

## FAT AND TOCOPHEROL CONTENT IN THE SEEDS OF AMARANTH IN CONDITIONS OF DIVERSIFIED FERTILIZATION WITH MACROELEMENTS

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### ABSTRACT

The aim of the study was to determine the content of fat and tocopherols in the seeds of Polish cultivars of amaranth – Rawa (*Amaranthus cruentus* L.) and Aztek (*Amaranthus hypochondriacus* × *Amaranthus hybridus*) – under the influence of varied macroelement fertilization in the environmental conditions of the Zamość region of south-eastern Poland. In a three-year field experiment (2013–2015) set up in a randomized split-plot design, amaranth was grown as a test plant at wide row spacing, on good wheat complex soil, in south-eastern Poland (N – 50°71', E – 23°04'). The field experiment had three variables: the weather in the years of research; four combinations of NPK application and two cultivars of the test plant – Rawa and Aztek. The research showed that the factor that most influenced the content of crude fat in the amaranth seeds was the cultivar, followed by the combination of NPK fertilization. The amount of α-tocopherol and total tocopherol depended significantly on the genetic factor and the fertilization combination, while the amount of β-tocopherol was determined more by fertilization than by the cultivar. The content of γ-tocopherol and δ-tocopherol was dependent only on the cultivar.

**Key words:** amaranth, fertilization, fat content, tocopherol content

### INTRODUCTION

Amaranth is counted among universal plants with seeds of very high nutritional value and leaves used as a valuable vegetable [Skwaryło-Bednarz et al. 2011]. There is increasing interest in amaranth as an alternative to currently cultivated crops [Skwaryło-Bednarz and Nalborczyk 2006]. At present, two cultivars of amaranth Rawa (*Amaranthus cruentus* L.) and Aztek (*Amaranthus hypochondriacus* × *Amaranthus hybridus*) are registered in Poland for seeds. The best soil and climate conditions for amaranth crops in Poland are found in the south-east of the country, especially the Zamość region. Interest in the cultivation of this plant is growing in this region as well, and this has prompted in-

depth research on the influence of the environment and agrotechnical treatments on the chemical composition of amaranth seeds and the possibility of increasing the content of particularly valuable nutrients – those with antioxidant properties. Research by Li et al. [2015] as well as López-Mejía et al. [2014] have shown that all parts of the amaranth plant, irrespective of the species, are good sources of antioxidants. Amaranth seeds have a high content of bioactive compounds with exceptional nutritional and nutraceutical potential for human health [Skwaryło-Bednarz et al. 2020]. They contain key proteins, several interesting essential amino acids, carbohydrates, dietary fibre, important phytochemi-

cals and, above all, fat. Amaranth contains from 50 to 90 g·kg<sup>-1</sup> fat, which not only has a favourable fatty acid composition, but most importantly contains dissolved nutrients such as squalene (50–80 g·kg<sup>-1</sup>), tocopherols and tocotrienols [Skwaryło-Bednarz 2012]. These compounds are particularly valuable owing to their antioxidant properties.

Due to the presence of bioactive substances in oil obtained from cold-pressed amaranth seeds, they act as functional food. The presence of essential unsaturated fatty acids, sterols and tocopherols means that they can help to prevent or control diseases of civilization, such as cardiovascular disease, cancer, or obesity, and to delay the ageing process. Numerous studies have been carried out to assess the quality and nutritional value of amaranth oil, which has a high content of tocopherols [Obiedzińska and Waszkiewicz-Robak 2012] and squalene [Lacatusu et al. 2018].

Amaranth seeds are also a valuable source of folic acid, irrespective of processing [Motta et al. 2017]. They are widely used for food production, including healthy, safe food and functional food [Szwejkowska and Bielski 2012]. In addition, the plant has a positive effect on the biological properties of soil, by stimulating the number and activity of soil microbes [Skwaryło-Bednarz and Krzepińko 2009a], and on the antioxidant properties of the soil [Skwaryło-Bednarz and Krzepińko 2009b].

The aim of this study was to evaluate the content of fat and tocopherols in the seeds of the Polish amaranth cultivars Rawa and Aztek under the influence of diversified macroelement fertilization in the environmental conditions of the Zamość region of south-eastern Poland.

## MATERIALS AND METHODS

**Grain sampling.** Seeds for analysis of the content of fat, homologues of tocopherol, and total tocopherol were obtained from the field experiment described below.

### Field experiment with amaranth as a test plant

The study was based on a three-year field experiment carried out in 2013–2015 on a field belonging to a private farmer, located in the village of Bodaczów (N 50°71', E 23°04'), near Zamość, on soil classified as good wheat complex. It was brown soil

formed from loess, with high content of P, K and Mg. The pH of the soil was slightly acidic (mean logarithm pH KCl 5.74), and the adsorption capacity as determined by the Kappen method was 125.8 mmol(+)·kg<sup>-1</sup>. The forecrop for amaranth was spring barley.

The experiment was set up in a randomized split-plot design with three variables:

1. Weather conditions in each year of the study
2. Four NPK fertilizer combinations:  
I – 40 kg N·ha<sup>-1</sup>, 30 kg P·ha<sup>-1</sup>, 30 kg K·ha<sup>-1</sup>  
II – 60 kg N·ha<sup>-1</sup>, 40 kg P·ha<sup>-1</sup>, 40 kg K·ha<sup>-1</sup>  
III – 80 kg N·ha<sup>-1</sup>, 50 kg P·ha<sup>-1</sup>, 50 kg K·ha<sup>-1</sup>  
IV – 120 kg N·ha<sup>-1</sup>, 70 kg P·ha<sup>-1</sup>, 70 kg K·ha<sup>-1</sup>

Nitrogen (N) was applied twice (before sowing and during intensive plant growth – six weeks after plant emergence) in the form of ammonium nitrate. Phosphorus (P) was applied before sowing in the form of granulated triple superphosphate, and potassium (K) in the form of potash. The results were compared to a control treatment without NPK fertilization.

3. Two cultivars of the test plant – Rawa and Aztek

Amaranth seeds were sown in the last third of May at wide row spacing (every 60 cm). The area of the plots for harvesting was 20 m<sup>2</sup>. Cultivation of the plants in the experiment was in accordance with agrotechnical requirements.

Every year after the amaranth seeds were harvested, their content of fat and tocopherols (total and individual homologues) was determined.

**Crude fat determination.** Determination of crude fat was carried out by the ether extract method with a Soxhlet extractor [Abolaji et al. 2017]. About 1 g of dried sample was wrapped in filter paper and placed in the thimble, which was then placed in the extraction tube. The receiving beaker was weighed, cleaned and dried and then filled with petroleum ether and placed in the extractor. Extraction was begun by turning on the water and heater. After siphoning six times, the ether was allowed to evaporate. The beaker was disconnected before the final siphoning. The extract was washed with ether, transferred to a clean glass dish and allowed to evaporate on a water bath. The dish was placed in an oven at 105°C for 2 h and cooled in a desiccator. The percentage of crude fat was determined by the following formula:

$$\begin{aligned} & \% \text{ crude fat} = \\ & = \text{wt. of ether extract} \times 100 / \text{wt. of sample} \end{aligned}$$

**Determination of tocopherol content.** To determine the tocopherol content, 3 g samples of seeds were ground and saponified using 60% KOH. Then the samples were extracted with peroxide-free diethyl ether. After the ether was distilled, HPLC was used for qualitative and quantitative determination of tocopherols in the residue [Nogala-Kalucka 1999, Gogolewski et al. 2000].

The equipment used for HPLC was a Waters Model 600 gradient pump, a LiChrosorb Si 60 column (200 mm, 5 µm Merck), a fluorometric detector, and the Waters Millennium 32 data acquisition system. Residue samples were dissolved in n-hexane and injected onto the column. A mixture of n-hexane and 2-propanol (99.5:0.5 v/v) was used as a mobile phase at a flow rate of 1.5 ml/min. Calibration curves prepared for each of the tocopherols were used to calculate the concentrations.

**Statistical analysis.** Analysis of variance (ANOVA) of the results was performed, and means were compared by the Tukey test using Statistica software, version 7.0. The significance level for rejection of the null hypothesis was 5% ( $p < 0.05$ ).

## RESULTS AND DISCUSSIONS

In the years of the research (2013–2015), the temperatures recorded in the study area were higher than the long-term average, although in May 2015 and June 2014 they were lower than the long-term average (Tab. 1). Each year in May, when amaranth was sown, rainfall was higher than the long-term average. Sow-

ing was particularly difficult in May 2014 due to the large amount of rainfall (Tab. 1). In 2013, excessive rainfall was also recorded in June and September. In 2014, September and October were dry months; the sum of precipitation was lower than the long-term average. In 2015 there was also excessive rainfall in September, while precipitation in the remaining months was lower than the long-term average (Tab. 1).

Analysis of the results of the three-year study revealed that the fat content in the amaranth seeds as well as the content of total tocopherols and their individual homologues were determined in varying degrees by the test factors – weather conditions, NPK fertilization rate, and cultivar (Tab. 2).

Although amaranth is not a typical oilseed plant, one of the predominant and very valuable constituents of amaranth seeds is fat, together with the compounds dissolved in it. The fat content in the years of the study ranged from 64.2 to 65.8 g·kg<sup>-1</sup>. This is less than the levels reported by Palombini et al. [2013] and Mburu et al. [2012].

The present study found no significant influence of temperature and moisture conditions in 2013–2015 on the amount of fat accumulated in the seeds (a difference of 1.6 g·kg<sup>-1</sup>) (Tab. 2). This may have been due to the very similar temperature and precipitation distribution in the years of research. The results of our research are supported in the literature. According to Ogrodowska et al. [2011], the location of the crop has no statistically significant influence on the content of tocopherols in amaranth seed oils. However, Kozak et al. [2011] reported that more variable weather, espe-

**Table 1.** Weather conditions in the years of the study

Months	Mean temperature (°C)			Long-term average 1971–2005	Total rainfall (mm)			Long-term average 1971–2005
	2013	2014	2015		2013	2014	2015	
May	17.2	14.2	13.6	14.1	108.2	241.6	91.1	65.5
June	20.3	16.3	17.7	16.8	120.5	91.3	36.2	78.9
July	21.5	20.7	21.1	18.4	44.2	146.1	60.4	98.4
August	21.9	18.7	22.3	17.8	11.2	82.8	11.3	54.3
September	13.5	14.8	16.0	12.9	79.3	48.1	54.2	52.2
October	11.8	9.5	7.7	7.4	5.7	34.4	34.4	40.3

**Table 2.** Content of fat ( $\text{g}\cdot\text{kg}^{-1}$ ), tocopherol homologues and total tocopherol ( $\text{mg}\cdot\text{kg}^{-1}$ ) (means for years and factors)

Factor	Fat	$\alpha$ -tocopherol	$\beta$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol	Total tocopherol
2013	65.8a	15.13a	40.43a	3.08a	8.51a	67.15a
2014	64.2a	14.50a	39.88a	2.77a	8.26a	65.41a
2015	64.6a	14.74a	39.95a	2.79a	8.40a	65.88a
HSD ( $\alpha = 0.05$ )	5.904	3.909	4.202	0.734	10.008	13.112
F <sup>0</sup> value	0.257	0.081	0.062	0.687	0.002	0.058
p value	0.776	0.922	0.940	0.512	0.998	0.944
Combination I	62.2 a	12.47a	37.52 a	2.73	6.93	59.65 a
Combination II	65.0 a	14.33a	40.37 a	2.85	9.33	66.88 a
Combination III	67.2 a	16,32 b	41.47 b	3.00	9.37	70.15 a
Combination IV	69.1b	18,77 b c	44.38 b	3.23	9.43	75.82 b
Control	60.8 a	12,07a	36.70 a	2.58	6.88	58.23 a
HSD ( $\alpha = 0.05$ )	7.584	4.16	4.27	1.121	15.744	16.96
F <sup>0</sup> value	3.497	7.762	9.107	0.856	0.128	3.222
p value	0.021	0.0003	0.0001	0.503	0.971	0.029
Rawa cv.	60.9 a	12.63 a	42.21 b	2.31 a	0.00 a	57.15 a
Aztek cv.	68.8 b	16.95 b	37.96 a	3.45 b	16.78 b	75.14 b
HSD ( $\alpha = 0.05$ )	2.528	1.99	2.25	0.226	1.33	5.22
F <sup>0</sup> value	40.285	19.94	14.96	107.58	664.83	49.88
p value	7.23E-07	0.0001	0.0006	4.27E-11	4.72E-21	1.11E-07

cially a greater amount of rainfall in the years of research (2006–2007), significantly affected the amount of stored fat. Years with a large sum of precipitation during seed formation and maturation are particularly beneficial for fat accumulation.

Many authors emphasize that the seeds of different varieties of amaranth may differ in their fat content [Bressani 1994, Tömösközi et al. 2009, Petkova et al. 2019]. *Amaranthus hypochondriacus* (Kharkiv variety – 7.81%) has a higher oil content than *Amaranthus cruentus* L. (Andijan variety – 6.39%) [Bozorov et al. 2018]. The fat content obtained in the present study is higher than that found in the same species by Tömösközi et al. [2009] –  $59.5 \text{ g}\cdot\text{kg}^{-1}$ , and lower than that reported by Dodok et al. [1994] –  $73.2 \text{ g}\cdot\text{kg}^{-1}$  and by Soriano-García and Aguirre-Díaz [2019] –  $7.02 \text{ g}\cdot\text{kg}^{-1}$ .

Fat accumulation in the amaranth seeds was significantly influenced by the cultivar and slightly less by

increasing rates of NPK application. The seeds of the Aztek variety accumulated  $7.9 \text{ g}\cdot\text{kg}^{-1}$  (13.0%) more fat than the seeds of the Rawa plants. Significantly more fat was accumulated in the seeds following NPK application at the highest rate (combination IV) as compared to lower application rates and the control treatment, which were not significantly different.

The statistical analysis shows that the amount of accumulated tocopherol homologues and total tocopherols in the amaranth seeds did not depend on temperature and moisture conditions in the years of research (Tab. 2). However, studies by Zubr and Matthäus [2002] show that the amount of tocopherols in the oil of *Camelia sativa* L. Crantz depends on weather and climatic conditions.

Tocopherols are compounds with a ring structure that constitute from 0.5% to 5% of the total fatty substances in oils. They have a significant influence on the biological value of oils because they are lipophilic

antioxidants. These are the most important and best known antioxidants [Nogala-Kałużka et al. 2003], and they include  $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol and  $\delta$ -tocopherol. The highest antioxidant efficiency is observed for  $\delta$ -tocopherol and  $\gamma$ -tocopherol, and the lowest for  $\alpha$ -tocopherol. The reverse pattern applies to biological activity, i.e.  $\alpha$ -tocopherol >  $\beta$ -tocopherol >  $\gamma$ -tocopherol >  $\delta$ -tocopherol. Scientific research indicates that due to the biological activity of vitamin E, determination of  $\alpha$ -tocopherol equivalent may be an important indicator of the quality of rape seeds [Nogala-Kałużka et al. 2003]. Tocopherols protect the phospholipids present in cell membranes, strengthen the walls of blood vessels, reduce the risk of inflammation, and are one of the causes of the hypocholesterolaemic effect of food products containing amaranth seeds [Januszewska-Jóźwiak and Synowiecki 2008].

The dominant tocopherols in amaranth seeds are  $\delta$ - and  $\alpha$ -tocopherol [Tang et al. 2016], while according to Ogródowska et al. [2011], the dominant tocopherols of amaranth oil are  $\alpha$ - and  $\beta$ -tocopherol. Our research shows that the amount of  $\alpha$ -tocopherol accumulated in the lipid fraction was determined to a much greater

extent by the cultivar than by fertilization. The Aztek cultivar contained significantly more  $\alpha$ -tocopherol (by 34.2%) than the Rawa cultivar. The results are supported by the research of other authors. According to Carraro-Panizzi and Erhan [2007] the cultivar is one of the most important factors determining the amount of tocopherols accumulated in the seeds of oilseed plants.

Analysis of the effect of increasing NPK application rates showed that the most  $\alpha$ -tocopherol was accumulated by amaranth grown using the highest level of macroelements – significantly more than in the case of the first and second application rates. We found no differences in the amount of this tocopherol homologue in seeds following the application of the second and third levels of NPK.

The amount of  $\beta$ -tocopherol was more dependent on the level of fertilization than on the cultivar. The third and fourth NPK application rates led to the accumulation of significantly more  $\beta$ -tocopherol than combinations I and II. The Rawa cultivar accumulated more  $\beta$ -tocopherol (by 11.2%) than the Aztek plants.

Czaplicki et al. [2012] have also found the dominant homologues in amaranth seeds to be  $\beta$ -tocopherol

**Table 3.** Content of fat ( $\text{g}\cdot\text{kg}^{-1}$ ), tocopherol homologues and total tocopherol ( $\text{mg}\cdot\text{kg}^{-1}$ ) (means for interaction of factors)

Interaction of factors		Fat	$\alpha$ -tocopherol	$\beta$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol	Total tocopherol
Year	Fertilizer combination	63.3	12.85	37.75	2.88	8.39	66.15
HSD ( $\alpha = 0.05$ )		2.088	11.56	11.87	3.044	44.06	47.31
F <sup>0</sup> value		0.0069	0.0013	0.0047	0.007	3.71E-07	0.0003
p value		0.999	0.999	0.999	0.999	0.999	0.999
Years	Cultivar	64.9	14.79	40.09	2.88	8.39	66.15
HSD ( $\alpha = 0.05$ )		2.690	2.149	2.44	0.202	1.45	5.64
F <sup>0</sup> value		0.0085	0.0069	0.0006	1.739	0.043	0.015
p value		0.992	0.993	0.999	0.197	0.958	0.985
Cultivar	Fertilizer combination	64.9	14.79	40.09	2.88	8.39	66.15
HSD ( $\alpha = 0.05$ )		0.710	0.257	0.260	0.165	0.144	0.736
F <sup>0</sup> value		6.54	17.33	44.26	0.725	309.81	14.62
p value		0.0016	2.78E-06	1.16E-09	0.585	1.11E-17	9.76E-06



and  $\delta$ -tocopherol, which accounted for 34% and 31% of all analysed tocopherols. Similar information and relation (percentage value) can be found in other studies [USDA 2019]. Tang et al. [2014] conducted an interesting study on the content of tocopherols in lipophilic phytochemicals of 14 varieties of amaranth leaves, and detected the presence of  $\alpha$  and  $\beta$ -tocopherols.

Our research shows that the amount of  $\gamma$ -tocopherol and  $\delta$ -tocopherol depended only on the cultivar. The Aztek cultivar accumulated more of these homologues than the Rawa cultivar.

Total tocopherol content depended more on the cultivar than on fertilization. Significantly more total tocopherol (31.5%) was found in the Aztek cultivar than in the Rawa variety. Gunstone et al. [2007] have reported that the average content of tocopherols in the seeds of various cultivars of *Amaranthus cruentus* L. is 4.94 mg·100 g<sup>-1</sup> (49.4 mg·kg<sup>-1</sup>) DW of seeds, and it can vary from 2.8 to 7.8 mg·100 g<sup>-1</sup> (28.0 to 78.0 mg·kg<sup>-1</sup>) DW. In the present study, the total content of tocopherol was within the range reported in the literature [Skwaryło-Bednarz 2012, USDA 2019]. Furthermore, it should be emphasized that the highest level of fertilization had a significant effect on the total content of tocopherols. Numerous studies indicate that the agrotechnical factor has a pronounced and significant effect on the concentration of total tocopherols in the seeds of typical oil plants [Egesel et al. 2008, Velasco et al. 2002], as well as other industrial crops, cereals and pseudocereals [Egesel et al. 2003, Marwede et al. 2005, Tarasevičienė et al. 2019].

In our research, the only significant interaction was between the cultivar and levels of macroelement fertilization for the quality parameters of the seeds, except for the content of  $\gamma$ -tocopherol (Tab. 3).

## CONCLUSIONS

In this work it was possible to evaluate that the factor that most influenced the content of crude fat in the amaranth seeds was the cultivar and followed by the combinations of macroelement fertilizer. The amount of  $\alpha$ -tocopherol and total tocopherol significantly depended on the genetic factor, i.e. the cultivar, and on the combination of NPK fertilization. In addition, the amount of  $\beta$ -tocopherol was more determined by the macronutrient fertilization combination than by the

cultivar. On the other hand the content of  $\gamma$ -tocopherol and  $\delta$ -tocopherol was dependent only on the cultivar. Due to their high content of the bioactive substances tested, amaranth seeds can be a valuable source of functional food.

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