Effect of Processing on the Flavonoid Content in Buckwheat (*Fagopyrum esculentum* Möench) Grain

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Six flavonoids have been isolated and identified in buckwheat grain. These are rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin. Rutin and isovitexin are the only flavonoid components of buckwheat seeds while hulls contain all six identified compounds. The total flavonoid concentration in the seeds was 18.8 and in the hulls 74 mg/100 g of dry matter. Dehulling the grain by using different temperature regimes resulted in drastic reductions of the total flavonoid concentration in the grain (by 75% of the control) and smaller but significant (15−20%) reduction in the hulls.

Keywords: *Buckwheat; Fagopyrum esculentum; flavonoids; temperature treatment; processing*

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Möench) in some countries such as China, Japan, and Poland is recognized as a valuable source of so-called "functional food". This can successfully replace rice or potatoes in the main menu. It is well-known that the grain of buckwheat can be stored for a long time without any symptoms of chemical changes. This is due to the content of several natural antioxidants including tocopherols (Keli et al., 1992), phenolic acids (Durkee et al., 1977), and flavonoids (Sato and Sakamura, 1975; Oomah and Mazza, 1996; Watanabe et al., 1997) stabilizing the grain during the storage.

The concentration of natural antioxidants may show strong variation depending on several factors including variety, location, and environmental conditions. Kitabayashi et al. (1995) reported that the rutin content in buckwheat varieties may range from 12.6 to 35.9 mg/100 g dry weight, while Keli et al. (1992) reported that the tocopherol concentration in Chinese germplasm varied from 0.09 to 8.15 mg/100 g (Tibet location), with an average for the country being 1.42 mg/100 g. According to Oomah and Mazza (1996), location influences the rutin concentration in the seed while the growing season has a significant influence on the total flavonoid in the hulls. The same authors reported extremely high concentrations of total flavonoids (387 and 1314 mg/100 g) and rutin (47 and 77 mg/100 g) in seeds and hulls, respectively. This remains in drastic disagreement with the data of Watanabe et al. (1997) reporting the rutin concentration of hulls as 4.3 mg/100 g of dry matter.

To obtain buckwheat groats of consumption quality, the grain needs to be dehulled. This is being done with two-step processing that includes dehulling and roasting. Dehulling involves raising the moisture content of the grain to 22% of dry matter followed by heating (10−20 min at 150−164 °C). The resulting brown seeds (light buckwheat groats) are ready for cooking or can be roasted (1−2 h, 100−150 °C) to produce dark brown groats. Temperature treatment may influence the phenolic concentration, but this has never been studied in detail. Thus, the aim of the present work was to isolate and identify flavonoids from buckwheat seeds and hulls, and to determine their concentrations in untreated grains and in the differently processed buckwheat groats.

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MATERIALS AND METHODS

Materials. Buckwheat (*F. esculentum* Möench var. Hruszowska) was obtained from the Breeding Station in Palikije, Poland. After harvest, the grains were dried at room temperature and finely powdered, and flavonoids were extracted. Some of the grains were dehulled manually and divided into seeds and hulls (control without heat treatment). The portions of the grains were processed with four processing procedures routinely used for producing buckwheat groats. The first procedure (I) involved raising the moisture content to 22%, followed by heating for 10 min to 150 °C. In procedure II, the heating process was prolonged to 1 h and 10 min, and in procedure III to 2 h and 10 min. The moisture content at the end of heating in procedures II and III was 13%. In procedure IV, the grains were treated with steam (pressure 0.35 MPa, 164 °C) for 20 min, followed by 50 min of treatment with steam (0.4 MPa, 150 °C) and final drying to 13% moisture content.

Extraction and Separation of Flavonoids. Powdered buckwheat grains (1 kg) were extracted by boiling under reflux with ethanol. The ethanol was evaporated to dryness, and the dry residue was suspended in water and loaded onto a C_{18} column (55 × 100 mm; 60 μm, Baker). The column was washed with water to remove carbohydrates, and flavonoids were removed with 70% methanol. Methanol was evaporated, and water solution was loaded onto the C_{18} column (40 × 300 mm; 25−40 μm, Merck). The column was washed with methanol−water (linear gradient 10−100% methanol), and 3 mL fractions were collected with a fraction collector. Fractions showing identical chromatographic characteristics (TLC on cellulose in 15% acetic acid and UV lamp detection) were combined. This afforded seven fractions containing two to three flavonoids. They were further separated on the C_{18} column (20 × 250 mm; 25−40 μm, Merck) using methanol−water solutions (ratio depending on the fraction) delivered isocratically. This yielded six flavonoids.

(**A**) 0.244 g; LSIMS (negative ion mode) *m*/*z* 609 $[M - H]^-$, 301 $[M - H -$ hexose $-$ deoxyhexose$]^-$; UV, λ_{max} (nm) (MeOH) 257, 269sh, 357; (MeONa) 272, 324, 411; ($AlCl_3$) 274, 303sh, 433; ($AlCl_3$/HCl) 268, 364sh, 397; (NaOAc) 273, 319, 384;

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($NaOAc/H_3BO_3$) 261, 378. On acid hydrolysis glucose and rhamnose were yielded.

(**B**) 0.048 g; LSIMS (negative ion mode) *m*/*z* 447 $[M - H]^-$; UV, λ_{max} (nm) (MeOH) 255, 269, 346; (MeONa) 267, 293sh, 402; ($AlCl_3$) 274, 368sh, 406; ($AlCl_3$/HCl) 276, 296sh, 363; (NaOAc) 274, 346; ($NaOAc/H_3BO_3$) 261, 372.

(**C**) 0.067 g; LSIMS (negative ion mode) *m*/*z* 431 $[M - H]^-$; UV, λ_{max} (nm) (MeOH) 269, 304sh, 326; (MeONa) 278, 328sh, 392; ($AlCl_3$) 275, 304, 348; ($AlCl_3$/HCl) 268, 302, 338; (NaOAc) 274, 370; ($NaOAc/H_3BO_3$) 269, 323, 368.

(**D**) 0.028 g; LSIMS (negative ion mode) *m*/*z* 301 $[M - H]^-$; UV, λ_{max} (nm) (MeOH) 255, 372; (MeONa) 275, 327; ($AlCl_3$) 271, 428; ($AlCl_3$/HCl) 266, 310sh, 360, 427; (NaOAc) 258sh, 272, 322sh, 382; ($NaOAc/H_3BO_3$) 260, 386.

(**E**) 0.005 g; LSIMS (negative ion mode) *m*/*z* 447 $[M - H]^-$; UV, λ_{max} (nm) (MeOH) 256, 270, 349; (MeONa) 267, 337sh, 406; ($AlCl_3$) 276, 307sh, 330sh, 424; ($AlCl_3$/HCl) 278, 302sh, 366sh, 386; (NaOAc) 272, 325, 367; ($NaOAc/H_3BO_3$) 262, 374.

(**F**) 0.013 g; LSIMS (negative ion mode) *m*/*z* 431 $[M - H]^-$; UV, λ_{max} (nm) (MeOH) 271, 334; (MeONa) 279, 331, 397; ($AlCl_3$) 279, 303sh, 351, 380; ($AlCl_3$/HCl) 279, 303, 346, 380; (NaOAc) 276, 301, 344; ($NaOAc/H_3BO_3$) 272, 337.

Thin Layer Chromatography (TLC). The isolated flavonoids were chromatographed in the presence of authentic standards of rutin, quercetin, vitexin, isovitexin, orientin, and isoorientin on cellulose ready to use plates (Merck) using the following solvent systems: S_1, 15% acetic acid; S_2, 1-butanol−acetic acid−water (4:1:5, upper layer); S_3, 50% acetic acid.

High-Performance Liquid Chromatography (HPLC). Flavonoids were chromatographed using a Waters system equipped with a model 616 pump, 600S controller, 996 photodiode array detector, and Millenium software. A C_{18} column (4.6 × 250 mm, 5 μm, Säulentechnik) at 50 °C was used. The solvent system was delivered at a 1 mL/min rate and consisted of a mixture of (A) 1% H_3PO_4 and (B) 40% acetonitrile in 1% H_3PO_4 formed according to the following gradient: 0 min, 20% B; 70 min, 100% B; 75 min, 20% B. Calibration curves for particular flavonoids were prepared for the solutions containing 0−1 mg/mL.

A 10 g sample of powdered buckwheat seeds and hulls was extracted three times with ethanol (50 mL each), and the combined extracts were evaporated to dryness. The dry residue was dissolved in water and passed through a Sep-Pak cartridge (C_{18}, Waters). The cartridge was washed with water, and flavonoids were removed with 70% methanol. The solid residue after evaporation of the solvent was dissolved in 10 mL of methanol and used for HPLC analysis.

Spectral Analyses. The spectra and bathochromic shifts were recorded with a spectrophotometer (Hewlett-Packard 8453) according to the method of Mabry et al. (1970). Mass spectra (LSIMS) were recorded with an AMD 402 spectrometer using glycerol as a matrix.

Statistical Analysis. Each sample was submitted to three replicate analyses, and the results were subjected to ANOVA variance and statistical significance by the *T*-test. The relative standard deviation for flavonoids was 6−7% ($n = 5$).

RESULTS AND DISCUSSION

Isolation of Flavonoids. Column chromatography on a C_{18} support allowed us to separate six flavonoid compounds from powdered buckwheat grain. Since these are not novel flavonoids and were previously identified in different buckwheat parts including hulls (Sato and Sakamura, 1975; Watanabe et al., 1997) and seedlings (Margna and Margna, 1982), in this study the structures of isolated compounds were confirmed with spectral (LSIMS, bathochromic shifts) and chromatographic (HPLC, retention time, UV−vis spectroscopy, cochromatography with appropriate standards) procedures. On the basis of spectral features and similarity to appropriate standards, these compounds were characterized as (**A**) rutin, (**B**) orientin, (**C**) vitexin, (**D**) quercetin, (**E**) isoorientin, and (**F**) isovitexin. In this way it was demonstrated that whole mature buckwheat grain contains flavonols and flavones as reported previously in seeds and seedlings, respectively.

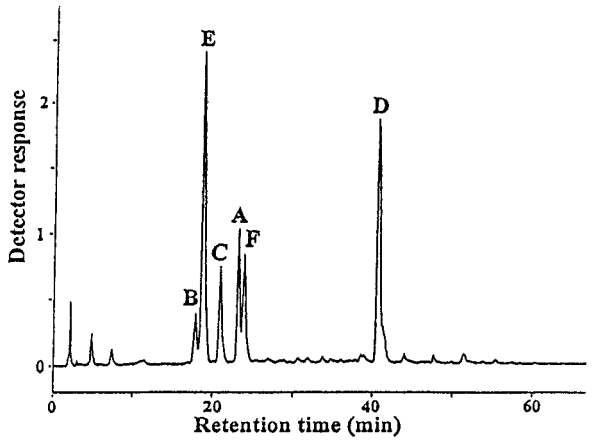

Figure 1. High-performance liquid chromatogram of flavonoid standards: (A) rutin, (B) orientin, (C) vitexin, (D) quercetin, (E) isoorientin, (F) isovitexin.

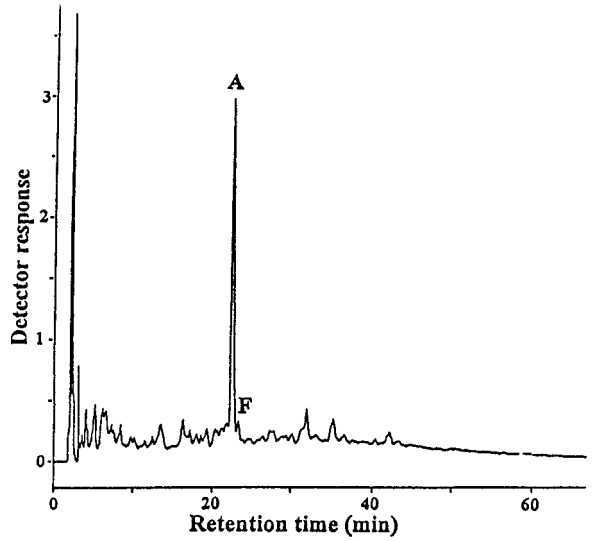

Figure 2. High-performance liquid chromatogram of a methanolic extract of buckwheat groat.

Concentration of Flavonoids in Groats and Hulls. The isolated compounds were used as standards for calibration of the HPLC procedure used for their determination (Figure 1). It was shown that the spectra of seed and hull flavonoids distinctly differ. While groats contain only two flavonoids, rutin and isovitexin (Figure 2), hulls include all six flavonoids **A**−**F** (Figure 3). The concentration of flavonoids in seeds (Table 1) dehulled manually was 18.8 mg/100 g, and rutin made up about 95% of the total. The concentration in the hulls (Table 2) was much higher than in seeds, and in manually removed hulls it was 74 mg/100 g. The rutin was also the dominant flavonoid in hulls; it made up about 45% of the total. These data fully support the results reported by Kitabayashi et al. (1995) where rutin concentrations in a number of buckwheat varieties ranged from 12.6 to 35.9 mg/100 g, but they are about half of those reported by Oomah and Mazza (1996). Total concentrations of flavonoids reported by Oomah and Mazza (1996) were 387 and 1314 mg/100 g in seeds and hulls, respectively, and seem to be overestimated, probably an artifact of the colorimetric procedure used

Table 1. Concentration of Flavonoids in Buckwheat Seed (mg/100 g ± Standard Deviation)[a,b]

	light groats	brown groats			
compound	0	I	II	III	IV
rutin	17.76 ± 0.40[e]	14.93 ± 0.30[d]	10.38 ± 0.32[b]	11.60 ± 0.54[c]	4.33 ± 0.07[a]
isovitexin	1.04 ± 0.01[d]	0.26 ± 0.01[a]	0.43 ± 0.02[c]	0.25 ± 0.01[a]	0.25 ± 0.01[b]
total	18.80	15.19	10.81	11.85	4.63

[a] 0, hulls removed manually; I−IV, dehulled with different temperature regimes; see Materials and Methods. [b] Means in a row followed by the same letter are not significantly different ($p = 0.05$).

Table 2. Concentration of Flavonoids in Buckwheat Hulls (mg/100 g ± Standard Deviation)[a,b]

	light groats	brown groats			
compound	0	I	II	III	IV
rutin	32.95 ± 0.92[c]	32.27 ± 0.38[c]	29.48 ± 2.50[b]	26.25 ± 1.20[a]	
orintin	8.13 ± 0.09[b]	10.22 ± 0.77[c]	7.07 ± 0.59[a]	10.00 ± 0.98[c]	
vitexin	14.92 ± 0.23[d]	14.20 ± 0.46[c]	9.68 ± 0.29[a]	13.48 ± 0.35[b]	
quercetin	2.72 ± 0.15[a]	3.24 ± 0.33[bc]	2.83 ± 0.29[b]	2.23 ± 0.26[a]	
isoorientin	8.08 ± 0.23[d]	6.97 ± 0.30[b]	5.21 ± 0.16[a]	7.73 ± 0.20[c]	
isovitexin	7.22 ± 0.38[c]	7.17 ± 0.35[c]	5.42 ± 0.29[a]	6.17 ± 0.20[b]	
total	74.01	74.07	59.69	65.86	

[a] 0, hulls removed manually; I−IV, dehulled with different temperature regimes; see Materials and Methods. [b] Means in a row followed by the same letter are not significantly different ($p = 0.05$).

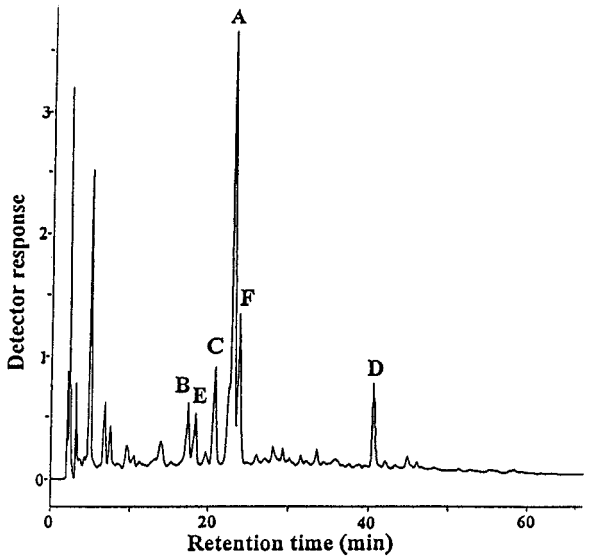

Figure 3. High-performance liquid chromatogram of a methanolic extract of buckwheat hull.

for determination, or because some ingredients in the sample matrix other than flavonoids gave an overlapping effect. Our present results show much higher concentrations of flavonoids in the hulls than in the seeds, but the concentrations are about one-tenth of those reported by Oomah and Mazza (1996). In contrast, the concentration in hulls was about 10 times higher than that reported by Watanabe et al. (1997). These discrepancies show that plant material can vary substantially, depending on the origin or on the method used for determination.

Influence of Heat Processing on Flavonoid Concentration in Groats and Hulls. The removal of hulls from the buckwheat grain by heat treatment resulted in a product which was both visually and chemically different. Groats manually dehulled or treated for 10 min at 150 °C were light brown, while those treated with temperature for longer than 1 h were all dark brown. Such groats besides visual changes were also chemically different. There are many tannins and crude fiber in the whole buckwheat grain. They can be found predominantly in hulls; thus, the dehulling process removes most of them from the seed. Also during the roasting process, the proteins and sugars of buckwheat may be changed into more easily available forms (Soral-Smietana, 1984). Roasting results also in color, flavor, and aroma changes. Regarding the total flavonoid concentration, even short temperature treatment (procedure I) caused a significant decrease (20% of total) in their concentration. Prolonged temperature treatment (procedures II and III) resulted in drastic reductions of flavonoid concentrations (about 40%) in comparison with groats manually dehulled. In the most severe temperature treatment (procedure IV), the reduction of flavonoid content was 75%. It is not well understood which processes are responsible for the observed flavonoid losses. This could be due to flavonoid breakdown during heating and/or extraction of glycosides by the steam. Such processes were reported in other research where flavonoid-containing plant material was thermally processed. Cooking both tomatoes and onions resulted in lowered quercetin content although less so following frying than boiling or microwave cooking (Crozier et al., 1997). Losses of quercetin mono- and diglucosides were observed during cooking of brown-skinned onion, but these losses were not translated into production of free quercetin (Price et al., 1997). A significant proportion of glucosides was leached unchanged from onion tissue into the cooking water. Similarly, red grape pomace peels dried at different temperatures (60, 100, and 140 °C) showed reduced levels of extractable polyphenols as compared to freeze-dried samples (Larrauri et al., 1997). As reported before, other compounds such as vitamins B_1, B_2, and E decreased during processing (Gromakov et al., 1983; Kirilenko and Sarkisova, 1977; Zalesskaya et al., 1979). These data clearly show that, during the dehulling process, depending on the temperature, the flavonoid level decreases. If flavonoid concentration is regarded as an indicator of the nutritional quality of buckwheat groats, then only light brown groats should be recommended for consumption.

The concentration of flavonoids in hulls was also affected during processing but to a much lesser degree. A 1 h treatment at high temperature (procedure II) did not influence flavonoid concentration, but increased duration of the temperature treatment reduced the level of flavonoids by about 15−20% relative to that of the manually dehulled control.

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