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THE EFFECTS OF ORGANIC AND CONVENTIONAL CULTIVATION SYSTEMS ON THE CONTENT OF BIOACTIVE SUBSTANCES IN HERBAL PLANTS

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Summary

Five species of herbs from organic and conventional cultivation (dropsy plant, lovage, mint, thyme and sage) were used for this experiment. The research material consisted of usable parts of fresh herbs, which were harvested in the first ten days of September 2009 and 2010. In order to avoid losses in the content of biologically active compounds in the plant material, the herbs were frozen at -80°C immediately after harvesting, and then subjected to freeze-drying; finally, analytical research was conducted. The results of two-year studies indicated that herbal plants from organic production contained a higher level of dry matter, vitamin C and flavonoids, while the conventional ones contained more phenolic acids. Regardless of the cultivation system, dropsy plant and mint were distinguished by the content of phenolic acids, the highest level of flavonoids was found in thyme, while lovage was the raw material containing the most vitamin C. The qualitative analysis of phenolic compounds contained in herbs showed that the most diverse composition of phenolic acids was attributed to sage and thyme, which also contained the largest number of identified flavonoids.

key words: herbs, organic cultivation, conventional cultivation, flavonoids, phenolic acids, vitamin C

INTRODUCTION

Herbal plants are an important component of diet as they contribute to the enhanced taste and aroma of foods and provide significant nutrients and bioactive substances. Thanks to that they enjoy a broad application in gastronomy, medicine as well as cosmetic and perfumery industries. They owe their unusual properties to the present essential oils, phenolic compounds, tannins, bitter compounds, organic acids and muci. Fresh herbs also contain enzymes, vitamins, phy-

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toncides and other active substances. They are a valuable source of antioxidants such as phenolic acids, flavonoids and vitamin C, which are important from the standpoint of prevention of many diseases caused by free radicals, including cardiovascular diseases, atherosclerosis, Alzheimer's disease, Parkinson's disease and tumours (Bartosz 1995, Grajek 2007).

Currently, among consumers there is observed the increased demand for food produced in a natural way, without the use of agricultural chemicals, resulting in rapid development of organic farming (Łuczka-Bakuła 2007). Its main purpose is to care about the environment, maintain an appropriate degree of soil fertility and produce high quality food. Crops are produced through natural methods which do not cause their contamination or loss of nutritional value and health properties (Worthington 2001). In accordance with the applicable laws, it is not permitted to use synthetic pesticides and artificial fertilizers in organic cultivation system. However, there are used natural fertilizers such as manure, compost and green manures as well as natural methods of protection, including the use of pathogens to fight natural enemies, the use of pheromone traps or glue boards (The Council Regulation 834/2007). That stimulates plants to a higher synthesis of phenolic compounds, which perform the function of natural pesticides (Young *et al*. 2005).

The results of many scientific studies on the quality of agricultural products from organic and conventional systems show a higher nutritional value and a higher content of biologically active compounds in organic raw materials (Brandt & Molgard 2001, Carbonaro *et al*. 2002, Hallmann & Rembiałkowska 2007, 2008, Bourn & Prescott 2002). At the same time, there are some results provided by other references which do not confirm such differences (Kazimierczak *et al*. 2009, Rembiałkowska & Hallmann 2007, Wunderlich *et al*. 2008, Asami *et al*. 2003). In numerous studies, the authors suggest that the variability of the quality parameters studied is hugely influenced by a crop season; therefore, the papers containing a comparison of quality parameters of plant raw materials should include at least two-year studies (Chassy *et al*. 2006, Pieper & Barrett 2009).

The purpose of this paper was to verify the hypothesis that herbal plants from organic production contain more bioactive compounds than the conventional ones.

MATERIAL AND METHODS

There were selected five species of herbal plants for the experiment: common mint, garden sage, dropsy plant, thyme and garden lovage. The choice of herbs was based on their high health value and frequent use by consumers. The herbs were grown in the years of 2009 and 2010, on a certified organic farm and a conventional farm located nearby in order to ensure similar climate and soil conditions. Table 1 presents the types of fertilizers and the nutrient content used in both years of cultivation. The study material consisted of fresh herbs collected from three- and four-year plantations. Herb harvesting was performed manually, during the phase of harvest ripeness corresponding to the utility ripeness of individual species.

Table 1. Characteristic of localization and fertilizers used for organic and conventional cultivation of herbs (mean value for 2009-2010)

Farm	Localization	Fertilizers	Fertilization range ($kg \cdot ha^{-1}$)		
			N	P	K
Organic	Rozalin 52° 10' N 18° 51' E	manure	80	45	105
Conventional	Szczytno 52° 56' N 20°48' E	mixed fertilizer	92.5	13.3	112

The weight of laboratory samples for testing was min. 0.2 kg. The usable parts of separate herb species (common mint, garden sage, dropsy plant, and thyme – leaves and leafy tops of shoots, and lovage garden – leaves) were used for analysis. In fresh herbs were determined: dry matter content by gravimetric method according to PN-R-04013:1988, the content of vitamin C by titration method according to PN-A-75101-11:1990, and the content of phenolic compounds by the method high performance liquid chromatography (HPLC) described by Hertog *et al*. (1992) with modifications. A weighted amount of freeze-dried herb sample (100 mg) was put into a plastic test tube, then 1 ml of methanol with 1% ascorbic acid were added, mixed thoroughly by vortex and incubated in an ultrasonic bath (15 minutes at 30°C). Then the samples were swirled at the speed of 5,000 rpm. From the test tube 1 ml of extract was collected and re-swirled at the speed of 12,000 rpm. The amount of 500 µl of extract was taken for HPLC vials and analyzed. To determine polyphenols there was used high performance liquid chromatograph HPLC Shimazu (Poland), consisting of two LC-20AD pumps, CMB-20A system controller, SIL-20AC autosampler, UV/VIS SPD-20AV detector, CTD-20AC oven, and Synergi Fusion-RP 80i column (250 x 4.60 mm). The gradient flow was applied along with two mobile phases - acetonitrile/deionized water (55% and 10%), pH 3.00. Time of the analysis - 36 minutes, flow - 1 ml/minute, wavelength - 250-370 nm. Polyphenols were identified based on Fluka and Sigma Aldrich external standards with purity of 99.5%. In order to avoid losses in the content of biologically active compounds in the plant material, the herbs were frozen at -80°C immediately after harvesting, and then subjected to freeze-drying; finally, analytical research was conducted.

For statistical analysis of results there was used two-way analysis of variance with the Tukey's test (α=0.05). A lack of differences between the examined groups was determined with the same letters. The p-value coefficient is given by figures and tables. If the coefficient calculated was statistically insignificant, the result of statistical analysis was determined as n.s. (not statistically significant). The factors tested was method of cultivation (organic and conventional) and plant species. Additionally, standard deviations (s.d.) for the examined sample were calculated. Each analysis was carried out in triplicate. The results were specified as a

mean of two-year experiment.

RESULTS AND DISCUSSION

The results of the studies showed a significantly higher content of dry matter in organic raw materials compared to conventional ones. This is confirmed by the studies on herbs, conducted by Seidler-Łożykowska *et al*. (2006), which reported the higher content of dry matter in organic plant material. The research on dried herbal spices having been purchased (Kazimierczak *et al*. 2010) also showed the higher dry matter content in the products which had a certificate of organic production compared to those coming from conventional system. The higher content of dry matter in organic raw materials compared to the conventional ones is also confirmed by the studies on other plant materials, including onions (Hallmann & Rembiałkowska 2006), apples (Rembiałkowska *et al*. 2004), beetroots (Sikora *et al*. 2007), and fresh carrot juice (Sikora *et al*. 2009). The opposite results were obtained in the studies on fruit and products of black currant (Kazimierczak *et al*. 2007, Kazimierczak *et al*. 2009) as well as two varieties of carrot - Perfekcja and Flacoro from organic and conventional cultivation systems (Rembiałkowska & Hallmann 2007), where conventional raw materials contained more dry matter.

The results obtained as well as the results of other authors, which confirm a higher content of dry matter in organic raw materials, can be explained by the fact of mineral fertilizers application in conventional crops, which results in the increased yield biomass; at the same time, the volume of water in plant cells grows, which, in turn, causes decline in dry matter content (Brandt & Mølgaard 2001).

In the own study, there was found that the herbs from organic farming contained more vitamin C compared to the conventional ones. Lovage was the richest in this vitamin (Table 2). Researchers engaged in studies on the quality of plant raw materials point out that the vitamin C content in plants is undoubtedly influenced by a cultivation method, where one of the decisive factors is fertilizing of plants. Their results generally confirm that the use of high doses of nitrogen fertilizer has a negative effect on the synthesis and accumulation of vitamin C in plants. It was proved, among others, by Toor *et al*. (2006) who ascertained that the tomatoes which were grown with the use of organic fertilizer were characterized by a higher content of vitamin C. These results correspond to the studies of organic and conventional fruit of blackcurrant (Kazimierczak *et al*. 2007), onion (Hallmann & Rembiałkowska 2006), pepper and tomato (Hallmann *et al*. 2005), potatoes (Rembiałkowska 2000) and beet roots (Sikora *et al*. 2008), as a result of which there was found a greater abundance of vitamin C in the case of organic raw materials, where no nitrogen fertilizer was applied. However, the studies on carrots from different cultivation systems presented the results of the vitamin C content in favour of conventional raw materials (Rembiałkowska & Hallmann 2007). With respect to the broccoli study, there was found no difference between the content of ascorbic acid in

organic and conventional products (Wunderlich *et al*. 2008), whereas conventional corn contained more vitamin C by 34% compared to the organic one (Asami *et al*. 2003).

Table 2. Content of dry matter and ascorbic acid in selected herbs from organic and conventional production systems (± standard deviation) (mean for 2009-2010)

Growing system	Herbs	Dry matter ($g \cdot 100\ g^{-1}$ f.m.)	Ascorbic acid ($mg \cdot 100\ g^{-1}$ f.m.)
Organic growing	dropsy plant	26.01±0.19	63.06±1.93
	mint	20.93±0.40	46.89±0.94
	lovage	19.14±0.09	48.60±2.10
	thyme	30.57±0.85	64.52±4.88
	sage	24.20±0.88	43.95±2.47
Conventional growing	dropsy plant	18.86±0.60	33.81±2.40
	mint	18.19±0.44	33.21±1.79
	lovage	17.27±0.15	95.61±3.65
	thyme	18.06±0.79	28.53±4.81
	sage	18.59±0.53	44.74±1.22
Mean for organic growing*		24.17 b	53.10 b
Mean for conventional growing		18.19 a	47.18 a
Mean for dropsy plant		22.43 c	48.44 b
Mean for mint		19.56 b	40.05 a
Mean for lovage		18.20 a	72.10 c
Mean for thyme		24.32 d	46.52 b
Mean for sage		21.40 c	43.60 ab
p-value			
Growing system		<0.0001	<0.0001
Herbs		<0.0001	<0.0001
Interaction		<0.0001	<0.0001

Note: Data followed by the different letters differ significantly at the 5% level of probability (α=0.05)

Ambiguity of the results regarding the vitamin C content in agricultural crops from conventional and organic farming confirms the thesis that the concentration of this vitamin in plants is affected by many non-cultivation factors connected with environmental conditions. It should also be noted that in the case of dropsy plant, mint and thyme the vitamin C content was significantly higher when they derived from organic farming, while the opposite situation concerned sage and lovage, which had bearing on the mean result.

According to data contained in Table 3, the method of cultivation had an impact on the accumulation of phenolic acids and flavonoids in herbs. It was confirmed that on average more phenolic acids were found in conventional raw materials, mainly due to dropsy plant, mint and thyme which were richer in these compounds when they derived from conventional cultivation. In the case of lovage and sage, a higher content of phenolic

acids was observed in organic raw materials. More flavonoids occurred in herbs from organic farming, and the dependence concerned most species. Only in the case of dropsy plant more flavonoids were found in the conventional herb. The results obtained correspond to the results of the studies on dried herbal spices from organic and conventional production (Kazimierczak *et al*. 2010). The results of blackcurrant and its products (Kazimierczak *et al*. 2007, Kazimierczak *et al*. 2009), onion (Hallmann & Rembiałkowska 2006), tomatoes (Rembiałkowska *et al*. 2003), beetroots (Sikora *et al*. 2008) and peaches (Carbonaro *et al*. 2002) also showed that the raw materials from organic cultivation system contained more flavonoids in relation to the conventional ones. Unfortunately, there are no reports on the analysis of phenolic acids in fresh herbs from different cultivation systems. The results similar to the ones presented in this paper were obtained in the studies on blackberries, strawberries, corn and Chinese cabbage, which contained significantly more phenolic acids when they derived from organic farming (Asami *et al*. 2003, Zhao *et al*. 2008).

Table 3. Total flawonoids and phenolic acids content in selected herbs from organic and conventional production systems (± standard deviation) (mean for 2009-2010)

Growing system	Herbs	Flavnoids (mg·100 g^{-1} f.m.)	Phenolic acids (mg·100 g^{-1} f.m.)
Organic growing	dropsy plant	71.72±1.56	34.01±1.47
	mint	274.13±18.45	216.64±197.49
	lovage	122.08±7.21	212.34±10.46
	thyme	385.22±55.80	124.78±12.48
	sage	182.90±12.76	184.79±8.15
Conventional growing	dropsy plant	90.98±9.64	724.84±15.79
	mint	96.56±2.61	509.53±7.47
	lovage	90.00±2.20	74.80±1.98
	thyme	361.10±23.40	206.56±29.83
	sage	86.80±2.73	103.42±2.25
Mean for organic growing*		207.21 b	154.51 a
Mean for conventional growing		145.09 a	323.83 b
Mean for dropsy plant		81.35 a	379.43 b
Mean for mint		185.35 c	363.09 b
Mean for lovage		106.04 ab	143.57 a
Mean for thyme		373.16 d	165.67 a
Mean for sage		134.85 b	144.10 a
p-value			
Growing system		<0.0001	<0.0001
Herbs		<0.0001	<0.0001
Interaction		<0.0001	<0.0001

Note: see table 2

In order to determine which of the herbal plants under examination are characterised by the richest and the most diverse composition, the plant material was subjected to qualitative analysis involving the identification of compounds based on external Fluka and Sigma Aldrich standards, with a purity of 99.5%. As a result of this analysis, there were revealed the following phenolic acids: gallic acid, chlorogenic acid, caffeic acid, p-coumarin acid and ferulic acid. In the group of flavonoids, in the material from both cultivation systems were identified: rutin, d-quercetin glucoside, quercetin, d-kaempferol glucoside, kaempferol, myricetin, luteolin and naringinin. The greatest abundance of the identified phenolic acids was typical for sage and lovage, where all detected substances occurred, while the poorest composition of phenolic acids was a property of dropsy plant and mint (from conventional cultivation). The highest amount of a gallic acid (163.64 mg·100 g^{-1} f.m.) and p-coumarin acid (225.44 mg·100 g^{-1} f.m.) contained dropsy plant from conventional production, in which there was no found others phenolic acids. The highest content of chlorogenic acid was identified in sage (24.20 mg·100 g^{-1} f.m.) from conventional production and in organic mint (18.89 mg·100 g^{-1} f.m.) and organic lovage (17.92 mg·100 g^{-1} f.m.). Caffeic acid was found in the largest quantity in organically produced mint (63.29 mg·100 g^{-1} f.m.) and lovage (52.19 mg·100 g^{-1} f.m.) and ferulic acid in conventionally produced thyme (43.46 mg·100 g^{-1} f.m.) (Table 4).

Table 4. Content of identified constituents of phenolic acids from selected herbs from organic and conventional production systems (in mg·100 g^{-1} f.m.) (mean for 2009-2010)

Growing system	Herbs	Gallic acid	Chlorogenic acid	Caffeic acid	P-coumarin acid	Ferulic acid
Organic growing	dropsy plant	8.30	0.00	0.00	12.13	0.00
	mint	7.17	18.89	63.29	2.68	0.00
	lovage	2.88	17.92	52.19	10.08	7.65
	thyme	7.18	0.00	3.08	16.35	14.26
	sage	12.80	20.54	2.95	26.88	31.61
	mean	7.67	14.77	24.30	13.62	10.70
Conventional growing	dropsy plant	163.64	0.00	0.00	225.44	0.00
	mint	6.62	0.00	0.00	201.63	0.00
	lovage	2.70	14.12	8.34	7.99	1.66
	thyme	8.38	2.44	5.64	19.88	43.46
	sage	3.27	24.20	2.44	14.56	15.89
	mean	36.92	10.07	3.28	93.90	12.20

Table 5. Content of identified constituents of flavonoids from selected herbs from organic and conventional production systems (in mg·100 g^{-1} f.m.) (mean for 2009-2010)

Growing system	Herbs	Rutin	D-quercetin glucoside	D-kaempferol glucoside	Myricetin	Quercetin	Kaempferol	Naringinin	Luteolin
Organic growing	dropsy plant	4.75	0.00	18.14	3.06	0.71	0.00	0.00	14.23
	mint	56.47	40.29	4.94	24.32	17.47	2.07	0.00	0.00
	lovage	9.98	24.86	0.00	4.79	9.46	0.66	35.98	0.00
	thyme	10.61	60.87	36.03	37.06	17.45	0.00	63.69	2.39
	sage	19.24	17.70	17.62	24.60	0.00	7.55	0.00	5.23
	mean	20.21	25.29	15.35	18.76	9.02	2.05	19.93	4.37
Conventional growing	dropsy plant	3.56	0.00	13.81	12.04	0.75	0.00	0.00	15.46
	mint	0.41	2.54	6.38	16.54	14.31	0.00	0.00	0.00
	lovage	6.92	20.84	0.00	3.96	7.72	0.64	21.66	0.00
	thyme	23.33	88.16	28.64	2.62	0.00	0.00	67.43	3.20
	sage	11.59	0.00	30.28	0.00	0.00	6.27	0.00	5.45
	mean	9.16	22.31	15.82	7.03	4.56	1.38	17.82	4.82

With regard to flavonoids, the largest content was associated with thyme from organic production, which contained all the flavonoids detected, but the lowest quantity of the compounds was typical for conventional sage. The results concerning the contents of individual flavonoids in herbs have shown the largest amount of rutin (56.47 mg·100 g^{-1} f.m.) in organic mint and d-kaempferol glucoside (36.03 mg·100 g^{-1} f.m.) and myricetin (37.06 mg·100 g^{-1} f.m.) in thyme from organic cultivation. Organic mint (17.47 mg·100 g^{-1} f.m.) and organic thyme (17.45 mg·100 g^{-1} f.m.) were richest in quercetin. In herbs was relatively few of kaempfrol and the richest was organic sage (7.55 mg·100 g^{-1} f.m.). Highest quantity of d-quercetin glucoside (88.16 mg·100 g^{-1} f.m.) and naringinin (67.43 mg·100 g^{-1} f.m.) contained a conventional thyme, while the richest in luteolin was dropsy plant (15.46 mg·100 g^{-1} f.m.) from conventional cultivation (Table 5).

As a result of analysis of profile of flavonoids and phenolic acids, there were not detected all compounds in some samples tested, and therefore statistical analysis related to the impact of cultivation methods and plant species on the content of individual compounds was impossible to perform.

CONCLUSIONS

1. There was confirmed the influence of a cultivation method on the content of biologically active substances in the herbal plants examined. The herbs from organic production exhibited a higher content of vitamin C, flavonoids and dry matter, while more phenolic acids were detected in the conventional herbs.
2. Regardless of the cultivation system which the herbs tested derived from, dropsy plant and mint were distinguished by the content of phenolic acids, the highest level of flavonoids was found in thyme, while lovage was the raw material containing the most vitamin C.
3. The analysis of phenolic compounds contained in herbs showed that the most diverse composition of flavonoids was attributed to thyme, while sage and thyme contained the largest number of the identified phenolic acids.

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WPŁYW EKOLOGICZNEGO I KONWENCJONALNEGO SYSTEMU UPRAWY NA ZAWARTOŚĆ SUBSTANCJI BIOAKTYWNYCH W ROŚLINACH ZIELARSKICH

Streszczenie

Badaniami objęto pięć gatunków roślin zielarskich: melisa lekarska, lubczyk ogrodowy, mięta pospolita, tymianek pospolity i szałwia lekarska, które pochodziły z uprawy ekologicznej i konwencjonalnej. Materiał do badań stanowiły części użytkowe

świeżych ziół, zebrane w pierwszej dekadzie września 2009 i 2010 roku. Aby uniknąć strat w zawartości związków biologicznie czynnych w materiale roślinnym, natychmiast po zbiorze zioła zostały zamrożone w temperaturze -80°C, a następnie poddano je liofilizacji, po czym przeprowadzono badania analityczne. Dwuletnie wyniki badań wykazały, że zioła z produkcji ekologicznej zawierały więcej suchej masy, witaminy C i flawonoidów, natomiast zioła konwencjonalne odznaczały się wyższą zawartością kwasów fenolowych. Niezależnie od systemu uprawy, pod względem zawartości kwasów fenolowych wyróżniały się melisa i mięta, najwięcej flawonoidów stwierdzono w tymianku, natomiast surowcem, który zawierał najwięcej witaminy C był lubczyk. Analiza profilu związków fenolowych zawartych w ziołach wykazała, że najbardziej urozmaiconym składem kwasów fenolowych odznaczały się szałwia i tymianek, który jednocześnie zawierał największą ilość zidentyfikowanych flawonoidów.