Acta Chromatographica 20(2008)4, 661–672
DOI: 10.1556/AChrom.20.2008.4.11

Analysis of Selected Phenolic Acids and Flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* Seeds and Sprouts by HPLC

P. PAŚKO[1], M. SAJEWICZ[2], S. GORINSTEIN[3], AND Z. ZACHWIEJA[1]

[1]Department of Food Chemistry and Nutrition, School of Medicine, The Jagiellonian University, 9 Medyczna Street, 30-688 Kraków, Poland
[2]Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland
[3]Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University – Hadassah Medical School, Jerusalem 91120, Israel
E-mail: paskopaw@poczta.fm

Summary. Red amaranth *(Amaranthus cruentus)* and quinoa *(Chenopodium quinoa*) are pseudocereals with particularly highly regarded nutritional value. Because of the high biological significance of the flavonoids and phenolic acids in these plants, qualitative and quantitative analysis has been performed by HPLC. Extracts from the seeds of two amaranth varieties *(A. cruentus* v. Rawa and v. Aztek) and quinoa seeds, and their sprouts grown in natural conditions and in the dark were analyzed. The main phenolic acid found both in seeds and sprouts was gallic acid. *p*-Hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, caffeic acid, and cinnamic acid were also found in the seeds and *p*-coumaric acid, syringic acid, and ferulic acid in the sprouts. The main flavonoid found in the sprouts was rutin. Vitexin, isovitexin, and morin were also detected in the sprouts, and orientin, vitexin, isovitexin, morin, and traces of hesperidin and neohesperidin in the seeds. Although sprouting conditions (daylight or darkness) had no effect on gallic acid content, light caused an increase in the amount of rutin and darkness resulted in increased amounts of isovitexin and vitexin.

Key Words: amaranth, quinoa, flavonoids, phenolic acids

Introduction

Although pseudocereals are valuable sources of nutrients, they are relatively rarely studied. Besides high levels of good quality proteins and fats they provide a wide range of microelements and some vitamins [1–4]. It should be also emphasized that pseudocereals do not contain gluten, and so can be introduced into the diet of children suffering from celiac disease. These plants are characterized by an ability to neutralize free radicals, because they contain flavonoids, phenolic acids, squalene, anthocyanins, and

vitamins with antioxidant activity [5, 6]. As far as we are aware, there is no information in the literature about the amounts of phenolic acids or flavonoids in amaranth and quinoa seeds. In addition, little is known about sprout growth, composition, and possible exploitation in nutrition, even though the sprouts have significantly greater antioxidant activity than the seeds. They can be used as an addition to a conventional diet and enrich it with vitamins, antioxidants, microelements, and valuable proteins.

Our interest has been the effect of sprouting conditions (natural conditions or complete darkness) on their antioxidant activity and on the amounts of the compounds discussed above. Qualitative and quantitative assessment of phenolic acids and flavonoids can be important in the use of plants and processed products in diet therapy. It is commonly known that these compounds have a wide variety of biological activity – anti-inflammatory, antiallergic, antioxidant, antitumor, and effects on the immune and cardiovascular systems [7–9]. Some rare compounds are also found in these plants, and may be important for prevention of cancerogenesis (for example morin) or cardiovascular diseases and radiation protection (for example orientin) [10, 11].

Experimental

Plant Material

Amaranth seeds (*Amarantus cruentus* varieties Aztek and Rawa) were cropped in eastern Poland and quinoa seeds (*Chenopodium quinoa*) were imported from Bolivia.

Chemicals

De-ionized water 18 MΩ cm was obtained from Milli RO and Q water purification systems, (Millipore). Methanol, acetone, hydrochloric acid (36%), and ferric chloride ($FeCl_3$) of analytical purity grade were from POCh (Gliwice, Poland). Apigenin, vitexin, isovitexin, orientin, isoorientin, kaempferol, quercetin, rutin, myricetin, morin, naringin, hesperidin, neohesperidin, gallic acid, cinnamic acid, vanillic acid, caffeic acid, ferulic acid, *p*-coumaric acid, and *p*-hydroxybenzoic acid were from Sigma–Aldrich and Merck (Germany).

The binary mobile phase was prepared from acetonitrile (ACN) of HPLC purity grade from Merck and water.

Sprouting

Seeds of the pseudocereals were placed in glass vessels and grown for 4, 6, or 7 days at a temperature of 20°C. They were watered every day. Half of the culture was kept under natural conditions (daylight) whereas the other was kept in the dark. Sprouting was very difficult to perform and, especially for quinoa, the efficiency was low.

Sample Preparation for Determination of Antioxidant Potential

Extract Preparation

Powdered samples of seeds and blended sprouts (1 g) were extracted with 40 mL 8:1:1 (*v*/*v*) methanol–0.16 molar hydrochloric acid–water for 2 h. The extracts were separated by decantation and the residues were extracted again with 40 mL 70% acetone for 2 h. The extracts were combined, separated by decantation, centrifuged, and stored in darkness in a freezer at −20°C.

Assessment of Antioxidant Activity by the FRAP Method

The FRAP (ferric reducing ability of plasma) assay was developed as a method for evaluation of plasma antioxidant capacity [12]. Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction causes formation of the intensely blue ferrous–tripyridyltriazine complex with an absorbance maximum at 593 nm. This assay, as modified by Bartoń et al. [13] can be used for the analysis of substances which do not absorb at the same wavelength. The FRAP method gives highly reproducible results.

Sample Preparation for HPLC

For extraction of seeds and sprouts the sample (0.200 g) was weighed into a small vial and 3 mL of a binary 1:1 (*v*/*v*) mixture of ethanol and 0.2 M HCl was added. The vial was closed with a septum and placed in a model RK 255 H Sonorex Super sonication bath manufactured by Bandelin (Berlin, Germany), at 40°C, for 40 min. During that time extraction of the flavonoids and polyphenols was combined with possible hydrolysis of the compounds of interest from the plant matrix [14]. Finally, the extract was separated

from solid matter by filtration, evaporated to 1 mL, and, without further purification, analyzed for flavonoid and polyphenol content by high-performance liquid chromatography with the diode-array detection (HPLC–DAD).

HPLC–DAD Analysis

HPLC–DAD was performed with a model P580A LPG liquid chromatograph equipped with a Gina model 50 autosampler and a model UVD340V DAD diode-array detector (Gynkotek/Dionex, Germering, Germany). Compounds were separated on a 250 mm × 4.6 mm i.d. Tosoh Biosep cartridge packed with 5-µm TSK gel ODS-80 TM (Tosoh, Tokyo, Japan; #08149). The mobile phase was a binary gradient prepared from acetonitrile (ACN) and water. The composition of the gradient is given in *Table I*. The flow rate was 1 mL min^{-1} and the column was thermostatted at 40°C.

Table I. The composition of the binary ACN–H_2O mobile phase gradient

0–2 min	Constant composition	5% CAN
2 – 22 min	Composition change	Increase from 5 to 25% ACN
22 – 32 min	Composition change	Increase from 25 to 55% ACN
32 – 50 min	Constant composition	55% CAN

The extracts (50 µL) were injected for HPLC–DAD analysis at, 50-min intervals, by use of the autosampler. Each analysis lasted for 50 min.

Under the same working conditions apigenin, vitexin, isovitexin, orientin, isoorientin, kaempferol, quercetin, rutin, myricetin, morin, naringin, hesperidin, neohesperidin, gallic acid, cinnamic acid, vanillic acid, caffeic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and syringic acid were analyzed as solutions in ethanol (concentration 0.1 mg mL^{-1}). Each analysis was repeated twice and the respective retention times were averaged.

Compounds of interest in the extracts obtained from the seeds and sprouts were identified by comparison of retention times (t_R) and UV spectra with those of standards. Quantification of the compounds was achieved by use of calibration plots obtained by injecting different volumes (5 to 30 µL at 5-µL intervals) of the individual standard solutions. (The plots were linear in the range investigated.) Each experiment was repeated three times. The numerical results (amounts of phenolic acids and flavonoids) listed in *Tables II* and *III* are means; experimental error was within the range ±1%.

Table II. Phenolic acid content of the seeds and sprouts of amaranth and quinoa

Phenolic acid	Phenolic acid content (mg kg^{-1} dry weight)								
	Amaranthus cruentus v. Aztek			*Amaranthus cruentus* v. Rawa			*Chenopodium quinoa*		
	Seeds	Sprouts (light)	Sprouts (darkness)	Seeds	Sprouts (light)	Sprouts (darkness)	Seeds	Sprouts (light)	Sprouts (darkness)
Gallic acid	440	370	360	400	360	350	320	70	70
p-Hydroxybenzoic acid	8.5	n.d.	n.d.	20.7	n.d.	n.d.	76.8	n.d.	n.d.
Vanillic acid	15.5	n.d.	n.d.	n.d.	n.d.	n.d.	43.4	n.d.	n.d.
p-Coumaric acid	n.d.	4.4	6.1	3.9	28.3	42.4	n.d.	n.d.	n.d.
Syringic acid	n.d.	6.3	4.2	n.d.	4.3	3.7	n.d.	n.d.	n.d.
Ferulic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	440	n.d.
Caffeic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	40	n.d.	n.d.
Cinnamic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10	6.3	n.d.
Total	464	380.7	370.3	424.6	392.6	396.1	490.2	516.3	70

n.d. – not detected

Table III. Flavonoid content of the seeds and sprouts of amaranth and quinoa

Flavonoids	Flavonoid content (mg kg^{-1} dry weight)								
	Amaranthus cruentus v. Aztek			*Amaranthus cruentus* v. Rawa			*Chenopodium quinoa*		
	Seeds	Sprouts (light)	Sprouts (darkness)	Seeds	Sprouts (light)	Sprouts (darkness)	Seeds	Sprouts (light)	Sprouts (darkness)
Rutin	n.d.	690	300	n.d.	620	460	360	2900	n.d.
Orientin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1076	n.d.	n.d.
Vitexin	n.d.	n.d.	n.d.	410	n.d.	n.d.	709	n.d.	240
Isovitexin	n.d.	n.d.	n.d.	266	n.d.	n.d.	n.d.	130	1455
Morin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	88.9	10.9	8.58
Hesperidin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.86	n.d.	n.d.
Neohesperidin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.93	n.d.	n.d.
Sum	–	690	300	676	620	460	2238	3041	1704

n.d. – not detected

As examples only, and to show the quality of the HPLC–DAD experiments, three examples of the chromatograms obtained are shown in *Figs 1–3*.

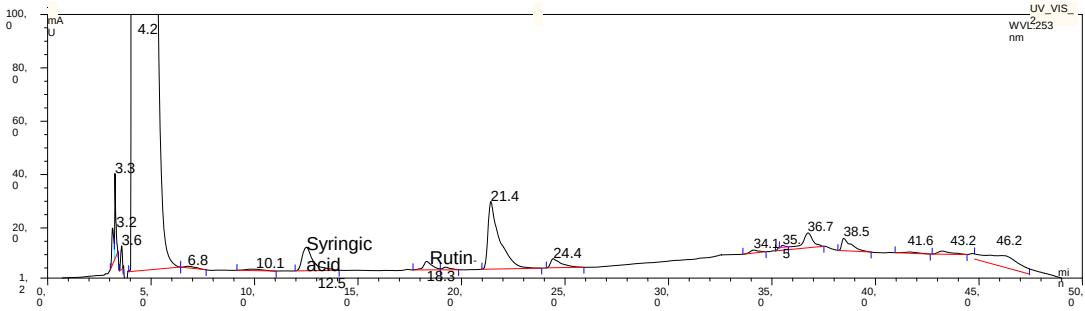

Fig. 1. Typical chromatogram obtained from an extract of *Amaranthus cruentus* v. Aztek sprouts grown under natural conditions

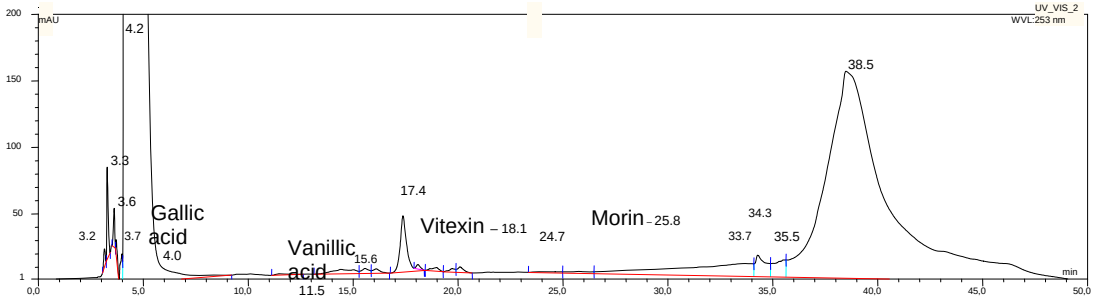

Fig. 2. Typical chromatogram obtained from an extract of *Amaranthus cruentus* v. Rawa seeds

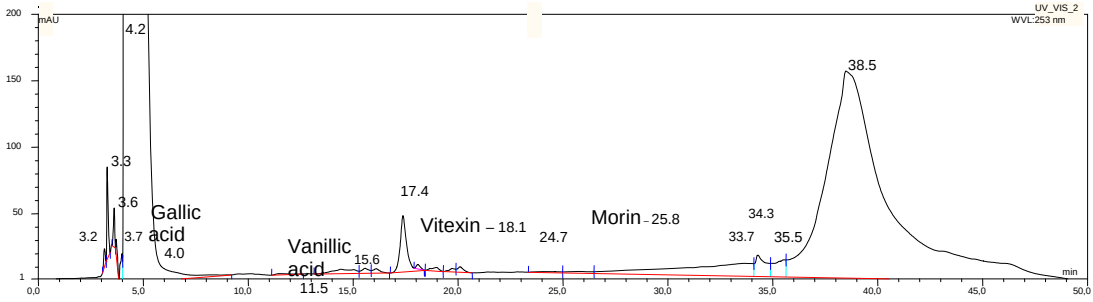

Fig. 3. Typical chromatogram obtained from an extract of the *Chenopodium quinoa* seeds

Results

Antioxidant Activity of Seeds and Sprouts

To select materials with the highest antioxidant activity (AA) for HPLC analysis, we investigated extracts of seeds and sprouts by the FRAP method. The results are compiled in *Table IV*.

Table IV. Antioxidant activity (AA) of the seeds and sprouts (mmol Fe^{2+} kg^{-1} dry weight, mean ± SD, n = 4), determined by use of the FRAP assay

Seeds		Sprouts							
		Days after seeding	4 days		6 days		7 days		
		Type of cultivation	Sprouts (light)	Sprouts (darkness)	Sprouts (light)	Sprouts (darkness)	Sprouts (light)	Sprouts (darkness)	
Amaranthus cruentus v. Aztek	3.37±0.40		248.1±5.6	127.4±0.8	148.0±2.3	80.6±1.7	163.2±2.5	91.3±2.4	
Amaranthus cruentus v. Rawa	3.73±0.20		149.4±4.6	126.2±2.5	111.9±2.0	85.4±1.1	103.8±5	61.1±8.6	
Chenopodium quiona	4.97±0.15		42.0±0.2	31.4±0.1	77.4±0.2	57.35±0.1	73.4±0.3	64.7±0.2	

The AA of sprouts was several times higher than that of seeds. Among the sprouts, the sprouts of amaranth v. Aztek had the highest AA; that of sprouts of amaranth v. Rawa AA was lower by nearly a half and the quinoa sprouts had the lowest AA. The activity of the sprouts depended on the time of growth; maximum values were reached on the fourth day for amaranth and on the sixth day for quinoa. The AA of sprouts grown in daylight was higher than that of those grown in darkness.

Amounts of Phenolic Acids in Seeds

Although the total phenolic acid contents of seeds was similar, the highest amount was in quinoa (490.2 mg kg^{-1} d.w.), slightly lower in amaranth v. Aztek (464 mg kg^{-1} d.w.), and lowest in amaranth v. Rawa (424.6 mg kg^{-1} d.w.) (*Table II*).

Gallic acid and *p*-hydroxybenzoic acid were detected in all the seeds. The prevalent acid was gallic acid – 440 mg kg^{-1} d.w. in amaranth v. Aztek, 400 mg kg^{-1} d.w. in amaranth v. Rawa, and 320 mg kg^{-1} d.w in quinoa. Levels of *p*-hydroxybenzoic acid were lower and, in contrast with gallic acid, the largest amount was in Quinoa seeds (76.8 mg kg^{-1} d.w.), lower in the seeds of amaranth v. Rawa (20.7 mg kg^{-1} d.w.), and the lowest in the seeds of Amaranth v. Aztek (8.5 mg kg^{-1} d.w.). The third acid present in the seeds of amaranth v. Aztek and quinoa was vanillic acid; the amounts were 15.5 mg kg^{-1} d.w. and 43.4 mg kg^{-1} d.w., respectively. Seeds of amaranth v. Rawa did not contain vanillic acid but did contain traces of *p*-coumaric acid (3.9 mg kg^{-1} d.w.). Quinoa seeds not only had the highest total content but also the greatest diversity of phenolic acids, because caffeic acid (40 mg kg^{-1} d.w.) and cinnamic acid (10 mg kg^{-1} d.w.) were detected in these seeds.

Amounts of Phenolic Acids in Sprouts

Total amounts of phenolic acids in sprouts (*Table II*) were similar in both amaranth varieties, whether grown in daylight or darkness, and ranged from 370.3 to 396.1 mg kg^{-1} d.w. Total amounts were lower than in seeds (424.6 and 464 mg kg^{-1} d.w., respectively). The opposite results were obtained for the quinoa sprouts grown in daylight, because the level of phenolic acids was higher in the sprouts (516.3 mg kg^{-1} d.w.) than in the seeds (490.2 mg kg^{-1} d.w.).

All sprouts, similar to seeds, contained mainly gallic acid, but the amount was lower than in the seeds. In both amaranth varieties the amounts were similar (350–370 mg kg^{-1} d.w.) whereas in quinoa the amounts was much lower (70 mg kg^{-1} d.w.). Sprouting conditions did not affect synthesis of gallic acid.

In addition to gallic acid, two other phenolic acids were identified in the sprouts – *p*-coumaric acid and syringic acid in amaranth sprouts and ferulic acid and cinnamic acid in quinoa sprouts grown in daylight.

The sprouts of both varieties of amaranth grown in the dark contained larger amounts of *p*-coumaric acid and lower amounts of syringic acid than sprouts grown in daylight. A substantial amount of ferulic acid (440 mg kg^{-1} d.w.) was detected in quinoa sprouts grown in daylight. This was the only material investigated in which ferulic acid was found.

No vanillic acid or *p*-hydroxybenzoic acid was found in sprouts.

Amounts of Flavonoids in Seeds

There was much variety in the amounts of flavonoids in the seeds (*Table III*). The seeds of amaranth v. Aztek contained none of the flavonoids whereas the total flavonoid content of amaranth v. Rawa seeds and quinoa seeds was 676 and 2238 mg kg^{-1} d.w., respectively. Vitexin (410 mg kg^{-1} d.w.) and isovitexin (266 mg kg^{-1} d.w.) were detected in the seeds of amaranth v. Rawa, and quinoa seeds contained at least flavonoids – mainly orientin (1076 mg kg^{-1} d.w.), vitexin (709 mg kg^{-1} d.w.) and rutin (360 mg kg^{-1} d.w.) but also morin (88,9 mg kg^{-1} d.w.) and traces of hesperidin (1.86 mg kg^{-1} d.w.) and neohesperidin (1.93 mg kg^{-1} d.w.).

Amounts of Flavonoids in Sprouts

Total amounts of flavonoids (*Table III*) in sprouts grown in daylight were higher than in sprouts grown in the darkness, and amounted to 690 mg kg^{-1} d.w. in amaranth v. Aztek, 460 mg kg^{-1} d.w. in amaranth v. Rawa, and 3041 mg kg^{-1} d.w. in quinoa. The prevalent flavonoid in the sprouts of quinoa and both varieties of amaranth was rutin; respective amounts in amaranth v. Aztek, amaranth v. Rawa, and quinoa were 620, 690, and as high as 2900 mg kg^{-1} d.w., respectively. Sprouts grown in the dark contained less rutin; for amaranth v. Aztek it was 56% less, for amaranth v. Rawa 26% less, and no rutin was found in quinoa. In the sprouts of both varieties of amaranth, rutin was the only flavonoid detected. Quinoa sprouts were significantly richer in flavonoids. Apart from rutin they contained vitexin, isovitexin, and morin. Quinoa sprouts grown in the darkness contained vitexin (240 mg kg^{-1} d.w.) and substantial amounts of isovitexin (1455 mg kg^{-1} d.w.), whereas those grown in daylight contained isovitexin only (130 mg kg^{-1} d.w.). It is remarkable that no isovitexin was present in quinoa seeds (at least not above the detection limit). Sprouting conditions had virtually no effect on the amount of morin, which was low (approx. 10 mg kg^{-1} d.w.). Some flavonoids present in the seeds were not found in the sprouts – vitexin and isovitexin for amaranth v. Rawa, and orientin, hesperidin, and neohesperidin for quinoa.

Discussion

We did not manage to find any literature reports of the phenolic acid and flavonoid content of amaranth and quinoa sprouts and seeds. A few reports discuss buckwheat seeds and true cereals and there were no data regarding

the presence of phenolic acids and flavonoids in the sprouts of cereals and pseudocereals.

The amount of gallic acid in the seeds of the pseudocereals (320–440 mg kg^{-1} d.w) was many times higher than in white corn seeds (4 mg kg^{-1} d.w.) [16] or in brown rice seeds (19 mg kg^{-1} d.w) [17], but lower than in dark buckwheat flour, in which the amount of gallic acid was greater than 1700 mg kg^{-1} d.w. [15].

p-Hydroxybenzoic acid was detected in all the seeds investigated; amounts were 76.8, 20.7, and 8.5 mg kg^{-1} d.w. in the seeds of quinoa, amaranth v. Rawa, and amaranth v. Aztek, respectively. The presence of this phenolic acid in wheat bran (3.4 mg kg^{-1} d.w.) has also been reported [18].

Cinnamic acid (10 mg kg^{-1} d.w.) and caffeic acid (40 mg kg^{-1} d.w.) were found in quinoa seeds only. The amount of caffeic acid was ten times higher than in rice seeds [17] and 50 times higher than in wheat bran [18].

p-Coumaric acid was present in the seeds of amaranth v. Rawa only; the amount was 3.9 mg kg^{-1} d.w., 20 times higher than in brown rice [17].

The presence of vanillic acid was detected in the seeds of amaranth v. Aztek and quinoa, at levels of 15.5 and 43.4 mg kg^{-1} d.w., respectively. The amount of vanillic acid in amaranth v. Aztek was similar to that in brown rice [17].

The total phenolic acid content of seeds is similar to that of brown rice [17].

The pseudocereal seeds investigated had diverse flavonoid profiles. Rutin was found only in quinoa seeds and its level was similar to that in buckwheat hulls (329.5 mg kg^{-1}) [19] and over 40% lower than in buckwheat seeds [20]. Vitexin was detected in the seeds of amaranth v. Rawa and quinoa, and the amounts (410 and 709 mg kg^{-1} d.w., respectively) were significantly higher than in buckwheat hulls (ca. 150 mg kg^{-1}). The presence of isovitexin in the seeds of amaranth v. Rawa was also demonstrated and the amount was three times higher than in buckwheat hulls (72 mg kg^{-1}). Orientin was present in quinoa seeds (13 times higher than in buckwheat hulls [19]), as also was morin, hesperidin, and neohesperidin.

Rutin was the prevailing flavonoid in the sprouts of amaranth and quinoa. The amount was reduced when the sprouts were grown in darkness (in v. Aztek by over 50%, and in v. Rawa by over 26%). The rutin content of sprouts grown under natural conditions was four times higher in quinoa than in amaranth v. Rawa and v. Aztek.

Conclusion

This HPLC method enabled qualitative and quantitative analysis of the phenolic acid and flavonoid content of the seeds and sprouts of amaranth (v. Aztek and Rawa) and quinoa. The results of this research led to the following conclusions:

1. The main phenolic acid present in sprouts and seeds was gallic acid. Gallic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric, caffeic acid, and cinnamic acids were also detected in the seeds whereas gallic acid, *p*-coumaric acid, syringic acid, and ferulic acid were found in the sprouts.
2. Rutin was the main flavonoid in the sprouts. We also found orientin, vitexin, isovitexin, morin, and the traces of hesperidin and neohesperidin in seeds. Vitexin, isovitexin, and morin were found in sprouts.
3. Sprouting conditions (daylight versus darkness) did not affect the level of gallic acid but the rutin content increased in the daylight and the vitexin and isovitexin content increased in darkness.
4. Quinoa seeds and sprouts were richer in phenolic acids and flavonoids, both quantity and quality, than the seeds and sprouts of both amaranth varieties.

Because of the substantial amounts of flavonoids present in the seeds and sprouts of quinoa, these products could be used as valuable dietary components, especially for the children suffering from gluten intolerance.

Acknowledgments

The authors wish to thank Professor H. Ekiert and Professor Z. Janeczko of the Medical College of the Jagiellonian University and Professor M. Piskuła of the University of Warmia and Mazury in Olsztyn for gifts of some of the standards.

References

[1] H. Khor and D. Chieng, Nutr. Res., **17**, 475 (1997)
[2] R. Teutonico and D. Knorr, Food Technol., **39**, 49 (1985)
[3] P. Paśko, M. Bednarczyk, and Z. Zachwieja, Żyw. Człow. Metab., **3/4**, 1256 (2007)
[4] J. Czerwieński, E. Bartnikowska, H. Leontowicz, E. Lange, M. Leontowicz, H. Katrich, S. Trakhtenberg, and S. Gorinstein, J. Nutr. Chem. **15**, 622 (2004)

[5] S. Gorinstein, O. Medina Vargas, N. Jaramillo, I. Arnao Salas, A. Martinez Ayala, P. Arancibia-Avila, F. Toledo, E. Katrich, and S. Trakhtenberg, Eur. Food Res. Technol., **225**, 321 (2007)
[6] P. Paśko, H. Bartoń, M. Fołta, and J. Gwiżdż, Roczn. PZH, **58**, 35 (2007)
[7] J. He, M. Klag, P. Whelton, J. Mo, J. Chen, M. Qian, P. Mo, and G. He, Am. J. Clin. Nutr., **61**, 366 (1995)
[8] P. Paśko and M. Bednarczyk, Brom. Chem. Toksykol., **2**, 217 (2007)
[9] K. Gonor, A. Pogozheva, S. Derbeneva, G. Mal'tsev, E. Trushina, and O. Mustafina, Vopr Pitan. **75**, 30 (2006)
[10] K. Kawabata, T. Tanaka, S. Honjo, M. Kakumoto, A. Hara, H. Makita, N. Tatematsu, J. Ushida, H. Tsuda, and H. Mori, Int. J. Cancer, **83**, 381 (1999)
[11] P. Uma Devi, A. Ganasoundari, B. Vrinda, K. Srinivasan, and M. Unnikrishnan, Radiation Res., **154**, 455 (2000)
[12] I. Benzie and J. Strain, Anal. Biochem., **239**, 70 (1996)
[13] H. Bartoń, M. Fołta, and Z. Zachwieja, Nowiny Lek, **74**, 510 (2005)
[14] R. Haruenkit, S. Poovarodom, H. Leontowicz, M. Leontowicz, M. Sajewicz, T. Kowalska, E. Delgado-Licon, N. Rocha-Guzmán, J. Gallegos-Infante, S. Trakhtenberg, and S. Gorinstein, J. Agric. Food Chem., **11**, 5842 (2007)
[15] I. Sensoy, R. Rosen, C. Ho, and M. Karwe, Food Chem., **99**, 388 (2006)
[16] D. Del Pozo-Insfran, C. Brenes, S. Serna Saldivar, and S. Talcott, Food Res. Int., **39**, 696 (2006)
[17] Z. Zhou, K. Robards, S. Helliwell, and C. Blanchard, Food Chem., **87**, 401 (2004)
[18] K. Kim, R. Tsao, R. Yang, and S. Cui, Food Chem., **95**, 466 (2006)
[19] D. Dietrych-Szostak and W. Oleszek, J. Agric. Food Chem., **47**, 4384 (1999)