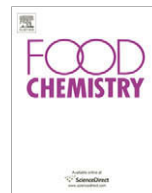
Food Chemistry 119 (2010) 1023–1029

Contents lists available at ScienceDirect

Food Chemistry

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



Effect of processing and cooking on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat

Clara Fares [a,*], Cristiano Platani [a], Antonietta Baiano [b,c], Valeria Menga [a]

[a] CRA, Centre for Cereal Research, S.S. 16, Km 675, 71100 Foggia, Italy
[b] Department of Food Science, University of Foggia, via Napoli, 25-71100 Foggia, Italy
[c] Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la valorizzazione dei Prodotti Tipici di Qualità (BIOAGROMED), Via Napoli, 71100 Foggia, Italy

ARTICLE INFO

Article history:
Received 28 April 2009
Received in revised form 16 June 2009
Accepted 4 August 2009

Keywords:
Phenolic acids
Trolox equivalent antioxidant capacity
Total phenolic content
Pasta processing
Cooking
Fibre
Debranning fractions

ABSTRACT

The effect of processing and cooking on phenolic acids profile and antioxidant properties of durum wheat (*Triticum turgidum* L. var. *durum*) pasta samples enriched with debranning fractions of an Italian wheat (*Triticum aestivum* L.) cultivar were studied.

Trolox equivalent antioxidant capacity (TEAC) by free radical scavenging activity against $ABTS^{\cdot+}$ cation radical, phenolic acid profile, total phenolic content (TPC) and fibre were determined. In the first debranning fractions of wheat, the highest concentration of phenolic acids, namely ferulic acid, and antioxidant capacity was found, but a diminishing trend was observed as the debranning levels proceeded. When semolina was processed into spaghetti, the main effect of processing was a reduction of the free phenolic acids content, chiefly caused by *p*-hydroxybenzoic acid decrease. In contrast an increase in bound phenolics fraction was observed. The boiling water could have enhanced the extraction of bound phenolics from the food matrix, primarily ferulic acid ester linked to cell walls. Cooking affected also the antioxidant capacity of pasta samples by enhancing its antioxidant properties *in vitro*.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Epidemiological studies indicate that whole grain consumption is related to a reduction in the incidence of coronary heart disease, diabetes and cancer (Slavin, Jacobs, & Marquart, 2001; Thompson, 1994). The potential health benefit of antioxidants is associated with protection against oxidative stress, which can result in chronic inflammation and possible insulin resistance (Willcox, Ash, & Catignani, 2004). Whole wheat flour has significant levels of antioxidants (Adom, Sorrells, & Liu, 2003; Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007) but they are unevenly distributed along the kernel. Recent literature has underlined that grain fractions (bran, flour and shorts) have different antioxidant capacities, wheat bran having the highest (Liyana-Pathirana & Shahidi, 2007). According to this study, in the outermost layers of two Canadian wheat grain (amber durum and hard red spring), total phenolic content and antioxidant capacity were found to be the highest, with respect to flour. Zhou, Su, and Yu (2004) showed that the antioxidant properties of the outermost layers of wheat kernel are due to the presence of the aleurone layer. These antioxidant properties are attributed to the relative abundance of phenolic compounds, primarily ferulic acid, in the aleurone layer, with respect to the other bran tissues (Esposito et al., 2005; Mateo Anson, Van Der Berg, Havenaar, Bast, & Haenen, 2008; Rhodes, Sadek, & Stone, 2002; Zhou et al., 2004). Aleurone also contains most of the lignans (Buri, von Reding, & Gavin, 2004), which are polyphenols whose metabolites act as antioxidants, exhibit antitumoral activities and have been reported to modulate the effects of estrogens (Qu, Madl, Takemoto, Baybutt, & Wang, 2005). This is the reason why industrial pretreatments and new debranning processes have been developed, in order to obtain novel wheat fractions for enriching cereal products (Hemery et al., 2007). In addition, it may be also interesting to remove only the outer layers, preserving the aleurone layer attached to starchy endosperm.

As phenolic acids and phytic acid were shown to be present in greater amounts in particular tissues, these compounds have been proposed as biochemical markers of the aleurone layer of soft wheat (Antoine, Peyron, Lullien-Pellerin, Abecassis, & Rouau, 2004; Hemery et al., 2009) and durum wheat (Peyron et al., 2002). Several authors have used these biochemical markers to determine the amounts of aleurone and pericarp in flours and other milling fractions (Greffeuille, Abecassis, Lapierre, & Lullien-Pellerin, 2006; Hemery et al., 2009) and in samples obtained from the wheat bran fractionation process (Antoine et al., 2004). Accordingly, the presence of aleurone layer in wheat fractions was evidenced by its high phenolic acids content, primarily ferulic acid

* Corresponding author. Tel.: +39 881 742972; fax: +39 881 713150.
E-mail address: clara.fares@entecra.it (C. Fares).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.08.006

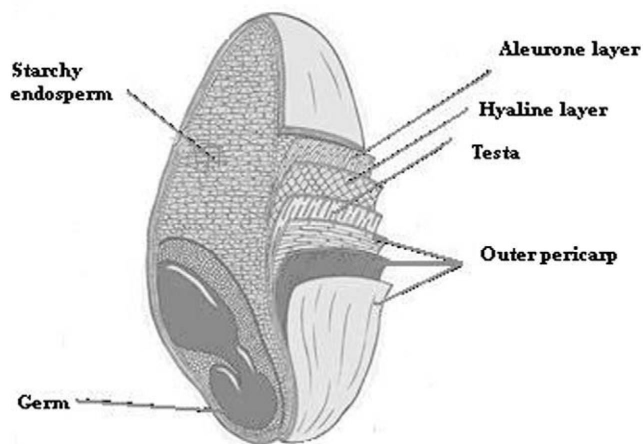


Fig. 1. Histological composition of wheat kernel.

(Mateo Anson et al., 2008). Antoine et al. (2004) have suggested that *p*-coumaric acid, which is known to be covalently bound to cell wall polysaccharides, could be considered a good indicator of the presence of aleurone cell walls.

Successive studies were conducted to assess the chemical composition of various dissected tissues, including hyaline layer, scutellum and testa (Barron, Surget, & Rouau, 2007) (Fig. 1). The authors found that the aleurone layer differed from the outer pericarp due to its lower content in ferulic acid dimers and trimers and the lower ratio arabinose/xylose. Moreover they found that about the 67% of *p*-coumaric acid found in the grain tissues was concentrated in the aleurone layer, even if its presence not excluded the presence of outer pericarp and intermediate layer as it was found not tissue-specific.

As phenolic compounds are concentrated in the outermost layers, the fractions resulting from the debranning process could be used as a natural source of antioxidants for the enrichment of food (Liyana-Pathirana & Shahidi, 2006). Based on these remarks, this study has (1) investigated the distribution of phenolic acids in the debranning fractions of an Italian wheat cultivar with a great antioxidant capacity; (2) identified the richest fraction in aleurone cell walls based on the presence of individual phenolic acids and antioxidant capacity; (3) evaluated the effect of processing and cooking on phenolic acids profile and some antioxidant properties of pasta samples enriched with the debranning fractions.

2. Materials and methods

2.1. Preparation of debranning fractions

An Italian wheat (*Triticum aestivum* L.) cultivar (Generale) was grown in Foggia during 2007–2008 and used to obtain enriched fractions in phenolic compounds and fibre. The cleaned grain (2 kg) was debranned by a friction debranning machine (Satake Toshiba Corp., Tokyo, Japan) for nine subsequent time intervals of 20 s, named debranning fractions 1, 2, 3, 4, 5, 6, 7, 8 and 9. Commercial semolina was obtained by Molino Tamma (Industrie Alimentari di Capitanata S.r.l., Foggia, Italy) and was enriched with the first, second and third fraction. With reference to Fig. 1, the first debranning fraction is composed of outer layers, testa and greater amounts of hyaline and aleurone layers. In the second and third fractions there are minor portions of hyaline and aleurone layers and slight amounts of endosperm. Wheat fractions were stored at −20 °C in the dark until analysis.

2.2. Pasta production

Pasta was prepared by mixing semolina or enriched semolina with 30% tap water to obtain a total dough water content of 44–45%. The dough was processed into spaghetti (1.70 ± 0.03 mm diameter) with a laboratory press (Serma) with a capacity of 1–2 kg. Extrusion conditions applied were temperature 50 ± 5 °C, pressure 70 ± 10 atm, and vacuum 700 mm Hg. Extruded spaghetti were dried in a laboratory pasta drier (NAMAD, Rome) using a low temperature drying procedure for 18 h at about 50 °C. Five kinds of spaghetti were produced, a control (C) made of 100% durum wheat semolina, and four enriched pasta samples: EP1 made with 6% of fraction 1, EP2 made with 10% of fraction 1, EP3 made with 6% of fractions 2 and 3, EP4 made with 10% of fractions 2 and 3.

2.3. Proximate analysis

Determination of total dietary fibre based on AOAC Method 991.43 (AOAC, 1997) as proposed by Prosky, Asp, Schweizer, De Vries, and Furda (1992), was carried out on debranning fractions of wheat, semolina and pasta samples (uncooked and cooked).

2.4. Cooking characteristics of pasta

To assess pasta cooking quality, each type of spaghetti (100 g) was cooked in 1 l of boiling tap water, and the optimal cooking time was taken when the white core in the strands disappeared after squeezing them between two glass plates. The sensorial judgement was performed on cooked pasta samples by three experienced panellists from the CRA–CER of Foggia. The following textural parameters were considered (D'Egidio, Mariani, Nardi, and Novaro, 1993): stickiness, the material adhering to surface of cooked pasta evaluated by visual inspection with the aid of standard reference samples and by handling; bulkiness, which is related to stickiness, the adhesion degree of spaghetti strands to each other evaluated visually and manually; firmness, the resistance of cooked pasta to chewing by the teeth. Each of these three parameters was evaluated using a score ranging from 10 to 100. The score of each sensory judgement component was the arithmetic mean of the values given by three experienced panellists; the final value, referred in the table, was the average of the means of stickiness, bulkiness and firmness. Total organic matter (TOM) was determined by the standard method of D'Egidio et al. (1981), and represents the amount of surface material released from cooked pasta into the washing water after rinsing. TOM values >2.1 g/100 g corresponded to low quality, between 2.1 and 1.4 g/100 g corresponded to good quality and <1.4 g/100 g indicated very good quality (D'Egidio and Nardi, 1996).

2.5. Extraction and determination of total extractable phenolic compounds (TPC)

Extraction was made according to the method proposed by Beta, Nam, Dexter, and Sapirstein (2005). Briefly, finely ground samples (1 g) were extracted twice by shaking them at room temperature in 4 ml of a methanol:water (80:20 v/v) solution acidified with 1% HCl, for 2 h. The mixtures were centrifuged at 1000g for 15 min. After centrifugation the supernatants were pooled. The extracts (0.2 ml) were added to 1.5 ml of 10-fold diluted Folin-Ciocalteau reagent (Singleton & Rossi, 1965). The mixture was allowed to equilibrate for 5 min and then neutralised with sodium carbonate. After incubation at room temperature for 90 min, the absorbance of the mixtures were measured at 725 nm. Acidified methanol was used as blank. Ferulic acid was used as the standard, and results are expressed in ferulic acid equivalents (μg of FAE/g sample dry matter basis). Determinations were performed in triplicate for

each extract and reported on dry matter basis (dm). The moisture content was determined by drying at 130 °C for 3 h in an oven.

2.6. Extraction and analysis of phenolic acids

Free and bound phenolic acids were extracted according to the method proposed by Mattila, Pihlava, and Hellstrom (2005). Briefly, 1 g sample was homogenised in 7 ml of a mixture of methanol containing 10% of a solution of acetic acid and water (85:15). The sample extract was ultrasonicated for 30 min and made to a volume of 10 ml with distilled water. After mixing, 1 ml was filtered for HPLC analysis of free phenolic acids. Next, 12 ml of distilled water and 5 ml of 10 M NaOH were added into the test tube, sealed, and stirred overnight at 20 °C using a magnetic stirrer. The solution was then adjusted to pH 2 and the liberated phenolic acids were extracted three times with 15 ml of a mixture of cold diethyl ether and ethyl acetate (1:1). The organic layers were combined, evaporated to dryness, dissolved in 1.5 ml of methanol, filtered and analysed by HPLC. In addition, after alkaline hydrolysis, an acid hydrolysis was also performed by adding 2.5 ml of concentrated HCl into the test tube and incubating in a water bath at 85 °C for 30 min. The sample was then cooled, and further sample handling was performed in the same way as after alkaline hydrolysis. The phenolic acids extracted with NaOH and HCl were labelled bound phenolics. The samples were run on an Agilent 1100 HPLC equipped with a diode array detector (DAD; Agilent, Waldbronn, Germany) according to the method proposed by Kim, Tsao, Yang, and Cui (2006). Phenolic acid separation was done with a 250 mm × 4.6 mm × 5 μm Zorbax SB-C18 column (Agilent). The temperature of the column oven was set at 35 °C. A gradient elution was employed with a mobile phase consisting of acetonitrile (solution **A**) and aqueous 2% acetic acid (solution **B**) as follows: at 0 min **B** = 100%; at 30 min **B** = 85%; at 50 min **B** = 50%; at 55 min **B** = 0%; at 60 min **B** = 100%; isocratic elution of 100% **B**, 60–70 min. The flow rate of the mobile phase was 1 ml/min, and the injection volume was 10 μl. The wavelengths used for the quantification of phenolic acids were 280 and 320 nm. All quantifications were based on the peak area of phenolic acid standards: *p*-hydroxybenzoic, chlorogenic, vanillic, syringic, *p*-coumaric, ferulic and salicylic acids and vanillin (Sigma–Aldrich Milano, Italy). The results of free, alkaline and acid hydrolysates were summed to present the total phenolic acid content (TPAC).

2.7. Trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacity (TEAC) was determined according to the procedure of Re et al. (1999) with some modifications. ABTS (2,2-azinobis-[3-ethylbenzothiazoline-6-sulphonic acid]) was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot +}$) was produced, allowing the reaction of the ABTS stock solution with potassium persulfate (2.45 mM, final concentration) for 12–16 h in the dark and at room temperature before use. $ABTS^{\cdot +}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. The absorbance was read two times exactly 1 and 6 min after the addition of 20 μl of the methanolic extracts to 2 ml of diluted $ABTS^{\cdot +}$. All tests were conducted in triplicate. The TEAC values of the extracts were calculated, using a Trolox standard curve, on the basis of the percentage inhibition of absorbance at 734 nm and expressed in μmol Trolox/g sample dm. The range of concentration of Trolox used for calibration curve was 0–15 μM.

2.8. Statistical analysis

Standard ANOVA procedure (one-factor randomised complete block design with three replicates) was applied on the data set with statistical package STATISTICA (StatSoft Italia Srl, Vers.7.1, 2005; Vigonza, Italy). Means were separated by LSD test at $p = 0.05$ probability level.

3. Results and discussion

3.1. Distribution of phenolic acids in the debranning fractions

According to several reports, the widespread phenolic acids in wheat are gallic, protocatechuic, *p*-hydroxybenzoic, gentisic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, salicylic, and *trans*-cinnamic acids (Wu, Haig, Pratley, Lemerle, & An, 1999). However only *p*-hydroxybenzoic, chlorogenic, vanillic, syringic, *p*-coumaric, ferulic and salicylic acids were detected in this study (Table 1). Besides these phenolic acids, vanillin, an aromatic aldehyde, was detected in the samples.

Several authors have suggested that the debranning process can be used to individually collect the peripheral layers of wheat grain, in order to obtain new food ingredients rich in antioxidants, fibre and micronutrients (Beta et al., 2005; Liyana-Pathirana, Dexter, & Shahidi, 2006), even if the composition of these fractions is a blend of different histological tissues (Findlay, 2006). In agreement with the above-mentioned literature, the debranning fractions collected from the Italian wheat cultivar Generale showed large amounts of phenolic compounds and fibre (Table 1).

In general, a diminishing phenolic acids trend was observed as the debranning levels proceeded, with the exception of *p*-hydroxybenzoic acid, which had the highest concentration in fractions 2 and 3. Ferulic acid was the most abundant in all fractions and it was always in the bound form. In agreement with previous research (Liyana-Pathirana et al., 2006), ferulic acid was predominant in the debranning fractions extracts. In the first fraction, the highest concentration of *p*-coumaric, salicylic and ferulic acids was also found. Except for *p*-hydroxybenzoic acid, which is mainly in the free form in all fractions analysed, the other phenolic acids are prevalent in the bound form. From the first fraction to the second, the concentration of ferulic acid decreased by about 21%, while *p*-coumaric acid decreased by two-thirds, and salicylic acid halved. Differences in phenolic acids amounts were observed also by Antoine et al. (2004) in hand dissected grain layers, and they indicated that *p*-coumaric acid could be considered a characteristic indicator of the presence of aleurone cell walls.

In contrast with other individual phenolic acids, *p*-hydroxybenzoic acid showed an increase of about 34% in fraction 2. Moreover it showed a steady content from fractions 5 to 9 and a reduction occurred only in debranned kernels, where the content decreased by 45% compared to the last fraction. In the first fraction ferulic acid accounted for 65% of the total phenolic acids content but it diminished as the debranning levels proceeded, down to a minimum level of 43% in debranned kernels. The same trend was observed in TEAC, which was unevenly distributed over the various fractions (Liyana-Pathirana & Shahidi, 2007). Accordingly, the highest values in antioxidant capacity were found in the first fraction, where ferulic acid had the highest concentration (from 4 to 10-fold higher in respect to the other phenolic acids). Therefore, this study confirms that ferulic acid is responsible for potential antioxidant properties of wheat grain fractions (Adom, Sorrells, & Liu, 2005; Martinez-Tome et al., 2004; Mateo Anson et al., 2008; Zhou et al., 2004). Interestingly, the assessment of TPC by Folin-Ciocalteu method always showed values lower than the total content of phenolic acids up to fraction 2 (about 10% of debranning degree); subsequently up to fraction 4 the differences become slight and subsequently the values are increasingly high. It appears that the extraction adopted for estimation of the TPC is not able to extract the bound phenolic esters linked to fibre that represent the greatest amount of antioxidants in the first fractions (1, 2 and 3).

Table 1
Phenolic acids, degree of debranning, fibre content, total phenolic acids content (TPAC, sum), total phenolic content (TPC) and Trolox equivalent antioxidant capacity (TEAC) in debranning fractions of Italian wheat cultivar Generale.

Phenolic acids[a] (μg/g dm)		Debranning fractions										Debranned kernel
		1	2	3	4	5	6	7	8	9	Whole wheat	
p-Hydroxybenzoic acid	Free	585 ± 12.5	887 ± 50.2	671 ± 37.7	577 ± 14.9	397 ± 29.3	397 ± 30.3	345 ± 33.5	326 ± 15.8	357 ± 21.2	275 ± 11.2	199 ± 44.1
	Bound	17.2 ± 0.54	9.70 ± 0.87	9.05 ± 0.19	4.79 ± 0.58	3.83 ± 0.20	3.05 ± 0.25	3.02 ± 0.28	3.11 ± 0.16	2.90 ± 0.09	5.59 ± 0.12	3.23 ± 0.05
	Total	602	897	679	582	400	400	348	329	360	281	202
Chlorogenic acid	Free	1.70 ± 0.03	1.46 ± 0.09	1.27 ± 0.03	1.14 ± 0.09	0.89 ± 0.05	0.9 ± 0.04	nd	nd	nd	nd	nd
	Bound	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Total	1.70	1.46	1.27	1.14	0.89	0.9	nd	nd	nd	nd	nd
Syringic acid	Free	3.59 ± 0.18	0.97 ± 0.02	0.62 ± 0.01	nd	nd	nd	nd	nd	nd	nd	1.13 ± 0.05
	Bound	42.6 ± 0.51	25.3 ± 2.71	15.7 ± 0.67	11.4 ± 0.83	8.93 ± 0.72	7.30 ± 0.57	7.08 ± 0.48	6.41 ± 0.15	4.83 ± 0.63	8.69 ± 0.33	4.27 ± 0.10
	Total	46.2	26.2	16.3	11.4	8.93	7.30	7.08	6.41	4.83	8.69	5.4
Vanillic acid	Free	12.2 ± 0.35	6.97 ± 0.31	3.73 ± 0.53	2.86 ± 0.53	2.21 ± 0.01	1.66 ± 0.04	1.79 ± 0.24	1.44 ± 0.03	1.59 ± 0.18	2.72 ± 0.09	1.13 ± 0.05
	Bound	41.1 ± 2.76	44.5 ± 12.2	28.3 ± 1.89	13.3 ± 0.13	14.4 ± 0.08	9.32 ± 0.01	7.04 ± 0.89	7.97 ± 0.15	7.57 ± 0.52	10.9 ± 0.52	2.68 ± 0.12
	Total	53.2	51.4	32.0	16.2	16.6	11.0	8.83	9.41	9.16	13.6	3.81
Vanillin	Free	10.8 ± 0.30	3.21 ± 0.2	2.22 ± 0.8	1.92 ± 0.25	1.29 ± 0.15	1.32 ± 0.09	1.33 ± 0.19	1.45 ± 0.04	1.55 ± 0.01	1.78 ± 0.02	1.79 ± 0.14
	Bound	35.9 ± 1.12	17.6 ± 1.55	7.11 ± 0.62	7.09 ± 1.36	7.10 ± 0.4	6.33 ± 1.23	6.87 ± 0.l9	4.67 ± 0.27	3.53 ± 0.30	5.34 ± 0.15	2.76 ± 0.01
	Total	46.7	20.8	9.33	9.01	8.39	7.65	8.20	6.12	5.08	7.12	4.55
p-Coumaric acid	Free	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Bound	147 ± 17.2	32.9 ± 1.55	46.9 ± 2.99	42.6 ± 2.78	34.7 ± 9.35	27.8 ± 6.03	23.9 ± 1.02	20.2 ± 0.38	17.3 ± 0.15	63.5 ± 11.1	24.5 ± 1.8
	Total	147	32.9	46.9	42.6	34.7	27.8	23.9	20.2	17.3	63.5	24.5
Ferulic acid	Free	7.47 ± 2.59	4.15 ± 0.19	4.8 ± 1.74	7.25 ± 0.18	5.55 ± 0.03	5.16 ± 0.53	5.43 ± 0.8	5.25 ± 0.32	5.70 ± 0.55	6.44 ± 10.5	5.07 ± 1.05
	Bound	2095 ± 56.8	1662 ± 69.8	1009 ± 17.8	786 ± 85.0	647 ± 23.2	539 ± 25.0	503 ± 11.1	474 ± 13.3	416 ± 7.36	465 ± 10.3	187 ± 3.85
	Total	2102	1666	1014	794	653	544	509	479	421	471	192
Salicylic acid	Free	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Bound	239 ± 7.42	114 ± 7.78	77.0 ± 13.6	83.4 ± 12.6	41.3 ± 17.1	34.0 ± 1.70	34.5 ± 3.01	28.6 ± 0.34	24.3 ± 1.1	53.3 ± 4.8	14.6 ± 1.05
	Total	239	114	77.0	83.4	41.3	34.0	34.5	28.6	24.3	53.3	14.6
Degree of debranning (%)		5.1	10.5	15.5	20.9	25.8	29.8	33.5	36.5	40.0		
Fibre content (% dm)		65.0 ± 0.10	34.4 ± 0.11	22.5 ± 0.11	17.5 ± 0.24	15.6 ± 0.18	14.1 ± 0.03	12.8 ± 0.08	11.6 ± 0.03	10.6 ± 0.20	13.3 ± 0.02	7.82 ± 0.28
TPAC (μg/g sample dm)		3238	2810	1876	1539	1164	1032	933	878	842	899	447
TPC (μg FAE/g sample dm)		2659 ± 0.15	2186 ± 3.09	1740 ± 6.20	1463 ± 8.02	1282 ± 18.6	1144 ± 3.08	1014 ± 12.4	952 ± 15.5	908 ± 9.28	934 ± 24.7	618 ± 9.27
TEAC (μmol Trolox/g sample dm)		5.8 ± 0.30	5.0 ± 0.32	3.9 ± 0.04	3.8 ± 0.14	3.4 ± 0.10	2.9 ± 0.20	2.5 ± 0.14	2.0 ± 0.26	1.9 ± 0.06	2.1 ± 0.12	1.1 ± 0.04

nd: not detectable.
[a] Values are means of three determinations ± standard deviation.

Consequently the TPC of the first fractions showed values lower than those of total phenolic acids, which were extracted by alkaline and acid hydrolysis. As the debranning process proceeds and growing parts of endosperm are collected in the debranning fractions, other compounds present in the kernel, such as tocopherols, carotenoids, amino acids and saccharides, interfered with the estimation (Liyana-Pathirana et al., 2006) and increased TPC with respect to TPAC.

3.2. Effect of processing and cooking on phenolic acids profile and antioxidant properties of pasta samples

To achieve the maximum benefits from diet it is critical to understand the bioactive factors and their distributions in food ingredients, as well as the effects of food formula, food processing and cooking on the availability of these beneficial components. So, this research has investigated the effect of processing and cooking on the antioxidant properties of pasta, a common Italian food, to elucidate its nutritional value as a potential contributor of antioxidants in the diet.

As the greatest amount of *p*-coumaric, salicylic and ferulic acids was found in the first fraction, this fraction could be that in which the aleurone cell walls were chiefly concentrated, as suggested by recent literature (Antoine et al., 2004; Hemery et al., 2009). Fractions 2 and 3 were also selected as containing still substantial amounts of ferulic, salicylic and *p*-hydroxybenzoic acids, although the antioxidant capacity was much lower (especially in fraction 3).

In Table 2 are shown the amounts of phenolic acids in semolina and corresponding pasta samples (cooked and uncooked). The main effect of processing was a decrease of the free phenolic acids

Table 2
Phenolic acids (μg/g sample dm) profile.

Sample		Semolina		Uncooked pasta		Cooked pasta	
		Free[b]	Bound[a]	Free[b]	Bound[a]	Free[b]	Bound[a]
Control	*p*-Hydroxybenzoic acid	90.0A	0.84a	61.1B	0.24c	55.5B	0.48b
	Vanillic acid	nd	nd	nd	nd	nd	nd
	Syringic acid	nd	0.49a	nd	0.19b	nd	0.28b
	Vanillin	nd	1.42a	nd	1.57a	nd	0.12b
	p-Coumaric	nd	1.74b	nd	1.23b	nd	5.77a
	Ferulic acid	1.08B	57.2b	1.90A	53.9b	2.01A	80.5a
	Salicylic acid	nd	2.27b	nd	4.00a	nd	2.28b
	Partial total	91.1	64.0	63.0	61.1	57.6	89.4
	TPAC	155		124		147	
EP1	*p*-Hydroxybenzoic acid	117A	2.08b	65.7B	2.18ab	66.5B	2.49a
	Vanillic acid	nd	0.66b	nd	1.97b	nd	5.03a
	Syringic acid	nd	3.15b	nd	3.55ab	nd	4.02a
	Vanillin	0.68	4.66a	nd	4.59a	nd	2.94b
	p-Coumaric	nd	6.76b	0.30	7.68b	nd	9.80a
	Ferulic acid	2.99B	154b	7.59A	160b	7.18A	215a
	Salicylic acid	nd	25.5a	nd	14.2b	nd	22.0a
	Partial total	121	197	73.6	194	73.7	261
	TPAC	318		268		335	
EP2	*p*-Hydroxybenzoic acid	132A	2.56b	111AB	2.84ab	78.3B	3.21a
	Vanillic acid	nd	1.92c	nd	4.41b	nd	6.77a
	Syringic acid	nd	4.44b	nd	5.08ab	nd	6.05a
	Vanillin	1.09	7.34a	nd	5.80ab	nd	4.59b
	p-Coumaric	nd	10.9b	1.77	10.82b	nd	15.3a
	Ferulic acid	3.27C	228b	11.0A	207b	8.69B	291a
	Salicylic acid	nd	24.5b	nd	23.7b	nd	44.4a
	Partial total	137	279	124	259	87.0	371
	TPAC	416		383		458	
EP3	*p*-Hydroxybenzoic acid	135A	1.28b	103B	1.27b	83.7B	1.73a
	Vanillic acid	nd	0.44b	nd	0.61b	nd	1.33a
	Syringic acid	nd	1.52b	nd	1.73b	nd	2.46a
	Vanillin	0.24	3.54a	nd	2.95b	nd	1.28c
	p-Coumaric	nd	4.82b	0.19	2.38c	nd	6.24a
	Ferulic acid	2.61A	105b	3.50A	115b	3.57A	156a
	Salicylic acid	nd	9.09b	nd	7.69b	nd	11.5a
	Partial total	138	125	107	131	87.3	180
	TPAC	263		238		267	
EP4	*p*-Hydroxybenzoic acid	166A	1.59b	114B	1.48b	106B	2.07a
	Vanillic acid	nd	1.51b	nd	1.28b	nd	1.80a
	Syringic acid	nd	2.26c	nd	2.93b	nd	3.26a
	Vanillin	0.36	6.41a	nd	3.68ab	nd	1.91b
	p-Coumaric	nd	5.60b	0.25	3.48c	nd	6.41a
	Ferulic acid	3.57B	157b	3.75B	164b	4.42A	199a
	Salicylic acid	nd	13.5a	nd	10.5b	nd	14.3a
	Partial total	170	188	118	187	110	228
	TPAC	357		305		339	

Abbreviations: EP1: enriched pasta with 6% of fraction 1; EP2: enriched pasta with 10% of fraction 1; EP3: enriched pasta with 6% of debranning fractions 2 and 3; EP4: enriched pasta with 10% of debranning fractions 2 and 3; TPAC (μg/g sample dm) is the result of the sum of each component. nd: not detectable.

[a] Different lower case letters for free phenolic acids in the same row for each sample (semolina, uncooked and cooked pasta) correspond to significant differences by LSD test ($p = 5\%$). Values are means of three determinations.

[b] Different capital letters for bound phenolic acids in the same row for each sample (semolina, uncooked and cooked pasta) correspond to significant differences by LSD test ($p = 5\%$). Values are means of three determinations.

fraction both in control and enriched samples (30.8%, 39%, 9.3%, 22.6% and 30.6% for control, EP1, EP2, EP3, and EP4, respectively). Conversely, the bound fraction did not change. The observed variation in free phenolic acids content was mainly due to *p*-hydroxybenzoic acid decrease. Only the pasta samples enriched with fraction 1 showed an increase in free ferulic acid, most evident in pasta with the highest addition (EP2). As expected, in the EP2 sample all values of phenolic acids were the highest (both ferulic acid in bound and free form, and total phenolic acids content) and this result is consistent also with the TEAC values of uncooked pasta (Fig. 2).

It is well documented that during pasta processing the presence of oxygen, water and heat treatment induces the oxidative degradation of antioxidants (carotenoids pigments, polyphenols) (Borrelli, Troccoli, Di Fonzo, & Fares, 1999; Fares et al., 2008). So the observed reduction in the content of phenolic acids during pasta processing may be due to the combined impact of the addition of water and oxygen during kneading. Indeed, in our study the loss was observed mainly in the free fraction of phenolic acids, which is more reactive in counterbalancing the action of oxygen and heat.

Cooking, however, affected almost of all the bound fraction of phenolic acids, which showed a consistent increase in all samples (46%, 34%, 43%, 37% and 22% for control EP1, EP2, EP3, and EP4, respectively; Table 2). The free phenolic acids did not vary, but in EP2 and EP3 a decrease was observed. Zielinski, Kozlowska, and Lewczuk (2001), on the other hand found that after a very strong hydrothermal processing all phenolic acids increased (both free and ester bound). The discrepancy between the two studies could be explained by the extreme conditions applied by those authors in comparison with our conditions. However in agreement with our data, the same authors have found that ferulic acid was the predominant compound after cooking. Regarding the individual phenolic acids content variation (Table 2), we found that for free phenolic acids it depended on the decrease of *p*-hydroxybenzoic acid, while for bound phenolic acids it was mainly ascribable to the increase of ferulic acid.

After cooking, pasta showed an increase in antioxidant capacity too, independently from the enrichment level. This variation agrees with the observed increase in bound phenolic acids, with respect to the uncooked samples. Such an increase is consistent with the observed variation of bound ferulic acid, which represents a confirmation that a part of the ferulic acid ester linked to cell walls (that was not measurable before cooking) is released by the pasta matrix during cooking and that, despite the heat, it preserved its antioxidant properties measured *in vitro*. Therefore, boiling water during cooking could enhance the extraction of bound phenolics from the food matrix and thereby increase the amount of them during the subsequent chemical extraction and determination. The observed variation of the TEAC in all cooked pasta samples is mainly ascribable to the increase in the extraction of bound ferulic acid. However, other mechanisms must be involved. It is known that during extrusion and drying the Maillard reaction takes place, giving rise to the production of different compounds with antioxidant activity (such as the Amadori compounds). In a recent paper (Rufián-Henares & Delgado Andrade, 2009) it has been demonstrated that *in vitro* gastrointestinal digestion is able to release Maillard reaction products from breakfast cereals, which lead to an increase of the antioxidant activity of the digested samples compared with the undigested ones. Then, it could be possible that the cooking process assayed in the present paper could release, or at least expose to the chemical environment, some Maillard reaction products that contribute to the overall antioxidant activity.

The sensorial evaluation of cooked pasta samples (Table 3) revealed the good performance of enriched samples, that above all

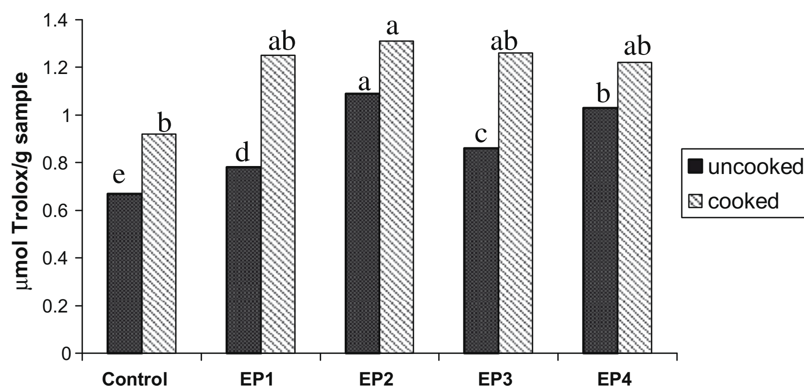


Fig. 2. TEAC measured on uncooked and cooked pasta samples. Values are mean of three determinations. Different lower case letters correspond to significant differences by LSD test ($p = 5\%$). Trolox equivalent antioxidant capacity (TEAC); EP1 = enriched pasta with 6% of fraction 1; EP2 = enriched pasta with 10% of fraction 1; EP3 = enriched pasta with 6% of debranning fractions 2 and 3; EP4 = enriched pasta with 10% of debranning fractions 2 and 3.

Table 3
Cooking behaviour and fibre content of pasta samples.

Sample	Optimal cooking time (min)	Weight after cooking (g)	TOM (g)	Organoleptic judgement	Fibre (% dm)	
					Uncooked	Cooked
Control	10	250b	1.06	66.70a	3.49e	4.48e
EP1	10	243b	1.20	58.30b	7.72b	8.53b
EP2	10	246b	1.00	66.70a	9.58a	11.31a
EP3	10	244b	1.00	63.30ab	5.25d	5.89d
EP4	10	262a	1.06	65.00ab	6.17c	6.99c

Abbreviations: EP1: enriched pasta with 6% of fraction 1; EP2: enriched pasta with 10% of fraction 1; EP3: enriched pasta with 6% of debranning fractions 2 and 3; EP4: enriched pasta with 10% of debranning fractions 2 and 3; TOM: total organic matter.
[a] Different lower case letters in the same column for each sample correspond to significant differences by LSD test ($p = 5\%$). Values are means of three determinations.

showed low stickiness as indicated by the low values of total organic matter (TOM). In accord with a previous study (Fares et al., 2008), fibre content was positively affected by cooking, showing increased values in all samples tested. The main phenomenon responsible for the observed increase of fibre was the gelatinisation and retro-degradation of starch during cooking, which changed part of it into resistant starch and led to an increase of total dietary fibre (Unlu & Faller, 1998; Vasanthan, Caosong, Yeung, & Li, 2002).

4. Conclusions

This study confirmed the presence of a phenolic acids gradient in wheat kernels, due to which the fraction richest in aleurone cell walls has been observed. To our knowledge, this research has been the first to document the relative changes in phenolic acids during pasta processing and cooking, and to relate such changes to the antioxidant capacity of products. During pasta processing, in which water, oxygen and heat triggered the oxidising reactions, a reduction of the free phenolic acids, which are recognised as free radical scavengers, occurred. Surprisingly, after cooking, pasta enhanced its antioxidant properties measured *in vitro*, independently of the enrichment level. It seemed that the antioxidant increase depended on the increased amount of ferulic acid released during cooking. But further studies are needed to elucidate the antioxidant activity of pasta under *in vivo* conditions. In addition the hypothesis that Maillard reactions products could be released after cooking should be checked.

Acknowledgement

The research was supported by the project "Qualità e sicurezza dei cereali" sponsored by MIUR (Minister for Scientific Research of Italy) and EU project DEVELONUTRI, contract number FP6-036296.

References

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry, 51*, 7825–7834.

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2005). Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *Journal of Agricultural and Food Chemistry, 53*, 2297–2306.

Antoine, C., Peyron, S., Lullien-Pellerin, V., Abecassis, J., & Rouau, X. (2004). Wheat bran tissue fractionation using biochemical markers. *Journal of Cereal Science, 39*, 387–393.

AOAC (1997). *Official Methods of Analysis* (16th ed). Gaithersburg, MD: AOAC international.

Barron, C., Surget, A., & Rouau, X. (2007). Relative amounts of tissues in mature wheat (*Triticum aestivum* L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science, 45*, 88–96.

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry, 82*, 390–393.

Borrelli, G. M., Troccoli, A., Di Fonzo, N., & Fares, C. (1999). Durum wheat lipoxygenase activity and other quality parameters that affect pasta colour. *Cereal Chemistry, 76*, 335–340.

Buri, R. C., von Reding, W., & Gavin, M. H. (2004). Description and characterization of wheat aleurone. *Cereal Foods World, 49*, 274–282.

D'Egidio, M. G., & Nardi, S. (1996). Textural measurement of cooked spaghetti. In J. E. Kruger, R. B. Matsuo, & J. W. Dick (Eds.), *Pasta and noodles technology* (pp. 133–156). St. Paul, MN: American Association of Cereal Chemists.

D'Egidio, M. G., De Stefanis, E., Fortini, S., Galterio, G., Nardi, S., & Sgrulletta, D. (1981). Influenza del tipo di acqua usata nella cottura sulla qualità delle paste. *Tecnica Molitoria, 32*, 505–511.

D'Egidio, M. G., Mariani, B. M., Nardi, S., & Novaro, P. (1993). Viscoelastograph measures and total organic matter test: Suitability in evaluating textural characteristics of cooked pasta. *Cereal Chemistry, 70*, 67–72.

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*, 1167–1173.

Fares, C., Codianni, P., Nigro, F., Platani, C., Scazzina, F., & Pellegrini, N. (2008). Processing and cooking effects on chemical nutritional and functional properties of pasta obtained from selected emmer genotypes. *Journal of the Science of Food and Agriculture, 88*, 2435–2444.

Findlay, C.J. (2006). Nutraceutical fractions from cereal grains. International patent application WO 2006/105651 A1. Applicant, 1289620 Ontario Inc.

Greffeuille, V., Abecassis, J., Lapierre, C., & Lullien-Pellerin, V. (2006). Bran size distribution at milling and mechanical and biochemical characterization of common wheat grain outer layers: A relationship assessment. *Cereal Chemistry, 83*, 641–646.

Hemery, Y., Lullien-Pellerin, V., Rouau, X., Abecassis, J., Samson, M. A., von Reding, W., et al. (2009). Biochemical markers: Efficient tools for the assessment of wheat grain tissue proportion in milling fractions. *Journal of Cereal Science, 49*, 55–64.

Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., & Abecassis, J. (2007). Dry process to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science, 46*, 327–347.

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

Liyana-Pathirana, C., & Shahidi, F. (2006). Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. *Journal of the Science of Food and Agriculture, 86*, 477–485.

Liyana-Pathirana, C., & Shahidi, F. (2007). Antioxidant and free radical scavenging activities of whole wheat and milling fractions. *Food Chemistry, 101*, 1151–1157.

Liyana-Pathirana, C., Dexter, J., & Shahidi, F. (2006). Antioxidant properties of wheat as affected by pearling. *Journal of Agricultural and Food Chemistry, 54*, 6177–6184.

Martinez-Tome, M., Murcia, M. A., Frega, N., Ruggieri, S., Jimenez, A. M., Roses, F., et al. (2004). Evaluation of antioxidant capacity of cereal brans. *Journal of Agricultural and Food Chemistry, 52*, 4690–4699.

Mateo Anson, N., Van Der Berg, R., Havenaar, R., Bast, A., & Haenen, G. (2008). Ferulic acid from aleurone determines the antioxidant potency of wheat grain (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry, 56*, 5589–5594.

Mattila, P., Pihlava, J. M., & Hellstrom, J. (2005). Content of phenolic acid and alkenylresorcinols and aventhramides in commercial grain products. *Journal of Agricultural and Food Chemistry, 53*, 8290–8295.

Peyron, S., Surget, A., Mabille, F., Autran, J. C., Rouau, X., & Abecassis, J. (2002). Evaluation of tissue dissociation of durum wheat grain (*Triticum durum* Desf.) generated by the milling process. *Journal of Cereal Science, 36*, 199–208.

Prosky, L., Asp, N. G., Schweizer, T. F., De Vries, J. W., & Furda, I. (1992). Determination of insoluble and soluble dietary fiber in foods and food products: Collaborative study. *Journal of AOAC, 75*, 360–367.

Qu, H., Madl, R. L., Takemoto, D. J., Baybutt, R. C., & Wang, W. (2005). Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. *Journal of Nutrition, 135*, 598–602.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 98*, 1231–1237.

Rhodes, D. I., Sadek, M., & Stone, B. A. (2002). Hydroxycinnamic acids in walls of wheat aleurone cells. *Journal of Cereal Science, 36*, 67–81.

Rufián-Henares, J., & Delgado Andrade, C. (2009). Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. *Food Research International, 42*, 394–400.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticolture, 16*, 144–158.

Slavin, J. L., Jacobs, D., & Marquart, L. (2001). Grain processing and nutrition. *Critical Reviews in Biotechnology, 21*, 49–66.

Thompson, L. U. (1994). Antioxidant and hormone-mediated health benefits of whole grains. *Critical Review in Food Science and Nutrition, 34*, 473–497.

Unlu, E., & Faller, J. F. (1998). Formation of resistant starch by twinscrew extruder. *Cereal Chemistry, 75*, 346–350.

Vasanthan, T., Caosong, J., Yeung, J., & Li, J. (2002). Dietary fiber profile of barley flour as affected by extrusion cooking. *Food Chemistry, 77*, 35–42.

Willcox, J. K., Ash, S. L., & Catignani, G. L. (2004). Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutrition, 44*(4), 275–295.

Wu, H., Haig, T., Pratley, J., Lemerle, D., & An, M. (1999). Simultaneous determination of phenolic acids and 2,4-dihydroxy-7-methoxy-3-one in wheat (*Triticum aestivum* L.) by gas chromatography-tandem mass spectrometry. *Journal of Chromatography Applied, 864*, 315–321.

Zhou, K., Su, L., & Yu, L. (2004). Phytochemicals and antioxidant properties in wheat bran. *Journal of Agricultural and Food Chemistry, 52*, 6108–6114.

Zielinski, H., Kozlowska, H., & Lewczuk, B. (2001). Bioactive compounds in the cereal grains before and after hydrothermal processing. *Innovative Food Science & Emerging Technologies, 2*, 159–169.