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THE QUANTITATIVE ANALYSIS OF POLIPHENOLIC COMPOUNDS IN DIFFERENT PARTS OF THE ARTICHOKE (*cynara scolymus* L.) DEPENDING ON GROWTH STAGE OF PLANTS

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Abstract. A diversity of active substances that are in the artichoke plants includes it into the group of medicinal plants of broad-spectrum performance. The research conducted in the years 2006–2008 included valuation of poliphenolic compounds content in different parts of artichoke plants during vegetative and generative growth (roots, petioles, leaves, immature and flower at beginning of flowering). The total content of poliphenolic compounds in the reduction on caffeic acid was marked in dried herb with the spectrophotometrical method with the Arnova reagent. The content of poliphenolic acids (caffeic, chlorogenic, ferulic and cynarine) was marked with high performance liquid chromatography (HPLC). The undertaken studies show that there are significant differences with respect to the content of poliphenolic compounds in different parts of artichoke plants. Definitely more total phenolic acids were accumulated in leaves during the vegetative growth (3.167% on average) and in young, immature buds during generative growth (3.730% on average). The chlorogenic acid and cynarine were the main compounds among poliphenolic acids. The content of poliphenolic acids was decreasing with age of plants as young immature artichoke buds had more chlorogenic acid and cynarine than mature heads at the beginning of flowering. The content of caffeic and ferulic acids in the artichoke herb depended on the growth phase of plants. Plants accumulated more caffeic acid in leaves during vegetative growth and ferulic acid in buds during generative growth.

Key words: artichoke, *Cynara scolymus* L., poliphenolic compounds, phenolic acids, growth stage

INTRODUCTION

An artichoke (*Cynara scolymus* L.) is perennial thistle belongs to the Asteraceae (Compositae) family and is a commonly cultivated plant in Southern Europe around the

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Mediterrean Sea region [Bianco 2005]. Due to the high temperature requirements, in the climate conditions of Poland artichoke is cultivated as an annual plant.

The edible parts of the artichoke are immature flower buds which include thick, fleshy 'heart' (base of a head) together with immature floret scales (involucral bracts). The artichoke is also a valuable herbal plant. A pharmaceutical material are dried leaves (*Cynarae folium*) or herb (*Cynarae scolymi herba*).

The diversity of active substances which are found in the artichoke classifies it as belonging to the group of medicinal plants having broad-spectrum pharamacological action. Substances found in the artichoke herb are assumed to effectively cure digestive and circulation disorders, they show strong antioxidant properties that protect organism against some tumours, lower the level of triglicerides and stimulate the immune system [Kirchoff et al. 1994; Kraft 1997; Llorach et al. 2002]. Phenolic acids found in the artichoke improve gall blader function, lower the the LDL cholesterol fraction, strenghten and regenerate liver cells [Wójcicki et al. 1981; Sidrach et al. 2005]. Compounds included in the artichoke plants have antiviral, antibacterial and antifungal properties [Llorach et al. 2002; Zhu et al. 2005].

According to many opinions, poliphenolic compounds found in artichoke plants, especially mono- and di-caffeoylquinic acids and flavonoids, decide about their health-enhancing and antioxidant properties [Wittemer et al. 2005; Fratianni et al. 2007].

The aim of the undertaken research, which results are shown in this work, was to estimate the content and chemical composition of poliphenolic compounds found in different parts of the artichoke depending on growth stage of plants.

MATERIAL AND METHODS

The experiment was conducted in the experimental field of the Departament of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin while phytochemical analysis were done in the laboratory of the Department, in the years 2006–2008. The experimental material were artichoke plants of the 'Green Globe' variety.

The artichoke plants were cultivated from seedlings, with spacing 1.0 × 1.0 m, in four replications. The transplants was planted outside in the phase 3–4 leaves in 1st decade of May in the seasons 2006–2008. In each year of research 3 plants in vegetative (height around 45 cm) and generative stage (young, not developed artichoke bud and at the beginning of head flowering) were harvested. Herb was collected in the vegetative phase in the 1st decade of July (90 days old) and generative phase: in the 1st decade of August (120 days old) – immature buds and 1st decade of September (150 days old) – flower at beginning of flowering. The whole plants were digged out and then prepared separating: roots, leaf nodes, whole leaves (node with a leaf blade) in vegetative stage and roots, leaf nodes, whole leaves, young immature buds and flower heads at the beginning of the flowering.

Directly after the harvest the fresh material was dried in a drying room in the temperature of 40°C. The 1 kg sample was prepared from the air dried herb of each combination directly grinded in a mill with sieve holes of 1 mm diameter. Samples of grinded

material were kept in hermetic containers for laboratory use. Chemical analysis involved dry plant material in each year of research.

The total content of phenolic acids was marked of spectrometric Arnova method (according to the Farmakopea Polska 1999) and expressed as caffeic acid equivalents. The chromatographic determination of phenolic acids was performed by HPLC, using LiChrom (Merck) chromatograph equipped with DAD detector (L-7450). A Lichrospher 100 RP-18 column (250 × 4 mm, 5µm) maintained 25°C. Eluent comprised: (A) acetonitryle (B) H_2O (1% acetic acid), in the following gradient: 0–20 min: 95% B to 64% B; 20 min to 25 min 64% B; 25 to 27 min 64% B to 95% B. The flow rate was 1 ml·min^{-1}, the iniection volume was 10 µm, and the spectra were recorded in the range 220–400 nm. The calibration curves were generated with concentration ranging from 0.01 to 1.0 mM of chlorogenic acid (5-caffeoylquinic), caffeic acid (3,4-dicaffeoylquinic), ferulic acid (4-hydro-3-methylcinnamale) and cynarin (1,3-dicaffeoylquinic).

The obtained results were evaluated statistically with analysis of variance and Tukey t-test at a 5% level of significance.

RESULTS AND DISCUSSION

There occurred the considerable differences regarding the general content of poliphenolic compounds in different parts of plants (tab. 1). Definitely more poliphenolic acids were marked in young, immature buds of artichoke than in leaves, which were harvested from plants in vegetative and generative stage. The least biologically active substances were found in roots and leaf nodes.

In an earlier research it was proved that artichoke plants accumulate more poliphenols in flower heads than in leaves [Romani et al. 2006; Fratianni et al. 2007]. Wide diversity of content of phenolic acids in edible parts of some alliums species observed Mysiak and Tendaj [2008].

Accumulation of active substances was influenced also by a development stage that the plants were harvested in. Roots of plants and leaves in vegetative stages contained considerably more total phenolic acids than at the beginning of flowering period, while in leaf nodes more poliphenolic compounds were marked during flowering than in the vegetative stage.

The dominant compound was chlorogenic acid, known to have oxireductive and anticarcinogenic properties [Rechner et al. 2002]. The large content of this substance explains its important role in biochemical reactions leading to form different poliphenolic compounds [Wittemer et al. 2005]. In roots, leaf nodes and leaves of plants the content of this substance was similar to the one observed before and during flowering period, but the highest amount was in leaves (vegetative stage 233 mg mg·100 g^{-1} dry weight; generative phase 229 mg·100 g^{-1} dry weight) than in other parts of the plant (fig. 1). The content of chlorogenic acid was lowered with the age of plants, as the young immature heads of artichoke had more of the substance (61 mg·100 g^{-1} dry weight) than mature heads at the beginning of the flowering period (26 mg·100 g^{-1} dry weight). No cynarin was marked in roots harvested in the vegetative and generative stage. The most cynarin was found in leaves (before flowering 156 mg·100 g^{-1} dry weight and during flowering

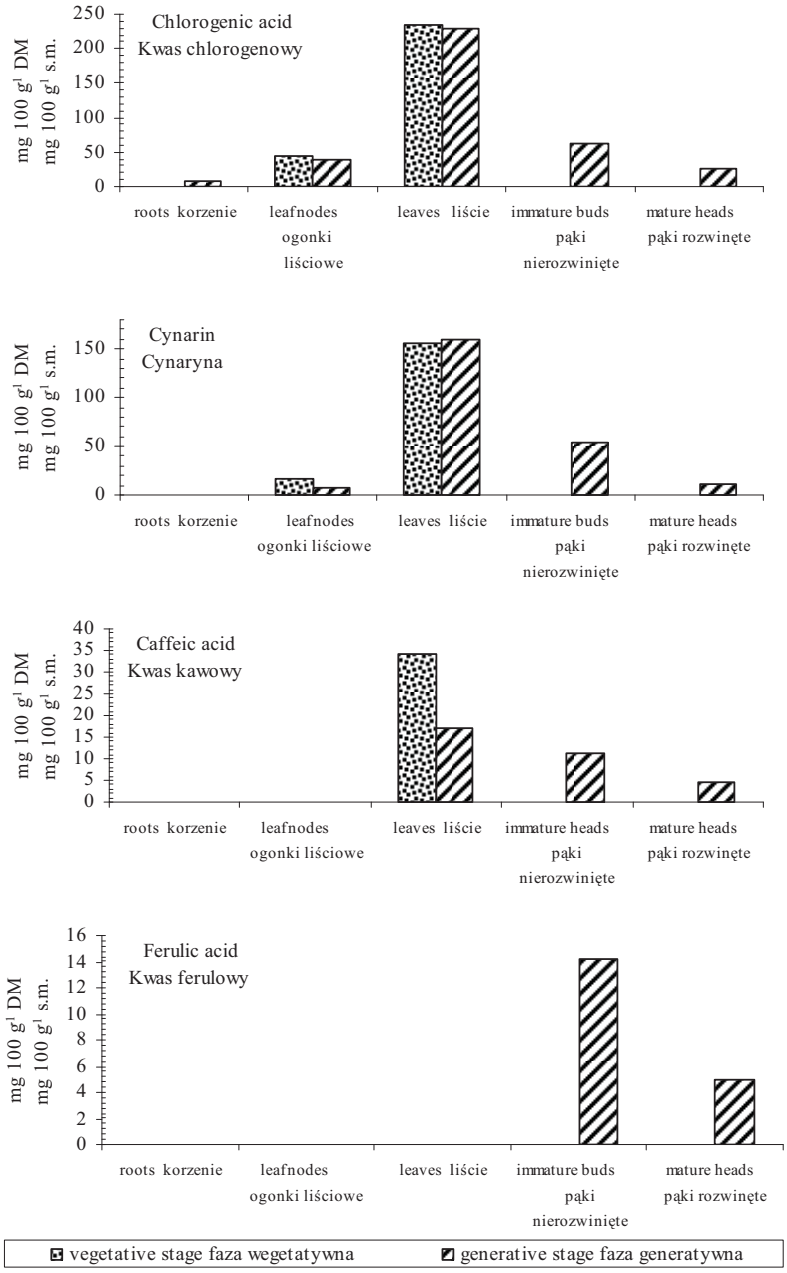

Fig. 1. Quantitative analysis of the major phenolic acids in different parts of artichoke at the vegetative and generative stage of plant growth

Rys. 1. Zawartość zidentyfikowanych fenolokwasów w różnych częściach roślin karczocha w fazie wzrostu wegetatywnego i generatywnego

Table 1. Total phenolic acids content (expressed as caffeic acid equivalents) in different parts of the artichoke plant (% DM)

Tabela 1. Zawartość kwasów polifenolowych ogółem (w przeliczeniu na kwas kawowy) w różnych częściach rośliny karczocha (% powietrznie suchej masy)

Part of plant Część rośliny	Years Lata	Vegetative stage Faza wegetatywna	Generative stage Faza generatywna
Roots Korzenie	2006	0.024a*	0.013b
	2007	0.007c	0.006c
	2008	0.014b	0.010b
	mean – średnia	0.015A	0.010B
Leaf nodes Ogonki liściowe	2006	0.067d	0.069d
	2007	0.155c	0.279ab
	2008	0.245b	0.309a
	mean – średnia	0.155B	0.219A
Leaves Liście	2006	2.330d	2.317d
	2007	3.722a	3.213c
	2008	3.450b	2.209e
	mean – średnia	3.167A	2.579B
Immature buds Pąki nierozwinięte	2006	-	3.746a
	2007	-	3.738a
	2008	-	3.706a
	mean – średnia	-	3.730
Flower at beginning of flowering Kwiatostan na początku kwitnienia	2006	-	1.355a
	2007	-	1.336a
	2008	-	1.316a
	mean – średnia	-	1.335

*Means values marked with the same letters among each part of artichoke do not differ significantly among each other at probability level of $\alpha = 0.05$.
Średnie oznaczone tymi samymi literami w obrębie każdej części rośliny nie różnią się istotnie między sobą na poziomie prawdopodobieństwa $\alpha = 0,05$.

159 mg·100 g^{-1} dry weight) and in young heads (53 mg·100 g^{-1} dry weight), while less in mature heads (12 mg·100 g^{-1} s.m.). Accumulation of caffeic acid and ferulic acid depended to a great degree on the plant development stage. Caffeic acid, which derivatives are used with success for insufficiency of vein circulation theraphy [Wittemer et al. 2005], was found in substantial amoung in artichoke leaves, especially those harvested during vegetative stage (34 mg·100 g^{-1} s.m.). In roots and leaf nodes the content of this substance was not observed at all. Ferulic acid was found in small quantities. It's presence was noted only in the generative stage, in young (14 mg·100 g^{-1} dry weight) and mature (5 mg·100 g^{-1} dry weight) artichoke heads.

Regarding the large diversity of poliphenolic compounds found in flower heads the interest in their use for production of health-enhancing functional food increases [Meijer and Mathijsen 1996; Romani et al. 2006; Kahle et al. 2007].

CONCLUSIONS

1. Content of phenolic acids (expressed as caffeic acid equivalents) of flowers heads as well as leaves of artichoke were high although varied depending of development stage of plants.

2. More phenolic acids were marked in immature buds of artichoke than in leaves, roots, leaf nodes as well as flowers at beginning of flowering.

3. The content of chlorogenic acid and cynarein were lowered with the age of plants, as immature heads of artichoke had more of the substance than mature heads at the beginning of the flowering period.

4. Artichoke leaves contained more caffeic acid especially those harvested during vegetative stage. Ferulic acid was found only in artichoke heads in the generative stage.

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ZAWARTOŚĆ ZWIĄZKÓW POLIFENOLOWYCH W RÓŻNYCH CZĘŚCIACH KARCZOCHA ZWYCZAJNEGO (*Cynara scolymus* L.) W ZALEŻNOŚCI OD STADIUM ROZWOJOWEGO ROŚLIN

Streszczenie. Różnorodność substancji aktywnych występujących w karczochu włącza go do grupy roślin leczniczych o szerokim spektrum działania. Badania przeprowadzone w latach 2006–2008 obejmowały ocenę zawartości związków polifenolowych w różnych częściach roślin karczocha w fazie wzrostu wegetatywnego i generatywnego (korzeniach, ogonkach liściowych, liściach, pąkach kwiatostanowych nierozwiniętych i na początku kwitnienia). Zawartość kwasów fenolowych ogółem w przeliczeniu na kwas kawowy oznaczono w suchej masie ziela metodą spektrofotometryczną z odczynnikiem Arnova. Skład fenolokwasów oznaczono metodą HPLC: kawowego, chlorogenowego, feulowego, cynaryny. Z przeprowadzonych badań wynika, że występują znaczne różnice pod względem zawartości związków polifenolowych w różnych częściach rośliny. Zdecydowanie więcej kwasów fenolowych ogółem rośliny zgromadziły w liściach w fazie wzrostu wegetatywnego średnio 3,167%, oraz w fazie generatywnej w młodych nierozwiniętych pąkach średnio 3,730%. Dominującym związkiem wśród kwasów fenolowych był kwas chlorogenowy i cynaryna. Zawartość kwasów fenolowych zmniejszała się wraz z wiekiem roślin gdyż młode nierozwinięte pąki karczocha zawierały więcej kwasu chlorogenowego i cynaryny niż pąki rozwinięte, na początku kwitnienia. Zawartość kwasu kawowego i ferulowego w zielu karczocha zależała od fazy rozwojowej rośliny. Więcej kwasu kawowego rośliny zgromadziły w liściach w fazie wzrostu wegetatywnego a kwasu ferulowego w pąkach w fazie wzrostu generatywnego.

Słowa kluczowe: karczoch, *Cynara scolymus* L., związki polifenolowe, kwasy fenolowe, faza wzrostu

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