NPC Natural Product Communications

2006
Vol. 1
No. 11
997 - 1002

The B Group Vitamins and Mineral Elements in the Selective Removal of Wheat Kernel Layers

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Received: August 4th; Accepted: August 24th, 2006

Dedicated to Professor E. Wollenweber on the occasion of his 65th birthday.

The objective of this study was to investigate how selective removal of the surface layers of whole wheat grains by abrasive scouring affects the distribution of mineral elements (macronutrients, micronutrients and trace elements) and the B group vitamins (thiamin, riboflavin pyridoxine, pantothenic acid and niacin) in three soft winter wheat varieties (Partizanka, Novosadska rana and Lasta). Although representing technologically different quality classes of wheat, the varieties were not significantly different in the B group vitamin contents, except for pyridoxine. Whole grains of all varieties exhibited very similar scouring behavior in reduction of vitamins: the most intensive investigated regime only slightly, but not significantly reduced the concentration of the B group vitamins. The nutritive value of scoured wheat grains with regard to the B group vitamins was preserved.

The investigated minerals were: Na, K, Ca, Mg, Mn, Fe, Cu, Zn, Ni, Cr, Se, Pb and Cd. The concentrations of minerals in scoured wheat grains were significantly reduced, dropping on average to 65% of the initial values for macro and 55% for microelements. The change of concentrations with the duration of applied treatment followed different patterns for different elements. Furthermore, a different pattern of the change for the same element in different wheat varieties was noticed. This might be due either to different mechanical properties of bran and bran layer interfaces or genotypic variability in distribution of elements in wheat grain. Severe reduction in Pb concentrations was achieved leading to improvement of whole-wheat food safety.

Keywords: B group vitamins, minerals, wheat, kernel, bran layers, scouring.

The biological value of wheat grains, in addition to the basic constituents of proteins, carbohydrates and lipids, is also contributed to by vitamins and mineral substances. Wheat grains are excellent sources of selenium, zinc, highly available iron, copper, manganese, molybdenum and boron and provide significant amounts of phosphorous, potassium, calcium, magnesium and nitrogen [1].

It is well established that vitamins and minerals are concentrated in the aleurone layer of the wheat kernel, whereas lignin and cellulose occur specifically in the pericarp. From a nutritional point of view, aleurone is a source of dietary fiber, minerals, B-vitamins, proteins, phytate, and phenolic compounds. In relation to their total amounts in the wheat kernel, over 60% of the minerals, 80% of niacin and 60% of pyridoxine are located in the aleurone [2].

A major proportion of the nutritionally important mineral elements is lost to the consumer by the milling process that involves degermination and removal of the pericarp and aleurone. On the other hand, this process removes a large portion of phytate, which is deleterious in the utilization of at least some of the trace elements, notably zinc [3].

The abrasive scouring prepares wheat grains for milling by removing the outer parts of whole grains, as well as physical and chemical contaminants. The milling process of wheat aims at isolating the starchy endosperm without contamination by peripheral layers of the grain (i.e. aleurone layer and pericarp). Processing generally reduces the contents of the nutrients and bioprotective substances. Recently, three wheat varieties were analyzed and it was reported that on average, the bran/germ fraction of wheat had 4-fold more lutein, 12-fold more zeaxanthin, and 2-fold more b-cryptoxanthin than the endosperm fraction. The hydrophilic antioxidant activity of the bran/germ fraction is 13–27-fold higher than that of the respective endosperm fractions. Similarly, lipophilic antioxidant activity is 28–89-fold higher in the bran/germ fractions [4].

Proper cultivation of wheat, as well as drying and subsequently keeping the wheat grain under safe storage conditions should either reduce or eliminate the risk of occurrence of mycotoxins. However, this has often been found difficult to achieve in practice, and surveys continue to find contaminated grain and cereal food products [5]. Abrasive scouring of the outer grain coat was found promising in eliminating physical, chemical and biological contaminants that are usually present on the surface of wheat grain [6].

In recent years, different approaches to incorporation of wheat bran in food products to optimize either the composition or physiological effects have been designed [7]. One of the approaches would be to optimize surface processing of the wheat kernel in a way to retain as high a portion of bran as possible in the whole grain products and in that way utilize its beneficial effects in consumption. Abrasive scouring of the outer wheat grain coat was found promising for that purpose. However, a high varietal variability in the mechanical properties of the aleurone layer, the adhesion forces between the aleurone layer and endosperm, the structural irregularity of aleurone layer – endosperm interface, and the mechanical properties of the grain peripheral layers [8] might influence the effect of wheat grain scouring. Therefore, better understanding of how selective removal of peripheral parts affects nutrients in whole wheat grain, coupled to the measurement of contaminants, would allow optimizing surface processing and utilizing it for enhancing nutritive as well as the bioprotective value of whole-wheat food products.

The aim in the present study was to investigate how selective removal of the surface layers of whole wheat grains by abrasive scouring affects the distribution of mineral elements (macronutrients, micronutrients and trace elements), and the B group vitamins (thiamin, riboflavin pyridoxine, pantothenic acid and niacin) in three different wheat varieties.

Selected results of the effects of the investigated scouring on grains from three wheat varieties are summarized in Table 1.

Table 1: Selected characteristics of untreated and scoured wheat grains of varieties Partzanka (P), Novosadska rana (NR) and Lasta (L).

Sample	Duration of scouring (min)	% of initial weight	Ash (%)	Protein (%)
P start	0	100.0	1.75	11.6
P I	2	94.9	1.56	11.8
P II	4	91.6	1.48	12.0
P III	6	86.1	1.42	12.2
NR start	0	100.0	1.77	10.5
NR I	2	96.2	1.62	10.7
NR II	4	91.0	1.44	10.9
NR III	6	86.9	1.40	11.2
L start	0	100.0	1.73	10.3
L I	2	93.2	1.49	10.5
L II	4	89.0	1.45	10.6
L III	6	85.1	1.34	10.9

Selective removal of wheat bran layers was followed by measuring the reduction of ash content coupled to the tracing of protein content enlargement in scoured grains. The reduction of ash content after the shortest scouring reflected the loss of minerals naturally occurring as bran constituents and the ones that are accumulated at the surface of the grains as impurities. Longer scouring further diminished ash values, thus indicating that mineral nutrients in scoured grains were reduced. Higher values for protein content in scoured grains suggested that only pericarp layers that are low in protein were removed to a different extent depending on the treatment duration and that the aleurone layer is not severely affected by the scouring.

The results obtained in this study for the B group vitamin contents are summarized in Table 2. They well fitted the range of values that were reported for the vitamin composition of wheat from 5 market classes, comprising 231 varieties and representing

Table 2: The B group vitamin contents (mean ± SD, n = 3, mg/100g) in untreated (start) and scoured (III) whole grains for three wheat varieties.

	Thiamin	Riboflavin	Pantothenic acid	Piridoxine	Niacin
Partizanka					
Start	0.67±0.07^{de}	0.15±0.02^{abcd}	2.32±0.21^{efg}	0.43±0.03^{ml}	4.78±0.09^{fg}
III	0.61±0.08^{cdef}	0.17±0.02^{bcde}	2.18±0.02^{defg}	0.36±0.02^{lp}	4.17±0.21^{efgh}
Novosadska rana					
Start	0.55±0.13^{rc}	0.13±0.02^{abc}	2.26±0.06^{uf}	0.24±0.02^{kjl}	5.19±0.07^{ghi}
III	0.51±0.07^{cd}	0.11±0.03^{ab}	2.03±0.08^{te}	0.19±0.01^{j}	4.84±0.31^{fgh}
Lasta					
Start	0.70±0.06^{sd}	0.15±0.01^{bc}	2.34±0.10^{ef}	0.48±0.01^{np}	5.28±0.08^{gh}
III	0.57±0.03^{cde}	0.16±0.00^{bcd}	2.15±0.02^{def}	0.51±0.04^{p}	4.91±0.09^{fghi}

Values in the same column with no letters in common are significantly different (P<0.05).

3 crop years and 49 growing locations. It was shown that the values for niacin were significantly influenced only by year, for riboflavin by class and location, for pyridoxine by year and class, whereas thiamin values were influenced by all of them [9]. Although representing technologically different quality classes of wheat, the varieties evaluated in this study were very similar in their B group vitamin contents. The highest content of B vitamins was found in whole grain of Lasta. The contents of thiamin, riboflavin and pyridoxine were 0.70, 0.15 and 0.48 mg/100g, respectively. Partizanka was the highest in pantothenic acid (2.32 mg/100g), whereas Novosadska rana was the highest in niacin (5.19 mg/100g). The content of B vitamins in the investigated wheat varieties was not significantly different at $P<0.05$. Statistically significant differences were found only for pyridoxine content, which ranged from 0.24 in Novosadska rana, 0.43 in Partizanka up to 0.48 mg/100g in Lasta. The removal of peripheral parts of the grains (14% on an average, by weight) by the most intensive scouring regime only slightly, but not significantly reduced the content of the B group vitamins. When compared with untreated wheat kernels, thiamin, riboflavin, pantothenic acid, pyridoxine and niacin contents in the scoured grains were not significantly different at $P<0.05$. A significant difference in pyridoxine content among the different wheat varieties was maintained after scouring most probably due to the starting varietal differences (almost a twofold lower content in Novosadska rana than in the others).

These results confirm that the outer bran layers (pericarp) of wheat kernels contain only small amounts of thiamin, riboflavin, pantothenic acid, pyridoxine and niacin, and that the major quantity of these vitamins are located in the aleurone layer. Also, it appears that the applied scouring removed mainly only the outer bran layers leaving the aleurone mostly intact. Furthermore, the applied treatment did not significantly affect the nutritive value for B vitamin contents of the investigated wheat varieties.

All three varieties were similar in the investigated macro and microelement contents. These were: Na, K, Ca, Mg, Mn, Fe, Cu, Zn, Ni, Cr, Se, Pb, and Cd (Tables 3 and 4). The trace elements, Hg and As were detected at levels lower than 0.05 mg/kg in all varieties and they were not followed further. The differences detected between the whole wheat grains among the samples with regard to mineral content were significant at $P<0.05$. The differences were expressed in contents of Mn, Zn, Fe, and Pb.

The concentrations of minerals in scoured wheat grains were reduced for most of them, but their change with the duration of the applied treatment followed different patterns for different elements. Furthermore, a different pattern of the change for the same element in different wheat varieties was noticed. This might be due either to different mechanical properties of bran and bran layer interfaces or genotypic variability in the distribution of elements in wheat grain [10].

Scouring kernels for two minutes removed impurities and outer bran layers (approx. 5% by weight) and slightly but significantly affected concentrations of nine elements. It significantly reduced contents of Na, Ca, Mg, Fe, Mn, Pb, Ni, Cr, and Cd.

Longer scouring removed on average 9% of the grain weight, which significantly affected the concentrations of Na, Ca, Mg, Mn, Fe, Se, Pb, and Cd. After the longest scouring regime, when approximately 14% of the grains weight was removed, concentrations of Na, K, Ca, Mg, Mn, Fe, Cu, Zn, Cr, Se, Pb, and Cd were significantly changed, dropping on average to 65% of the initial

Table 3: Macro and microelement contents (mean, n = 2, mg/kg) in untreated (start) and scoured (I, II and III) whole grains for three wheat varieties. Values in the same column within each wheat variety with no letters in common are significantly different (*P*<0.05).

	Na	K	Ca	Mg	Mn	Fe	Cu	Zn
Partizanka								
Start	193.0 ^{li}	2130.2 ^{fghi}	191.5 ^{kg}	723.0 ^{kf}	35.5 ^{p}	40.1 ^{def}	4.6 ^{cde}	1.7 ^{abc}
I	158.3 ^{ghi}	2120.3 ^{lh}	159.0 ^{fgh}	577.1 ^{efg}	28.0 ^{lkj}	31.5 ^{ke}	3.5 ^{bcde}	1.0 ^{lc}
II	129.5 ^{jg}	1735.1 ^{kg}	141.5 ^{efgh}	517.0 ^{defg}	17.0 ^{kj}	23.5 ^{jd}	2.7 ^{bcd}	0.8 ^{kjb}
III	143.0 ^{kh}	1515.3 ^{jf}	133.1 ^{jf}	451.5 ^{je}	13.5 ^{j}	17.1 ^{cdef}	2.4 ^{abcd}	0.3 ^{ja}
Novosadsaka rana								
Start	152.2 ^{ti}	2050.3 ^{fghi}	173.5 ^{defg}	764.5 ^{def}	14.0 ^{sd}	31.5 ^{u}	2.2 ^{sc}	1.2 ^{sb}
I	109.3 ^{ghi}	1455.2 ^{efgh}	148.5 ^{tf}	861.1 ^{se}	10.0 ^{rc}	25.5 ^{t}	0.9 ^{bcd}	1.0 ^{abcd}
II	94.3 ^{srh}	1265.3 ^{efg}	162.5 ^{ste}	592.2 ^{cdef}	12.5 ^{cde}	18.5 ^{s}	1.2 ^{bcde}	0.7 ^{abc}
III	75.3 ^{rg}	1680.2 ^{fgh}	95.1 rd	583.3 rd	12.5 ^{cde}	12.1 ^{r}	1.4 ^{rb}	0.8 ^{ta}
Lasta								
Start	143.5 ^{yf}	1630.3 ^{ywf}	184.5 ^{z}	829.0 ^{z}	29.0 ^{zp}	21.0 ^{cdef}	2.7 ^{wb}	1.6 ^{abcd}
I	78.5 ^{vd}	1585.2 ^{we}	160.0 ^{y}	717.5 ^{ywv}	19.0 ^{wpt}	16.0 ^{bcde}	2.3 ^{bcd}	2.3 ^{yc}
II	86.1 ^{we}	1115.4 ^{def}	87.1 ^{v}	557.5 ^{wv}	17.0 ^{vzs}	20.0 ^{cde}	1.4 ^{abc}	1.1 ^{vab}
III	114.2 ^{defg}	590.2 ^{vd}	101.5 ^{wyv}	523.5 ^{v}	22.5 ^{yst}	15.5 ^{bcde}	1.1 ^{vc}	1.2 ^{wb}

Table 4: Trace element contents (mean, n = 2, mg/kg) in untreated (start) and scoured (I, II and III) whole grains for three wheat varieties. Values in the same column within each wheat variety with no letters in common are significantly different (*P*<0.05).

	Ni	Cr	Se	Pb	Cd
Partizanka					
Start	0.40 ^{hc}	0.96 ^{cdef}	0.50 ^{bcde}	0.24 ^{j}	0.06 ^{abc}
I	0.81 ^{gd}	1.18 ^{de}	0.44 ^{bcd}	0.17 ^{ihg}	0.05 ^{hb}
II	0.79 ^{cd}	1.06 ^{hd}	0.33 ^{abcd}	0.12 ^{hg}	0.05 ^{ab}
III	0.68 ^{cde}	1.27 ^{ge}	0.36 ^{bc}	0.08 ^{g}	0.05 ^{ga}
Novosadska rana					
Start	0.65 ^{def}	1.55 ^{mf}	0.54 ^{md}	0.22 ^{mlc}	0.06 ^{mc}
I	0.59 ^{cde}	1.28 ^{kle}	0.46 ^{bcd}	0.19 ^{kb}	0.04 ^{klb}
II	0.54 ^{bcde}	0.85 ^{defg}	0.49 ^{kc}	0.20 ^{abcd}	0.04 ^{abc}
III	0.60 ^{cdef}	0.80 ^{ld}	0.40 ^{lb}	0.13 ^{la}	0.04 ^{la}
Lasta					
Start	0.70 ^{cdef}	1.02 ^{def}	0.44 ^{cd}	0.21 ^{bcd}	0.06 ^{rb}
I	0.59 rd	0.59 ^{cdef}	0.40 ^{bcde}	0.20 ^{rb}	0.06 ^{abc}
II	0.40 ^{pc}	0.11 bc	0.45 ^{cde}	0.06 ^{abcd}	0.05 ^{pa}
III	0.45 ^{cde}	0.78 de	0.40 ^{bcde}	0.07 ^{pa}	0.05 ^{ab}

values for macroelements, and 55% for microelements. Modest variation of values for Cd and Se concentrations suggested that these elements were uniformly distributed in the wheat grain, as was reported for Se in dissection and milling studies [10].

Severe reduction of Pb concentration by, on average, 30% in Partizanka and Lasta grains was registered after scouring II, then dropping to 32% of the initial Pb concentration after scouring III. Also, in Novosadska rana grains, Pb concentrations were reduced, but to a lower extent (61% of the initial value). This is important for enhancing the safety of whole-wheat products.

Experimental

Samples of three Serbian soft winter wheat cultivars, grown in 2002, were used, each of them representative of a different quality class with regard to their technological baking parameters (e.g. protein content, sedimentation value, flour extraction, yield of bread, yield of bread volume, and bread crumb quality number). These were: cultivar Partizanka (1^{st} quality class), cultivar Novosadska rana (2^{nd} quality class) and Lasta (3^{rd} quality class), grains containing 11.8, 11.7 and 11.9% moisture, respectively.

Scouring: Bulk wheat was divided into 4 × 5 kg lots. One 5 kg lot was left untouched (designated as start) while the others were subjected to a scouring regime using a pilot plant tangential scourer constructed at the Faculty of Agriculture, University of Belgrade fitted with a special abrasive carborundum drum [11]. One lot was scored for 2 minutes (designated as I), another lot for 4 minutes (designated as II), and the third lot for 6 minutes (designated as III). The scourings were collected and weighed. Depending on the investigated wheat variety, the approximate weight of wheat removed was 5.2, 9.4 and 13.9% by weight, respectively, or 262, 473 and 698 g. The air dried grains were ground to a fine powder (wholemeal flour) in a mill (Retsch) using a 0.5 mm screen size and then mixed thoroughly. Moisture, ash and protein (N × 6.25) were determined by AOAC methods [12]. All results are expressed on a dry weight basis.

Vitamin extraction: The samples of wholemeal flour were defatted with light petroleum (40-70°C) using a Soxhlet apparatus.

Acid-ethanolic extract: A 5g wholemeal flour sample was extracted for 5 hours with a mixture of 10 mL 0.1 M acetic acid and 40 mL ethanol using a Soxhlet apparatus. The extract was decanted and the same procedure repeated using new portions of solvents. Extracts were combined, filtered, evaporated near to dryness, redissolved in 5 mL distilled water and used for the analysis of thiamin, pantothenic acid, pyridoxine and niacin [13].

Extraction of riboflavin: A 10g sample of wholemeal flour was refluxed with 51 mL of solvent mixture containing methanol, pyridine, H_2O and glacial acetic acid (30:10:10:1). The obtained extract was clarified by centrifugation for 15 minutes at 3500 RPM. All operations were performed in subdued light [14].

Apparatus and conditions: A chromatographic 8100 Spectra-Physics HPLC System equipped with a Waters 484 UV/VIS detector was connected to a computed integrator Maxima 820 work station. The detection wavelength was 220 nm. Chromatographic conditions with slight modifications regarding the flow rate and ratio of solvents in the mobile phase were as in ref. [15]. Separation of 10-20 μL samples was accomplished on a Lichrosorb NH_2 10 μm 250 × 4.6 mm column (Merck). The mobile phase was 0.005 M KH_2PO_4 (pH 4.4) / acetonitrile in a ratio 14:86, with a 2.5 mL/min flow rate at 65°C. The concentration of standards was 0.1 mg/mL.

Minerals: For recovery of metal elements, 5 g wholemeal flour was dry-ashed at 600°C for 15 hours. Concentrated HCl (3 mL) was added and the suspension was evaporated to dryness, extracted with 20 mL 25% HCl and brought up to a volume of 100 mL with demineralized water. Metal ion concentrations were determined, as three replicates, using a Pye Unicam SP-9 atomic absorption spectrophotometer.

Statistical analysis: Experiments were performed in triplicate. The data were analyzed using Statistica software version 5.0 (StatSoft Co.,Tulsa, OK). The significance of differences between means was determined by a t-test procedure for independent samples at $P < 0.05$.

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