Extraction of Rutin from Buckwheat (*Fagopyrum esculentum* Moench) Seeds and Determination by Capillary Electrophoresis

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The content of the flavonoid rutin was determined in different milling fractions of buckwheat seeds and in buckwheat stems, leaves, and flowers. The extraction was performed by using a solvent containing 60% of ethanol and 5% of ammonia in water. The extracts were analyzed by capillary electrophoresis (running buffer of 50 mM borate (pH 9.3), 100 mM sodium dodecyl sulfate; determination at 380 nm). In bran fractions the concentration of rutin was 131−476 ppm, and in flour fractions 19−168 ppm. On average, about 300, 1000, and 46000 ppm of rutin were found in leaves, stems, and flowers, respectively. The results indicate that buckwheat could be an important nutritional source of flavonoids, especially in countries with a low mean daily flavonoid intake.

Keywords: *Fagopyrum esculentum; flavonoids; rutin; nutraceuticals; capillary electrophoresis*

INTRODUCTION

Rutin (quercetin 3-rhamnosylglucoside) is a flavonol glycoside plant metabolite able to antagonize the increase of capillary fragility associated with hemorrhagic disease or hypertension in man (Griffith et al., 1944; Schilcher et al., 1990). Rutin extracted from buckwheat groats also showed antioxidant activity; however, it was lower than that of catechins isolated from the same material (Watanabe, 1998).

Buckwheat may be used as a good source of dietary rutin (Ohsawa and Tsutumi, 1995; Kitabayashi et al., 1995a,b; Watanabe et al., 1997; Watanabe, 1998). To our knowledge no rutin has been found in other pseudocereals or cereals. Buckwheat is interesting for the production of nutraceutical preparations because, besides the rutin, it also contains proteins with a well-balanced amino acid composition (Eggum et al., 1981), a relatively high level of dietary fiber (He et al., 1995), soluble carbohydrate fagopyritols (Obendorf, 1998; Steadman and Obendorf, 1998), retrograded starch in groat products (Skrabanja and Kreft, 1998), and interesting levels of zinc, copper, and manganese (Ikeda and Yamashita, 1994).

According to the published information, rutin can be analyzed by using a spectrophotometric method (AOAC, 1990), high-performance liquid chromatography (HPLC) (Ohara et al., 1989), or capillary electrophoresis (CE) (Pietta et al., 1991, 1994; McGhie et al., 1994). The drawback of the spectrophotometric method could be the interference of the rutin spectrum with the spectra of other polyphenolic compounds, which could be present in buckwheat grain samples (Luthar and Kreft, 1996; Watanabe et al., 1997; Watanabe, 1998). The accuracy of spectrophotometric methods for the determination of flavonoids in the presence of other flavonoids was questioned by Askal et al. (1992). The available HPLC method is time-consuming and demands considerable amounts of mobile-phase constituents, so it is less suitable for quick determination of a great number of samples (e.g., in plant breeding). CE is a high-resolution technique, which enables the quick and accurate determination of rutin from complex samples. In both HPLC and CE, one problem could be the extraction of rutin from samples and separation from other soluble grain constituents, such as the soluble starch, which could interfere with the analytical procedure.

There are no literature data on the adaptation of the CE method for the analysis of rutin in buckwheat seeds, which is specific because of low content of rutin in some samples and the presence of starch. Additionally, there are no reports on the variability of rutin content in different milling fractions from one seed lot.

The purpose of our study was largely threefold. The first aim was to define optimal conditions for the extraction of rutin from buckwheat flours and to prepare samples for CE. The second was to establish the applicability of CE determination of rutin in buckwheat flour samples. Finally, the intention of the present work was also to estimate the ranges of rutin content in different fractions of buckwheat flour and bran and, thus, to evaluate the nutraceutical value of buckwheat with special emphasis on the content of rutin.

MATERIALS AND METHODS

Plant Material. Samples. Flour, bran, and husk fractions were obtained by milling the buckwheat grains (cv. Siva, harvested in Slovenia) on a roller mill (Modic, 1998).

All milling fractions analyzed resulted from one seed lot from Domzale, Slovenia. These fractions are produced by consecutive milling and sieving in such a way that allowed us to produce as many different milling fractions as possible. The milling fractions differ in content, depending on the different parts of the seed. From flowering plants (the stage beginning of flowering) of cv. Siva and cv. Petra, grown in Ljubljana, flowers, stems, and leaves were collected and immediately after collection were frozen and freeze-dried. The freeze-dried samples were milled by using a Udy laboratory mill to pass through a 0.5 mm sieve.

Methods. *Extraction*. Two hundred and fifty milligrams of milling fractions was macerated in 5 mL of extraction solution

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10.1021/jf990186p CCC: $18.00
Published on Web 10/28/1999

Table 1. Rutin Content of Buckwheat Milling Fractions ($n = 3$)

sample	milling fraction	rutin (ppm) ± SD
1	husk	29.5 ± 10.34
2	flour 1	74.2 ± 0.97
3	flour 2	22.5 ± 2.01
4	flour 3	19.2 ± 1.93
5	flour 4	57.3 ± 5.13
6	flour 5	22.9 ± 3.01
7	flour 6	37.6 ± 9.40
8	flour 7	77.9 ± 6.43
9	flour 8	19.4 ± 7.60
10	flour 9	81.6 ± 17.74
11	flour 10	168.2 ± 14.66
12	bran 1	159.7 ± 5.71
13	bran 2	131.9 ± 20.58
14	bran 3	192.2 ± 24.77
15	bran 4	385.1 ± 48.76
16	bran 5	259.5 ± 17.63
17	bran 6	439.4 ± 61.32
18	bran 7	194.9 ± 45.78
19	bran 8	332.8 ± 79.20
20	bran 9	236.2 ± 35.98
21	bran 10	476.9 ± 5.33
22	bran 11	326.8 ± 83.91
23	bran 12	475.5 ± 82.60

(water/ethanol/ammonium hydroxide = 35:60:5) for 3 h, at 30 °C during shaking. After extraction, the suspensions were centrifuged for 15 min at 3300*g*. A 1 mL aliquot (2.6 mL for samples with low content of rutin) of supernatant was evaporated under reduced pressure.

To check if the extraction was complete, the extraction of the milling fraction was repeated once more as follows: The remaining supernatant was removed and replaced with a fresh extraction solution to the same total volume as during the first extraction. The samples were extracted during shaking for another 3 h and centrifuged, and an aliquot of supernatant was taken and evaporated.

To analyze the rutin content in leaves, stems, and flowers, samples of 100, 100, and 50 mg, respectively, were extracted according to the same procedure. Prior to analysis, all evaporated samples were dissolved in 100 μL of running buffer.

CE. Separations were performed on a Hewlett-Packard HP 3D capillary electrophoresis system with uncoated capillaries (Hewlett-Packard, Waldbronn, Germany) (i.d. = 50 μm, length = 57 cm, with a bubble cell), at 18 °C, by applying a voltage of 25 kV. The running buffer was 50 mM borate (pH 9.3), with 100 mM sodium dodecyl sulfate (SDS) (Hewlett-Packard). The expected electric current is 85 mA. Prior to analyses, the capillary was flushed for 3 min with the running buffer. The buffer was replenished after every 10 analyses. The samples were injected by 20 mbar pressure for 20 s. The electropherogram measured at 380 nm was quantified by integration. All samples were three times independently extracted and analyzed. The concentration was determined by comparison with standard solutions. Standard solutions were prepared by dissolving rutin (Fluka AG, Buchs SG, Switzerland) in the running buffer. The identity of the peak of rutin was confirmed by the spectroscopic analysis and by the proportional increase of the electropherographic peak caused by the addition of rutin to the samples.

Statistical Analysis. The data were statistically evaluated (ANOVA) and the standard deviation (SD) was determined using Statgraphics 5.0.

RESULTS AND DISCUSSION

Using the extraction procedure described, in most cases the total rutin was extracted in the first extraction, but in some milling fractions up to 20% of rutin was not extracted until the second extraction. In such cases the values measured in both extractions were summed to obtain the results presented in Tables 1 and 2. In the third repeated extraction no rutin was found.

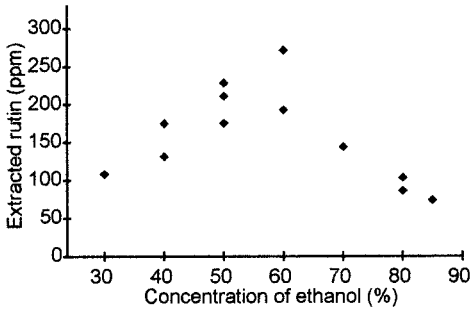

Figure 1. Connection between ethanol concentration in extraction solvent and efficiency of rutin extraction from buckwheat bran (independent subsamples from bran 11) in 2 h of extraction. At concentrations of >60% of ethanol (all solvents contained 5% of ammonium hydroxide), a clear trend of decreasing extraction efficiency can be observed.

Table 2. Rutin Content ($n = 3$) of Different Parts of Individual Buckwheat Plants Cv. Siva (Plants 1 and 2) and Tetraploid Cv. Petra (Plants 3 and 4)

	rutin (ppm) ± SD			
	Siva		Petra	
sample	plant 1	plant 2	plant 3	plant 4
stem	658 ± 266	889 ± 141	1456 ± 465	1212 ± 52
leaves	138 ± 39	211 ± 42	654 ± 78	166 ± 49
flowers	39260 ± 2040	50440 ± 1080	58891 ± 490	36178 ± 4450

A slightly better extraction was achieved using methanol instead of ethanol in the extraction procedure, but because of the toxicity of the methanol, ethanol was used in routine extractions.

An appropriate ethanol concentration in the extraction solution is important for effective extraction. Although the solubility of rutin increases with the increased ethanol concentration, the efficacy of extraction decreases if the ethanol concentration rises to >60% (Figure 1). This could be the result of stronger hydrogen bonds between rutin and starch in the less polar solvent. As the polarity of the solvent increases with the addition of water, the hydrogen bonds might become weaker. If the ethanol content in the extraction solution is <50%, the starch is swelled to such an extent that it cannot be sufficiently removed by centrifugation. The optimal ethanol concentration in the extraction solution is therefore between 50 and 60%. It is important to note that the rutin content in the extract containing 5% of ammonia gradually decreased due to oxidation, if it was not immediately evaporated. The evaporated samples were stable. On the electropherogram, the rutin was well separated from other compounds (Figure 2). The retention time was 12.4 min. The calibration curve had a slope value of 477 mAU/(mg/mL) and the correlation was $R = 0.999$. The quantification limit for rutin in this CE system is 25 mg/L, and by using the extraction procedure described, it is possible to determine a rutin content as low as 15 mg/kg of flour. With the application of more flour and the evaporation of more extract, the method can easily accommodate the analysis of even lower contents of rutin. The main advantage of the described CE analysis, compared to HPLC, is in the very low consumption of chemicals. Only 2 mL of water-based buffer is required for 10 analyses, whereas for each HPLC analysis of rutin, at least 20 mL of methanol or acetonitrile would be needed.

The content of the rutin measured in milling fractions is presented in Table 1. In bran fractions there are 131−476 ppm of rutin, in flour fractions 19−168 ppm of rutin,

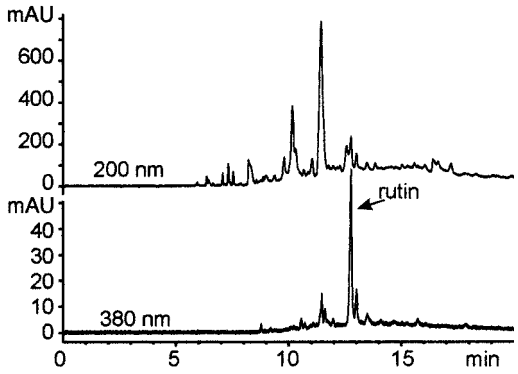

Figure 2. Electropherograms of the extract of buckwheat bran (bran 11) detected at 200 and 380 nm. At least 34 compounds can be seen on the electropherogram at 200 nm. The peak of rutin is well separated on the electropherogram at 380 nm.

and in the husk fraction 29 ppm of rutin. Differences among fractions are statistically significant at the $p < 0.0001$ level.

Therapeutic doses of rutin, with clinically clearly demonstrated effects, were reported for daily doses of rutin between 180 and 350 mg (Schilcher et al., 1990). Thus, the daily intake of 100 g of buckwheat flour or bran in food would therefore cover ~10% of the therapeutic doses. These are well below the therapeutic doses but could meet the demands of preventive nutrition and post-therapeutic nutritional treatment, especially with the consideration that, besides buckwheat, other foods and/or beverages could also be consumed as natural nutritional sources of rutin and other flavonoids. However, the recommended daily intake of rutin has not yet been established and is not within the scope of this research.

The intake of rutin at 18 mg/day (equivalent to ~10 mg/day of the aglycon quercetin), with 100 g of particular buckwheat bran in the product, could increase the average doses of flavonols and flavons otherwise consumed (through vegetables, fruits, red wine, and tea) by 4-fold in Finland and by almost 2-fold in the United States [total intake = 2.6 and 13 mg/day of flavonoids, respectively (Hertog et al., 1995)]. According to the results of Velioglu et al. (1998), in two products from buckwheat seed the antioxidant activity was proportional to their total phenolic content. Buckwheat bran and some types of flour can be considered as a food with a high content of flavonoids, because their level is higher than that, for example, in cabbage, apple, red wine, or tea (Hertog et al., 1992, 1993). The results on rutin content of buckwheat materials provide, in addition to the data on the content of flavonoids in other food materials (Hertog et al., 1992, 1993), a base for epidemiological studies investigating the relationship between flavonoid intake and the risk of coronary heart disease and cancer, especially for countries with high buckwheat consumption (Nepal, Japan) or some areas and populations such as in China (He et al., 1995).

The substitution of some wheat flour in the diet with buckwheat bran could also be important because of the content of fagopyritols, a group of phytochemicals, that may have an important use in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) (Steadman and Obendorf, 1998).

In all plants studied, more rutin was found in stems than in leaves, and the most rutin was found in flowers (Table 2). Differences are statistically significant at the $p < 0.0001$ level. Results (Table 2) confirm that buckwheat "green flour" obtained by milling dried flowering buckwheat plants—which is sometimes used in Japan as a natural food colorant for pasta, ice cream, and other food products—could be an interesting source of dietary rutin. The same is valid for buckwheat tea products, obtained and marketed in Europe as a source of rutin. However, the possible dietetic and/or physiological role of the dianthrones profagopyrin and fagopyrin—another group of substances isolated from flowering buckwheat plants (Brockmann et al., 1952)—is not yet clear.

ABBREVIATIONS USED

CE, capillary electrophoresis; HPLC, high-performance liquid chromatography; NIDDM, non-insulin-dependent diabetes mellitus; SD, standard deviation; SDS, sodium dodecyl sulfate.

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Received for review February 19, 1999. Revised manuscript received September 15, 1999. Accepted September 20, 1999. This study was partially funded by the Ministry of Science and Technology of the Republic of Slovenia and was coordinated in COST 916 of the European Union.

JF990186P