

## **Influence of agroecological factors on biologically active compounds in globe artichoke heads**

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**Abstract.** The composition of biologically active compounds in plants depends on the climate and growing conditions, cultivar properties, plant development stage, harvesting time and other factors. The research aimed to evaluate the effect of agroecological factors on the composition of biologically active compounds in globe artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori) heads. The experiment was carried out under open field conditions in the Institute of Horticulture, in Pūre Research centre during the vegetation period of 2015 and 2016. The experiment was arranged in two different soils: brown soil with residual carbonates and the soil strongly altered by cultivation and used two types of seed treatment (without vernalisation, with vernalisation). The quality was evaluated at each harvest time during the all vegetation period. Significant influence of the tested factors on the biochemical content of heads was stated on the content of phenols (73–213 mg GAE per 100 g fresh weight) and vitamin C in artichoke heads (5–20 mg per 100 g fresh weight). Pigments, flavonoids and antiradical activity was not significantly influenced by the tested factors. A slight tendency on producing a higher content of biologically active compounds in more harsh conditions was observed, particularly for phenols and flavonoids.

**Key words:** *Cynara cardunculus*, flavonoids, pigments, soil.

### **INTRODUCTION**

The globe artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori), (Fam. *Asteraceae*) is widely distributed all over the world and especially in South Europe, the Middle East, North Africa, South America, the United States and China (Pandino et al., 2013). Artichokes have a long history as a medicinal plant, used in folk medicine since Roman times. All parts of the plant can be used for medicinal purposes (Christaki et al., 2012). The medicinal uses of artichokes are very wide. The edible part of the plant is the immature inflorescence, commonly called capitulum or head. Artichoke heads are of low protein and fat content, and a high content of minerals, vitamins, carbohydrates, inulin and polyphenolic compounds (Kolodziej & Winiarska, 2010; Pandino et al., 2013).

Artichokes have a higher polyphenol content compared to other vegetables. They have beneficial effects on consumer health (Turkiewicz et al., 2019). Polyphenolic compounds have strong antioxidant properties, although their content varies between different artichoke varieties and plant parts (Ciancolini et al., 2013). Phenols are characterised by a 90% higher antioxidant activity compared to, for example, vitamin C and carotenoids (Correa et al., 2015). Although the description of artichoke cultivation can be found in the oldest books on horticulture dating back to the 1st half of the 20th century (Gailītis, 1946), nowadays they are not widely grown in Latvia - only in home gardens for experimental and/or ornamental purposes.

Globe artichoke is a perennial herbaceous plant (Cult et al., 2002). In Latvia, there are hard wintering conditions for artichoke to overcome winter periods characterized by fluctuating and often too low soil and air temperature damaging plants. Long periods without snow or often thaws are typical in the period under changing climate conditions. It has a negative influence on the plant's overwintering ability (Bratch, 2014). Therefore, artichokes are considered as an annual plant in Latvia conditions. Also in Poland artichokes are grown as an annual crop (Salata et al., 2012). For the first year of production, vernalized seeds are used or the plants are planted before frost to ensure a 10-day cold period below 10 °C (Fernandez & Curt, 2005). Artichokes are characterised by high genetic variability, resulting in inhomogeneous cultivar material. It is referred that even 15–25% of the plants may be unproductive in the first year, even if they have received the necessary cold conditions (Bratsch, 2014).

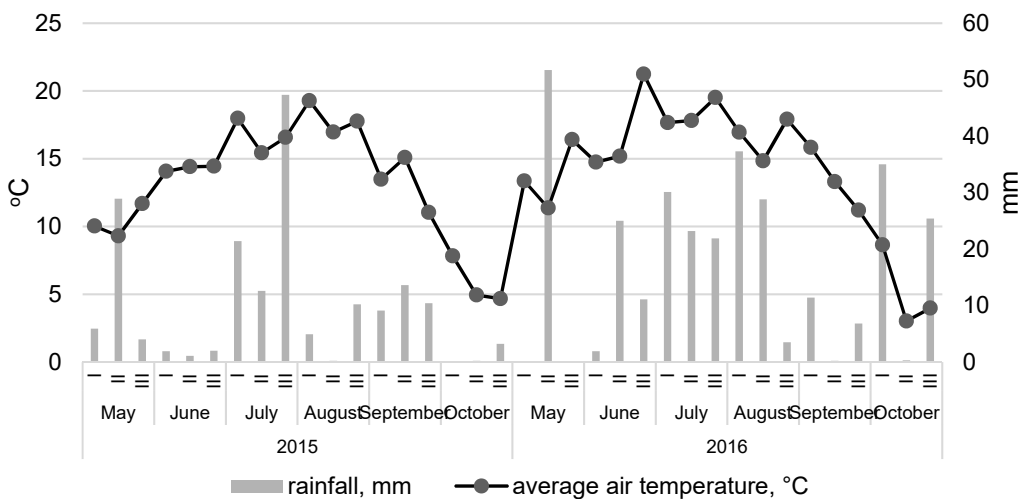
Seed germination is strongly influenced by temperature and substrate. Different substrates and materials can be used for seed germination: filter paper, sand, soil, compost, peat (Lekič et al., 2011). Artichokes are well adapted to different soil conditions. Soils with good water drainability, fertile, high nutrient content and deep tilled are the most suitable for artichoke cultivation, as they have a deep root system. Heavy clay soils and light sandy soils are not preferred for artichoke cultivation (Bratsch, 2014; Colla et al., 2012). The optimal temperature for artichokes growth and development is day temperatures between 20–25 °C and night temperatures between 12–14 °C for good quality and high yields (Ciancolini, 2012; Bratsch, 2014). The plants are also tolerant to higher temperatures, but the quality of the heads decreases at 30 °C (Smith et al., 2008). Drought is one of the main abiotic stress factors affecting plant growth and development. It is reported that at least 500 mm of total water is required (Shinohara et al., 2011). Limited water supply is one of the main factors that also affect plant physiological and metabolic processes. Water stress can significantly reduce plant height, shoot and root dry mass. Abiotic stress has a significant effect on the biochemical content of the plant itself and on the artichoke heads. During the long period of drought stress, photosynthetic activity in the plant is reduced, as well as antioxidant activity and vitamin C content are negatively influenced (Tanha et al., 2014).

The influence of agroecological conditions on the growth and biochemical composition of globe artichoke in Latvia is not investigated until now. The research aimed to evaluate the effect of agroecological factors on the content of biologically active compounds of globe artichoke in Latvia. Research hypothesis - ensuring suitable agroecological factors can increase the content of biologically active compounds in globe artichoke heads.

## MATERIALS AND METHODS

The investigation was carried out in Pūre (Tukums district, Latvia 57°2'9"N 22°54'25"E) in the vegetation seasons of 2015 and 2016. The trial was set up in a two-factorial design, with four replications, where factor A - soil (strongly altered by cultivation soil - A<sub>1</sub>, brown soil with residual carbonates - A<sub>2</sub>) and factor B - the type of treatment of germinating seeds (without vernalisation - B<sub>1</sub>, with vernalisation - B<sub>2</sub>). In the beginning at March, the seeds of artichokes variety 'Green Globe' were germinated in Petri dishes on filter paper saturated with water. After 10 days, half of the sprouts were planted in trays and half placed in the refrigerator at 2–3 °C and after two weeks transplanted in 12 cm diameter plastic pots. Two different soils were compared for artichoke growing: strongly altered by cultivation soil (Ant), with high content of nitrogen and phosphorus, low content of potassium (total N 0.21%, P<sub>2</sub>O<sub>5</sub> - 352.1 mg kg<sup>-1</sup>, K<sub>2</sub>O - 133.5 mg kg<sup>-1</sup> and organic matter 5.4%) and brown soil with residual carbonates (BRk), with optimal content of nitrogen, phosphorus and potassium (total N 0.10%, P<sub>2</sub>O<sub>5</sub> - 190.4 mg kg<sup>-1</sup>, K<sub>2</sub>O - 191.8 mg kg<sup>-1</sup> and organic matter 2.8%). In the middle of May, the plants were planted on the field, in 4 replicates, with a planting scheme of 0.7×0.9 m. The first artichoke heads were harvested at the beginning of August. As the artichoke plant develops heads continuously through the vegetation period, and they mature gradually, all heads were harvested from each plot continuously every week until October for both years.

Meteorological conditions during the investigation period (precipitation and average air temperature) were collected by an automatic meteorological station 'Lufft' located at Pūre (Fig. 1). Overall the years 2015 and 2016 were suitable for artichokes yield production, although they were not optimal in terms of humidity and temperature, especially 2015 was too dry for good artichoke yield production. In 2015 rainfall was only 177 mm and in 2016 - 314 mm.



**Figure 1.** Meteorological conditions of vegetation seasons of 2015 and 2016, in 10-day periods (I, II, III).

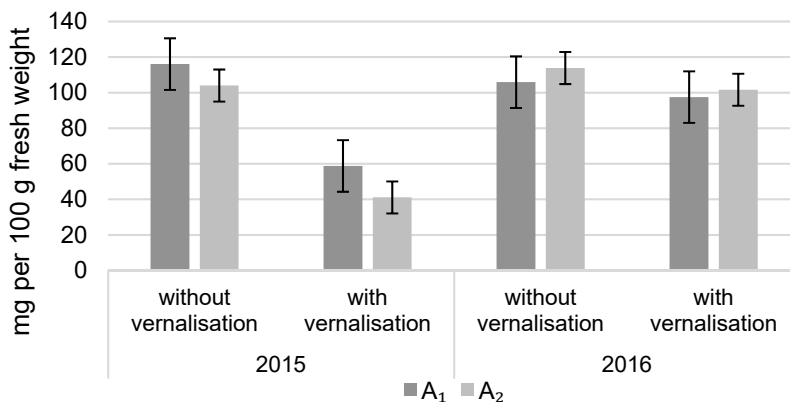
Biochemical content analyses for artichoke heads were performed at the Latvia University of Life Sciences and Technologies, Institute of Soil and Plant Sciences. All biochemical parameters were determined for each harvest time for the average sample from the variant in three replications. The data were analysed by averaging over the vegetation period.

The chlorophyll and carotenoid contents were determined spectrophotometrically in an ethanol solution, the light absorbance of the solution was read at 665 nm, 649 nm and 440 nm wavelengths. The content of chlorophyll and carotenoid in the plant material was expressed as mg per 100 g fresh weight (Linchtenthaler, Buschmann, 2001). The ascorbic acid content was determined by titration of filtrate of the acidified (HCl+ HPO<sub>3</sub>) extract with 0.001 M iodine. The content of vitamin C (ascorbic acid) in the plant material was expressed as mg per 100 g fresh weight (Moor et al., 2005). The anthocyanin content was determined spectrophotometrically and the light absorbance of the solution was read at 535 nm. The content of anthocyanin in the plant material was expressed as mg per 100 g fresh weight (Moor et al., 2005). The phenolic content was determined spectrophotometrically in acidified ethanol solution; the light absorbance of the solution was read at 320 nm. The content of phenols in the plant material was expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight (Singleton et al., 1999). Flavonoids were determined spectrophotometrically in ethanol solution; the light absorbance of the solution was read at 415 nm. Flavonoid content in the plant material was expressed as mg quercetin equivalent (QE) per 100 g fresh weight (Kim et al., 2003). The antiradical activity was determined spectrophotometrically in methanol extract using 1.1. - diphenyl - 2 picrylhydrazyl radical (DPPH\*) the light absorbance of the solution was read at 517 nm. The antiradical activity was calculated as a percentage (%) of DPPH discoloring (Barros et al., 2007). The spectrophotometer used for the analyses was a 'SHIMAZU UV-1800'. The yield from each plot was weighted by using the weighting scales KERN at each harvest time.

The results were analysed using Microsoft Excel 2016 and STATISTICA TM, at the significance level of  $\alpha = 0.05$ . The data were processed using ANOVA. The data were processed using dispersion analysis. A significant difference (*LSD*) between individual factor values is indicated in the graphs. A 95% confidence level was used to determine the significance of the difference between the variables.

## RESULTS AND DISCUSSION

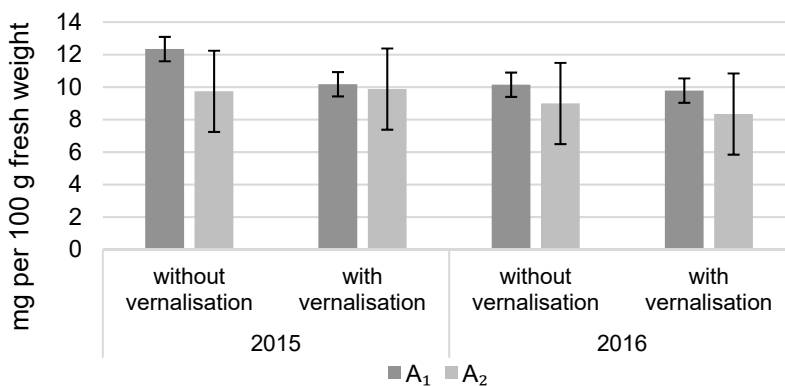
The chlorophyll content is often measured to assess plant growth intensity, as it is closely related to photosynthetic rate. Differences in biochemical content of plants between all variants were evaluated in both years of harvest. The average chlorophyll content of artichoke heads significantly differed between years ( $p = 0.000$ ) and between seeds treatments ( $p = 0.000$ ) when evaluated several times per season. It was not significantly affected by the soil ( $p = 0.37$ ). In both years, the average chlorophyll content in all experimental variants ranged from 41 to 114 mg per 100 g fresh weight (Fig. 2). In the first year, the higher chlorophyll content was determined in plants grown in the strongly altered by cultivation soil, while in the second year the opposite tendency was observed: the higher chlorophyll content was in the artichoke heads grown in the brown soil with residual carbonates, but it should be stressed that the differences were not statistically significant.



**Figure 2.** Chlorophylls content in the analysed artichoke heads: A<sub>1</sub> – strongly altered by cultivation soil; A<sub>2</sub> – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A<sub>1</sub> – 29, A<sub>2</sub> – 18).

In both years, the chlorophyll content was higher in the variants where the seeds germinated without vernalisation, but this difference was significant only in 2015. A notable difference between years in agroecological conditions was observed - the lower temperature and precipitation in June and July of 2015 in comparison to 2016. This leads to an assumption that plants grown from vernalised seeds are more responsive to unfavourable conditions during the period of intensive plant growth and heads formation (June - July). In Spain, the evaluation of artichoke heads of two varieties and three hybrids showed chlorophyll contents ranging from 16–55 mg per 100 g fresh weight (Turkiewicz et al., 2019). In comparison to this reference, our plants had higher chlorophyll content.

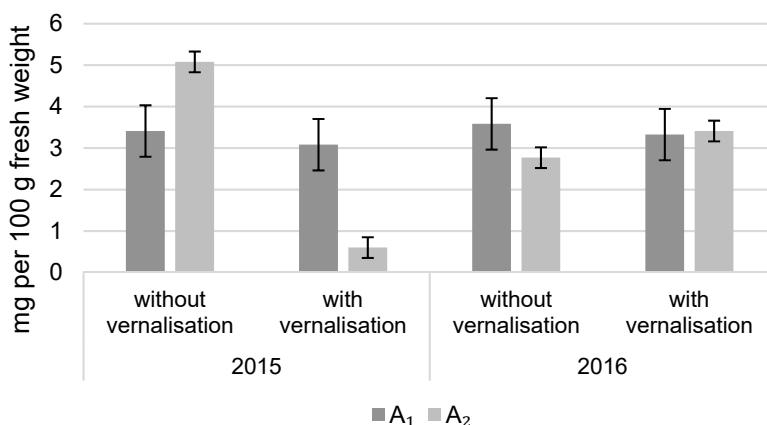
The average carotenoid content of artichoke heads over the growing season was significantly different between years ( $p = 0.02$ ) and between soils ( $p = 0.01$ ), but was not significantly affected by the type of seed treatment ( $p = 0.13$ ). In both years, the carotenoid content on average in all experimental variants ranged from 8.3 to 12.3 mg per 100 g fresh weight (Fig. 3).



**Figure 3.** Carotenoid content in the analysed artichoke heads: A<sub>1</sub> – strongly altered by cultivation soil; A<sub>2</sub> – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A<sub>1</sub> – 1.5, A<sub>2</sub> – 5).

In both years, there was observed a common tendency for the carotenoid content to be insignificantly higher in the cultivated soil, except in 2015 for the variant without vernalization treatment, when there were observed significant differences between the soils. This leads us to assume that in rich soil more carotenoids are produced by the plant in comparison to less fertile soil. The carotenoid content of artichoke heads in the cultivars and hybrids evaluated in Spain was similar to the values obtained in the described trial, ranging from 3.5 to 8.5 mg per 100 g fresh weight (Turkiewicz et al., 2019).

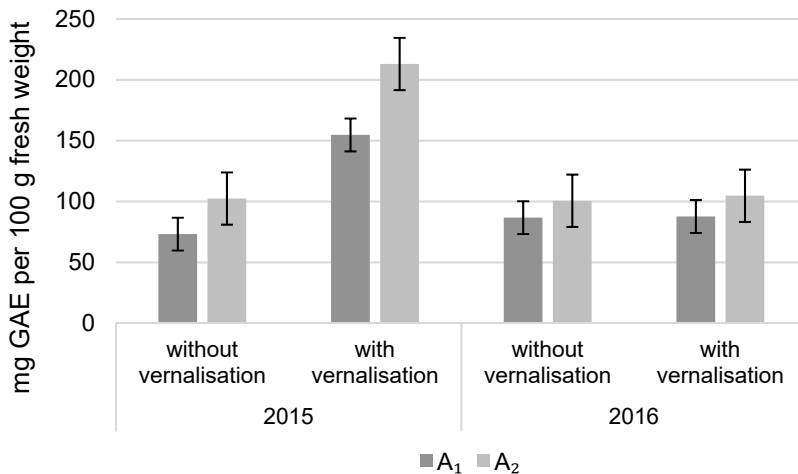
The high genetic variability of the artichoke varieties resulted in visual differences in the intensity of the blue colour hue produced by the anthocyanins (Bekheet & Sota, 2019). When compared statistically, only the seed treatment had a significant effect on the anthocyanin content in the artichoke heads throughout the growing season ( $p = 0.000$ ), although a clear tendency for one particular investigated factor influence on the anthocyanin content in the plants was not found. In both years, the anthocyanin content averaged over all experimental variants ranged from 0.6 to 5.1 mg per 100 g fresh weight (Fig. 4), which is similar to plants grown in Tunisia, where anthocyanin content varied between 2.5 and 3.7 mg per 100 g fresh weight, depending on the variety (Dabbou et al., 2017).



**Figure 4.** Anthocyanin content in the analysed artichoke heads: A<sub>1</sub> – strongly altered by cultivation soil; A<sub>2</sub> – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A<sub>1</sub> – 1.1, A<sub>2</sub> – 0.5).

The two main phenolic compounds found in artichoke heads are chlorogenic acid and cynarin. They have strong antioxidant activity. The phenolic content and their activity in artichokes may vary depending on the plant part and variety, the ripeness of the head, storage and processing (Shinohara et al., 2011). In both years of the trial, the phenolic content varied from 73 to 213 mg GAE per 100 g fresh weight (Fig. 5). All factors in the trial had a significant influence ( $p = 0.00$ ) on the phenolic content of artichoke heads. In both harvest years, there was a common tendency when more phenolics were synthesised in plants grown on less fertile brown soil with residual carbonates (differences were not significant). In 2015, regarding the phenolic content, there were stated significantly higher values in the heads of plants grown by vernalisation treatment.

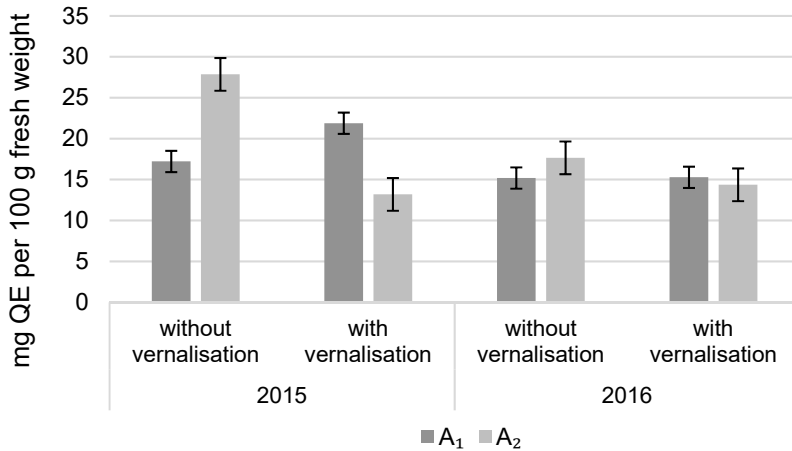
A study in Texas has shown that under drought stress, more phenolic compounds are synthesised (Shinohara et al., 2011). The data of 2015 somehow is in agreement with the finding of Shinohara (2011), that less favourable conditions promote the synthesis of phenols. Especially it was observed for vernalised plants, which are assumed as being in light stress in 2015 also in accordance with chlorophyll data.



**Figure 5.** Phenols content in the analysed artichoke heads: A<sub>1</sub> – strongly altered by cultivation soil; A<sub>2</sub> – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A<sub>1</sub> – 27, A<sub>2</sub> – 43).

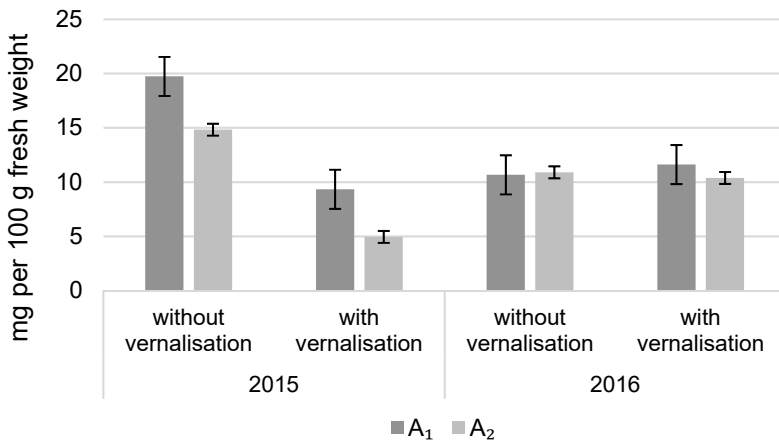
The flavonoids in the plant affect its colour, taste and aroma (Sergejeva et al., 2018). Flavonoids are the largest group of polyphenols and their content is also influenced by various factors such as genotype, environmental conditions (temperature, light, soil), cultivation system, and storage. Several methods are used to determine polyphenols in plants, which play an important role in data interpretation. Consequently, it is often difficult to compare data from different studies. Genotype and analysed plant parts (head, stem, leaves) are the main factors influencing the content of polyphenol compounds in the sample. In a study carried out in Italy, evaluating 17 globe artichoke cultivars, their total polyphenolic content varied from 12 to 59 mg quercetin equivalent (QE) g<sup>-1</sup> fresh weight (Pandino et al., 2013). The values of flavonoid content obtained in the described trial varied from 13 to 28 mg QE per g fresh weight (Fig. 6).

Statistically significant differences in the flavonoid content of artichoke heads were observed between trial years as well as between seeds treatment ( $p = 0.000$ ). The soil type did not have a significant effect. A common tendency was observed in both crop years: plants grown without vernalization had a higher flavonoid content in brown soil with residual carbonates (significant only in 2015), while in the variant with vernalisation more flavonoids were synthesised in the plants grown in strongly altered by cultivation soil (significantly only in 2015). Comparing the flavonoid content of the plants by year, the highest flavonoid content in artichoke heads was found in 2015. Which supports previous statements in our research and also refers to others about the plants defence mechanisms in unfavourable conditions by increased production of physiologically active compounds (Kolodziej & Winiarska, 2010).



**Figure 6.** Flavonoid content in the analysed artichoke heads: A<sub>1</sub> – strongly altered by cultivation soil; A<sub>2</sub> – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A<sub>1</sub> – 2.6, A<sub>2</sub> – 4).

The differences in the content of vitamin C in artichoke heads were significant between all tested factors ( $p < 0.05$ ). From all harvests over the two years, the average vitamin C content of the heads varied between 5 and 20 mg per 100 g fresh weight per variant (Fig. 7). In 2015, the content of vitamin C was significantly higher in non-vernalised plants in both soils. However, no significant differences were observed in 2016.



**Figure 7.** Vitamin C content in the analysed artichoke heads: A<sub>1</sub> – strongly altered by cultivation soil; A<sub>2</sub> – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A<sub>1</sub> – 3.6, A<sub>2</sub> – 1.1).

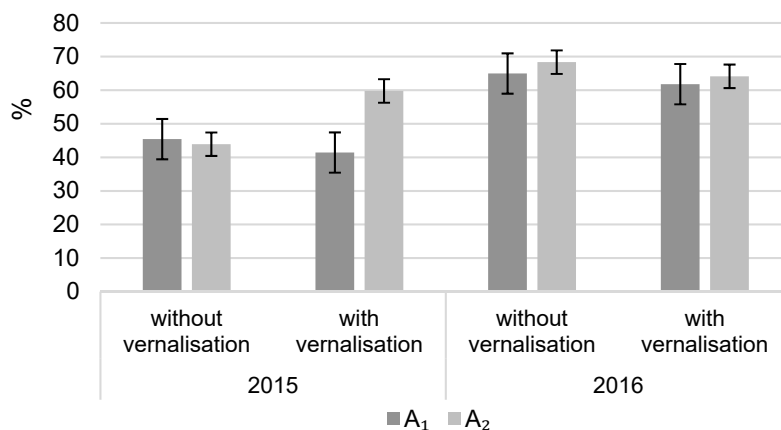
Two varieties of globe artichoke were grown in Poland over for three years and the vitamin C content of the petals and the central part of the heads was determined. One of the varieties ('Symphony') had an average vitamin C content of 14.5 mg in the central



part of the heads and 11.3 mg the outer petals per per 100 g fresh weight over the three years. The other variety ('Madrigal') showed a significant difference between the vitamin C content in the central part of the head and in the outer petals, 15.8 and 6.5 mg per 100 g fresh weight respectively (Salata et al., 2012).

From the results over the two trial years, it is not possible surely determine which conditions promote the synthesis of vitamin C. Also in southern Italy, differences were found between genotypes and harvest times. The four genotypes were evaluated for vitamin C content between 3 and 6 mg per 100 g fresh weight and were higher at later harvest times, but the meteorological conditions were not specified in the description of the experiment to compare with the results of the designed study (Melilli et al., 2013). In another study in Italy, the content of vitamin C in artichoke heads was 14 mg per 100 g fresh weight (Dosi et al., 2013).

The antiradical activity differs from the colorimetric method used (Dabbou et al., 2017; Mabeau et al., 2007). In Poland, it was found that, depending on the method, the antiradical activity of an extract obtained from globe artichoke leaves of the cultivar 'Green Globe' ranged from 44% (DDPH method) to 80% (ABTS method) (Biel et al., 2019). In the other trial in Tunisia, when two cultivars were grown and analysed by the DPPH method, antiradical activity was determined to be 63 and 70% (Dabbou et al., 2017). In the established trial, the antiradical activity (as determined by the DPPH method) varied on average between 41 and 68% over the two years (Fig. 8). However, the results are not exactly comparable as different stock solutions and reagent volumes were used in each trial, resulting in different dilutions.



**Figure 8.** Antiradical activity in the analysed artichoke heads: A<sub>1</sub> – strongly altered by cultivation soil; A<sub>2</sub> – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A<sub>1</sub> – 12, A<sub>2</sub> – 7).

There were stated significant differences between years ( $p = 0.000$ ) and between soils ( $p = 0.01$ ), while the type of seed treatment did not have a statistically significant influence on the antiradical activity ( $p = 0.57$ ). In general, there was an observed tendency for having higher antiradical activity in plants grown on less fertile brown soil with residual carbonates, but overall the differences were not significant. In 2016, there were not found significant differences between any factors. After two years of the trial,

it seems that in less favourable meteorological conditions (2015) antiradical activity of plants is lower. Further trials are necessary to certainly approve the observed tendencies.

## CONCLUSIONS

The content of phenols and vitamin C in artichoke heads was significantly influenced by all the factors evaluated in the trial. The common influence was stated for the vegetation season - both components were higher in the harshest year - 2015. The chlorophyll and flavonoid contents were significantly affected by the conditions of the year and type of seed treatment, when chlorophyll was higher in better meteorological conditions in 2016, but flavonoids were higher in not so favourable conditions - in drier 2015 and less fertile soil. Both parameters were negatively influenced by the seed treatment - vernalisation. The carotenoid content and antiradical activity were significantly affected by the differences in the years and soils - higher carotenoid content was stated in the dry vegetation period of 2015 and more fertile soil, but ARA was higher in 2016 in less fertile soil. Only the vernalisation had a significant effect on the anthocyanin content. Often no significant effect was found for a single factor, but the effect was significant in combination with other factors. There was no observed common tendency for particular conditions to ensure a higher level of all bioactive compounds in artichokes. A slight tendency on producing a higher content of biologically active compounds in more harsh conditions was observed, particularly for phenols and flavonoids. Further trials are necessary in a longer period time to cover more different agroclimatic conditions to get a clear understanding of the influence of different factors on the content of biochemically active compounds in artichoke plants.

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