PAPER

NUTRITIONAL CONTENT AND ANTIOXIDANT PROPERTIES OF SELECTED SPECIES OF *AMARANTHUS* L.

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ABSTRACT

The aim was to assess the entire plant of three species of *Amaranthus* L. with regard to their chemical content, determination of the most valuable genotype, and the optimum harvest time in relation to the species' nutritional value. The amaranth harvested later was found to contain less protein and ash, but clearly more fibre, and nitrogen-free extracts. The highest content of mineral compounds was observed at the beginning of blossoming. The genotypes were characterized by very high levels of radical scavenging activity, which was dependent on harvest timing. Betanine and amaranthine concentration decreased with the delay in harvesting.

Keywords: amaranth, antioxidant activity, betacyanins, chemical composition, genotype, harvest time

1. INTRODUCTION

The effects of globalization and the observed unification of food production around the world have limited the number of cultivated plant species. To counteract this trend, new plants - previously not used on a massive scale, but with a proven nutritional value - have been introduced in commercial agriculture. This group includes one of the oldest cultivated plants, belonging to the genus *Amaranthus*. The value of amaranth depends mainly on the unique chemical composition of its seed (MOTA *et al*., 2016). The chemical composition of the foliage is also very interesting and presents significant nutritional value. The content of high quality protein in the leaves amounts to 19,4%-30% of dry matter (ANDINI *et al*., 2013; NGUGI *et al*., 2017). The entire amaranth plant contains biologically active components such as anthocyanins, flavonoids, and phenyl acids, which are responsible for the plants' pharmacological properties (LI *et al*., 2015).
Apart from the aforementioned nutritional value of the whole amaranth plant, there are other reasons justifying efforts to increase its cultivation volume. Two such economic factors are the relatively undemanding cultivation and the high yield. In order for the plants to produce valuable flowers and seeds, a relatively low crop density is recommended (from 10 to 30 plants per m^2) (YARNIA, 2010). In the case of forage crop cultivation the plants should produce delicate and thin shoots (O'BRIEN and PRICE, 2008). An increase in crop density to 140 plants per m^2 results in better parameters for green foliage (required ratio of leaf mass to shoot mass). A lower crop density results in higher shoot mass and higher fibre content. In the case of high crop density the produced shoots are narrower, and tend to drop older leaves that receive less light.
The choice of the best time for forage crop harvesting is related to the size of the harvest and its nutritional value, which could be determined in two ways: by choosing a specific day after sowing (ABBASI *et al*., 2012) or by following the plant's development phase (POSPIŠIL and POSPIŠIL, 2008). Regardless of the harvest method, all the mentioned papers report that the most beneficial period for harvest in terms of the nutritional value and digestibility of the plant is the end of its vegetative stage, and if the size of the collected yield is concerned – full bloom stage. A delay in harvest quite negatively influences the quality of the collected mass.
In this study, we attempted to assess whether the species of the genus *Amaranthus* L. differentiates chemical composition of the whole plants, and whether the nutritional value whole plants *of Amaranthus* L. is dependent on the harvest time.
Therefore, the aim of study was to assess the chemical composition of the whole plant of three species of the genus *Amaranthus* L. and to determine the most valuable genotype, as well as the optimum harvest time with regard to the plants' nutritional value.

2. MATERIALS AND METHODS

2.1. Study sites

The experiment was conducted in 2014 at the Mochełek Experimental Station near Bydgoszcz, Poland (53°12′24″N, 17°51′40″E). The experiment was conducted in a totally random system and was repeated three times. The soil in which the crops were grown is typical Haplic Luvisols, created from fluvioglacial sands on sandy loam, class IVa. The soil pH was slightly acidic (pH 5.6 in KCl). The content of silt and clay in the arable layer stood at 15%. Analysis of soil mineral content showed high levels of phosphorus (20.9 mg/100g), very low levels of magnesium (2.2 mg/100g), and low levels of potassium (8.0 mg/100g). Sowing was carried out on 15th May with a spacing of 0.45 m, 5 kg per ha, at 1-2 cm depth

by spacing seeder. The area of the harvest plot was 10 m^2. No mineral fertilization was used. The plant maintenance included mechanical weeding of the interrows. The fore crop for amaranth was spring barley.

2.2. Plant material

The first experimental factor (A) was genotype: (A1) *Amaranthus cruentus* L. 'Rawa' cultivar, (A2) *Amaranthus hypochondriacus* L. 'Aztek' cultivar, (A3) *Amaranthus caudatus* L. 'Oscar Blanco', and 'Phule Kartiki' cultivar. The second factor (B) was harvest time – the number of days after sowing: (B1) the beginning of blossoming, (B2) full bloom, and (B3) full seed development. The choice of experimental factor (harvest time) was made based on observation of the developmental stage of the majority of plants in the plot field. The term of specific agrophenophases was established *a priori* and not using the calendar. On the day prior to harvest, random plant samples were collected for chemical analysis (20 samples of each cultivar).

2.3. Climatic conditions

The atmospheric conditions for the year of the study are presented in Table 1. In comparison with the mean values of multi-year meteorological parameters (1996-2013) the humidity during sowing and germination periods was higher (the sum of precipitation was higher by 45%), but in the following months it was relatively lower. In addition, very high temperatures were registered over the entire vegetative phase. As a result, the values of Seljaninov hydrothermal indicator in June, July, and August were lower than the mean, which confirms the occurrence of drought. Despite this, based on long-term research on amaranth cultivation conducted at the same location, it could be assumed that the weather during the year 2014 was particularly beneficial for the growth and development of all the chosen amaranth species.

Table 1. Temperature, precipitation and hydrothermal index during the experiment as compared with multiyear average [1996-2013].

Years	The meteorological parameter	Decade of the month	Month				
			April	May	June	July	August
2014	The average air temperature (°C)	I	7.3	10.4	17.6	21.1	21.8
		II	8.7	12.8	15.8	20.6	16.1
		II	13.7	16.3	14.5	22.8	14.1
	Average monthly temperature		9.9	13.3	16.0	21.5	17.2
	Precipitation (mm)	I	17.6	32.1	10.2	14.4	18.9
		II	18.6	10.6	12.0	7.7	16.2
		II	4.5	23.0	22.7	33.3	22.2
	Monthly amounts of precipitation [mm]		40.7	65.7	44.9	55.4	57.3
	Hydrothermal index		1.37	1.59	0.94	0.83	1.07
1996-2013	The average air temperature (°C)		8.0	13.2	16.3	18.5	17.8
	Rainfall (mm)		28.0	60.9	53.5	88.8	67.0
	Hydrothermal index		1.17	1.49	1.09	1.55	1.21

2.4. Chemical analyses

The chemical composition of samples were determined according to procedures of the Association of Official Analytical Chemists (AOAC, 2012): dry matter was determined by drying at 105ºC to a constant weight, crude fat by Soxhlet extraction with diethyl ether, crude ash by incineration in a muffle furnace at 580ºC for 8 h. Crude protein (N × 6.25) by Kjeldahl method using a Büchi Distillation Unit B - 324 (Büchi Labortechnik AG, Switzerland). Crude fibre was determined in an ANKOM 220 fibre analyzer (ANKOM Technology, USA). Nitrogen-free extract (NFE_s) was calculated as: $NFE_s = 100 - (\text{moisture} + \text{crude protein} + \text{crude fat} + \text{crude ash} + \text{crude fibre})$. The fibre components were determined using the detergent method according to VAN SOEST *et al*. (1991) performed with the ANKOM 220 fibre analyzer. Determination of neutral detergent fibre (NDF) was conducted on an ash-free basis and included sodium dodecyl sulphate (Merc 822050). Determination of acid detergent fibre (ADF) included hexadecyl-trimethyl-ammonium bromide (Merc 102342), while acid detergent lignin (ADL) was determined by hydrolysis of ADF samples in 72% sulphuric acid. Hemicellulose content was calculated as the difference between NDF and ADF, while cellulose content as the difference between ADF and ADL.

The material for analyses of the major dietary element concentrations was subjected to mineralization in concentrated H_2SO_4 and $HClO_4$ acids, whilst the material for analyses of the micro-compound concentrations was subjected to mineralization in a mixture of HNO_3 and $HClO_4$. The concentration of phosphorus (P) were determined by colorimetric method, with ammonium molybdate, at wavelength 660 nm, using a Specol 221 apparatus. An Atomic Absorption Spectrometer apparatus (iCE 3000 Series, Thermo Fisher Scientific) was used to determine potassium (K), sodium (Na) and calcium (Ca) by means of emulsion flame spectroscopy, whilst magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) by means of absorption flame spectroscopy.

Assessment of betacyanins was carried out using a spectrophotometer; UV-1800r. Exactly 2g of ground plant material was placed in a mortar before homogenization with 10cc demineralized water to ease extraction. The pH of the resulting solution was adjusted to 5.4 (STINTZING *et al*., 2004). Samples were diluted in a 0.05 M phosphate buffer (pH 6.5) as described by STINTZING *et al*. (2003) using the extinction coefficients of betanin (ε = 60000 $dm^3/mol/cm$; λ = 538 nm; molecular weight = 550) (WYLER and MEUER, 1979) and of amaranthine (ε = 56600 $dm^3/mol/cm$; λ = 538 nm; molecular weight = 726) (PIATTELLI *et al*., 1969).

The level of polyphenols was determined by the POLI-SWAIN and HILLIS (1959) method, using Folin-Ciocalteu reagent. The extract used for determination of polyphenols was obtained by grinding dried samples of amaranth green forage in the lab sample mill (OG 109), followed by extraction with 40 mL 0.08 M HCl in 80% methanol, at a temperature of 18-22ºC for two hours. The extract from 5g of green forage was centrifuged at 1500 g for 15 minutes and the remains were re-extracted twice with 40 mL 70% acetone for two hours. The content of polyphenols was expressed in mg of chlorogenic acid in 100g of the product. The extract's capacity to eliminate free radicals was assessed using the method described by RE *et al*. (1999) using ABTS•+. ABTS•+ free radical diluted in a solution of potassium persulfate adjusted to provide an absorbance of 0.74-075 at 734 nm. The methanol-acetone extract (0.8 mL) was topped up to 1 mL with a 1:1 mixture of acetone and methanol. Subsequently, 2 mL of ABTS•+ free radicals were added to the mixture. The extract was incubated at 3ºC for 6 minutes and the absorption measurement was subsequently carried out at 734 nm.

The capacity to eliminate free RSA (Radical Scavenging Activity) was derived from the following equation:

$$RSA = (E_1 - E_2) / E_1$$

where:
E_1 – sample absorbance before incubation,
E_2 – sample absorbance after incubation.

2.5. Statistical analysis

The data were subjected to statistical analysis using ANOVA in a randomized block design. The separate factors in the analysis were genotype and harvest time. The sum of the errors and the interaction of genotype × harvest date were used to test the significance of the main effects. The significance of differences between means shown in the form of homogeneous groups was assessed using a Duncan test at P = 0.05. Results are presented as mean±SD (standard deviation). All chemical analyses were performed in three replications.

3. RESULTS AND DISCUSSIONS

3.1. Chemical composition

Traditional vegetables provide low-cost quality nutrition for large parts of the population in both rural and urban areas. One such example is amaranth, containing more nutrients than typical leafy vegetables (VENSKUTONIS and KRAUJALIS, 2013) In this study, we found statistically significant differences were found in the dry matter content, crude ash, crude protein, crude fibre, and nitrogen-free extract (NFE) in the whole plant between the studied genotypes (Table 2). The harvest time influenced amaranth chemical composition. Similar to AMADUCCI *et al*. (2000) and FRASER *et al*. (2001), who showed that the delay in harvesting of plants caused changes in their chemical content, in our study a later harvesting time caused an increase in dry matter and crude fibre content (Table 3), but a decrease in crude protein content. Green leafy vegetables have long been recognized as the cheapest and most abundant potential source of protein because of their ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as water, CO_2, and atmospheric nitrogen (ALETOR *et al*., 2002). Our results concerning crude protein in amaranth leaves do not provide a clear indication of statistical difference between the studied species, with protein content ranging between 95.9 and 101 g per kg of dry matter, similar to the results of other authors (AKUBUGWO *et al*., 2007; MODIL, 2007).
However, the results of our analysis permit the claim that *Amaranthus hypochondriacus* L. ('Aztek') tends to have a slightly higher protein level than the other studied genotypes. The highest amounts of protein were found in plants harvested at the beginning of blossoming (128 g/kg DM), being 37% and 32% higher than the samples from the two consecutive terms, respectively. Even higher crude protein levels in amaranth biomass (152 to 216·kg DM) were reported by POSPIŠIL *et al*. (2009), which depended on the cultivar and the year. Crude fat content in the whole plant differed between the studied amaranth cultivars, with the highest amount found in the 'Rawa' cultivar. Levels depended not only on the genotype but also on the harvest time.

Table 2. The tested the whole plant of amaranth composition [g/kg DM].

Specification	Dry matter	Crude ash	Crude protein	Crude fat	Crude fibre	NFEs
			Cultivar (A)*			
(A1) 'Rawa'	939±0.26	146±1.15	95.9±0.79	22.6^{a}±0.12	235±0.18	499±0.65
(A2) 'Aztek'	936±0.40	144±0.20	101.0±0.74	17.6^{ab}±0.17	240±1.27	495±0.16
(A3) 'Phule Kartiki'	941±0.75	163±1.37	98.0±0.81	11.3^{b}±0.31	219±0.34	508±2.15
(A4) 'Oscar Blanco'	938±0.38	154±0.58	97.9±0.02	12.2^{b}±0.45	249±1.00	486±0.05
			Term of the mowing - no. of days after sowing date (B)**			
(B1) - 60	934±0.12	183^{a}±0.83	128.0^{a}±0.66	15.4^{b}±0.31	203^{b}±0.90	470^{c}±0.76
(B2) - 90	942±0.25	145^{b}±0.26	80.3^{b}±0.53	11.5^{b}±0.35	268^{a}±0.86	494^{b}±0.78
(B3) - 120	939±0.41	128^{b}±0.67	86.9^{b}±0.57	20.8^{a}±0.27	236^{ab}±1.36	527^{a}±18.9

*The cultivar's means denoted by different letters differ statistically at (for all columns separately).
**The term of mowing's means denoted by different letters differ statistically at (for all columns separately).

3.2. Fibre Fractions

Recently, increasing attention has been paid to the components that are difficult to digest in the gastrointestinal tract of humans, belonging to the dietary fibre group. The source of the dietary fibre and the ratios of its fractions determine its properties and applications; the chemical and physical properties of its many structures have various effects on the physiology of the human body (MANN *et al*., 2009). In our study, the content of crude fibre in the selected species was affected only by harvest time; a delay in harvest increased the content of crude fibre in the whole plant. The levels of specific fibre fractions were also determined (Table 3) and indicated that a later harvest time caused a increase in NDF, ADF, ADL and cellulose content. HCEL are best at binding ions of heavy metals (HU *et al*., 2010). Celluloses and lignin also have these properties, but to a lesser degree and are dependent on the fraction's origin. Cellulose does not have good ion exchange properties, nor does it bind bile acids or salts (KAHLON *et al*., 2007). Cellulose fibres are virtually undigested in the gastrointestinal tract; however, they aid intestine peristalsis.

Table 3. The tested the whole plant of amaranth dietary fibre [g/kg DM].

Specification	NDF***	ADF	ADL	HCEL	CEL
		Cultivar (A)*			
(A1) 'Rawa'	440±3.02	304±1.06	51.2^{b}±1.99	136±4.08	253±0.93
(A2) 'Aztek'	440±2.57	332±0.16	63.1^{a}±2.42	109±2.73	269±2.57
(A3) 'Phule Kartiki'	415±0.29	291±3.91	54.5^{ab}±0.88	126±4.20	236±3.03
(A4) 'Oscar Blanco'	453±3.39	329±2.61	55.1^{ab}±0.16	124±5.99	274±2.76
		Term of the mowing - no. of days after sowing date (B)**			
(B1) - 60	408^{b}±1.83	288^{b}±2.20	51.8^{b}±0.30	121±0.38	236^{b}±2.50
(B2) - 90	460^{a}±0.83	347^{a}±0.01	54.1^{b}±1.05	113±0.83	293^{a}±1.05
(B3) - 120	444^{a}±0.01	307^{ab}±1.90	62.1^{a}±1.18	137±1.90	245^{b}±0.72

*The cultivar's means denoted by different letters differ statistically at (for all columns separately).
**The term of mowing's means denoted by different letters differ statistically at (for all columns separately).
***NDF, neutral detergent fibre, ADF, acid detergent fibre, ADL, acid detergent lignin, CEL, cellulose, HCEL, hemicelluloses.

In our study, the highest relative amount of cellulose was found at the second harvest time (almost 20% more in comparison to the first harvest term), which is the result of an increase in content in tissues during the development and aging of the plant. Lignin accumulates in the cell wall at the end of cell growth, after the formation of polysaccharide scaffolding of the wall is completed. The highest level of ADL fraction was present in the dry matter from the last harvest term. This fraction does not present a high capacity for binding heavy metals. However, similar to cellulose, it also aids in intestine peristalsis.
NDF had a highest share in dietary fibre, followed by ADF, which includes lignin and cellulose. A later harvest time increased the amount of these fractions in the whole amaranth plant, which was probably caused by the production of cell wall components, as well as more polyphenols as a result of temperature stress. The delay in harvest promoted lignification as well as an increase of cell wall participation in cells of plant tissue (AMADUCCI *et al*., 2000).

3.3. Macronutrients

A special role is assigned to mineral components due to their participation in numerous anti-oxidation processes. The enzyme peroxide dismutase is activated by copper, zinc, and manganese. Deficiency of the mentioned elements, or magnesium, calcium and potassium, decreases the efficiency of internal anti-oxidant mechanisms causing an increased risk of degenerative diseases (AMES, 2010; PRASHANTH *et al*., 2015). In our study, we assessed the level of selected elements in the amaranth samples and found that they were influenced by the experimental factors (Tables 4 and 5). The content of the tested mineral components in the studied cultivars of amaranth differed, which indicates various capacities to absorb and accumulate the mentioned components in the biomass by the different cultivars growing in similar conditions. The highest content of calcium and magnesium were found in the 'Aztek' cv., while the lowest calcium was detected in 'Rawa' cv., and the lowest magnesium in 'Phule Kartiki' cv. (Table 4). 'Rawa', 'Phule Kartiki' and 'Oscar Blanco' cultivars had higher phosphorus concentrations than 'Aztek' cv. (6.8 g/kg DM). The content of potassium in 'Oscar Blanco' cv. (63.7 g/kg DM) was the highest and differed from other cultivars. Present research confirmed that amaranth was a rich source of potassium. The richest in potassium was 'Oscar Blanco' cv., containing 10 g/kg more of this element than spinach (MATRASZEK *et al*., 2002), which belongs to the superfood group and is considered a rich source of potassium. 'Aztek' cultivar contained the highest level of magnesium (4.4 g/kg DM). The highest concentration of sodium was recorded for 'Rawa' cv. (111 g/kg DM) and 'Oscar Blanco' cv. (95.8 g/kg DM). Phosphorus was the only element not influenced by the time of harvest, and its mean concentration in the studied genotypes of amaranth amounted to 8.9 g/kg DM. Differences in Ca and Na content were recorded between the two harvest times. A tendency was found for less of the elements in the green mass of amaranth in the consecutive harvest terms (Table 4). The amaranth is a valuable product and could be an important source of necessary nutrients in the human and the animal diet (AKUBUGWO *et al*., 2007; ONWORDI *et al*., 2009; MLYNEKOVÁ *et al*., 2014). The application of food minerals in an organism largely depends on the ratios. The requirements regarding particular mineral components and their ratios in the animal diet depend, *inter alia*, on the species of animal, age, and physiological stage. The potassium to sodium ratio (K:Na) in humans plays an important role in the regulation of blood pressure; it should be less than one to avoid adverse effects (YUSUF *et al*., 2007). As shosphorus metabolism is linked to calcium metabolism in the system, calcium-phosphate homeostasis crucially depends on an appropriate Ca:P ratio in food (close to 1). An inappropriate Ca:P ratio decreases calcium absorption, which negatively affects the skeletal system and physiological

processes (DRIVER *et al*., 2006; YE *et al*., 2006). A ratio lower than 1:2 impairs calcium absorption and vitamin D synthesis. This results in higher levels of parathyroid hormone and accelerates bone resorption processes (KEMI *et al*., 2010). 'Rawa', 'Phule Kartiki', and 'Oscar Blanco' cultivars all fulfilled the aforementioned requirements, which confirms the results of AKUBUGWO *et al*. (2007). In addition, the 'Phule Kartiki' and 'Aztek' cultivars had a K:Na ratio close to 1, which qualifies their use in poultry feeds.

Table 4. The tested the whole plant of amaranth macronutrients [g/kg DM].

Specification	Ca	P	K	Mg	Na	Ca:P	K:Na
			Cultivar (A)*				
(A1) 'Rawa'	10.4^{b}±0.13	9.6^{a}±0.01	55.6^{b}±0.33	3.8^{b}±0.01	111.8^{a}±0.56	1.1^{b}±0.01	0.5^{c}±0.01
(A2)' Aztek'	13.7^{a}±0.07	6.8^{b}±0.05	54.3^{b}±0.41	4.4^{a}±0.01	65.4^{b}±1.83	2.0^{a}±0.01	0.8^{a}±0.02
(A3) 'Phule Kartiki'	10.7^{ab}±0.10	9.1^{ab}±0.09	54.6^{b}±0.14	3.6^{b}±0.01	60.9^{b}±1.17	1.2^{b}±0.02	0.9^{a}±0.01
(A4) 'Oscar Blanco'	10.8^{ab}±0.13	10.1^{a}±0.06	63.7^{a}±0.51	3.7^{b}±0.01	95.8^{a}±0.69	1.0^{b}±0.02	0.7^{b}±0.01
			Term of the mowing - no. of days after sowing date (B)**				
(B1) - 60	14.6^{a}±0.16	10.3±0.01	65.6^{a}±0.26	4.7^{a}±0.02	90.4^{a}±0.25	1.5^{a}±0.01	0.8^{a}±0.01
(B2) - 90	9.7^{b}±0.08	8.2±0.04	62.1^{b}±0.51	3.1^{c}±0.01	70.1^{b}±0.46	1.3^{ab}±0.01	0.9^{a}±0.01
(B3) - 120	9.9^{b}±0.07	8.3±0.13	43.5^{c}±0.27	3.8^{b}±0.01	89.5^{a}±0.79	1.2^{b}±0.02	0.5^{b}±0.01

*The cultivar's means denoted by different letters differ statistically at (for all columns separately).
**The term of mowing's means denoted by different letters differ statistically at (for all columns separately).

3.4. Micronutrients

Iron and zinc are both responsible for the proper functioning of specific and non-specific immune responses. The studied amaranth genotypes turned out to be a rich source of iron. The harvest time did not affect the content of this component (Table 5). The whole plant of amaranth was found to contain on average 236.4 mg/kg of iron, which confirms the results of KAMGA *et al*. (2013). By comparison, MATRASZEK *et al*. (2002) recorded 30% and 7% less iron in the respective dry matter of lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.). As confirmed in the samples in the presented research, FUNKE (2011) found 40% more iron in amaranth leaves cultivated in Nigeria than in lettuce. The daily diet should also provide proper amount of zinc, since it is not accumulated in tissues, but excreted from the system. Zinc has a beneficial effect on the production, maturation and activity of leukocytes. However, excessive consumption may limit iron and copper absorption, which can lead to anaemia. In our study, both the cultivar and the harvest time had an effect on the content of Zn. 'Phule Kartiki' cv. biomass had the highest content of Zn. The later the harvest, the lower the amount of Zn in the selected amaranth cultivars. Iron metabolism also involves copper, and the highest content of copper was found in 'Aztek' cv. The highest levels of copper were also found in the amaranth at the beginning of blossoming (Table 5). Manganese is an essential trace element, necessary for development and growth of the organism. It is a component of metalloenzymes such as superoxide dismutase, arginase, and pyruvate carboxylase, and is involved in amino acid, lipid and carbohydrate metabolism. Disturbances in manganese absorption and retention may play a role in the etiopathogenesis of several diseases and disorders. There has been no specific manganese deficiency syndrome described in humans (ZABŁOCKA-SŁOWIŃSKA and GRAJETA, 2012; PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES, 2013). Manganese content in the studied plants was significantly

correlated to both cultivar type and harvest time. The highest content of manganese was reported in the 'Phule Kartiki' cv. (177.5 mg/kg DM). The mean content of manganese in the studied cultivars of amaranth stood at 150.8 mg/kg DM. The highest manganese content was reported in amaranth collected during the full bloom period. Manganese concentration in vegetable leaves, including amaranth, varies between 2.54 mg/kg DM in indian spinach (*Basella alba* L.), 5.46 mg/kg DM in bush buck (*Gongronema latifolium* L.), 6.14 mg/kg DM in roselle plant (*Hibiscus sabdariffa* L.) and 10.6 mg/kg DM in smooth amaranth (*Amaranthus hybridus* L.) (ASAOLU *et al*., 2012).

Table 5. The tested the whole plant of amaranth micronutrients [g/kg DM].

Specification	Zn	Fe	Mn	Cu
Cultivar (A)*				
(A1)' Rawa'	37.8^{b}±0.36	231.1^{ab}±1.23	132.6^{b}±0.01	2.8^{c}±0.03
(A2) 'Aztek'	44.4^{b}±0.04	237.4^{ab}±0.17	140.4^{b}±0.20	3.7^{a}±0.30
(A3) 'Phule Kartiki'	82.4^{a}±0.06	272.3^{a}±0.14	177.5^{a}±0.10	3.5^{ab}±0.10
(A4) 'Oscar Blanco'	41.0^{b}±0.09	205.0^{c}±1.03	152.7^{ab}±0.18	3.0^{bc}±0.02
Term of the mowing - no. of days after sowing date (B)**				
(B1) - 60	60.0^{a}±0.25	248.4±1.11	147.9^{ab}±0.02	4.3^{a}±0.20
(B2) - 90	48.6^{b}±0.04	219.7±0.47	171.9^{a}±0.22	2.5^{b}±0.01
(B3) - 120	45.6^{b}±0.04	241.1±1.07	132.7^{b}±0.12	2.8^{b}±0.09

*The cultivar's means denoted by different letters differ statistically at (for all columns separately).
**The term of mowing's means denoted by different letters differ statistically at (for all columns separately).

3.5. Polyphenol, pigments and scavenging ability

Betanins are water-soluble nitric herbal dyes and can be found in the cell fluids. Their stability within a wide pH range from 3.5 to 7.0 contributes to the fact that they are excellent food dyes and good substitutes for anthocyanins (MORENO *et al*., 2008). In a collective summary for the *Amaranthaceae* family, KHAN and GIRIDHAR (2015) found the betanin content between 7.6 and 117.0 mg/kg DM (in *Celosia* spp., *Achyrranthes* spp., *Aerva sanguinolenta* spp., *Alternanthera* spp., *Iresine herbstii* spp., *Gomphrrena globose* spp.). In the studied whole plant of amaranth, betanin levels of 11.4-17.3 mg/kg DM and amaranthine of 106.5-161.1 mg/kg in air dried mass of different cultivars of amaranth were found. In amaranth shoots, VENSKUTONIS and KRAUJALIS (2013) recorded 1.77 mg/100g of betanin. They also showed that amaranthine and isoamaranthine are the dyes found in the highest quantities, at 15.3 and 5.87 mg/100g, respectively. The presence of amaranthine and the absence of isoamaranthine were reported in *Celosia argentea* L. (SCHLIEMANN *et al*., 2001). In our study, a content of betanin in the whole plant of amaranth ranged between 1.19 and 1.54 mg/100g and depended on genotype (Table 6). The highest content was reported in the leaves of the 'Aztek' and 'Oscar Blanco' cultivars. Analysis of the effect of harvest time on betanin content showed that a delay in harvest decreased the content of this compound in the whole plant. The highest content of amaranthine was reported in dry matter of plants from the first harvest, as well as in the dry matter of the 'Aztek' and 'Oscar Blanco' cultivars. Betacyanins also display a free radical scavenging capacity, and betanin and its metabolites maintain their properties in acidic conditions (TAIRA *et al*., 2015). The free radical scavenging activity (RSA) of bethanidine is comparable to that of vitamin E (TESORIERE *et al*., 2009). The concentrations found in the

various cultivars fell within the range 2.9 mg/g to 35 mg/g DM. Free radial scavenging activity is also correlated with the content of polyphenols in raw material. The statistical analysis of the genotypes allowed their classification into two homogenous groups. The group with the highest content of polyphenols was the 'Phule Kartiki' cv. (2792.5 mg/100g DM). Such a high content of total polyphenols in the whole plant of this cultivar did not result in statistically significantly higher antioxidant activity compared to other cultivars. The high capacity to remove free radicals in the studied cultivars oscillated within a very narrow range (97.5% to 98.4%), which can be ascribed to harvest time, with RSA slightly higher in the second and third terms. Although the highest levels of polyphenols were found in the second harvest term (2884.2 mg/kg DM), and the lowest in the third term (1482.3 mg/kg DM), free radical scavenger properties were statistically unchanged. Differences in phenol compounds content between leafy vegetables, including *Amaranthus cruentus* L. and *Amaranthus hybridus* L. were shown by ADEMOYEGUN *et al*. (2013). Of these two species, it was *A. hybridus* that had higher phenol content and higher anti-radical activity. Due to the fact that these compounds play an important role both for these plants' ontogenesis, and their health promoting and sensory properties, it seems they should be considered as potential food supplements. In this regard, amaranth is more beneficial than sprouts and leaves of oat and buckwheat, commonly known for their antioxidant potential; no less important is the presence of betacyanines in amaranth (PIĄTKOWSKA *et al*., 2015; WITKOWICZ *et al*., 2015).

Table 6. Polyphenol content [mg/100g DM], pigments content [mg/kg DM] and scavenging ability RSA [%] in the tested the whole plant of amaranth.

Specification	Polyphenol	RSA	Betanine	Amaranthine
		Cultivar (A)*		
(A1) 'Rawa'	1669.7^{b}±49.3	98.0±0.22	11.9^{b}±0.31	111.3^{b}±2.96
(A2) 'Aztek'	1846.3^{b}±53.3	98.1±0.22	14.4^{a}±0.76	134.5^{a}±7.07
(A3)' Phule Kartiki'	2792.5^{a}±182.8	97.8±0.31	12.1^{b}±0.35	113.3^{b}±3.33
(A4) 'Oscar Blanco'	1768.3^{b}±61.73	98.0±0.22	15.4^{a}±0.02	143.7^{a}±0.18
		Term of the mowing - no. of days after sowing date (B)**		
(B1) - 60	1690.9^{b}±55.6	97.5^{b}±0.09	17.3^{a}±0.09	161.1^{a}±0.82
(B2) - 90	2884.2^{a}±172.2	98.1^{a}±0.09	11.7^{b}±0.13	109.5^{b}±1.30
(B3) - 120	1482.3^{b}±32.6	98.4^{a}±0.21	11.4^{b}±0.10	106.5^{b}±0.93

*The cultivar's means denoted by different letters differ statistically at (for all columns separately).
**The term of mowing's means denoted by different letters differ statistically at (for all columns separately).

4. CONCLUSIONS

The weather in the vegetative season in 2014 was particularly beneficial for the growth and development of the cultivated amaranth species. The factor that determined the nutritional value of the studied plants was the harvest time. A delay in harvest time decreased the amount of crude protein and mineral compounds (determined in ash). The amaranth from later harvests (full bloom and full seed development) had increased concentration of crude fibre and total carbohydrates. The content of mineral compounds was different, which indicated differences in the abilities to absorb and accumulate particular elements by the studied cultivars. With a delay in harvest the concentrations of the macro and micro-compounds decreased. The studied amaranth demonstrated very

high free radical scavenging activity, with the highest RSA values recorded in the two later harvest terms. The content of pigments in amaranth was correlated to both the genotype and the harvest term. The highest concentrations of pigments were observed in 'Oscar Blanco' cv. and 'Aztek' cv. This content then decreased with the delay in harvest. The amaranth is a valuable product and may be an excellent source of essential nutritional components in the human and animal diet. However, based on the presented results, it is difficult to unequivocally indicate the most valuable genotype and optimal harvest term with reference to the nutritional value.

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