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CHANGES IN AMARANTH POLYPHENOL CONTENT DURING THE DIFFERENT VEGETATION PHASES

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ABSTRACT

Total content of polyphenols was investigated in different anatomical parts of amaranth during different growth periods. Five amaranth cultivars were included in the experiment (*Amaranthus hypochondriacus* L.: cultivars Annapurna and Koniz, *Amaranthus caudatus* L.: cultivar Oscar Blanco, *Amaranthus cruentus* L.: cultivars Golden Giant and Rawa). Analysis were done in 4 growth phases: phase I. – intensive stem growth, phase II. – formation of the flowers and pollination, phase III. – milky ripeness, phase IV. – full ripeness. Based on the determined total polyphenol content in amaranth it is possible to create this anatomical part order: leaves > flowers > seeds > stems. No statistically significant differences were confirmed between phases I., III. and IV. On the other hand the total polyphenol content in amaranth determined in growth phase II. was significantly different in comparison to other growth phases. Statistically significant differences in polyphenolic content were confirmed between all investigated anatomical parts of amaranth.

Keywords: Amaranth, polyphenolics, anatomical parts, growth phase

INTRODUCTION

Amaranths are plants of the genus *Amaranthus*. The cultivated forms are useful for producing nutritious grain and foliage, and as colorful ornamentals (**Brenner *et al.*, 2000**). *Amaranthus hypochondriacus*, *Amaranthus cruentus* and *Amaranthus caudatus* are among the most widespread species of the family *Amaranthaceae*. In spite of the fact that the genus *Amaranthus* has been the subject of many taxonomic studies, it is still poorly understood and is widely considered to be a "difficult" genus. It constists of about 70 species, of which about 40 are native to the Americas and the rest to Australia, Africa, Asia and Europe (**Costae, Demason, 2001**).
Amaranth is a very versatile crop that is grown in a wide range of agro-climatic conditions; it resists drought, heat, and pests, and adapts readily to new environments (**Rana *et al.*, 2007**).
Cultivated amaranth species can be used, not only as a source of edible seeds, leafy vegetables, and forage, but also as ornamentals (**Mlakar *et al.*, 2009**). Amaranth contains between 15 and 22% protein, with a significantly higher content of lysine, and acceptable levels of tryptophan and methionine, and it contains 58 and 66% starch as well as 9 to 16% dietary fiber (**Tosi *et al.*, 2001**). Lipid contents range between 7 and 8.5% with is higher than most other cereals. Amaranth oil contains approximately 77% unsaturated fatty acids and is high in linoleic acid, which is necessary for human nutrition. The lipid fraction is unique due to the high squalene content (**Sun *et al.*, 2005**). High concentrations of calcium, phosphorus, iron, potassium, zinc, vitamin E and B complex, as well as low levels of antinutritional factors, make this grain a product of high interest for food formulation. The very low glyadine content, less than 0.01% in some varieties (*Amaranthus cruentus*, *A. mantegazzianus*), makes useful for those who suffer from celiac disease (**Tosi *et al.*, 2001**). Amaranth is a rich source of polyphenols (flavonoids) with relative high antioxidant activity. Caffeic acid, p-hydroxybenzoic acid and ferulic acid are the main phenolic compounds in amaranth grains (**Klimczak *et al.*, 2002**). **Barba de la Rosa *et al.* (2009)** also detected low levels of quercetin glycoside, rutin (4.0–10.1 µg/g DM) in amaranth seeds. Vegetable amaranth has received significantly less research attention than grain amaranth (**Alegbejo, 2014**). However, it has been rated considerably higher in minerals, such as calcium, iron, phosphorous (**Makus, 1984**; **Igbokwe *et al.*, 1988**) and carotenoids (**Martirosyan, 2001**) than most vegetables. Amaranth leaves, stems and entire plants may be eaten raw or cooked. Cooking and discarding the water removes potentially harmful oxalates and nitrates (**Babalola *et al.*, 2010**). Amaranth leaves have similar nutritional quality to spinach and other green materials. Except for the Fe content, pasta made from amaranth green leaves or spinach green leaves flour did not differ significantly in the amount of moisture, fat, protein, crude fiber, and total carbohydrate present (**Borneo, Aguirre, 2008**).
The aim of this study was to investigate the dynamics of total polyphenolics creation in different anatomical parts of five amaranth cultivars during different growth periods.

MATERIAL AND METHODS

Plant material

Five cultivars from amaranth (Golden Giant, Rawa, Annapurna, Oscar Blanco, Koniz) were obtained from Plant Production Research Center in Piešťany. All cultivars are registered in the EU. The anatomical parts of amaranth were manually separeted, dried at 105 °C to constant weight (WTC Binder, Germany) and powdered (Fritsch Pulverisette, Germany).

Extract preparation

Plant extracts were prepared by adding 25 mL of 80% methanol (Sigma-Aldrich, USA) to 1 g milled sample. The mixture was shaken at room temperature for 8 h at 250 rpm. Samples were then filtered through filter paper (130 g/m^2, Filtrak, Germany) and kept at 8 °C for further analysis.

Determination of total polyphenol content (TP)

The total polyphenol content (TP) was estimated using Folin-Ciocalteau reagent (Merck, Germany) according **Lachman *et al.* (2003).** Sample extract (0.05 to 1 mL to the expected polyphenol content), 2.5 mL Folin-Ciocalteau reagent and 3 –

5 mL H_2O were added to a 50 mL flask. After 3 min. 7.5 mL of 20% Na_2CO_3 (Sigma-Aldrich, USA) were added to the flask and diluted to 50 mL with H_2O. The mixture was incubated for 2 h at laboratory temperature and the absorbance was measured at 765 nm on the spectrophotometer Shimadzu 710 (Shimadzu, Japan) against the blank sample. The total polyphenol content was expressed as gallic acid equivalents (GAE) in mg/kg DM (dry matter). The linearity range for this assay was determined at 200 – 1000 mg/mL (R^2 = 0,998).

Statistical analysis

All determinations were done in six repetitions. The data were analysed using the package Statgraphics (multifactorial analysis of variance, LSD-text contrasts, $p < 0.05$).

RESULTS AND DISCUSSION

Total content of polyphenols was investigated in different anatomical parts of amaranth during different growth periods (stems. leaves, flowers and seeds). Five amaranth cultivars were included in the experiment (*Amaranthus hypochondriacus* L.: cultivars Annapurna and Koniz, *Amaranthus caudatus* L.: cultivar Oscar Blanco, *Amaranthus cruentus* L.: cultivars Golden Giant and Rawa). Analysis were done in 4 growth phases: phase I. – intensive stem growth, phase II. – formation of the flowers and pollination, phase III. – milky ripeness, phase IV. – full ripeness (Table 1).

Table 1 Total content of polyphenols in different anatomical parts of amaranth cultivars in different growth phases (mg GAE/kg DM)

Cultivar	Plant material	Phase I.	Phase II.	Phase III.	Phase IV.
		mg GAE/kg DM	mg GAE/kg DM	mg GAE/kg DM	mg GAE/kg DM
Golden Giant	steams	969.03	1450.10	1449.75	1723.73
	leaves	4333.79	4042.35	3093.69	4610.92
	flowers	0.00	2532.19	3261.44	0.00
	seeds	0.00	0.00	0.00	2548.75 b
Rawa	steams	1464.75	1716.46	1373.42	1935.41
	leaves	3605.35	8854.52	3302.83	3763.96
	flowers	0.00	4707.67	4835.50	0.00
	seeds	0.00	0.00	0.00	1381.05 a
Annapurna	steams	1539.96	1413.23	1390.78	1530.33
	leaves	3619.15	4117.35	4158.58	3338.27
	flowers	0.00	3374.63	3578.54	0.00
	seeds	0.00	0.00	0.00	2869.90 b
Oscar Blanco	steams	1115.30	1343.07	1623.38	1161.52
	leaves	5775.25	8457.29	4998.13	4202.33
	flowers	0.00	3856.40	3938.31	0.00
	seeds	0.00	0.00	0.00	1634.10 a
Koniz	steams	1122.90	1092.29	1274.02	1924.42
	leaves	3605.77	6740.68	4613.15	4481.79
	flowers	0.00	4096.08	4029.67	0.00
	seeds	0.00	0.00	0.00	1807.81 a

The different letter in column denotes a statistically significant difference (P < 0.05); TP – total polyphenol content; SD – standard deviation, DM – dry mater.

The data relating to total polyphenol content in seeds of amaranth in the literature are very diverse. While determined values of TP in amarath seeds by **Mošovská *et al.* (2010), Gorinstein *et al.* (2007), Klimczak *et al.* (2002)** and **Czerwinski *et al.* (2004)** were 1048.8, 430, 391 – 562 and even 149 mg GAE.kg^{-1} DM respectively, **Dlamini *et al.* (2010)** reported significantly higher amount of TP in amaranth seeds (17600 mg GAE/kg). Our results are in correlation to those presented by **Paśko *et al.* (2008)** who analysed seeds of 2 amaranth cultivars and determined the similar values of total polyphenol content (2950 and 3000 mg GAE/kg DM respectively) and **Guzmán-Maldonado., Paredes-Lûpez (1998)**, who presented total polyphenol content in amaranth seeds in interval 2000 – 4000 mg GAE/kg DM.

Total polyphenol content (TP) in stems and leaves was determined in all four growth phases (Table 1). The obtained results confirmed in all growth phases a higher polyphenol content in leaves of all cultivars compared with stems. The determined values of total polyphenols (mg GAE/kg) in stems of observed amaranth cultivars in I., II., III. and IV. growth phases were intervals 969.03 – 1539.96, 1092.29 – 1716.4, 1274.02 – 1623.38 and 1161.52, while in leaves were the determined values of TP (mg GAE/kg) in growth phases I., II., III. and IV. in intervals 3605.35 – 5775.25, 4042.35 – 8854.52, 3093.69 – 4998.13 and 3338.27 – 4610.92 respectively.

Table 2 Total Average values of total polyphenol content in stems and leaves of amaranth in different growth phases (mg GAE/kg DM)

Anatomical Part	Growth phase	TP	SD
		mg GAE/kg DM	
Stems	I.	1242.22 a	246.48
	II.	1403.03 ab	224.00
	III.	1422.27 ab	128.99
	IV.	1655.08 b	322.10
Leaves	I.	4187.86 c	941.10
	II.	6442.44 d	2298.54
	III.	4033.28 c	821.47
	IV.	4079.45 c	526.38

The different letter in column denotes a statistically significant difference (P < 0.05); TP – total polyphenol content; SD – standard deviation, DM – dry mater.

Statistically significant differences in total polyphenol content was determined in amaranth stems only between growth phases I. and IV. and in leaves between growth phase II. and the other investigated growth phases of amaranth plant (Table 2). In leaves the determined TP content was almost 3.3 times higher compared with stems.

Table 3 Average values of total polyphenol content in stems, leaves and flowers of amaranth in growth phases II. and III. (mg GAE/kg DM)

Anatomical Part	Growth phase	TP	SD
		mg GAE/kg DM	
Stems	II.	1403.03 a	224.00
	III.	1422.27 a	128.99
Leaves	II.	6442.44 c	2298.54
	III.	4033.28 c	821.47
Flowers	II.	3739.39 b	816.32
	III.	3928.69 b	591.70

The different letter in column denotes a statistically significant difference ($P < 0.05$); TP – total polyphenol content; SD – standard deviation, DM – dry mater.

In growth phases II. and III. the total polyphenol content in stems, leaves and flowers was determined (Table 1). The highest polyphenol content in amaranth flowers in phases II. and III. was detected in cv. Rawa (4707.67 and 4835.50 mg GAE/kg respectively). The highest value of TP in stems and leaves in growth phase II. was determined in cv. Rawa (1716.46 and 8854.52 mg GAE/kg respectively) while in growth phase III. (milky ripeness) in cv. Oscar Blanco (1623.38 and 4998.13 mg GAE/kg respectively).

Based on the results the followed order of total polyphenol content in investigated amaranth anatomical parts can be created: leaves > flowers > stems. Statistically significant differences in polyphenolic content were confirmed between all investigated anatomical parts of amaranth (Table 3).

Table 4 Average values of total polyphenol content in stems, leaves and seeds of amaranth in growth phase IV. (mg GAE/kg DM)

Anatomical Part	Growth phase	TP	SD
		mg GAE/kg DM	
Stems	IV.	1655.08 a	322.10
Leaves	IV.	4079.45 b	526.38
Seeds	IV.	2048.32 a	632.47

The different letter in column denotes a statistically significant difference ($P < 0.05$); TP – total polyphenol content; SD – standard deviation, DM – dry mater.

In growth phase IV. the total polyphenol content was determined in three anatomical parts of amaranth plants (stems, leaves, seeds). In leaves 2.76 times higher values compared with stems and 1.9 times higher values of TP content compared with seeds were determined (Table 1). The highest TP content in seeds was determined in cv. Annapurna, the lowest one in cv. Rawa. The determined values of TP content in leaves were statistically significantly different from the other investigated anatomical parts of amaranth plant (Table 4).

Based on the determined total polyphenol content in amaranth it is possible to create this anatomical part order: leaves > flowers > seeds > stems.

No statistically significant differences were confirmed between phases I., III. and IV. On the other hand the total polyphenol content in amaranth determined in growth phase II. was significantly different in comparison to other growth phases. Statistically significant differences in polyphenolic content were confirmed between all investigated anatomical parts of amaranth.

CONCLUSION

Amaranth as a pseudocereal is an excellent source of highly appreciated nutritional components as well as other bioactive compounds such as polyphenolics, beta-carotene and lutein. Grain amaranth species that have high level of proteins should be promoted in developing countries as a source of proteins. On the other hand also amaranth leaves, stems and entire plants may be eaten raw or cooked. Amaranth leaves have similar nutritional quality (moisture, fat, protein, crude fiber, and total carbohydrate content) to spinach and other green vegetables. Leaves of amaranth contain also high amount of polyphenols with benefit on the human health due to their antioxidant activity. Based on the determined total polyphenol content in investigated amaranth cultivars it is possible to create this anatomical part order: leaves > flowers > seeds > stems.

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