ISSN 1644-0692
www.acta.media.pl

ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol. Hortorum Cultus, 14(4) 2015, 155-167*

THE POLYPHENOLIC COMPOUNDS CONTENT OF A CARDOON HERB DEPENDING ON LENGTH OF THE VEGETATION PERIOD

Andrzej Sałata[1], Halina Buczkowska[1],
Salvador Vicente Lopez Galarza[2], Hector Moreno-Ramon[2]

[1] University of Life Sciences in Lublin, Lublin, Poland

[2] Universitat Politecnica de Valencia, Valencia, Spain

Abstract. The influence of vegetation period length of cardoon plants (*Cynara cardunculus* L.) on herb yield and its pharmacological value conditioned by chemical contents in air dried herb was examined in the research. The research conducted in the years 2009–2011 included valuation of total phenolic acids as equivalent to caffeic acid, flavonoids and tannins content in plants during their vegetative growth. The effect of the vegetation period length on cardoon plants yield was observed. The content of polyphenolic acids (caffeic, chlorogenic and cynarine) was marked with a performance liquid chromatography (HPLC).The contents of biologically active compounds depended on plants age – the most phenolic acids were noted in herb harvested from plants 120-days and 150-days old (1.86–2.58%). Herb obtained from plants of different age contained from 0.38 to 0.43% flavonoids. More tannins were accumulated in young cardoon plants after 90–120 days of cultivation (3.72–3.43%) in comparison to plants 150-days old (3.25%). The content of caffeic acid, chlorogenic acid and cynarin in cardoon herb depended on length of vegetation period of plants. The content of phenolic acids in leaves increased with time. Values of correlation coefficients indicate significant correlation of total phenolic acids and tannins content in cardoon herb. The higher content of total phenolic acids the lower content of tannins (R = -0.88). Strong correlation was noted between the content of total phenolic acids and the content of chlorogenic acid (R = 0.67) and caffeic acid (R = 0.78). On the basis of the results of the presented work it could be stated that cardoon leaves are a valuable material for herb industry.

Key words: Cynara cardunculus, phenolic acids, flavonoids, tannins, cynarin, chlorogenic acid, caffeic acid

Corresponding author: Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin, Lublin, Leszczyńskiego 58, 20-068, Lublin, e-mail: andrzej.salata@up.lublin.pl

INTRODUCTION

Cynara cardunculus L. is a member of the Asteraceae family including the globe artichoke (*C. cardunculus* L. var. *scolymus* (L.) Fiori), the cultivated cardoon (*C. cardunculus* var. *altilis* DC), and the wild cardoon (*C. cardunculus* L. var. *silvestris* (Lamk) Fiori) [Sonnante et al. 2002].

In cardoon, cropleaf petioles are harvested at the end of the bleaching operation, when heads still have not been developed. The crop plays an important role in the Mediterranean countries. In Central Europe a cardoon is mainly treated as a valuable herbal plant. The pharmacological material are dried leaves. Cardoon contains a high level of phenolic compounds, especially in leaves, as reported by several authors [Fratianni et al. 2007, Falleh et al. 2008, Pandino et al. 2011]. In available literature the wide therapeutic spectrum of polyphenolic compounds included in cardoon herb is emphasized [Balasundram et al. 2006].

The phenolics include cynarin (1,3-di-O-caffeoylqunic acid), luteolin, cynaroside (luteolin-7-O-glucoside), scolymoside (luteolin-7-rutinoside) and phenolic acids such as caffeic, coumaric, hydroxycinnamic, ferulic, and caffeoylqunic acid derivatives, mono- and dicaffeoylqunic acids [Pandino et al. 2011].

Diversity of active substances found in cardoon includes it into a group of medicinal plants of a broad spectrum of pharmacological activity. Compounds included in cardoon herb are credited with efficient activity in cure of disorders of the digestive and circulatory systems, they protect organism against cancer and stimulate immune system [Rossoni et al. 2005]. Phenolic acids included in cardoon are cholagogic, they increase transport of bile to duodenum [Gebhardt and Fausel 1997]. Phenolic compounds strengthen and regenerate liver cells [Bundy et al. 2008], they also protect liver [Gebhardt 2005] and increase volume and good glow of bile, what allows to remove harmful substances dangerous to liver [Speroni et al. 2003]. An increased secretion of bile caused by cardoon extract decreases level of triglycerides in blood serum [Rossoni et al. 2005]. A decrease in total cholesterol and LDL fraction were confirmed in patients with lipid metabolism disorders [Kraft 1997].

Flavonoids and caffeic acid derivatives originate from phenylpropanoid pathway. This biosynthesis pathway, starting from phenyloalanine, is induced upon biotic (i.e. pathogen attack) and abiotic (UV irradiation, wounding) stresses [Benlloch-Gonzàlez et al. 2005]. According to Pandino et al. [2011], phenolic compounds accumulate in specific parts of the plant of cardoon can protect leaf cells from photo-oxidative damage from excess ultraviolet (UV) light. Content of phenolic compounds in cardoon leaves depends, among other things, on variety features [Pinelli et al. 2007], plant organs [Schütz et al. 2004], date of harvest [Bano et al. 2003, Lutz et al. 2011].

Actual state of research on cardoon concerns mainly on cultivation of this species in Mediterranean climate. There are no works available on cultivation of cardoon in continental climate, in less favourable conditions for this thermophilous plant.

The aim of the undertaken research was to evaluate the influence of the length of vegetation period of plants on biologically active compounds contents in cardoon herb.

MATERIALS AND METHODS

The research was conducted in the years 2009–2011 in the Department of Vegetable Crops and Medicinal Plants of the University of Life Sciences in Lublin, Poland (51º14'N; 22º34'E). The plant material were cardoon plants (*Cynara cardunculus* L.) imported from the Rijnsburg seed company (the Netherlands), for purpose of obtaining raw material for pharmaceutical industry.

The field experiment was established according to random blocks method with four replications. Cardoon achenes were sown directly in the field, three achenes per spot, with spacing of 0.4 × 0.4 m. The plot area was 10 m^2 and there were 60 plants per plot (6.25 plants per 1 m^2). Thinning was done in a phase of 2–3 proper leaves, leaving 1 plant per spot. In the years 2009–2011 seeds were sown in the field in the first decade of May each year.

The experiments were conducted on a loessal soil of mechanical composition of loamy sand containing 1.6% of organic matter. Runner bean was a forecrop for cardoon in the successive years of cultivation. The nitrate, phosphorus and potassium fertilization in the form of ammonium nitrate, superphosphate and potassium chloride were carried out according to the results of the chemical analysis of the soil in order to obtained final concentration of: 120 mg N ($NO_3^+NH_4^-$), 50 mg P, 190 mg K, 55 mg Mg, in 1 dm^3 of the soil. The pH of the soil was 7.1. While harvesting plants during vegetative growth, leaves were cut 2 cm above the ground level. Cardoon herb harvests were done in the following dates: from 90-days old plants – in the first decade of August, 120-days old plants – in the first decade of September and 150-days old plants – in the first decade of October. In the years of research, cardoon herb was harvested from each plot, from 25 randomly selected plants of each replication, what constituted the yield of fresh herb. Directly after harvest fresh plant material was dried in a drying room in the temperature of 40°C. On the basis of the herb weight after being dried, the yield of air dry herb was counted. Samples of 1 kg were prepared from air dried herb from each combination. They were grinded in a grinder with sieve diameter of 1 mm. Samples of grinded material were stored in hermetical containers for laboratory use.

The extraction procedure, performed for samples under study, was adapted from Pinelli et al. [2007]. Power plant material (10 g)was extracted with methanol (1:10) in a reflux condenser in the temperature of the solvent boiling point for 3 hours, then after percolation, material was again treated with 80% of methanol and extracted twice for 2 hours. Methanol extracts were joined, solvent was evaporated, and the remains were eluted with hot water (50 ml). Water solutions were left for 24 hours in a refrigerator. Separated tarry residues containing ballasts were filtered and rinsed with distilled water. Obtained in this way filtrate was degreased by shaking 3 times with light petroleum (30 ml each), then purified water solutions were extracted 10-times with diethyl ether (20 ml each). Joined ether extracts were concentrated to 100 ml volume and shaken 10 times with 5% water solution of $NaHCO_3$ (10 ml each) in order to transform phenolic acids into salts readily soluble in water. Bicarbonate fractions, including phenolic acids salts, were acidified with 35% HCl to pH = 3 and in this way free phenolic acids were obtained which were again extracted with diethyl ether by 10 times shaking with

this solvent (10 ml each). Ether extracts were joined and dried with anhydrous Na_2SO_4. The solvent was then distilled till dry, giving free phenolic acids fractions.

The total phenolic acids equivalent to caffeic acid content in plant material was determined using the spectrophotometric method with Arnov's reagent according to the procedure described in the Polish Pharmacopoeia VI [2002].

The methods was adapted from Pinelli et al. [2007]. The chromatographic determination of phenolic acids was performed by HPLC, using LiChrom-Merck chromatograph equipped with diode-array detector DAD (L-7450), pump (L-7100), decongestant (L-7612), dosing loop 20 µl, thermostat (L-7360). ALiChrospher 100 RP C 18 of 250 mm × 4 mm filled with stationary phase of particles diameter of dp = 5 µm. Mobile phase was acetonitryl-water solution (20 + 80 v/v) with addition of 1% (v/v) of acetic acid. The flow rate was 1.0 ml/min, while the injection was 20 µl. Identification of phenolic acids was conducted on the basis of comparison of their retention times (t_R) with the standard and by determining their UV spectrum (220–400 nm). The content of separate phenolic acids in the examined material was calculated on the basis of calibration curve determined for each identified phenolic acid: caffeic (3.4-O-dicaffeoylquinic), chlorogenic (5-O-caffeoylquinic) and cynarin (1.3-O-dicaffeoylquinic). To identify and determine the content of separate phenolic acids in plant material standards obtained from Roth company were used. The content of phenolic acids in each sample were marked in 4 replications.

The total content of flavonoids was marked with a modified Christ-Müller method [1960], calculated for quercetin and followed the procedure described in the Polish Pharmacopoeia VI [2002].

The content of tannins was marked with gravimetric titration method according to the Polish Pharmacopoeia IV [1970] and AOAC method [1965], after some modification.

The obtained results were evaluated statistically with the analysis of variance with the use of SAS statistical software [2003]. After ANOVA examination the means with significant differences ($\alpha < 0.05$) were separated by Tukey HSD test.

RESULTS

The yield of cardoon herb depended on length of the vegetation period of plants, expressed as a number of days from the date of seeds germination to the harvest done after 90, 120 and 150 days (tab. 1). The yield of fresh herb and air dry herb obtained from older plants, 120 and 150-days old, was higher by 6.6–6.9 and 1.1 $t \cdot ha^{-1}$ respectively, than the one obtained from plants 90-days old.

The fresh and air dry yield of herb differed in the years of the research. Higher yield of fresh and air dry herb was obtained from plants in the years 2009 and 2010 in comparison to the one harvested in the year 2011. The tendency in changing the yield of herb depending on the length of vegetation period of plants was similar and statistically significant in the years of research. The years 2009–2011 were of favourable thermal conditions for cardoon cultivation (fig. 1). Since the beginning of plants vegetation, throughout the following months until cultivation, until to August, the mean temperatures of each month were higher than mean temperatures on many years. Considering

the precipitation, the year 2011 was very unfavourable period for growth of cardoon. In May the monthly sum of precipitation was less than many years sum by 37%, and in June was higher only by 3%. In July, the monthly sun of precipitation was higher than many years sum by 123%. The two successive months, August and September, were period of sporadic rainfalls.

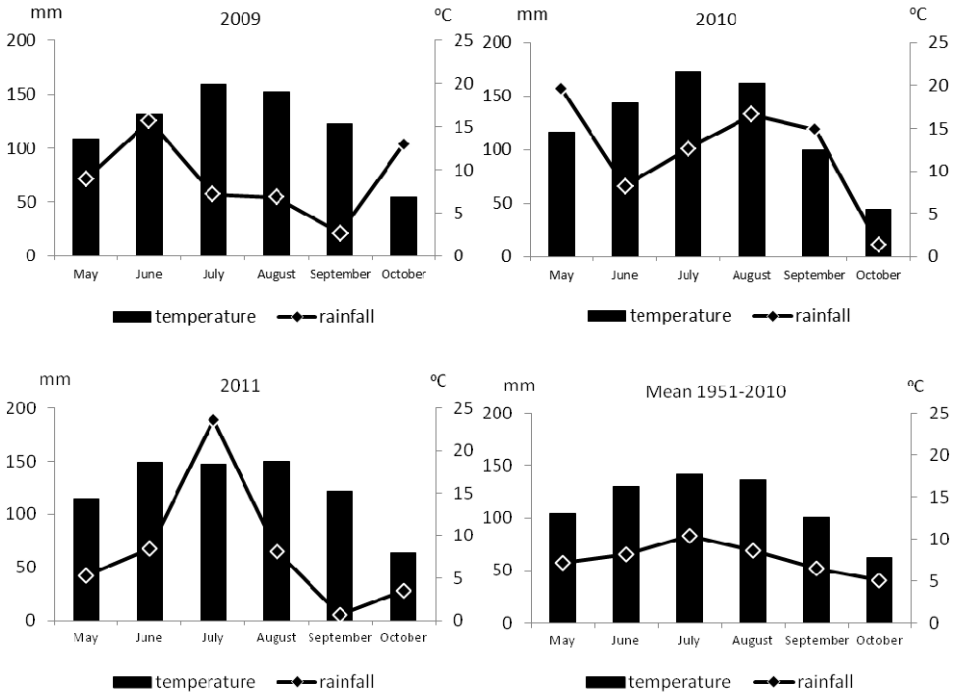

Fig. 1. The total decade and monthly rainfall and average air temperature during research, according to Felin Meteorological Station of the University of Life Sciences in Lublin

In order to estimate the influence of the length of vegetation period on contents of biologically active compounds in raw material, a yield of phenolic acids and flavonoids was calculated (tab. 1). The highest yield of phenolic acids and flavonoids per area unit was obtained from 120- and 150-days old plants, while the lowest one from 90-days old plants. In case of calculated yield of phenolic acids, a tendency in the quantity of yield depending on the length of plants vegetation period was similar in the years of research. Together with extending the length of vegetation period the yield of phenolic acids increased. The yield of flavonoids calculated for plants of different age was less differentiated in the years of research. Evaluating the effect of the length of vegetation period of plants in the second year of the research (2010), the differences in the calculated yield were statistically insignificant. Independently on the length of vegetation period, definitely higher yield of flavonoids was calculated in raw material in the years 2010 and 2011.

Table 1. The yield of fresh herb, air dry herb, yield of phenolic acids and yield of flavonoids depending on the plants vegetation period length (mean from the years 2009–2011)

Treatment	Vegetation period, days		Fresh herb yield ($t \cdot ha^{-1}$)	Air dry herb yield ($t \cdot ha^{-1}$)	Yield of phenolic acids ($kg \cdot ha^{-1}$)	Yield of flavonoids ($kg \cdot ha^{-1}$)
Vegetation period, days (A)		90	14.9a	2.3a	24.4a	9.9a
		120	21.5b	3.3b	61.9b	13.0b
		150	21.8b	3.4b	87.1b	12.7b
Year (B)		2009	20.7a	3.1a	64.2a	16.6a
		2010	19.9a	3.2a	58.3a	10.5b
		2011	17.5b	2.7b	51.0a	8.5b
(A×B)	90	2009	17.2a	2.6a	32.0a	12.4a
		2010	15.9a	2.5a	24.7a	10.4a
		2011	11.6b	1.8a	16.5a	7.0b
	120	2009	23.1c	3.5b	68.3b	19.8c
		2010	21.2c	3.4b	61.1b	9.8a
		2011	20.2c	3.1b	56.4ab	9.4c
	150	2009	21.9c	3.3b	92.3b	17.7a
		2010	22.7c	3.6b	89.0b	11.3a
		2011	20.8c	3.2b	80.0b	9.0ab

* – means values for vegetation period (A), for year mean value (B) and interaction mean values (A × B) within columns with the same letter are not significantly different at $\alpha = 0.05$

The contents of biologically active substances, that are total phenolic acids, flavonoids and tannins in air dry cardoon herb obtained from plants after 90, 120 and 150 days of vegetation are presented in Table 2. Herb harvested from the oldest plants, 150-days old, contained more total phenolic acids in comparison to 120-days plants and to 90-days old plants. Regardless of the length of the vegetation period of plants, the differences in the contents of total phenolic acids in each year were observed. Plants accumulated definitely more phenolic acids in total in the first year of the research and less in the second and the third ones. On the basis of the analyses of the obtained results regarding the contents of the total phenolic acids in the air dry herb, the regularity was observed, that in the years of the research their content increased together with the extending the vegetation period of plants.

In case of herb harvested from plants after 90, 120 and 150 days of vegetation, the contents of flavonoids was similar and differences were statistically insignificant. Irrespective of the vegetation period, herb obtained in the first year of the research characterized with the highest content of flavonoids in comparison to those harvested in the second and the third year. Significant differences in the content of flavonoids were also noted when they were evaluated in respect of correlation between the length of vegetation period and years. Definitely higher content of flavonoids was marked in herb harvested in the first year of the research from plants that had grown for 90, 120 and 150 days, in comparison to the remaining years.

Table 2. The content of total phenolic acids, flavonoids and tannins, in % of air dry weight, in herb depending on the vegetation length (mean from the years 2009–2011)

Treatment	Vegetation period, days		Total phenolic acids	Flavonoids	Tannins
Vegetation period, days (A)		90	1.04*a	0.38a	3.72a
		120	1.86b	0.39a	3.43b
		150	2.58c	0.43a	3.25c
Year (B)		2009	2.09a	0.54a	3.66a
		2010	1.74b	0.33b	3.27b
		2011	1.73b	0.32b	3.46c
(A×B)	90	2009	1.24a	0.48ab	4.04a
		2010	0.97a	0.41b	3.41b
		2011	0.92a	0.39bc	3.71c
	120	2009	1.97b	0.57a	3.80c
		2010	1.81b	0.29c	3.36c
		2011	1.80b	0.30c	3.13b
	150	2009	2.81c	0.54a	3.55bc
		2010	2.45d	0.31c	3.05d
		2011	2.48d	0.28c	3.15d

* – means values for vegetation period (A), for year mean value (B) and interaction mean values (A × B) within columns with the same letter are not significantly different at $\alpha = 0.05$

Statistical evaluation of the data regarding the contents of tannins in the herb of the cardoon showed significant differentiation depending on the length of the vegetation period in the years of the research. It was noted that the longer vegetation period of plants the lower content of total tannins. Herb obtained from the youngest plants, 90-days old, contained more tannins (3.72%) than the one harvested from plants 120-days and 150-days old (respectively 3.43 and 3.25%).

Significant differences between the values of the studied feature were noted also when they were evaluated in the years of the research. The herb harvested in the first year of the research characterized with the highest content of the tannins and it was significantly lower in the second one.

Analyzing the effects of correlation between the length of the vegetation period of the plants in the years of the research, the relation that 90-days plants contained highest tannins than 120- and 150-days ones was observed.

The presence of derivatives of caffeolyquinic acid was noted in the cardoon herb, while only three of them: caffeic, chlorogenic and cynarin were marked in higher quantity (tab. 3). The concentration of the caffeic acid in air dry herb depended only on the length of the vegetation period of plants. Significantly more caffeic acid contained herb harvested from the oldest plants, 150-days old, than from plants 120-days and 90-days old. No significant differences in the contents of the caffeic acid between the years of

research was observed. In all years of the experiment, the content of the caffeic acid increased together with extending the vegetation period of plants.

All examined factors had a significant influence on the mean content of the chlorogenic acid in air dry cardoon herb. The results of the comparisons depending on the length of the vegetation period proved that statistically less chlorogenic acid contained herb obtained from 90-days old plants and significantly more from 150-days old ones. Significantly more of this compound plants accumulated in the first and the third years of the research, and significantly less in the second one.

Table 3. The content of phenolic acids in mg·g^{-1} of air dry herb, depending on the vegetation length of plants (mean from the years 2009–2011)

Treatment	Vegetation period, days		Caffeic acid	Chlorogenic acid	Cynarin
Vegetation period, days (A)		90	1.03*a	3.50a	2.28a
		120	1.36b	4.68b	2.54b
		150	1.93c	5.29c	2.95c
Year (B)		2009	1.38a	4.58a	2.45a
		2010	1.44a	4.29b	2.56a
		2011	1.51a	4.59a	2.77b
(A×B)	90	2009	0.90a	3.31a	2.12a
		2010	1.05a	3.45ab	2.32ab
		2011	1.15a	3.75b	2.40b
	120	2009	1.42b	4.79c	2.45b
		2010	1.31b	4.56d	2.61bc
		2011	1.36b	4.68cd	2.56b
	150	2009	1.81c	5.65e	3.34e
		2010	1.95c	4.87c	2.75c
		2011	2.03c	5.35e	2.77c

* – means values for vegetation period (A), for year mean value (B) and interaction mean values (A × B) within columns with the same letter are not significantly different at $\alpha = 0.05$

Statistical analysis of the calculated and mean values of the cynarin showed differentiation of its contents in herb harvested after 90, 120 and 150 days of the vegetation. Clearly visible was the increase of the contents of the cynarin in herb together with extending the vegetation period of plants. Significantly higher content of cynarin characterized herb harvested from plants 150-days old in comparison to 120- and 90-days old ones. The significant differences in the contents of this compound were observed between the years of the research. Herb harvested in the third year of the experiment had higher content of cynarin in comparison to the first and the second ones.

The influence of the correlation of the length of the vegetation period and years of the research on the examined feature was significant. In all years of the research, significantly higher contents of cynarin was marked in herb harvested from 150-days old plants. There was a tendency observed, that together with extending the vegetation period, the contents of cynarin in herb was increasing. Plants 150-days old accumulated more of this compound in the first year of the research, while the contents of the phenolic acids in total and flavonoids were the highest.

Table 4. The correlation coefficients between phenolic compounds marked in artichoke herb (mean from the years 2009–2011)

Phenolic compounds	Total phenolic acids (%)	Flavonoids (%)	Tannins (%)
Total phenolic acids (%)	–	0.05	-0.88**
Flavonoids(%)	0.05	–	-0.15
Tannins (%)	-0.88**	-0.15	–
Caffeic acid ($mg \cdot g^{-1}$)	0.78**	-0.51**	-0.45**
Chlorogenic acid ($mg \cdot g^{-1}$)	0.67*	-0.29	-0.44*
Cynarin ($mg \cdot g^{-1}$)	0.39*	-0.25	-0.65**

* – significant at 0.05 level to probability
** – significant at 0.01 level to probability

The values of correlation coefficients indicate significantly high correlation between content of total phenolic acids and tannins in cardoon herb (tab. 4). The negative value of correlation coefficient confirms dependence that the higher content of phenolic acids the lower content of tannins (R = -0.88). Negative and at the same time significant value of coefficient was noted between the content of tannins and caffeic and chlorogenic acids and cynarin. Significant strong correlation between the content of total phenolic acids and the content of caffeic acid (R = 0.78) and chlorogenic acid (R = 0.67) was indicated. The relation between the content of flavonoids and caffeic acid was significant and it was a negative value. It means, that the higher content of flavonoids in cardoon herb the lower content of caffeic acid.

DISCUSSION

In the presented research, a significant influence of the length of plants vegetation period on cardoon yield was noted. The highest yield of fresh and air dry herb was obtained from older plants, 120- and 150-days old, and lower one from plants 90-days old. A differentiation of yield quantity was observed already at early stage, after 90 days.

In the available literature on the agronomy of cardoon in cultivation for pharmaceutical industry there is no data on the effect of the term of harvesting the herb on the

contents of biologically active compounds. In the presented work, during the research conducted for 3 years, harvesting the herb in later terms (in the first decade of October and September) increased the yield of phenolic acids and flavonoids. Similar relationships were observed by Göttman and Honermeier [2003] who, in the first date of harvest, obtained herb of low contents of active substances of around 0.49–0.68%, below the established norm of 1% for phenolic acids and 0.2% for flavonoids [Polish Pharmacopeia VI 2002].

An extraordinary medicinal features of a cardoon result from the high content of phenolic compounds, which may vary from 0.9 to 2.7% of dry weight [Sanchez-Moreno et al. 1998]. In studied air dried herb harvested from plants of different age the content of total phenolic acids converted into caffeic acid ranged from 1.04 to 2.58% of dry air weight. Different value of total phenolic acids indicated significant influence of plants vegetation period on their content. Earlier works show that the content of phenolic acids depends on metabolic processes during vegetation and increases in leaves together with the age of plants [Pinelli et al. 2007].

Cardoon herb characterized with average content of flavonoids converted into quercetin (0.38–0.43%) in comparison to 2.86 mg·100 g^{-1} of dry weight converted into rutin marked by Gouveia and Castilho [2012] in extract from *C. cardunculus* var. *silvestris* from Madeira, as well as by Pinelli et al. [2007] in extract from *C. cardunculus* var. *altilis* DC in Sardinia. Important information is that more flavonoids were marked in air dry herb harvested from plants 150-days old and less from 90 and 120-days old ones. The obtained results indicate the correlation between the content of flavonoids in cardoon herb and the age of plants. The dependence is similar to results obtained by other authors [Fratianni et al. 2007]. Other works show that in conditions stressful for cardoon plants, under UV irradiance, the content of flavonoids increases in cell fluids of epidermis [Romani et al. 2006]. Leaves obtained from plants whitened contained 14% less flavonoids than not whitened ones when cardoon was cultivated in conditions of high level of sunshine [Pinelli et al. 2007].

In the presented work herb obtained from plants of different age was reach in tannins (3.25–3.75%) which are valued due to the astringent, antibacterial and anti-inflammatory properties. Lattanzio et al. [2005] noted, that in edible part of artichoke the amount of soluble fractions of tannins is lower and it is 2.22% of dry weight.

In cardoon herb there are flavonoids (apigenin, luteolin) and chlorogenic acids which belong to compounds of the highest anti-oxidative activity [Djeridane et al. 2006].

The quality of herb material depends to a large degree on the content of phenolic acids [Kukić et al. 2008]. In studied plant material among identified acids dominated: chlorogenic acid, caffeic acid and cynarin. Definitely noticeable was the increase of phenolic acids content with the extending the vegetation period of plants. Significantly more caffeic acid accumulated plants 150-days old in comparison to 120 and 90-days old ones. In studies of Sharaf-Eldin et al. [2007] conducted in Germany, the content of chlorogenic acid in artichoke herb was higher and ranged from 2.59–5.36 mg·100 g^{-1}, and the content of cynarin was 1.85–2.0 mg·100 g^{-1}. In cardoon leaves of Madeira variety the caffeic acid was not noted, while leaves contained more chlorogenic acid (73.4 mg·g^{-1}) and less cynarin at the same time (1.17 mg·g^{-1}), [Fernandez et al. 2005].

All derivatives of caffeic acid, among them chlorogenic acid and cynarin, as secondary metabolites undergo continuous changes in cell fluid [Wagenbreth and Eich 2005]. In stressful conditions for plants, at low air temperature or under UV irradiance, the amount of cynarin and chlorogenic acid increases [Gouveia and Castilho 2012]. Morales et al. [2005] observed that the content of chlorogenic acid decreases and the content of ferrulic acid increases with the age of leaf.

In the conditions of conducted experiment, after 150 days of plants growth the positive correlation between the content of total phenolic acids and caffeic and chlorogenic acids was observed. The research carried up to now confirm that cultivated forms of cardoon and plants growing in natural sites are an excellent source of polyphenolic acids and polyphenolic anti-oxidants [Wang et al. 2003].

CONCLUSIONS

On the basis of the results of the presented work it could be stated that cardoon leaves are a valuable material for herb industry. The content of biologically active substances in air dry herb depended on the age of plants at the moment of harvest. In comparison to young plants (90-days old), older plants (120 and 150-days old) contained more total phenolic acids and less tannins. The content of caffeic acid, chlorogenic acid and cynarin in cardoon herb depended on the length of vegetation of plants. The longer vegetation the higher content of phenolic acids in leaves. As a herb species, *C cardunculus* appears not only interesting with respect to its secondary metabolites but also as a good source of health promoting polyphenols.

REFERENCES

AOAC Official Method (1965). Spectrophotometric Metod.

Balasundram, N., Sundram, K., Sammar, S. (2006). Phenolic compounds in plants and agri-industrial by-products. Antioxidant activity, occurrence, and potential uses. Food Chem., 1, 191–203.

Bano, M.J., Lorente, J., Castillo, J., Benavente-Garcia, O., Rio, J.A., Otuno, A., Qurin K.W., Gerard, D. (2003). Phenolic diterpenes, flavones and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis* and antioxidant activity. J. Agric. Food Chem., 51, 4247–4253.

Benlloch-Gonzàlez, M., Fournier, J.M., Ramos, J., Benlloch, M. (2005). Strategies underlying salt tolerance in halophytes are present in *Cynara cardunculus*. Plant Sci., 168, 635–659.

Bundy, R., Walker, A.F., Middleton, R.W., Wallis, C., Simpson, H.C.R. (2008). Artichoke leaf extract (*Cynara scolymus*) reduced plasma cholesterol in otherwise healthy hypercholesterolemic adults: A randomized, double blind placebo controlled trial. Phytomedicine, 15, 668–675.

Christ, B., Müller, K.H. (1960). Determination of the amount of flavonol derivatives in drugs. Arch. Pharm., 293, 1033–1042.

Djeridane, M., Yousfi, B., Nadjemi, D., Boutassouna, P., Stocker, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem., 97, 654–660.

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., Abdelly, C. (2008). Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. Comp. Ren. Biol., 331, 372–379.

Fernandez, J.A., Esteva, J., Gonzalezs, A., Revetre, J.A., Vincente, F., Lopez, A. (2005). New tendencies in the techniques of artichoke production in SE Spain. Acta Hort., 681, 215–220.

Fratianni, F., Tucci, M., De Palma, M., Pepe, R., Nazzaro, F. (2007). Polyphenolic composition on different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). Food Chem., 104, 1282–1286.

Gebhardt, R. (2005). Choleretic and anticholestatic activities of flavonoids of artichoke (*Cynara cardunculus* L. subsp. *scolymus* (L.) Hayek). Acta Hortic., 681, 429–435.

Gebhardt, R., Fausel, M. (1997). Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. Tox. in Vitro, 11, 669–672.

Gouveia, S.C., Castilho, P.C. (2012). Phenolic composition and antioxidant capacity of cultivated artichoke, Madeira cardoon and artichoke-based dietary supplements. Food Res. Inter., 48, 712–724.

Göttman, S., Honermeier, B. (2003). Einfluss von bastandesdichte und standraumverteilung der pflanzen auf ertrag und qualität der blattdrogen bei der artischocke (*Cynara scolymus* L.). Mitt. Ges. Pflanzenbauwisse, 15, 211–214.

Kraft, K. (1997). Artichoke leaf extract-recent findings reflecting effects on lipid metabolism, liver, and gastrointenstinal tracts. Phytomedicine, 4, 369–378.

Kukić, J., Popović, V., Petrović, S., Mucaji, P., Ćirić, A., Stojković, D., Soković, M. (2008). Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts. Food Chem., 107, 861–868.

Lattanzio, V., Cicco, N., Linsalata, V. (2005). Antioxidant activities of artichoke phenolics. Acta Hort., 681, 421–427.

Lutz, M., Henríquez, C., Escobar, M. (2011). Chemical composition and antioxidant properties at mature and baby artichoke (*Cynara scolymus* L.) raw and cooked. J. Food Comp. Anal., 24, 49–54.

Morales, F., Cartelat, A., Alvarez-Fernandez, A., Moya, I., Cerovic, Z.G. (2005). Time-resolved spectral studies of blue-green fluorescence of artichoke (*Cynara cardunculus* L. var. *scolymus*) leaves: Identyfication of chlorogenic acid as one of the major fluorophores and age-mediated changes. J. Agric. Food Chem., 53, 9668–9678.

Pandino, G., Lombardo, S., Mauromicale, G., Williamson, G. (2011). Phenolic acids and flavonoids in leaf and floral stem of cultivated and wild *Cynara cardunculus* L. genotypes. Food Chem., 126, 417–422.

Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E., Romani, A. (2007). Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. Food Chem., 105, 1695–1701.

Polish Farmacopoeia IV (1970). Polish Farmaceutical Society, Warszawa, 41–42.

Polish Farmacopoeia VI (2002). Polish Farmaceutical Society, Warszawa, The Netherlands, 150.

Romani, A., Pinelli, P., Cantini, C., Cimato, A., Heimler, D. (2006). Characterization of Violetto di Toscana, a typical Italian variety of artichoke (*Cynara scolymus* L.). Food Chem., 65, 221–225.

Rossoni, G., Grande, S., Galli, C., Visioli, F. (2005). Wild artichoke prevents the age associated loss of vasomotor function. J. Agric. Food Chem., 53, 10291–10296.

Sanchez-Moreno, C., Larrauri, J.A., Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric., 76, 270–276.

Schütz, K., Krammerer, D., Carle, R., Schieber, A. (2004). Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice and pomace by HPLC-DAD-ESI/MS. J. Agric. Food Chem., 51, 601–608.

Sharaf-Eldin, M.A., Schnitzler, W.H., Nitz, G., Razin, A.M., El-Oksh, I.I. (2007). The effect of gibberellic acid (GA_3) on some phenolic substances in globe artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori). Sci. Hortic., 111, 326–329.

Sonnante, G., De Paolis, A., Lattanzio, V., Perrino, P. (2002). Genetic variation in wild and cultivated artichoke revealed by RAPD markers. Genet. Resour. Crop. Evo., l49, 247–252.

Speroni, E., Cervellati, R., Covoni, P., Guizzardi, S., Renzulli, C., Guerra, M. (2003). Efficacy of different *Cynara scolymus* preparation of liver complaints. J Ethnopharm., 86, 203–211.

Version 9.1 (2003). SAS Institute Inc., Cary, NC, USA.

Wagenbreth, D., Eich, J. (2005). Farmaceutically relevant phenolic constituents in artichoke leaves are useful for chemical classification of accessions. Acta Hortic., 681, 467–474.

Wang, M., Simon, J., Aviles, I., He, K., Zheng, Q., Tadmor, Y. (2003). Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). J. Agric. Food Chem., 51, 601–608.

ZAWARTOŚĆ ZWIĄZKÓW POLIFENOLOWYCH W ZIELU KARDA W ZALEŻNOŚCI OD DŁUGOŚCI OKRESU WEGETACJI ROŚLIN

Streszczenie. W pracy badano wpływ długości okresu wegetacji roślin karda (*Cynara cardunculus* L.) na jego wartość farmakologiczną, uwarunkowaną składem chemicznym surowca. Badania przeprowadzono w latach 2009–2011, oceniając zawartość kwasów fenolowych w przeliczeniu na kwas kawowy oraz flawonoidów i garbników w zależności od fazy rozwojowej roślin. Zawartość kwasów polifenolowych (kawowego, chlorogenowego i cynaryny) oznaczano z wykorzystaniem HPLC. Zawartość substancji biologicznie czynnych zależała od wieku roślin – największą ilość fenolokwasów stwierdzono w zielu zebranym z roślin 120- i 150-dniowych (1,86–2,58%). Ziele zebrane z roślin w różnym wieku zawierało 0,38–0,43% flawonoidów. Więcej garbników zgromadziły młodsze rośliny karda po 90–120 dniach uprawy (3,72–3,43%) w porównaniu z roślinami 150-dniowymi (3,25%). Zawartość kwasu kawowego, chlorogenowego i cynaryny w zielu karda zależała od długości okresu wegetacji roślin. Wraz z jego wydłużaniem zwiększała się zawartość tych kwasów fenolowych w liściach. Wartości współczynników korelacji wskazują na istotnie wysoką współzależność zawartości fenolokwasów ogółem i garbników w zielu karda. Większej zawartości fenolokwasów ogółem towarzyszy mniejsza zawartość garbników (R = -0,88). Silną korelację odnotowano pomiędzy zawartością fenolokwasów ogółem a zawartością kwasu chlorogenowego (R = 0,67) i kwasu kawowego (R = 0,78). Na podstawie uzyskanych wyników tej pracy można stwierdzić, że liście karda stanowią cenny surowiec dla przemysłu zielarskiego.

Słowa kluczowe: *Cynara cardunculus* L., kwasy fenolowe, flavonoidy, garbniki, cynaryna, kwas chlorogenowy, kwas kawowy

Accepted for print: 30.04.2015

For citation: Sałata, A., Buczkowska, H., Galarza, S.V.L., Moreno-Ramon H. (2015). The polyphenolic compounds content of a cardoon herb depending on length of the vegetation period. Acta Sci. Pol. Hortorum Cultus, 14(4), 155–167.