International Journal of
Food Science & Technology
www.ifst.org

Original article

Can the sprouting process applied to wheat improve the contents of vitamins and phenolic compounds and antioxidant capacity of the flour?

Slađana Žilić,[1]* Zorica Basić,[2] Vesna Hadži-Tašković Šukalović,[3] Vuk Maksimović,[3] Marijana Janković[1] & Milomir Filipović[4]

1 Department of Technology, Maize Research Institute, Slobodana Bajića 1, Belgrade-Zemun 11085, Serbia
2 Military Medical Academy, Institute of Hygiene, Crnotravska 17, Belgrade, Serbia
3 Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, Belgrade, Serbia
4 Department of Breeding, Maize Research Institute, Slobodana Bajića 1, Belgrade-Zemun 11085, Serbia

(Received 1 July 2013; Accepted in revised form 20 September 2013)

Summary The content of tocopherols, B vitamins, as well as free soluble, soluble conjugated and insoluble bound phenolic compounds was determined in untreated, steeped and sprouted wheat grains. Antioxidant capacity of whole wheat grains and their phenolic fractions was also evaluated. Sprouting significantly increased the levels of tocopherols, niacin, riboflavin, as well as free and bound phenolic compounds improving nutritional value and antioxidant capacity of wheat grains/flour. After sprouting for 5 days, the content of total phenolics, flavonoids and ferulic acid calculated as the sum of its fractions was increased by 9.9, 30.7 and 21.6%, respectively. The content of α-, β+γ- and δ-tocopherols was increased for 3.59-fold, 2.33-fold and 2.61-fold respectively, while the content of niacin, as predominant B vitamin, was increased for 1.19-fold after sprouting. The total antioxidant capacity of untreated, steeped and sprouted whole wheat grains was 19.44, 20.37 and 22.70 mmol Trolox Eq/kg, respectively. Sprouted wheat, as a rich source of bioavailable phytochemicals, should be used to improve the nutritional quality of food.

Keywords Antioxidant capacity, phenolics, sprouted wheat, steeped wheat, vitamins.

Introduction

Many epidemiological studies have demonstrated that health beneficial effects of whole wheat were attributed to the bioactive factors in bran, such as nondigestible carbohydrates and phytochemicals (Jensen *et al.*, 2004). However, the importance of wheat has mainly been attributed to its ability to be ground into flour and semolina that form the basic ingredients of bread and pasta, respectively, while bran is mainly used for animal feeding. Due to low nutritional quality of bread, its vitamin fortification has become mandatory in many countries. In contrast to fortification, adding of sprouted wheat flour is a natural way to increase vitamin levels. Although sprouting has been known for a very long time mainly in the Eastern countries, in recent years, sprouted grains have become very popular and widely accepted as a functional food because of their nutritious and health benefits. Hydrolytic enzymes activated during the process of wheat sprouting result in the degradation of starch and nonstarch polysaccharides, as well as proteins, leading to an increase in reducing sugars, soluble dietary fibres, peptides and amino acids, as well as the release of the insoluble phenolic compounds covalently bound to cell wall polysaccharides (Hung *et al.*, 2012). Further, the biochemical processes, occurring during sprouting, can generate bioactive components, such as riboflavin, thiamine, biotin, pantothenic acid, niacin, vitamin C, tocopherols and phenolic compounds, and also increase their availability (Moongngarm & Saetung, 2010). Sprouting of wheat has been suggested as an inexpensive and effective method to enhance the antioxidant capacity of flour through the increase of low-molecular weight antioxidants (Alvarez-Jubete *et al.*, 2010). Improving the hydrolytic enzymes efficiency, sprouting supports the biochemical mechanisms in humans and therefore can be considered as a kind of predigestion that helps breaking down the high-molecular complex compounds into their building blocks that are considered to have many physiological

*Correspondent: Fax: +381 11 37 54 994;
e-mail: szilic@mrizp.rs

doi:10.1111/ijfs.12397

benefits related to life-threatening diseases (Komatsuzaki *et al*., 2007). In contrast to the increase in bioactive compounds, the baking performance of wheat drastically decreases during sprouting, making the use of sprouted wheat for baking restricted. Additionally, due to the increased content of free amino acids and reducing sugars, sprouted wheat can potentially promote the Maillard reaction (Abderrahim *et al*., 2012). On the other hand, the study of Hussain & Uddin (2012) indicated that sprouting improved wheat flour functional properties such as water absorption capacity that is important in the development of ready-to-eat cereal foods.

In recent years, efforts have been made to improve plant raw materials that are widely used as food ingredients through the increase in the content of bioactive compounds, as well as processing methods to achieve a higher bioavailability of nutrients naturally present in the plant materials. There are a number of detailed studies showing the nutritional and antioxidant properties of sprouted wheat. However, studies on quantification of B vitamins and characterisation of all three differently bioavailable chemical forms of phenolic compounds of steeped and sprouted wheat flours are very limited. Therefore, in this study, we investigated the effects of steeping and sprouting on the content of bioactive components such as riboflavin, thiamine, pyridoxine, niacin and tocopherols in wheat flours and also how this approach affected the health considering the development of phenolic phytochemicals in terms of content and chemical form and contribution to the total antioxidant capacity of whole wheat flours.

Material and methods

Plant materials, steeping and sprouting conditions

The grains of bread wheat genotype harvested in Serbia in 2012 were used. The wheat grains were washed with distilled water in the flow for 60 min with air continuously bubbled through the system. The grains were then immersed in distilled water (1:1.5, w/v) and steeped for 24 h at room temperature (21 °C). After steeping, the water was decanted. A portion of imbibed wheat grains were transferred into a plastic container and incubated in the dark for 5 days in an incubator at 20 °C and humidity of 50%. The grains steeped for 24 h and grains sprouted for 5 days were air-dried at 55 °C for 8 h to remove the moisture of approximately 11%. For the control sample, untreated whole wheat grains were used. All samples were milled into whole-grain flour using a Perten 120 lab mill (Perten, Sweden) to a fine powder (particle size < 500 μm). Steeped and sprouted whole wheat grains were produced in a mill company (Bread Line d.o.o., Belgrade-Zemun, Serbia).

Analytical procedures

Analysis of tocopherols

The content of tocopherols was determined by the HPLC method (Sánchez-Pérez *et al*., 2000). A n-hexane extraction was applied. The wheat flour sample (1 g) was mixed with 10 mL of n-hexane, and the mixture was rigorously shaken at 4 °C for 30 min. After centrifugation at 7000 ***g*** for 15 min, the upper layer was separated and evaporated under N_2. The dried sample was then redissolved in 5 mL of methanol, vortexed, centrifuged at 5000 ***g*** for 10 min, and the clean upper layer was collected. A HPLC system with the Waters M600 E pump, thermostat and Rheodyne 7125 injector was used. The separation of tocopherols was performed on the Nucleosil 50-5 C18 column (250 × 4 mm, i.d., 5 μm) at flow rate of 1.0 mL min^{-1}. The mobile phase consisted of 95% methanol. The detection was performed with the Shimadzu RF-535 fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Identified peaks were confirmed and quantified by data acquisition and spectral evaluation using the 'Clarify' chromatographic software. The content of tocopherols is expressed as μg per g of d.m.

Analysis of thiamine (vitamin B_1), riboflavin (vitamin B_2) and pyridoxine (vitamin B_6)

The thiamine, riboflavin and pyridoxine were released from wheat flour by alkaline and enzymatic hydrolysis as described by Sánchez-Machado *et al*. (2004). Specifically, 1 g of a finely ground wheat sample was treated with 15 mL of 0.1 M HCl at 100 °C for 30 min. After cooling to room temperature, sample was brought to pH 4.3–4.7 with 2.5 M sodium acetate, then 1.25 mL of a 10% aqueous solution of taka-diastase was added, incubated in an oven at 45 °C for 4 h, filtered through Whatman No. 41 paper and diluted to 25 mL with Milli-Q water. For thiamine determination, it was necessary to perform its oxidation to thiochrome. One mL of the solution prepared as mentioned was treated with 0.5 mL of a 1% solution of potassium ferricyanide in cold 20% aqueous NaOH, vortexed for 10 s and used. All extracts were injected in the HPLC system. The separation of B vitamins was performed on the Nucleosil 50-5 C18 column (250 × 4 mm, i.d., 5 μm). The mobile phases were a 450 mL of methanol + 650 mL of 5 mM ammonium acetate for B1 and B2 vitamins, and a 250 mL of methanol + 770 mL of 5 mM hexanesulphonic acid for B6 vitamin. The flow rate was set at 1.0 mL min^{-1}. The detection was performed with the Shimadzu RF-535 fluorescence detector at the excitation wavelength of 370, 450 and 286 nm, and at the emission wavelength of 430, 530 and 392 nm for vitamin B1, B2 and B6, respectively. Identified peaks were confirmed and quantified by data

acquisition and spectral evaluation using the 'Clarify' chromatographic software. The content of B vitamins is expressed as μg per g of d.m.

Analysis of niacin (vitamin B_3)
Total niacin was extracted according to the modified AOAC (1984) method. One g of wheat flour was hydrolysed with 25 mL of 4% calcium hydroxide for 2 h in an autoclave at 8.27 kPa. Cooled hydrolysates were centrifuged for 15 min at 7000 ***g***. After the pH was adjusted to 5.0–5.2 with 1% phosphoric acid and diluted to 50 mL, the extracts were filtered through 0.45-μm membrane filters and injected in the HPLC system consisting of Waters M600 E binary pumps, thermostat and Rheodyne 7125 injector connected to the Waters 2996 diode array detector (Waters, Milford, MA, USA). Niacin was separated on the LiChroCART 125-4 Purospher C-18 RP column (125 × 4 mm, i.d., 5 μm) at a temperature of 20 °C using an isocratic elution programme with a mobile phase containing 50 mM phosphate buffer pH 3.0, at a flow rate of 1 mL min^{-1}. Identified peaks were confirmed and quantified by data acquisition and spectral evaluation using the 'Clarify' chromatographic software. The content of niacin is expressed as μg per g of d.m.

Extraction of free soluble, soluble conjugated and insoluble bound phenolic compounds
Free soluble, soluble conjugated and insoluble bound phenolic compounds in wheat samples were extracted according to the procedure described by Antoine *et al.* (2004). Twenty mL of acetone/methanol/water mixture (7:7:6, v/v/v) was used to extract free and soluble conjugated phenolic compounds from 0.5 g of flour. Insoluble phenolic compounds in the residue and conjugated phenolic compounds in the acetone/methanol/water extract were released by alkaline hydrolysis for 4 h at room temperature using 4 M NaOH before extraction. After the pH was adjusted to 2.0 by 6 M HCl, all the hydrolysates were extracted with ethyl acetate and diethyl ether (1:1, v/v) for four times. Five mL of combined extracts were evaporated under N_2 stream at 30 °C to dryness. The final residues were redissolved in 1.5 mL of methanol. After filtering through a 0.45-μm nylon filter, samples were kept at −40 °C prior to the HPLC analysis. Such prepared methanolic solutions of phenolic fractions (phenolic extract) were used for the analyses of phenolic acids, total phenolic compounds, flavonoids and antioxidant capacity.

Measurement of total phenolics content (TPC)
The total phenolic content was determined according to Singleton *et al.* (1999) and expressed as mg of gallic acid equivalent (GAE) per kg of d.m.

Measurement of total flavonoids content
The flavonoid content was determined according to Eberhardt *et al.* (2000) and expressed as mg of catechin equivalent (CE) per kg of d.m.

Analysis of individual phenolic acids
Quantification of phenolic acids was performed by the HPLC system. Prior to injection, methanolic extracts were filtered through 0.22-μm nylon syringe filters (Phenomenex, Torrance, CA, USA). Filtered extracts were injected in the Waters HPLC system consisting of 1525 binary pumps, thermostat and 717 + autosampler connected to the Waters 2996 diode array detector (Waters). Phenolic acids were separated on the Symmetry C-18 RP column (125 × 4 mm, i.d., 5 μm) using the linear gradient elution programme with a mobile phase containing solvent A (0.1% phosphoric acid) and solvent B (acetonitrile), at a flow rate of 1 mL min^{-1} with the following gradient profile: 20 min from 10 to 22% B, 20 min with a linear rise to 40% B, 5 min reverse to 10% B and additional 5 min equilibration time. The identification of phenolic acids was accomplished by comparing the retention time and absorption spectra of peaks in wheat samples to those of standard compounds. The quantification of phenolic acids was based on calibration curves built for each of the compounds identified in the samples. The content of phenolic acids is expressed as μg per g of d.m.

Analysis of total antioxidant capacity (TAC)
Measuring the total antioxidant capacity of phenolic fractions and wheat flour samples was done based on QUENCHER method described by Serpen *et al.* (2008) using 7 mM aqueous solution of ABTS (2,2-azino-bis/3-ethyl-benothiazoline-6-sulphonic acid) with 2.45 mM $K_2O_8S_2$ as the stock solution. The working solution of $ABTS^+$ was obtained by diluting the stock solution in water/ethanol (50:50, v/v). The wheat flour samples (10 mg), as well as phenolic extracts (500 μL evaporated to dryness), were mixed with 20 mL of $ABTS^+$ working solution, and the mixture was rigorously shaken at 4 °C for 25 min. After centrifugation at 6200 ***g*** for 5 min (10 °C), the absorbance measurement was performed at 734 nm. The total antioxidant capacity was expressed as the Trolox equivalent antioxidant capacity (TEAC) in mmol of Trolox per kg of d.m.

Statistical analysis

The analytical data were reported as mean ± SD of at the least two independent extractions. Significance of differences between wheat samples were analysed by the Fisher's least significant differences test, after variance analysis for trials was set up according to the

randomised complete block design. Differences with $P < 0.05$ were considered significant. Statistical evaluation was carried out by MSTATC statistical software (Michigan State University, East Lansing, MI, USA).

Results and discussion

The content of tocopherols (α, β+γ and δ), thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3) and pyridoxine (vitamin B_6) in the wheat samples is given in Table 1. Tocopherol contents in untreated wheat grains were low, with 0.94 μg g^{-1} of α-tocopherol, 1.71 μg g^{-1} of β+γ-tocopherol and 1.26 μg g^{-1} of δ-tocopherol. α-Tocopherol concentration, quantified in this study, was a fivefold lower than that (4.37 μg g^{-1}) reported by Yang *et al.* (2001), but threefold higher than that (0.3 μg g^{-1}) reported by Seibold (1990). According to Lampi *et al.* (2008) in genotypes rich in tocols, there was usually a high proportion of β-tocotrienols and low proportion of α-tocopherol. However, the content of α-, β+γ-, as well as δ-tocopherol was significantly affected by sprouting. The content of aforementioned tocopherols was increased for 3.59-fold, 2.33-fold and 2.61-fold respectively, in relation to that in untreated wheat grains. These results are in agreement with Yang *et al.* (2001). Although the wheat samples contain all tocopherol forms, only α-tocopherol is the most biologically active form of vitamin E because in biological systems it is the major lipid-soluble chain-breaking antioxidant. Besides, bioaccessibility of α-tocopherol from wheat bread is very high, being 99.6%, and that from wheat germ almost as good, being 53.29% (Reboul *et al.*, 2006). These results indicate that sprouted wheat grains and their products could be important sources of α-tocopherol.

Table 1 Content of tocopherols and B vitamins in whole untreated, steeped and sprouted wheat grains/flours

Vitamins	Untreated wheat	Steeped wheat	Sprouted wheat
E vitamins (μg g^{-1})			
α-Tocopherol	0.94 ± 0.09[b]	0.96 ± 0.08[b]	3.38 ± 0.26[a]
β+γ-Tocopherol	1.71 ± 0.05[b]	1.51 ± 0.08[b]	3.98 ± 0.26[a]
δ-Tocopherol	1.26 ± 0.06[b]	1.06 ± 0.08[b]	3.29 ± 0.23[a]
Total tocopherols	3.91 ± 0.14[b]	3.53 ± 0.18[b]	10.65 ± 0.49[a]
B vitamins (μg g^{-1})			
Niacin	72.81 ± 1.69[b]	70.87 ± 1.99[b]	86.74 ± 1.74[a]
Thiamine	5.81 ± 0.17[a]	5.73 ± 0.14[a]	5.18 ± 0.14[b]
Riboflavin	0.27 ± 0.02[b]	0.28 ± 0.02[b]	0.41 ± 0.02[a]
Pyridoxine	0.38 ± 0.02[a]	0.37 ± 0.01[a]	0.30 ± 0.02[b]

Means followed by the same letter within the same row are not significantly different, according to Fisher's last significance difference test ($P = 5\%$).

Although wholegrain cereals are a rich source of many vitamins, thiamine, riboflavin and niacin (vitamins B_1, B_2, and B_3) have been normally added to white flour and other refined grains since the 1940s to replace the nutrients that are removed with refining. In untreated wheat sample, the 92% of total content of group B vitamins (79.27 μg g^{-1}) was mainly represented by niacin (72.81 μg g^{-1}; Table 1). Unfortunately, it should be noted that some diseases, such as pellagra, occur because niacin in cereals, being incorporated in large molecules (probably glycoproteins), is mainly present in a nutritionally unavailable form (Combs, 2008). Prodanov *et al.* (1997) have reported increments in available niacin brought about by sprouting. According to our study, after sprouting, the content of total niacin was increased by 19.13% than in untreated wheat grains, while that in steeped wheat grains was slightly reduced. Our results have confirmed early research of Nason (1950) according to which the increase occurred in the maize embryo while the endosperm lost niacin. Tarr & Arditti (1982) have suggested that tryptophan from the endosperm acts as a niacin precursor when translocated to the embryonic axis. Our results indicate that sprouting also significantly improved the content of riboflavin by about 1.5-fold compared with the untreated wheat grains. However, in accordance with the results of Moongngarm & Saetung (2010) for germinated brown rice, sprouting of the wheat brought a slight reduction of thiamine and pyridoxine contents probably due to leaching out of water-soluble vitamins during the soaking. Thiamine and pyridoxine contents decreased from 5.81 and 0.38 μg g^{-1} of nontreated to 5.18 and 0.30 μg g^{-1} of sprouted wheat grains, respectively (Table 1).

The changes in the total phenolic content (TPC) of wheat during sprouting determined by the Folin–Ciocalteu method are shown in Table 2. The present study demonstrated that bound phenolics were a predominant fraction in untreated grains, and its content was higher by about 2.12 and 1.24-fold as compared to those of soluble free and conjugated phenolics, respectively. The level of total soluble conjugated phenolics in wheat grains was higher by about 1.72 times than those of its total soluble free phenolics. The similar phenolic fractions relation was obtained by Liyana-Pathirana & Shahidi (2006) in whole grains of soft white winter and hard red spring wheat. According to our study, both steeping and sprouting significantly ($P < 0.05$) increased the content of total soluble free and insoluble bound phenolic fractions, while decreased the conjugated component. Food components to exert their biological effects must be in some form bioavailable. Compared with untreated wheat grains, the content of free phenolic compounds, that may be declared bioavailable, was increased by 5.7 and 28% after steeping for 24 h and sprouting for

Table 2 Content of free soluble, soluble conjugated and insoluble bound total phenolics (mg GAE kg^{-1}) and flavonoids (mg CE kg^{-1}) in whole untreated, steeped and sprouted wheat grains/flours

Phenolics	Phenolic fractions*	Untreated wheat	Steeped wheat	Sprouted wheat
Total phenolics	Free soluble	673.56 ± 0.17^{c} (20.65)	712.22 ± 2.99^{b} (23.58)	862.46 ± 29.89^{a}
	Soluble conjugated	1156.58 ± 36.34^{a} (35.47)	818.62 ± 47.51^{c} (27.09)	1094.96 ± 6.64^{b} (30.55)
	Insoluble bound	1430.70 ± 17.99^{c} (43.87)	1490.67 ± 23.34^{b} (49.33)	1626.89 ± 16.16^{a} (45.39)
	Total	3260.84 ± 58.13^{a}	3021.51 ± 67.86^{b}	3584.31 ± 52.70^{a}
Total flavonoids	Free soluble	18.41 ± 0.06^{c} (7.31)	20.69 ± 0.22^{b} (8.54)	27.39 ± 0.84^{a} (8.32)
	Soluble conjugated	37.96 ± 1.05^{a} (15.08)	27.64 ± 1.56^{c} (11.41)	31.09 ± 0.19^{b} (9.45)
	Insoluble bound	195.43 ± 2.36^{b} (77.61)	193.81 ± 2.99^{b} (80.04)	270.66 ± 2.68^{a} (82.23)
	Total	251.80 ± 4.11^{b}	242.14 ± 5.41^{c}	329.14 ± 4.77^{a}

Means followed by the same letter within the same row are not significantly different, according to Fisher's last significance difference test ($P = 5\%$).

*Values in parentheses represent the percentage contribution of each phenolic fraction to its total.

5 days, respectively. This result can be caused by the hydrolysis of conjugated phenolic compounds that contributes to increase the free phenolics content after sprouting. As regards the 1.14-fold increase in insoluble bound phenolic compounds found in sprouted wheat compared with untreated, this result can be explained by phenols biosynthesis *de novo* in the embryonic axis of sprouted wheat grains. It is known that bound wheat phenolics associated with the cell walls may survive upper gastrointestinal tract digestion and finally reach the colon, where colonic digestion by intestinal microflora may release the bulk of them. Thus, our results suggest that the majority of the wheat phenolic compounds are those which can be released in the colon to exert their healthful benefits locally and beyond after absorption. Based on the literature data, it can be concluded that the content of different fractions of total phenolics is highly dependent on sprouting conditions. Although our results are consistent with the studies of Hung *et al.* (2012), these authors reported reduction of waxy wheat bound phenolic compounds after 12 and 24 h of sprouting, but then a significant increase after 36 and 48 h. Yang *et al.* (2001) suggested that wheat grains steeped for 24 h and then sprouted for 7 days would produce the most desirable sprouts with respect to concentration of antioxidants, such as phenolics.

The total flavonoid content (TFC) of untreated, steeped and sprouted wheat is shown in Table 2. In accordance with the study of Dinelli *et al.* (2009), insoluble bound flavonoids were predominant fraction in all samples. The content of total soluble flavonoids (free + conjugated fractions) was low being 56.37, 48.33 and 58.48 mg CE kg^{-1} in untreated, steeped and sprouted wheat grains, respectively. The obtained range was slightly lower than the total soluble flavonoids contents of 10 durum wheat evaluated by Dinelli *et al.* (2009). However, our results indicated that a significant increase in the content of soluble free and insoluble bound flavonoids, respectively by 48.77 and 38.49%, occurred after sprouting for 5 days. Flavonoids are polyphenolic compounds that occur ubiquitously in plant tissues in relatively high concentrations as sugar conjugates. They occur mostly in O-glycosidic form with a number of sugars such as glucose, galactose, rhamnose, arabinose, xylose and rutinose. However, major cereal crops such as wheat predominantly synthesise flavone-C-glycosides, which are stable to hydrolysis (Brazier-Hicks *et al.*, 2009). It follows that can be assumed that a low degree of flavone-C-glycosides hydrolysis may be reason for the low values of conjugated flavonoids in all wheat samples, given that the applied spectrophotometric assay is based on the aluminium chloride complex formation with flavonoid aglycones. Among bioactive compounds, flavonoids are particularly important in the human diet as antioxidants and antiviral agents. Also, the epidemiological studies have indicated that their consumption is associated with a reduced risk of cancer and cardiovascular disease (Wang *et al.*, 2012).

Four individual phenolic acids, ferulic, isoferulic, *p*-coumaric and caffeic acid, were detected in the wheat samples. The contents of soluble free, soluble conjugated, insoluble bound, as well as the total phenolic acids calculated as the sum of these fractions, are given in Table 3. As shown, ferulic acid was the major compound, followed by isoferulic, *p*-coumaric and caffeic acids. In addition, the insoluble bound form

Table 3 Content of free soluble, soluble conjugated and insoluble bound phenolic acids (μg g^{-1}) in whole untreated, steeped and sprouted wheat grains/flours

Phenolic acids	Phenolic acid fractions*	Untreated wheat	Steeped wheat	Sprouted wheat
Ferulic	Free soluble	11.08 ± 0.22^{b} (1.82)	2.93 ± 0.05^{c} (0.44)	30.5 ± 0.42^{a} (4.13)
	Soluble conjugated	34.69 ± 0.69^{c} (5.71)	38.53 ± 1.01^{b} (5.75)	65.22 ± 0.33^{a} (8.83)
	Insoluble bound	561.45 ± 5.61^{c} (92.46)	628.48 ± 0.69^{b} (93.87)	670.05 ± 3.35^{a} (90.75)
	Total	607.22 ± 5.64^{c}	669.94 ± 6.11^{b}	738.32 ± 4.43^{a}
Isoferulic	Free soluble	4.92 ± 0.10^{a} (2.89)	2.31 ± 0.06^{b} (1.49)	1.66 ± 0.01^{c} (0.91)
	Soluble conjugated	19.84 ± 0.39^{b} (11.68)	15.51 ± 0.61^{c} (9.98)	34.36 ± 0.58^{a} (18.88)
	Insoluble bound	145.14 ± 2.87^{a} (85.43)	137.64 ± 2.11^{b} (88.53)	145.48 ± 2.89^{a} (79.94)
	Total	169.90 ± 3.01^{b}	155.47 ± 2.57^{c}	181.99 ± 3.26^{a}
p-Coumaric	Free soluble	n.d. (0.00)	0.31 ± 0.01 (1.36)	n.d. (0.00)
	Soluble conjugated	1.50 ± 0.02^{b} (7.60)	1.63 ± 0.06^{a} (7.13)	n.d. (0.00)
	Insoluble bound	18.24 ± 0.30^{c} (92.40)	20.91 ± 0.46^{b} (91.51)	39.73 ± 0.79^{a} (100.0)
	Total	19.74 ± 0.39^{c}	22.85 ± 0.52^{b}	39.73 ± 0.79^{a}
Caffeic	Free soluble	n.d. (0.00)	n.d. (0.00)	n.d. (0.00)
	Soluble conjugated	n.d. (0.00)	n.d. (0.00)	n.d. (0.00)
	Insoluble bound	5.53 ± 0.13^{b} (100.0)	7.11 ± 0.25^{a} (100.0)	4.79 ± 0.39^{c} (100.0)
	Total	5.53 ± 0.13^{b}	7.11 ± 0.25^{a}	4.79 ± 0.39^{c}

n.d., not detected.

Means followed by the same letter within the same row are not significantly different, according to Fisher's last significance difference test (P = 5%).

*Values in parentheses represent the percentage contribution of each phenolic acid fraction to its total.

Table 4 ABTS radical-scavenging capacity (mmol Trolox Eq kg^{-1}) of phenolic fractions from whole untreated, steeped and sprouted wheat grains/flours

Fractions	Untreated wheat	Steeped wheat	Sprouted wheat
Free soluble	11.61 ± 0.19^{b}	11.17 ± 0.03^{c}	12.40 ± 0.36^{a}
Soluble conjugated	10.85 ± 0.27^{ab}	10.67 ± 0.60^{b}	11.10 ± 0.13^{a}
Insoluble bound	24.66 ± 0.11^{b}	24.61 ± 0.86^{b}	26.93 ± 0.45^{a}
Total	47.12 ± 0.57^{b}	46.45 ± 1.53^{b}	50.43 ± 1.02^{a}

Means followed by the same letter within the same row are not significantly different, according to Fisher's last significance difference test (P = 5%).

was the major form of the phenolic acids in all wheat samples. Several previous studies have found increases in free and total phenolic acids of sprouted wheat (Yang *et al*., 2001; Hung *et al*., 2011). In our study, the germination process increased the content of ferulic acid compared with that in untreated wheat grains by about 175, 88 and 19% in soluble free, conjugated and insoluble bound phenolic fractions, respectively. The accumulation of the ferulic acid was due to the phenolic biosynthesis and hydrolysis of polyphenolic compounds bound to cell walls, as reported by Yang *et al*. (2001). The increase in the free ferulic acid content found in sprouted wheat suggests an improved bioavailability and a higher antioxidant potential. However, the contents of free isoferulic as well as bound caffeic acid were decreased by sprouting.

Here, the QUENCHER method with the ABTS reagent was used to determine the antioxidant capacity of phenolic fractions (Table 4) and whole untreated, steeped and sprouted wheat grains (Fig. 1). Total antioxidant capacity of free and bound phenolics correlates very well with their contents in phenolic extracts of untreated, steeped and sprouted wheat samples (r^2 = 0.72 and r^2 = 1, $P < 0.05$, respectively). In our study, after sprouting, antioxidant capacity of free, conjugated and bound phenolic was increased by 6.8, 2.3 and 9.2%, respectively, in relation to that in phenolic extracts of untreated wheat grains (Table 4). These results are in agreement with those reported by

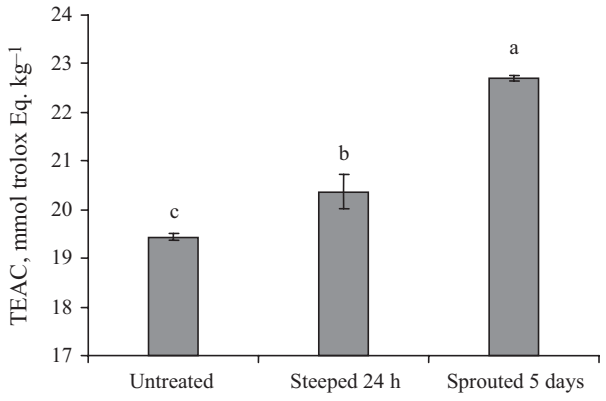

Figure 1 Total antioxidant capacity of whole untreated, steeped and sprouted wheat grains/flours obtained by the QUENCHER method with ABTS. The vertical bars represent the standard deviation of each data point. Bars with different letters are statistically significantly different, according to Fisher's last significance difference test (P = 5%).

Hung *et al.* (2011). The results of Lee *et al.* (2005) showed that flavonoid aglycones had greater antioxidant activities than their glucosides when the potency was assessed using the LDL oxidation assay. As mentioned, in our study, the total antioxidant capacity of whole grains of wheat samples was determined with the direct measurement procedure that skips all time-consuming solvent extraction and hydrolysis steps. As both soluble and insoluble compounds simultaneously come into contact with the ABTS radical cations, the direct procedure is able to measure accurately the total antioxidant capacity of whole wheat grains. Our results indicate that sprouting significantly improved the antioxidant capacity of whole wheat grains. Although other antioxidant compounds such as tocopherols, carotenoids, proteins, vitamin C, sugars and lignans might increase the antioxidant capacity of cereals grains (Žilić *et al.*, 2011), the fact is that the antioxidant capacity of untreated, steeped and sprouted whole wheat grains when compared with the sum of antioxidant capacity of phenolic fractions in the samples was considerably lower. So, the total antioxidant capacity of untreated, steeped and sprouted whole wheat grains was 19.44, 20.37 and 22.70 mmol Trolox Eq kg^{-1}, respectively (Fig. 1), while the sum of antioxidant capacity of phenolic fractions in the samples was 47.12, 46.45 and 50.43 mmol Trolox Eq kg^{-1}, respectively (Table 4). Furthermore, our results indicate that wheat grain phenolic compounds especially in bound form, as dominant, exert lower antioxidant capacity in comparison with its hydrolysed and isolated free forms in analysed extracts. This behaviour could probably be explained by the chemical nature and reactivity of the aforementioned compounds present in the wheat grain. The individual compounds can suffer polymerisation and other reactions that promote important structural changes and as a consequence, variations in their antioxidant capacity. For instance, when the degree of phenolic compounds polymerisation exceeds a critical value, the reduced availability of its hydroxyl groups and the increased molecular complexity promotes a decrease in antioxidant capacity (Pinelo *et al.*, 2004).

Conclusions

The present study shows that sprouting enhances the nutritional value of whole wheat flours through biosynthesis of tocopherols, niacin and riboflavin in the embryonic axis. In addition to other antioxidants, enhancement in phenolic compounds caused by either biosynthesis *de novo* or release of these compounds by induced enzymatic hydrolysis during sprouting contributed to the increase in antioxidant capacity of whole wheat grains/flour. Although whole sprouted flour is a negative quality parameter with regard to standard baking processes, it should be taken into consideration for human diets as a food ingredient that could provide positive health effects. Interests in incorporating bioactive ingredients such as phenolic antioxidants and vitamins from sprouts into popular foods such as bread have grown rapidly, due to the increased consumer health awareness. Common dietary compounds, such as bread, in the form of functional foods, can deliver a high concentration of antioxidants that may play a role in protection from diseases, such as cancer, cardiovascular diseases and degenerative diseases (Arts & Hollman, 2005).

Acknowledgments

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants No. TR-31069) and supported by COST action FA1005 (Infogest).

References

Abderrahim, F., Huanatico, E., Repo-Carrasco-Valencia, R., Arribas, S.M., Gonzalez, M.C. & Condezo-Hoyos, L. (2012). Effect of germination on total phenolic compounds, total antioxidant capacity, Maillard reaction products and oxidative stress markers in canihua (*Chenopodium pallidicaule*). *Journal of Cereal Science*, **56**, 410–417.

Alvarez-Jubete, L., Wijngaard, H., Arendt, E.K. & Gallagher, E. (2010). Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chemistry*, **119**, 770–778.

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.

Arts, I.C.W. & Hollman, P.C.H. (2005). Polyphenols and disease risk in epidemiologic studies. *The American Journal of Clinical Nutrition*, **81**, 317S–325S.

Association of Official Analytical Chemists. (1984). *Official Methods of Analysis*. Washington, DC: AOAC.

Brazier-Hicks, M., Evans, K.M., Gershater, M.C., Puschmann, H., Steel, P.G. & Edwards, R. (2009). The *C*-glycosylation of flavonoids in cereals. *Journal of Biological Chemistry*, **284**, 17926–17934.

Combs, G.F. (2008). *The Vitamins: Fundamental Aspects in Nutrition and Health*. Ithaca, NY: Elsevier Academic Press.

Dinelli, G., Carretero, A.S., Di Silvestro, R. *et al*. (2009). Determination of phenolic compounds in modern and old varieties of durum wheat using liquid chromatography coupled with time-of-flight mass spectrometry. *Journal of Chromatography A*, **1216**, 7229–7240.

Eberhardt, M.V., Lee, C.Y. & Liu, R.H. (2000). Antioxidant activity of fresh apples. *Nature*, **405**, 903–904.

Hung, P.V., Hatcher, D.W. & Barker, W. (2011). Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. *Food Chemistry*, **126**, 1896–1901.

Hung, P.V., Maeda, T., Yamamoto, S. & Morita, N. (2012). Effects of germination on nutritional composition of waxy wheat. *Journal of the Science of Food and Agriculture*, **92**, 667–672.

Hussain, I. & Uddin, M.B. (2012). Optimization effect of germination on functional properties of wheat flour by response surface methodology. *International Research Journal of Plant Sciences*, **3**, 31–37.

Jensen, M.K., Koh-Banerjee, P., Hu, F.B. *et al*. (2004). Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. *The American Journal of Clinical Nutrition*, **80**, 1492–1499.

Komatsuzaki, N., Tsukahara, K., Toyoshima, H., Suzuki, T., Shimizu, N. & Kimura, T. (2007). Effect of soaking and gaseous treatment on GABA content in germinated brown rice. *Journal of Food Engineering*, **78**, 556–560.

Lampi, A.-M., Nurmi, T., Ollilainen, V. & Piironen, V. (2008). Tocopherols and tocotrienols in wheat genotypes in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, **56**, 9716–9721.

Lee, C.H., Yang, L., Xu, J.Z., Yeung, S.Y.V., Huang, Y. & Chen, Z.-Y. (2005). Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chemistry*, **90**, 735–741.

Liyana-Pathirana, C.M. & Shahidi, F. (2006). Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Journal of Agricultural and Food Chemistry*, **54**, 1256–1264.

Moongngarm, A. & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chemistry*, **122**, 782–788.

Nason, A. (1950). The distribution and biosynthesis of niacin in germinating corn. *American Journal of Botany*, **37**, 612–623.

Pinelo, M., Manzocoo, L., Nuñez, M.J. & Nicoli, M.C. (2004). Interaction among phenolics in food fortification. Negative synergism on antioxidant capacity. *Journal of Agricultural and Food Chemistry*, **52**, 1177–1180.

Prodanov, M., Sierra, I. & Vidal-Valverde, C. (1997). Effect of germination on the thiamine, riboflavin and niacin contents in legumes. *European Food Research and Technology*, **205**, 48–52.

Reboul, E., Richelle, M., Perrot, E., Desmoulins-Malezet, C., Pirisi, V. & Borel, P. (2006). Bioaccessibility of carotenoids and vitamin E from their main dietary sources. *Journal of Agricultural and Food Chemistry*, **54**, 8749–8755.

Sánchez-Machado, D.I., López-Cervantes, J., López-Hernández, J. & Paseiro-Losada, P. (2004). Simultaneous determination of thiamine and riboflavin in edible marine seaweeds by high-performance liquid chromatography. *Journal of Chromatographic Science*, **42**, 117–120.

Sánchez-Pérez, A., Delgado-Zamerreno, M.M., Bustamante-Rangel, M. & Hernández-Mendez, J. (2000). Automated analysis of vitamin E isomers in vegetable oils by continuous membrane extraction and liquid chromatography-electrochemical detection. *Journal of Chromatography A*, **881**, 229–241.

Seibold, R.L. (1990). *Cereal Grass: What's in it for you?* Lawrence, KS: Wilderness Community Education Foundation.

Serpen, A., Gökmen, V., Pellegrini, N. & Fogliano, V. (2008). Direct measurement of the total antioxidant capacity of cereal products. *Journal of Cereal Science*, **48**, 816–820.

Singleton, V.L., Orthofer, R. & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, **299**, 152–178.

Tarr, J.B. & Arditti, J. (1982). Niacin biosynthesis in seedlings of Zea mays. *Plant Physiology*, **69**, 553–556.

Wang, W.-X., Si, H. & Zhang, Z. (2012). Quantitative structure – activity relationship study on antitumour activity of a series of flavonoids. *Molecular Simulation*, **38**, 38–44.

Yang, F., Basu, T.K. & Ooraikul, B. (2001). Studies on germination conditions and antioxidant contents of wheat grain. *International Journal of Food Sciences and Nutrition*, **52**, 319–330.

Žilić, S., Hadži-Tašković Šukalović, V., Dodig, D., Maksimović, V., Maksimović, M. & Basić, Z. (2011). Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *Journal of Cereal Science*, **54**, 417–424.