found the alkaline conditions as more efficient than acidic hydrolysis. Also Adom *et al.* (2003) preferred an alkaline hydrolysis.

Table 4. Content of phenolic compounds (PC) extracted from commercial wheat bran in various conditions.

Samples No.	Extracting conditions	Extracted fraction	Content of PC (min-max)	Reference
1	Methanol/acetone (M/A)	Free and soluble-conjugated PC	1.62 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
1	Acidic hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent M/A extraction	Insoluble-bound PCs	15.89 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
1	Alkaline hydrolysis of extraction residue of free and soluble-conju-gated fraction and subsequent M/A extraction	Insoluble-bound PC	3.72 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
4	Methanol/water (80:20 v/v)	Free and soluble-conjugated PC	0.18–0.34 mg/g f.w. as gallic acid	Kim *et al.*, 2006
4	Alkaline hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent ethyl ether extraction	Insoluble-bound PC	2.14-2.33 mg/g f.w. as gallic acid	Kim *et al.*, 2006
4	Acidic hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent ethyl ether extraction	Insoluble-bound PC	0.65-1.07 mg/g f.w. as gallic acid	Kim *et al.*, 2006
1	Ultrasound-assisted extraction with 64% ethanol	Free and soluble-conjugated PC plus an aliquote of bound phenolics mobilised by ultrasounds	3.12 mg/g f.w. as gallic acid	Wang *et al.*, 2008
51	Aqueous ethanol 80% (v/v)	Free and soluble-conjugated PC	0.85-1.75 mg/g f.w. as gallic acid	Verma *et al.*, 2008
51	Alkaline hydrolysis of extraction residue of free and soluble-conju-gated fraction and subsequent ethyl ether and ethyl acetate extraction	Insoluble-bound PC	2.31-5.38 mg/g f.w. as gallic acid	Verma *et al.*, 2008

On the contrary, Arranz and Saura Calixto (2010), by performing HPLC analyses of methanol-acetone extracts, as well as of alkali and sulphuric acid hydrolysates of bran, found higher amounts of phenolic compounds in acidic (15.89 mg/g f.w.) than in alkaline hydrolysates (3.72 mg/g f.w.).

To enhance the efficiency of the release of phenolic compounds from bran it has also been proposed an ultrasound-assisted extraction, by using ethanol as solvent, with good results (Wang *et al.*, 2008). The effects of acoustic cavitations produced in the solvent by the passage of ultrasonic waves exert a mechanical effect, allowing greater penetration of solvent into the

sample matrix and increasing the contact surface area between solid and liquid phase; as a result the solute quickly diffuses from solid phase to the solvent (Wang *et al.*, 2008).

Many studies evidenced the antioxidant properties of bran, mainly attributable to its phenolic content (Zhou and Yu, 2004). Bran has been reported to be able to inhibit lipid oxidation catalyzed by either iron or peroxyl radicals (Baublis *et al.*, 2000). Bran extracts exert LDL protective effects in biologic systems (Yu *et al.*, 2005), and reduce lipid peroxidation of liposomes (Zielińsky and Kozłowska, 2000). Durum wheat bran has been the object of selections aimed to identify fractions with different functional and nutritional properties. It has been observed that the antioxidant activity is higher in the most internal bran fractions and increases in fractions having thinner granulometry (Esposito *et al.*, 2005). Durum bran extracts were observed to inhibit seed oil oxidation (Onyeneho and Hettiarachchy, 1992).

In the last decades, a large number of studies focussed their attention towards the employ of natural antioxidants to substitute synthetic molecules, and various agri-industry by-products have been proposed as source to extract antioxidant compounds (Moure *et al*., 2001; Fernàndez-Bolanos *et al.*, 2002). In this framework, the use of durum wheat bran to produce edible phenolic extracts has been proposed, with the final aim of enriching fresh pasta. The use of an edible solvent, such as aqueous ethanol, coupled to an alkaline hydrolysis by addition of either NaOH or KOH, with subsequent neutralization, has been experimented with good yields (Delvecchio *et al.*, 2012; Delvecchio and Pasqualone, 2012).

VIII – Conclusions

The varietal variability observed in the levels of wheat phenolic compounds suggest the possibility of identifying cultivars and wild accessions with higher levels of these secondary metabolites. Moreover, the outer layers of the caryopsis are particularly rich in phenolic compounds. These materials could be the starting point to prepare phenolic extracts useful in the formulation of wheat-based functional foods with enhanced antioxidant activity. Novel value-added utilisations of wheat milling by-products would enhance their marketing potential, and benefit the agricultural economy.

Acknowledgments

This work has been carried out in the framework and with the financial support of the Project PON ISCOCEM.

References

Abdel-Aal E.S.M., Hucl P., Sosulski F.W., Graf R., Gillott C., Pietrzak L., 2001. Screening spring wheat for midge resistance in relation to ferulic acid content. *J. Agricult. Food Chemistry*, 49, pp. 3559-3566.

Adom K.K., Sorrells M.E., Liu R.H., 2003. Phytochemical profiles and antioxidant activity of wheat varieties. . *J. Agricult. Food Chemistry*, 51, pp. 7825-7834.

Adom K.K., Sorrells M.E., Liu R.H., 2005. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties". . *J. Agricult. Food Chemistry*, 53, pp. 2297-2306.

Arranz S., Saura Calixto F., 2010. Analysis of polyphenols in cereals may be improved performing acidic hydrolysis: a study in wheat flour and wheat bran and cereals of the diet. *J. Cereal Sci.*, 51, pp. 313-318.

Bartolome B., Gomez-Cordoves C., 1999. Barley spent grain: release of hydroxycinnamic acids (ferulic and p-coumaric acids) by commercial enzyme preparations. *J. Sci. Food and Agriculture*, 79, pp. 435-439.

Baublis A.J., Decker E.A., Clydesdale F.M., 2000. Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. *Food Chemistry*, 68, pp. 1-6.

Bravo L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews,* 56, pp. 317-333.

Delvecchio L.N., Bianco A.M., Paradiso V.M., Summo C., Caponio F., Pasqualone A., 2012. Estrazione di sostanze fenoliche dalla crusca e loro impiego nella formulazione di impasti. *Tecnica Molitoria*, 63, pp. 1238-1244.

Delvecchio L.N., Pasqualone A., 2012. Production trials of fresh pasta enriched with phenolic compounds extracted from wheat by KOH-induced hydrolysis. *Progress in Nutrition*, 4, pp. 247-251.

Dixon R.A., 2001. Natural products and plant disease resistance. *Nature*, 411, pp. 843-847.

Esposito F., Arlotti G., Bonifati A.M., Napolitano A., Vitale D., Fogliano V., 2005. Antioxidant activity and dietary fibre in durum wheat bran by-products. *Food Research International*, 38, pp. 1167-1173.

Feillet P., Autran J.C., Icard-Vernière C., 2000. Pasta brownness: an assessment. *J. Cereal Sci.,* 32, pp. 215–233.

Fernàndez-Bolanos J., Rodrìguez G., Heredia A., 2002. Production in large quantities of highly purified hydroxityrosol from liquid-solid waste of two-phase olive oil processing or Alperujo. *J. Agricult. Food Chemistry*, 50, pp. 6804-6811.

Fuerst E.P, Anderson J.V., Morris C.F., 2006. Delineating the role of polyphenol oxidase in the darkening of alkaline wheat noodles. *J. Agricult. Food Chemistry*, 54, pp. 2378-2384.

Graf E., 1992. Antioxidant potential of ferulic acid. *Free Radical Biology and Medicine*, 13, pp. 435-448.

Hammerschmidt R., 1999. Phytoalexins: what have we learned after 60 years? *Annual Review of Phytopathology,* 37, pp. 285-306.

Heimler D., Vignolini P., Isolani L., Arfaioli P., Ghiselli L., Romani A., 2010. Polyphenol content of modern and old varieties of Triticum aestivum L. and T. durum Desf. grains in two years of production". *J. Agricult. Food Chemistry*, 58, pp. 7329-7334.

Kim D.H., Jung E.A., Sohng I.S., Han J.A., Kim T.H., Han M.J., 1998. Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. *Archives of Pharmaceutical Research*, 21, pp. 17-23.

Kim K.H., Tsao R., Yang R., Cui S.W., 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry*, 95, pp. 466-473.

King A., Young G., 1999. Characteristics and occurrence of phenolic phytochemicals. *J. American Dietetic Association,* 99, pp. 213-218.

Klepacka J., Fornal L., 2006. Ferulic acid and its position among the phenolic compounds of wheat. *Critical Reviews in Food Science and Nutrition*, 46, pp. 639-647.

Lempereur I., Rouau X., Abecassis J., 1997. Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (*Triticum durum* L.) grain and its milling fractions. *J. Agricult. Food Chemistry,* 25, pp. 103-110.

Mateo Anson N., Berg V.D.R., Havenaar R., Bast A., Haenen G.R.M.M., 2008. Ferulic acid from aleurone determines the antioxidant potency of wheat grain (*Triticum aestivum* L.). *J. Agricult. Food Chemistry,* 56, pp. 5589-5594.

Mc Keehen J.D., Busch R.H., Fulcher R.G., 1999. Evaluation of wheat (Triticum aestivum L.) phenolic acids during grain development and their contribution to *Fusarium* resistance. *J. Agricult. Food Chemistry,* 47, pp. 1476-1482.

Menga V., Fares C., Troccoli A., Cattivelli L., Baiano A., 2010. Effects of genotype, location and baking on the phenolic content and some antioxidant properties of cereal species. *Internat. J. Food Science and Technology,* 45, pp. 7-16.

Miller A., Engel K.H., 2006. Content of oryzanol and composition of steryl ferulates in brown rice (Oryza sativa L.) of European origin. *J. Agricult. Food Chemistry*, 54, pp. 8127–8133.

Moure A., Cruz J.M., Franco D., Domínguez J.M., Sineiro J., Domínguez H., Núñez M.J., Parajó J.C., 2001. Natural antioxidants from residual sources. *Food Chemistry*, 72, pp. 145-171.

Nicolas J., Cheynier V., Fleuriet A., Rouet-Mayer M.A., 1993. Polyphenols and enzymatic browning. In: *Polyphenolic phenomena*. Sca bert A. (ed), Paris: INRA Editions, pp. 165-175.

Onyeneho S.N., Hettiarachchy N.S., 1992. Antioxidant activity of durum wheat bran. *J. Agricult. Food Chemistry,* 40, pp. 1496-1500.

Pasqualone A., Clodoveo M.L., Simeone R., 2004. Polyphenol content and polyphenol oxidase activity in tetraploid wheat: effects on brown index of semolina and dough. In: *Polyphenol communications.* Hoikkala A. *et al.* (eds). Helsinki: University of Helsinki, pp. 337-338.

Ramos S., 2008. Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. *Molecular Nutrition and Food Research*, 52, pp. 507-526.

Renger A., Steinhart H., 2000. Ferulic acid dehydrodimers as structural elements in cereal dietary fibre. *European Food Research and Technology*, 211, pp. 422-428.

Smith M.M., Hartley R.D., 1983. Occurrence and nature of ferulic acid substitution of cell wall polysaccharides in gramineous plants. *Carbohydrates Research*, 118, pp. 65-80.

Taranto F., Delvecchio L.N., Mangini G., Del Faro L., Blanco A., Pasqualone A., 2012. Molecular and physic-chemical evaluation of enzymatic browning of whole meal and dough in a collection of tetraploid wheats. *J. Cereal Sci.*, 55, pp. 405-414.

Thomasset S.C., Berry D.P., Garcea G., Marczylo T., Steward W.P., Gescher A.J., 2007. Dietary polyphenolic phytochemicals - promising cancer chemopreventive agents in humans? A review of their clinical properties. *International J. Cancer*, 120, pp. 451-458.

Verma B., Hucl P., Chibbar R.N., 2008. Phenolic content and antioxidant properties of bran in 51 wheat cultivars. *Cereal Chemistry*, 85, pp. 544-549.

Wang J., Sun B,. Cao Y., Tian Y., Li X., 2008. Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry*, 106, pp. 804-810.

White P.J., Xing Y. 1997. Antioxidants from cereals and legumes. In: *Natural antioxidants, chemistry, health effects, and application.* F. Shahidi (ed.), Champaign IL: AOCC press, pp. 25–63.

Wink M., 2010. Biochemistry of plant secondary metabolism. 2nd ed., Oxford: John Wiley & Sons Ltd - Blackwell Publishing Ltd, pp. 481.

Yu L., Haley S., Perret J., Harris M., Wilson J., Qian M., 2002. Free radical scavenging properties of wheat extracts. *J. Agricult. Food Chemistry*, 50, pp. 1619-1624.

Yu L., Zhou K., Parry J.W., 2005. Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. *LWT- Food Science and Technology*, 38, pp. 463-470.

Zielińsky H., Kozłowska H., 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *J. Agricult. Food Chemistry*, 48, pp. 2008-2016.

Zhou K., Yu L., 2004. Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebensmittel-Wissenschaft und Technologie,* 37, pp. 717-721.