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ORIGINAL PAPER

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LEAF PETIOLES BLANCHING INFLUENCE ON THE YIELD AND CHEMICAL COMPOSITION OF CARDOON (*Cynara cardunculus* L.)

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ABSTRACT

The influence of leaf blanching with black polyethylene non-woven (PP) or white and black polypropylene foil (PE) used as soil mulching and blanching of leaves on yield, usefulness and biological value of cardoon leaf petioles were evaluated in the presented work. The effect of blanching duration of petioles (30, 25 and 10 days before leaves' harvest) on the content of bioactive compounds was also estimated. The aim of the research was to evaluate the total yield of leaves, yield structure, dry weight, content of crude fibre, total sugars, L-ascorbic acid, chlorophyll, phenolic acids in conversion to caffeic acid, total flavonoides and DPPH activity in leaf petioles depending on the method and duration of leaves blanching. The content of apigenin, chlorogenic acid and cynarin in leaf petioles were marked with HPLC. Petioles of non blanched plants showed more dry weight and contained more L-ascorbic acid, chlorphyll, total phenolic acids and flavonoides than the uncovered ones. As a tendency it was observed that together with lengthening the blanching time from 10 to 30 days before harvest, the level of dry weight, crude fibre, total sugars, L-ascorbic acid and chlorophyll decreased. A reverse relationship was observed related to the blanching duration, as extending the time from 10 to 30 days before harvest, the content of total phenolic acids increased from 0.144 to 0.155% of fresh weight, while the content of flavonoides decreased from 0.662 to 0.352% of fresh weight. Lengthening blanching from 10 to 30 days before harvest of leaves decreased the content of apigenin and cynarin in petioles while increased the content of chlorogenic acid. The antioxidant activity DPPH did not depend on the method and duration of blanching. Unusual nutritional and medicinal benefits of cardoon petioles come from its rich and valuable chemical composition.

Key words*:* black non-woven, polyethylene film, flavonoids, phenolic acids, apigenin, chlorogenic acid, cynarin

INTRODUCTION

Cardoon (*Cynara cardunculus* L.), called also Spanish artichoke, is a perennial plant. The cardoon is native to the Mediterranean region, it is traditionally cultivated in the south of Europe, just like an artichoke (*Cynara scolymus* L.) [Ierna and Mauromicale 2010]. Edible parts are whole leaves or only thickened petioles.

Cardoon is a valuable vegetable of a low caloric value. According to the United States Department of Agriculture [USDA 2016], 100 g of fresh cardoon

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petioles provide only 17 kcal (71 kJ). Cardoon petioles contain bitter compounds (sesquiterpenes lactones), which give a characteristic bitter taste. Consumption of cardoon petioles positively influences digestion. Cardoon added to dishes may increase their digestibility [Nazni et al. 2006, Guarrara and Savo 2013].

Cardoon leaf petioles may be eaten when cooked in salted water with addition of lemon juice or wine vinegar until tender. Addition of lemon juice or vinegar prevents enzymatic browning [Vaughan and Geissler 2009]. Fresh cardoon petioles contain 1.6 g·100 g^{-1} of polysaccharides insoluble in water in fresh weight, which are important compounds from a dietetic point of view [USDA 2016].

Cardoon is also considered a medicinal plant. It's therapeutic effect is determined with a high content of many active compounds: polyphenols, flavonoids, sesquiterpenes lactones, phytosterols, triterpenes, tannins [Venere et al. 2005, Gouveia and Castilho 2012, Pandino et al. 2012]. Phenolic acids and their esters are a broad group of secondary metabolites, of which cardoon plants contain mainly of chlorogenic acid (5-O-dicaffeoylquinic acid) and cynarin (1,3-O--dicaffeoylquinic acid). Cynarin is a chemical substance of a clinically proven bile and hypolipidemic action [Bundy et al. 2008, Lattanzio et al. 2009]. Polyphenolic acids contribute to neutralisation of free radicals, chelating metal ions, changing the activity of enzymes and availability of proteins [Pandino et al. 2011]. It was proven, that phenolic acids counteract coronary artery disease, cancer, inflammation and diabetes [Rossoni et al. 2005]. A large group among phenols are flavonoids. In regard to variations in chemical structure of these compounds different groups are identified: flavanones, flavanols, flavones, isoflavones, flavonols and anthocyanins. Cardoon contains the most of flavones, mainly apigenin and luteolin and their derivatives [Pandino et al. 2012]. Flavonoides play an important role in protection of liver cells and they show antioxidant and hipolipidemic activities [Schütz et al. 2004].

Studies conducted so far show that luteolin has the strongest antioxidant and hepatoprotective activities of all active compounds present in cardoon [Gebhardt 2001]. Another activity of luteolin is vasodilatation, what contributes to blood pressure regulation [Rice-Evans et al. 1995, Pinelli et al. 2007]. A presence of polyphenolic compounds in cardoon gives a possibility to use this valuable vegetable to control cardiovascular diseases and cancer [Kukić et al. 2008, Christaki et al. 2012, Pandino et al. 2012].

Under unfavourable conditions of cultivation, when it is cool or dry, cardoon leaves might become bitter as a result of sesquiterpenes lactones accumulation. It is usually accompanied by overcapacity of fibre, what also lowers culinary qualities of petioles. In order to increase taste properties of cardoon leaf petioles intended for direct consumption, a blanching of leaves is used in practice. This process reduces access of leaves to light what increases tenderness of flesh and taste values. Blanching is conducted through covering aboveground parts of plants with cardboard, foil or non-woven to completely stop light accessibility to the part of plant being blanched. Usually the treatment is started 30 days before a planned harvest [Fernandez et al. 2006].

There is no data available concerning usefulness of plastics for direct blanching of edible parts of plants. A type of material used for blanching and soil mulching influences climate around plants in a different way. Mulching of soil with black foil (PP) or white and black one (PE) improves thermal conditions of soil air, humidity and soil structure, more effective usage of nutritional compounds by plants, increases concentration of CO_2 both in soil air and around plants, decreases soil erosion, eliminates weeds, improves cleanliness and quality of products [Zhou et al. 2011, Zhang et al. 2013, Filipović et al. 2016].

Covers made of polyethylene foil and polypropylene non-woven reduce evapotranspiration and condensation of water on the inner side of the cover [Gosar et al. 2010]. A better air exchange under non-woven PP than under PE foil encourages fast water evaporation from a covered soil surface. On the other hand, black non-woven (PP) provides better drainage for water from precipitation than foil (PE) [Gosar and Baričevič 2011, Muñoz et al. 2017].

The aim of the studies was to evaluate the influence of leaves blanching on yield, usefulness and biological value of leaf petioles of cardoon. The pur-

pose of this work was an assessment of suitability of synthetic materials for soil mulching and leaves blanching, as well as explanation how duration of blanching affects content of bioactive compounds.

MATERIAL AND METHODS

Description of the field experiment

Location of the experiments. The experiments were conducted in the years 2011–2013 in research units of the University of Life Sciences in Lublin. Field experiments were conducted in the Felin Experimental Station (51°11'N, 22°28'E), laboratory analyses of the plant material were done in the Laboratory of Vegetable and Herbal Materials Quality in the Department of Vegetables and Medicinal Plants of the University of Life Sciences in Lublin (Poland).

Soil condition and plants fertilization. The cultivation was conducted on a loess soil with light soil chemical composition, containing 1.6% of organic matter and with a soil pH of about 6.5. Before the experiment was set up, in the autumn of the preceding year, a following mineral fertilization had been done: P – 40 kg·ha^{-1}, K – 100 kg·ha^{-1}, and during spring before planting transplants fertilization with N – 100 kg·ha^{-1} was applied. The content of mineral compounds in soil was on average as following: 30 mg N·dm^{-3} (NO_3^- + NH_4^+), 40 mg P·dm^{-3}, 100 mg K·dm^{-3}, 25 mg Mg·dm^{-3}. In the successive years of studies the content of nutrients in soil was completed to the level of: 120 mg N·dm^{-3} (NO_3^- + NH_4^+), 60 mg P·dm^{-3}, 160 mg K·dm^{-3}, 60 mg Mg·dm^{-3}. A mineral fertilization was applied in the form of granular triple superphosphate, potassium sulphate, ammonium nitrate and magnesium sulphate. A tillage was done in the autumn and a land leveller and a cultivation unit (a cultivator with roller tillers) were used.

Plant material and preparation of seedlings. Plant material were plants of cardoon (*Cynara cardunculus* L.), obtained from seeds imported through a Rijnsburg seeds company (the Netherlands). Plants showed a quick rate of growth and profuse foliage, forming big leaves, green on the upper side and greenish-grey and covered with tomentose hair underside. Leaf blades were situated on long, fleshy petioles.

Plants were cultivated from seedlings. For the purpose of the experiment, during the second decade of March each year, seeds were sown in a glasshouse into multipots filled with peat substrate, each single pot volume was 90 cm^3. During the first three weeks the air temperature was 20°C (±2°C) during the day and 16°C at night. From the fourth week onwards the temperature was decreased to 14–16°C until the time of planting the plants to field. Plants were fertilized twice with 0.1% of nitrogen. The transplants were hardened a week before planting, by reducing irrigation and intensive ventilation of the glasshouse. In each year of the experiment plants were planted outside in the second decade of May.

Description of the experiment and field studies

The usefulness of different methods of soil mulching and blanching of cardoon petioles were evaluated in the experiment. There were 18 plants per plot of an area of 9.0 m^2, with a spacing of 1.0 × 0.5 m. The experiment was set up in a two-factorial design with a completely randomized blocks, in four replications.

Factors of the experiment: (1) method of leaf blanching: black non-woven (PP), white and black foil (PE), without blanching; (2) blanching duration before harvest: 30 days, 25 days, 10 days.

Soil mulching and leaves blanching were done with: polypropylene black non-woven (PP) of 60 g·m^{-2} weight and 120 cm wide; white and black polyethylene foil (PE) with light reflection of 0.085 mm thickness and 200 cm wide, which were spread on a ground surface 14 days before planting forming strips of 100 cm wide. Then the surface of a cover was cut every 50 cm to insert plants into soil. The edges of the material were folded and covered with soil.

The dates of planting were the same each year – the first decade of May. Transplants were planted in a phase of 3–4 leaves.

In the experiment the usefulness of different methods of cardoon leaf blanching was evaluated. Plants were covered during 30 days (25th of July), 25 days (31st of July) or 10 days (15th of August) before harvest. The treatment was applied through lifting up the black non-woven (PP) or white and

black foil (PE) to the height of leaf petioles, so that they had no access to light.

The harvest of cardoon leaves was done once. Whole rosettes were collected from each plot on: 25th of August in the years 2011 and 2012, and 26th of August in the year 2013. On the basis of weight of 10 plants, the total yield was determined for 1 m^2. Commercial yield in this experiment were leaves (petioles and leaf blades) of the minimal length of 60 cm. That yield was separated into two fractions: leaves 60–80 cm long and over 80 cm long. Leaves shorter than 60 cm were treated as a non-commercial. The following features were estimated: number of leaves per plant, length of petioles, width of petioles at the base (cm) and the mean weight of leaf.

Laboratory analyses

Plant material. Plant material samples were collected from each object for analyses. The analyzed materials were mixed standard samples from 1 kg of commercial yield leaf petioles of each combination. Directly after harvest, taking an edible part of cardoon of the fresh plant material, the content of the following compounds was estimated:

– dry weight (%) with oven-drying method with a temperature of 105°C,

– crude fibre (% of fresh weight) marked according to the Henneberg and Stohmann method [Jakubowski 1980];

– total sugars (g·100 g^{-1} of fresh weight) marked according to the Luff -Schoorl method [PN-A-79011-5:1998];

– L-ascorbic acid (mg·100 g^{-1} of fresh weight) marked according to the Roe method modified by Evelyn [Roe 1961];

– total chlorophylls (mg·100 g^{-1} of fresh weight) marked according to the Arnon method [Arnon 1960].

Sample preparation for HPLC analysis. Phenolic extract was obtained by solvent extraction with methanol (1 : 10) in a reflux condenser in the temperature of the solvent boiling point for 3 h. Then after percolation, material was treated again with 80% of methanol and extracted twice for 2 h. Methanol extracts were joined, solvent was evaporated, and the remains were eluted with hot water (50 ml). Water solutions were left for 24 h in a refrigerator. Separated tarry residues containing ballasts were filtered and rinsed with distilled water. Filtrate obtained in this way 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 readily soluble in watersalts. 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 content in plant material was determined with the use of the Folin Ciocateu reagent based on the reduction of phosphowolframate-phosphomolybdate complex by phenolic blue reaction products following the method described by Dewanto et al. [2002]. An aliquot of diluted sample extract was added to 0.5 ml of distilled water and 0.125 ml of the Folin Ciocateu reagent. The mixture was shaken and allowed to stand for 5 min, before addition of 1.25 of 7% Na_2CO_3. The solution was then adjusted to final volume of 3 ml with distilled water and mixed thoroughly. After incubation in dark, the optical density of blue-coloured samples was measured at 760 nm. The total phenolic content of plant parts was expressed as caffeic acid (%) equivalents of fresh weight through the calibration curve with caffeic acid. All samples were analyzed in three replications.

HPCL determination of polyphenols. The HPLC separation was performed on a UFLC Shimadzu series instrument (Japan) coupled to a diode-array detector (DAD). The separation was performed on a PhenomenexSynergi Fusion-RP column (4 µm, 250×4.6 mm i.d.; Phenomenex) with a sample injection volume of 20 µl. The mobile phase was composed of acetonitrile (A) and water/formic acid (100/0.1, v/v) (B). A gradient program was used as follows: 20% A (0 min), 25% A (10 min), 25% A

(20 min), 50% A (40 min), 100% A (42–47 min), 20% A (49–55 min). The mobile phase flow rate was 1 mL/min; the chromatogram was recorded at 280 nm and 320 nm and spectral data for all peaks were accumulated in the range of 190–400 nm. Column temperature was controlled at 30°C [Gouveia and Castilho 2012].

The content of separate phenolic acids in the examined material was calculated on the basis of calibration curve determined for each identified phenolic acid: chlorogenic (5-O-caffeoylquinic), cynarin (1,3-O-dicaffeoylquinic) and apigenin (apigenin-7--O-glucoside). Standards obtained from Roth Company were used to identify and determine the content of separate phenolic acids in plant material.

The markings were done in three replications in each of samples, expressing the content in mg per 100 mg of fresh weight (mg $100^{-1}$of fresh weight).

Marking flavonoids content. The content of flavonoids was marked with the spectrophotometric method according to Christ Muller [Singleton and Rosi 1965]. To get the percentage content of flavonoids they were converted into quercetin. 3 × 1.0 g of powdered material was placed in round-bottomed flasks. Then 20 ml of acetone, 2 ml of 25% HCl solution and 1 ml of 0.5% hexamethylenetetramine were added. Then the content of flask was heated over a boiling water bath in a reflux condenser for 30 minutes from the boiling start. Obtained hydrolysate was filtered into a graduated flask of 100 ml capacity and then the residue together with cotton was placed in a round-bottom flask, 20 ml of acetone was added and it was boiled for 10 minutes. This way of digestion was repeated one more time and obtained extracts were filtered to a graduated flask and the acetone was added to capacity of 100 ml. Then 20 ml of hydrolyzed extract was moved to a funnel, 20 ml of water was added and it was shaken with 15 ml of ethyl acetate. Joined organic phases were washed twice with 40 ml of water, filtered through cotton wool into a graduated flask and completed with ethyl acetate to 50 ml capacity. The 10 ml of acetate extract containing flavonoids extracted from the studied material were placed into 3 graduated flasks of 25 ml capacity. 2 ml of 2% solution of aluminium chloride was added to two flasks and all three flasks were made up to the mark with solution of methanol and acetic acid. After 45 min the absorbance of the solution with aluminium chloride was measured with a spectrophotometer Uvikon-932 in comparison to a model, in 1-cm trays at the wavelength of $\lambda = 425$ nm.

Antioxidative activity against DPPH radical. Marking and counting the percentage of inhibition of DPPH (2.2-diphenyl-1-picryl-hydrazyl hydrate) was done according to the method given by Sánchez-Moreno et al. [1998]. The agent containing solution of radicals was prepared before the analysis were done. 0.012 g of DPPH was weighted and transferred quantitatively to a graduated flask of 100 ml capacity, made up to volume with methanol, then dissolved in ultrasonic bottle for 15 minutes. Blind test [A_R]. 1 ml of distilled water, 3 ml of methanol and 1 ml of DPPH solution were measured to the test tube. They were stirred and after 40 min read spectrophotometrically. Studied sample [A_T]. 1 ml of diluted sample, 3 ml of methanol and 1 ml of DPPH solution were put into the test tube. Sample was stirred and after 40 min read spectrophotometrically. Percentage of inhibition [% DPPH] = $100 - [A_T/A_R \times 100]$

Statistical analyses

The obtained data was analyzed statistically with the use of the analysis of variance (One-Way ANOVA), with the Statistica 13.1 software (StatSoft Inc.). To estimate the significance of differences (LSD) between the means the Tukey test was used at the $p = 0.05$ and $p = 0.01$ levels of significance.

DESCRIPTION OF THE RESULTS

Soil temperature. The soil temperature at the depth of 20 cm under covers of black non-woven (PP) or white and black foil (PE) as well as in an uncovered ground was measured (fig. 1).

The soil temperature depended on weather conditions present during the studies. During all years of the experiment higher soil temperatures were noted on plots with mulches of black non-woven (PP) and white and black foil (PE) in comparison to an uncovered soil. Depending on the years of the experiment, the differences in the temperature on the plots covered and uncovered ranged between 0.5 to 1.2°C.

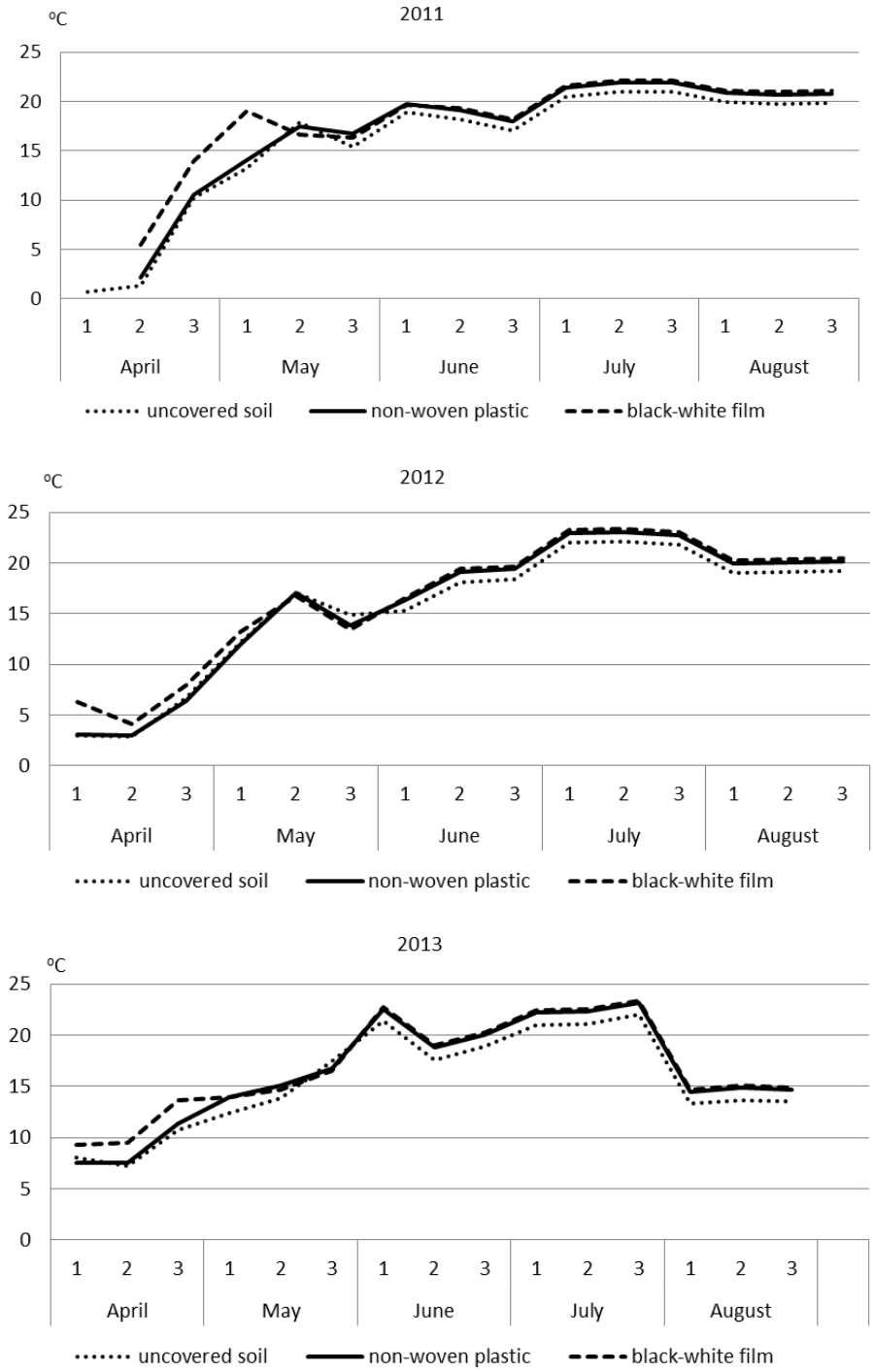

Fig. 1. Soil temperature at the depth of 20 cm under covers of black non-voven, white and black foil and uncovered ground

Table 1. Mean daily average temperatures of air and precipitation and multiannual means during research according to the Felin Meteorological Station

Month	2011		2012		2013		Multiannual mean	
	Temp. (°C)	Rainfalls (mm)	Temp. (°C)	Rainfalls (mm)	Temp. (°C)	Rainfalls (mm)	Temp. (°C)	Rainfalls (mm)
May	14.3	42.2	15.0	56.3	15.3	101.6	13.0	57.7
June	18.6	67.8	17.3	62.8	18.5	105.9	16.2	65.7
July	18.4	189.0	21.4	52.3	19.2	126.1	17.8	83.5
August	18.8	65.3	19.2	37.6	19.2	17.8	17.1	68.6
Mean	17.5	–	18.2	–	18.1	–	16.0	–
Sum	–	364.3	–	209.0	–	351.4	–	275.5

Table 2. Analysis of variance of the experimental factors as a function of a cultivation season, method of cultivation and duration of blanching

Description	Season of cultivation (SW)	Blanching method (B)	Blanching duration (D)	SW × B	SW × D	B × D
Total yield of leaves (kg m^{-2})	*	NS	NS	*	NS	NS
Yield of leaves longer than 60 cm (kg m^{-2})	*	*	NS	*	NS	NS
Yield of leaves longer than 80 cm (kg m^{-2})	NS	*	NS	*	NS	NS
No. of leaves per plant	*	NS	NS	*	NS	NS
Mean weight of leaves (g)	NS	NS	*	*	NS	NS
Length of leaf petioles (cm)	*	NS	NS	NS	NS	NS
Width of leaf petioles (cm)	*	NS	NS	NS	NS	NS
Dry weight (%)	**	*	*	*	*	NS
Crude fibre content (fresh weight %)	**	*	*	*	*	NS
Total sugars content (g $100g^{-1}$ of fresh weight)	*	**	**	*	*	NS
L-ascorbic acid content (mg $100g^{-1}$ of f.w.)	*	*	*	*	*	NS
Total chlorophyll content (mg g^{-1} of f.w.)	*	*	*	*	*	NS
Phenolic acids content (fresh weight, %)	*	**	*	*	NS	NS
Flavonoids content (fresh weight, %)	**	**	*	*	NS	NS
DPPH activity (%)	*	NS	NS	NS	NS	NS

NS – unsignificant, * significant at the level of 0.05, **significant at the level of 0.01

It should be emphasized that the way of mulching had a significant effect on the temperature only during the first period when the covers were used, in April. In the successive years of the experiment, from the 2nd decade of May, the differences were smaller.

Meteorological conditions. Years with thermal condition characterized with high air temperature during cultivation of cardoon plants in field, should be treated as favourable for cultivation of thermophilic plants, such as cardoon (tab. 1). The mean temperature during the vegetation period (May–August) in the years 2011–2013 was 17.9°C and exceeded the multiannual average in the year 2011 by 1.5°C, by 2.2°C in the year 2012 and by 2.1°C in the year 2013. The highest mean air temperature was noted in 2011 in August (18.8°C), in July in the year 2012 (21.4°C) and in July and August in the year 2013 (19.2°C).

The precipitation in the years of the experiment was differentiated in comparison to the multiannual average. From May to August, in comparison to the multiannual average, the sum of precipitation was lower by 66.5 mm in the year 2012 and in the years 2011 and 2013 it was higher by 88.8 and 75.9 mm respectively. Individual vegetation periods were characterized by a big changeability. The highest precipitation was noted in July in the year 2011 (monthly sum 189.0 mm), in 2012 in May, June and July (56.3, 62.8 and 52.3 mm respectively) and in 2013, in May, June and July (101.6, 105.9, 126.1 mm respectively). The least precipitation was noted in the year 2013 in August (monthly sum 17.8 mm).

RESULTS

There were differences identified in the yield and its structure between the years of the experiment (tab. 3). Higher total yield of leaves and yield of leaves longer than 60 cm were obtained in the year 2013, when plants formed more leaves of higher mean weight and leaf petioles characterized with higher mean length and width.

The content of dry weight, crude fibre, total sugars and L-ascorbic acid in leaf petioles of cardoon were higher in the year 2012, when the mean air temperature during vegetation was 18.2°C and the precipitation was 209 mm in comparison to the year 2011, when the mean air temperature was 17.5°C and precipitation was 364 mm.

The plants accumulated slightly more chlorophyll in leaf petioles in 2012 and 2013, when the mean air temperature during vegetation was higher than in the year 2011.

Different weather conditions had an influence on the content of total phenolic acids expressed as caffeic acid and total flavonoids expressed as quercetin as well as on antioxidant activity DPPH. In the year 2013 plants accumulated more phenolic acids and less flavonoids. Higher antioxidant capacity of juice obtained from leaf petioles was noted in the year 2012.

Both the type of a cover and duration of blanching had no significant influence on total yield of leaves (tabs 2 and 3). The black non-woven (PP) used as soil mulching and blanching of cardoon plants increased yield of leaves longer than 60 cm by 24% on average and at the same time decreased the yield of leaves longer than 80 cm by 35% on average in comparison to white and black foil (PE). Leaves covered during the longest period, that is for 30 days before harvest, showed a higher mean leaf weight.

The highest content of dry weight (8.94%), L-ascorbic acid (1.76 mg·100 g^{-1} of fresh weight) and chlorophyll (94.17 mg·g^{-1} of fresh weight) characterized non-blanched fresh leaf petioles of cardoon in comparison to the content of those compounds in petioles covered with black non-woven (PP) or white and black foil (PE). Leaf petioles covered with black non-woven (PP) showed higher dry weight and content of L-ascorbic acid but at the same time they contained less crude fibre and total sugars than those covered with white and black foil (PE). The materials used for blanching had an influence on the content of chlorophyll, as leaf petioles covered with white and black foil (PE) contained more of that compound than covered with black non-woven (PP).

As a tendency it was observed that lengthening blanching of the leaf petioles from 10 to 30 days before harvest, decreased the content of dry weight, crude fibre, total sugars, L-ascorbic acid and chlorophyll.

Table 3. Yield quantity and structure, chemical composition of leaf petioles of cardoon depending on a method and duration of blanching in the years 2011–2013

Description	Years			Blanching method			Duration of blanching, days			Mean
	2011	2012	2013	black non-woven	white and black foil	not covered	30	25	10	
Total yield of leaves (kg m^{-2})	7.46b	6.40c	8.03a	7.08	7.46	7.87	7.40	7.34	7.19	7.36
Yield of leaves longer than 60 cm (kg m^{-2})	4.75b	3.51c	5.08a	5.00a	3.80b	4.32a	3.56	3.50	3.42	4.10
Yield of leaves longer than 80 cm (kg m^{-2})	1.21	1.21	1.31	0.98b	1.53a	1.81a	1.98	1.93	1.95	1.55
No. of leaves per plant	25.2b	22.5b	28.8a	25.9	25.9	24.9	24.0	26.7	25,8	25.5
Mean weight of leaves (g)	130.0	127.4	135.2	133.3	129.6	138.1	183.2a	162.5b	162.2b	144.6
Length of leaf petioles (cm)	56. 1b	73.7a	70.8a	70.0	66.2	71.1	69.1	65.4	66.1	67.6
Width of leaf petioles (cm)	1.9c	2.1b	3.3a	2.9	2.9	3.1	2.6	2.6	2. 8	2.7
Dry weight (%)	8.04c	8.18a	8.13b	8.02b	7.84c	8.94a	7.63c	7.68b	8.22a	8.08
Crude fibre content (fresh weight %)	7.23b	7.74a	7.73a	7.31b	7.82a	7.49b	7.51c	7.57b	7.65a	7.56
Total sugars content (g 100g $^{-1}$ of fresh weight)	0.57c	0.68a	0.61b	0.72b	0.89a	0.36c	0.62b	0.61b	0.64a	0.63
L-ascorbic acid content (mg 100g $^{-1}$ of f.w.)	1.55c	1.87a	1.62b	1,62b	1.48c	1.76a	1.32c	1.75b	1.89a	1.65
Total chlorophyll content (mg g^{-1} of f.w.)	63.81b	66.00a	66.60a	53.85c	67.89b	94.17a	47.32c	54.65b	65.74a	64.45
Phenolic acids content (fresh weight, %)	0.174b	0.176b	0.216a	0.166b	0.155c	0.304a	0.155a	0.152b	0.144c	0.182
Flavonoids content (fresh weight, %)	0.666a	0.554b	0.443c	0.541b	0.534c	0.650a	0.352c	0.454b	0.662a	0.528
DPPH activity (%)	63.04b	82.75a	64.14b	69.56	64.92	63.31	66.92	65.04	64.23	67.10

Values in a single line marked with different letters differ significantly

Table 4. Apigenin, chlorogenic acid and cynarin content in cardoon leaf petioles depending on duration of blanching in the years 2011–2013 (mg 100 g of fresh weight)

Years	Blanching duration, days	Apigenin	Chlorocenic acid	Cynarin	Mean
2011	10	184 ±28	574 ±34	1.83 ±0.16	253c
	20	163 ±28	630 ±30	1.51 ±0.11	265b
	30	118 ±37	689 ±28	1.32 ±0.10	269a
2012	10	224 ±33	524 ±37	0.83 ±0.14	250a
	20	212 ±22	540 ±29	0.81 ±0.16	251a
	30	233 ±24	699 ±28	0.82 ±0.16	311b
2013	10	102 ±26	588 ±19	1.73 ±0.18	231a
	20	86 ±25	605 ±18	1.61 ±0.15	231a
	30	40 ±25	645 ±18	1.62 ±0.14	229b
2011–2013	10	170 ±18	562 ±25	1.46 ±0.16	244c
	20	154 ±16	592 ±22	1.31 ±0.22	249b
	30	130 ±19	678 ±18	1.25 ±0.19	270a
Mean		151	610	1.34	254

Every three values in the last column marked with different letters differ significantly

The content of phenolic compounds depended both on the way and duration of blanching. Higher content of total phenolic acids and total flavonoids characterized leaf petioles covered with the non-woven than foil. There was a diverse relationship concerning a duration of blanching, as with exceeding the period from 10 to 30 days before harvest, a content of phenolic acids increased from 0.144 to 0.155% of fresh weight, while the content of flavonoids decreased from 0.662 to 0.352% of fresh weight. In case of the content of total phenolic acids and total flavonoids the interaction between the seasons of cultivation and duration of blanching was not observed (tab. 2). The antioxidant capacity DPPH did not depend on the way or duration of blanching.

The main compounds of poliphenols were apigenin, chlorogenic acid and cynarin (tab. 4). The duration of cardoon leaves blanching influenced the mean content of poliphenols in leaf petioles. On average, in the years 2011–2013 a tendency was noted, that together with extending the blanching from 10 to 30 days before harvest, the content of poliphenols increased. Such a relationship that leaf petioles of cardoon blanched for 30 days before harvest contained more poliphenols in comparison to those blanched for 10 days was observed only in 2011, in contrast to the years 2012 and 2013, when leaves blanched for 10 and 20 days before harvest contained more apigenin, chlorogenic acid and cynarin than leaf petioles blanched for 30 days. Extending blanching from 10 to 30 days before harvest of leaves decreased the content of apigenin and cynarin in leaf petioles while increased the content of chlorogenic acid. In case of apigenin and chlorogenic acid such a tendency was noted in all years of the experiment.

DISCUSSION

In the experiment conducted in the years 2011–2013 in the process of cardoon leaves blanching a positive effect was obtained both with the use of black non-woven (PP) and white and black foil (PE), as a respectively high total yield of leaves was obtained (7.08–7.46 kg m^{-2}), while in case of non-blanched plants it was 7.87 kg m^{-2}. It was noted [Wojciechowska and Siwek 2011], that the white colour

of foil increases light reflection from the surface and extends the duration of high photosynthetic activity in the life of leaves. According to Sugimura [2001], a white and black foil used to soil mulching promotes high refelection of photosynthetic radiation (PAR), what might improve light and thermal conditions around plants, thereby increasing yield. The results of our experiment did not prove such a positive influence of white and black foil on yield in comparison to black non-woven. It should be emphasized that in the conditions of the presented experiment, lower soil temperatures were noted under white and black foil (PE), what is connected with different physical features of the materials. Soil covered with the foil having as external side the white one increased soil temperature during low air temperatures, and lowered soil temperature during high air temperatures [Filipović et al. 2016].

In the presented work mulching of soil and blanching of plants with black non-woven (PP) increased yield of leaves longer than 60 cm, while use of white and black foil increased yield of the longest leaves (over 80 cm). The relationship between the yield structure and the type of mulching material was described earlier by other authors: Golian et al. [2012] and Michalik [2008] in cultivation of celery (*Apium graveolens* var. *dulce*), Golian and Anyszka [2015] in cultivation of celery cabbage (*Brassica pekinensis*) and leek (*Allium ampeloprasum* var. *porrum*).

In a cardoon cultivation with the use of covers, the plants formed similar number of leaves with similar mean weight. In comparison to uncovered plants a significant influence of the used materials for soil mulching and blanching on morphological features of cardoon leaves, length and width of leaf petioles was not proven. The positive influence of the used materials for mulching, increasing number of leaves per plant as well as their mean weight was described in works of other authors: in studies on cultivation of celery and butter head lettuce (*Lactuca sativa*) covered with polypropylene foil of different colours [Siwek et al. 2009] and in the experiment concerning the effect of mulching with foil (PE) and paper on a butter head lettuce [Brault and Stewart 2002].

Cardoon is a plant of a high dietetic and medicinal importance, so that a primary significance is not only yield, but also a quality expressed with chemical composition. Leaf petioles obtained from non-blanched plants contained more dry weight, L-ascorbic acid, chlorophyll, total phenolic acids and total flavonoids, and at the same time less total sugars in comparison to plants covered. On the contrary, a limitation to light exposure in case of plants covered with white and black foil (PE) in comparison to black non-woven (PP) caused that leaf petioles had less dry weight, L-ascorbic acid, total phenolic acids and total flavonoids, and at the same time more crude fibre, total sugars and chlorophyll. Different influence of the used materials for soil mulching and blanching was connected with different soil temperature and microclimate around plants. Similar tendencies that limitation of light exposure through covering edible parts of plants with foil reduces the content of dry weight accompanied by increase of total sugars was noted in cultivation of celery [Michalik 2008, Siwek et al. 2009], kohlrabi (*Brassica oleracea* var. *gongylodes*) [Biesiada 2008], melon (*Cucumis melo*) [Majkowska-Gadomska 2010], chicory (*Cichorium endivia*) [Kowalczyk et al. 2015] and lettuce [Kleinhenz et al. 2003, Siwek and Ambroszczyk 2009]. The effect of reduction of light to edible parts of plants through blanching with different materials also decreases the content of L-ascorbic acid, chlorophyll and anthocyanins, what was observed in cultivation of celery [Siwek and Libik 2005, Wojciechowska and Siwek 2006, Wojciechowska et al. 2007].

In the presented work, together with extending duration of leaf petioles blanching from 10 to 30 days, the content of dry weight, crude fibre, chlorophyll, total sugars, L-ascorbic acid and flavonoids decreased. The results indicate an especially high sensitivity of parenchyma tissues of cardoon leaf petioles to intensive light. As a consequence of extending the time of reduction in light availability through blanching of leaf petioles with foil and non-woven (till 30 days before harvest), there was a destruction of chlorophyll in leaf petioles, leading finally to their decolourisation. The lack of light under covers resulting in low photosynthesis activity in leaves of many plants species decreases the content

of total sugars, L-ascorbic acid, chlorophyll and crude fibre [Rekowska and Skupień 2007, Michalik 2008].

Extending the blanching period up to 30 days before leaves harvest slightly decreased the content of total sugars in edible parts of cardoon. According to data obtained by Kowalczyk et al. [2015], in hydroponic cultivation of endive, blanching of leaves with white and black foil for 10 days before planned harvest caused significantly higher decrease in total sugars content, even by 88%, in comparison to plants non-blanched.

Extending the blanching of leaf petioles from 10 to 30 days before harvest was accompanied by decrease in content of total flavonoids (respectively 0.662–0.352% of fresh weight) together with decrease in apigenin quantity (170–130 mg 100 g of fresh weight). Blanching leaves for 30 days before harvest increased the content of total phenolic acids (from 0.144 to 0.155% of fresh weight) and chlorogenic acid (from 562 to 678 mg 100 g of fresh weight), while decreased the content of cynarin (from 1.46 to 1.25 mg 100 g of fresh weight) in comparison to 10 days blanching of cardoon leaves. The obtained results are coincide with the work of Pinelli et al. [2007], where a significantly lower content of flavonoids and their glycosides, luteolin and apigenin, was noted in blanched leaf petioles of cardoon (2.7–6.3% of dry weight) in comparison to non-blanched ones (25.0–35.2% of dry weight), wherein apigenin was not noted in non-blanched plants. In the available literature [Edreva et al. 2005, Tsormpatsidis et al. 2008] it is clearly indicated that flavonoids protect plant cells against high insolation, especially against harmful UV-B radiation (280–320 nm). A similar relationship was proved by Schmidt et al. [2010] in cultivation of white mustard in different light conditions, lower amount of light in January decreased the content of flavonoids in leaves in comparison to October, when light conditions were definitely more favourable. Tattini et al. [2005] in case of *Ligustrum vulgare* woody plants observed, that at the low sunlight intensity, the share of apigenin decreased and the content of quercetin and luteolin increased among the flavonoids present in leaves. In the work of Reinfenrath and Müller et al. [2007] a similar analogy could be found as plants of white mustard (*Synapis alba*) cultivated in field contained more flavonoids than those cultivated at the same time in a glasshouse in conditions of low light intensity.

A high dependence of antioxidant properties of extracts obtained from artichoke and cardoon leaves from phenolic compounds showed Llorach et al [2002] and Wang et al. [2003] in their research. Such a relationship was not proven in this work. The materials used for leaves covering as well as blanching duration had no influence on the degree of the DPPH radical inhibition. The extended period of leaves blanching decreased content of total flavonoids and at the same time increased the content of total phenolic acids. To some extent, these contradictions are explained by articles, in which it was noted that an ability to surpress a free DPPH radical, therefore antioxidant capacity, does not directly depend on the content of phenolic compounds but from a biological activity [Falleh et al. 2008, Velez et al. 2012]. Among caffeoylquinic acids, cynarin and chlorogenic acids are included in the most important ones, such as luteolin and apigenin from the group of flavonoids [Di Venere et al. 2005]. Falleh et al. [2008] expressed an opinion that all derivatives of caffeic acid, and among them chlorogenic acid and cynarin, as secondary metabolites in cell fluid undergo transformations, and the content of these two compounds in case of cardoon decide about biological activity in a significant way. It might be supposed that in the presented experiment, an antioxidant activity depended mainly on the content of chlorogenic acid and cynarin but not on total phenolic compounds content, which amount decreased as a result of leaves blanching.

The results of the experiment presented in this work confirm the influence of weather conditions on the content of chemical compounds and secondary metabolites in leaf petioles of cardoon. In the year 2012 the level of dry weight, crude fibre, total sugars, L-ascorbic acid in leaf petioles of cardoon was higher, and in the year 2013, plants accumulated more phenolic acids and less flavonoids than in the year 2011. In the literature there is a common opinion that the differences in the contents of phenolic compounds in cardoon leaves result from different weath-

er conditions, especially light intensity and temperature during cultivation [Falleh et al. 2008, Schmitd et al. 2010, Gouveia and Castilho 2012].

CONCLUSION

On the basis of the results of the experiment it was stated, that both way of leaf petioles blanching as well as duration of blanching had no influence on total yield of leaves.

Leaf petioles obtained from plants covered with black non-woven (PP) and white and black foil (PE) contained slightly less dry weight, L-ascorbic acid, chlorophyll, total phenolic acids and total flavonoids, and at the same time more total sugars in comparison to non-blanched ones. Limitation in light availability to plants with the use of black non-woven (PP) on the contrary to white and black foil (PE), made the leaf petioles more juicy and less coloured, containing less crude fibre, chlorophyll and more dry weight, L-ascorbic acid, total flavonoids and total phenolic acids.

The blanching duration of the plants had no significant effect neither on yield nor on morphological features of cardoon, only the leaves obtained from plants blanched for 30 days before harvest showed a higher mean weight. Leaf petioles harvested from plants blanched for shorter time, 10 days before harvest, contained more dry weight, crude fibre, total sugars, L-ascorbic acid, chlorophyll, total flavonoids, and less total phenolic acids in comparison to those blanched for 30 days.

The materials used for leaf blanching as well as duration of the treatment had no influence on a degree of the DPPH radical inhibition.

In the presented experiment an influence of the weather conditions on content of chemical compounds and secondary metabolites in leaf petioles of cardoon was confirmed. In the year 2012, when the mean air temperature during plants growth was higher and a precipitation lower in comparison to 2011, leaf petioles had more dry weight, crude fibre, total sugars and L-ascorbic acid.

In the year 2013, when mean diurnal air temperatures and precipitation were higher than multiannual mean, plants accumulated more phenolic acids and less flavonoids.

Due to high palatability, medicinal and dietetic values, resulting from high content of total sugars, L-ascorbic acid, crude fibre, chlorophyll, carotenoids, mineral salts, polyphenols and many other compounds of mainly medicinal activity, it deserves a spread in cultivation and consumption.

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