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THE COMPARISON OF THE BIOACTIVE COMPOUNDS CONTENT IN SELECTED LEAFY VEGETABLES COMING FROM ORGANIC AND CONVENTIONAL PRODUCTION

Summary

Four species of leafy vegetables from organic and conventional production (coriander, leaf mustard, rocket salad and watercress) were used in this experiment. The research material consisted of edible parts of vegetables harvested in the beginning of September 2015. After harvest, dry matter content of plant samples was determined. Then plant material was freeze-dried and stored at -80°C until further laboratory analyses (for chlorophylls, total polyphenols, phenolic acids, flavonoids, carotenoids) were performed. The experiment indicated no differences in dry matter and chlorophylls content between plants from organic and conventional production. At the same time organic vegetables contained, on average, significantly more polyphenols (total) and phenolic acids in comparison to conventional ones. In contrast, plants from conventional production were more abundant in total flavonoids and carotenoids. The effect of agricultural production methods on the content of individual phenolic compounds and carotenoids in vegetables was also observed. Among the examined species, coriander (especially the organic one) was the richest in total polyphenols, in that phenolic acids and flavonoids, as well as carotenoids and chlorophylls. The obtained results provide confirmation that the leafy vegetables, especially those from organic farming, serve as important sources of valuable compounds, i.e. antioxidants with beneficial effects on the human body. Thus, they should be recommended in a preventive healthcare.
***Key words**: coriander, leaf mustard, rocket salad, watercress, organic, conventional, agriculture, polyphenols, phenolic acids, carotenoids, chlorophylls*

PORÓWNANIE ZAWARTOŚCI SKŁADNIKÓW BIOAKTYWNYCH W WYBRANYCH WARZYWACH LIŚCIOWYCH POCHODZĄCYCH Z PRODUKCJI EKOLOGICZNEJ I KONWENCJONALNEJ

Streszczenie

W eksperymencie wykorzystano cztery gatunki warzyw liściowych pochodzących z produkcji ekologicznej i konwencjonalnej (kolendra, musztardowiec, rukola i rukiew wodna). Materiał badawczy stanowiły części użytkowe warzyw, które zebrano na początku września 2015. Po zbiorze w warzywach oznaczono zawartość suchej masy. Następnie materiał roślinny poddano liofilizacji i przechowywano w temperaturze minus 80 °C celem ustabilizowania składu chemicznego, a następnie przeprowadzono analizy laboratoryjne. W wyniku przeprowadzonych badań nie wykazano różnic w zawartości suchej masy i chlorofili pomiędzy roślinami z produkcji ekologicznej i konwencjonalnej. Jednocześnie warzywa z produkcji ekologicznej zawierały średnio istotnie więcej polifenoli ogółem i kwasów fenolowych w porównaniu do warzyw konwencjonalnych. Natomiast warzywa z produkcji konwencjonalnej były zasobniejsze we flawonoidy i karotenoidy. System produkcji wpływał również, choć nie tak wyraźnie, na zawartość poszczególnych związków fenolowych i karotenoidów w warzywach. Wśród badanych gatunków najbogatszą pod względem zawartości polifenoli ogółem, w tym kwasów fenolowych i flawonoidów, a także karotenoidów i chlorofili była kolendra, która pochodziła z produkcji ekologicznej. Uzyskane wyniki stanowią wyraźne potwierdzenie, że warzywa liściowe, a zwłaszcza te z upraw ekologicznych, są istotnym źródłem cennych składników, m.in. przeciwutleniaczy wykazujących korzystny wpływ na organizm ludzki. Dlatego też powinny być one zalecane w profilaktyce zdrowotnej.
***Słowa kluczowe**: kolendra, musztardowiec, rukola, rukiew wodna, produkcja ekologiczna i konwencjonalna, polifenole, karotenoidy, chlorofile*

1. Introduction

Leafy vegetables represent a valuable source of minerals, fiber, and vitamin C, as well as vegetable pigments (carotenoids, chlorophylls) and polyphenolic compounds, with high biological activity. Most of these compounds are known to act as strong antioxidants that protect human body against harmful effects of free radicals, such as heart diseases, tumors and aging processes [1, 2, 3, 4]. As shown by many research studies, organic production system promotes the synthesis of the higher amounts of antioxidant substances by plants when compared to the conventional system. The reason for the differences in the chemical composition of plant raw materials from organic and conventional production is a different approach to the use of fertilizers and plant protection agents in these systems. Mineral fertilizers, especially nitrogen, used in conventional production stimulates production of plant biomass. This can down regulate other important processes in plants, resulting in depletion of the chemical composition of plant organs. This phenomenon can be explained by the growth and differentiation balance hypothesis - GDBH [5, 6]. According to this hypothesis, plants regulate their metabolism in order to maximize the biomass production (growth) and/or stimulate differentiation of produced substances depending on the availability of minerals in the soil. In situation when the concentration of available minerals (nitrogen) in the soil is high, the growth direction predominates, and when it is low, differentiation is stimulated. Differentiation means the production of greater amounts of various secondary metabolites based on carbon, such as starch, cellulose, vitamin C and other secondary metabolites not containing nitrogen, e.g. terpenoids, flavonoids and phenolic acids.

As Stefanelli et al. [7] highlighted in their study that higher accumulation of polyphenol compounds in plants grown with a deficit of nitrogen can also be explained by the theory of the carbon to nitrogen (C / N) ratio, published for the first time by Bryant et al. [8] and confirmed in the Coley' et al. [9], Lerdau and Coley' [10], and Gayler' et al. [11] studies. According to this theory, limited availability of nitrogen causes its lower assimilation, limiting plant growth and photosynthesis, and thus reducing the synthesis of metabolites based on nitrogen, while increasing the synthesis of carbon-based secondary metabolites. The release of nutrients per time unit from easily assimilable mineral fertilizers differs much from the release of nutrients from organic fertilizers, where macro- and microelements become available to plants gradually, after mineralization by soil fauna and flora [12]. Thus, the differences in the availability of nutrients for plants during the growing period can cause differences in the plant composition, even at similar levels of yields.

In the conventional system readily available nutrients in high doses are provided to the plants with mineral fertilization, and at the same time chemical protection against diseases and pests is used. At the same time plants grown in organic system collect nutrients much slower from fertilizers with their lower availability (e.g. manure, compost), and disease and pest control in organic farms is carried out with the use of non-chemical protection methods, such as biological and mechanical control [13, 14].

Although the number of evidence on the differences in the chemical composition of plants from organic and conventional production is growing, the question whether organic products, including vegetables, are characterized by a higher nutritional and biological value in comparison to their conventional counterparts, is still a discussion subject of the scientists around the world. Therefore, the aim of this study was to compare the content of selected antioxidants in leafy vegetables produced under organic and conventional conditions.

2. Materials and methods

The research was carried out in 2015 in Warsaw University of Life Sciences. Research material consisted of four species of leafy vegetables (coriander, leaf mustard, rocket salad, watercress) coming from organic and conventional production. Plants were cultivated in two farms located in close proximity, to ensure similar climate and soil conditions. Both farms are located in Mazovian Voivodship (Poland), in that organic certified farm in Rozalin (52.0483°N, 20.7468°E), and conventional farm in Nadarzyn (52.1000°N, 20.8000°E). The plants were harvested manually in the best vegetative stage of growth from the culinary point of view. Usable parts of the each of vegetable species were collected for laboratory analyses. The size of samples was min. 0,2 kg.

In both farms vegetables were grown on soil quality class III. The organic farm used organic fertilization in the form of compost, with N:P:K ratio of 90kg:45kg:105kg per hectare. In the conventional farm mineral fertilizer was applied; N:P:K ratio amounted to 95.2kg:42.kg:133.7kg per hectare.

Dry matter content of the examined vegetables was determined using gravimetric method, according to the Polish standard [15]. Before the chemical analyses, leaf samples were freeze-dried using a Labconco 2.5 freeze-drier (Labconco Corporation, Kansas City, Misouri, USA) at the temperature of -40 °C and under a pressure of 10 Pa. A freeze-dried samples were kept in plastic test tubes at -80°C, in order to prevent losses of biologically active compounds.

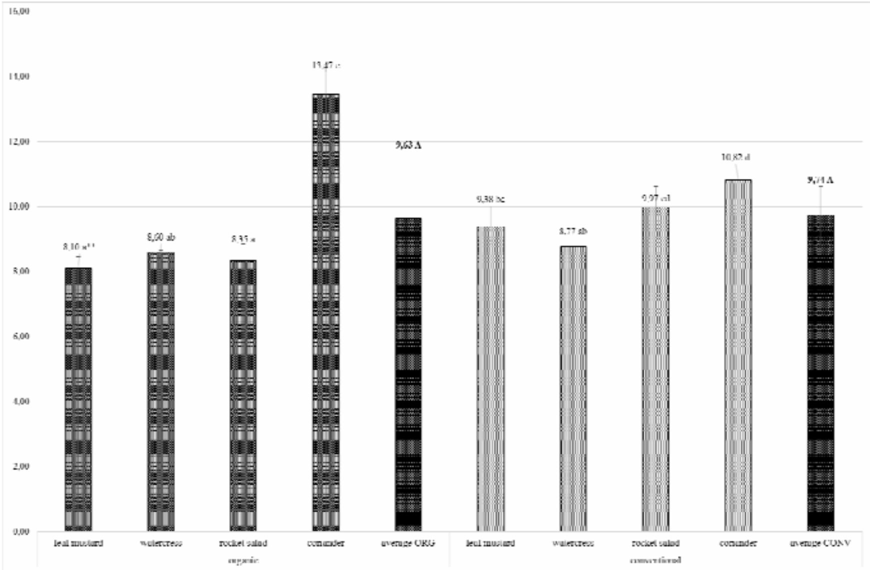
The content of polyphenols (flavonoids and phenolic acids) was determined by HPLC method, with identification of individual phenolic compounds according to the Fluca and Sigma Aldrich standards with a purity of 99.98% (Shimazu equipment, USA Manufacturing Inc, USA: two pump LC-20AD, controller CBM-20A, column oven SIL-20AC, spectrometer UV/Vis SPD-20 AV) [16].

The content of carotenoids and chlorophylls in plants were determined by HPLC method (Shimazu equipment as described above) [17]. Individual carotenoids (lutein, beta-carotene and alpha-carotene) and chlorophylls (a and b) were identified on the basis of the Fluca standards with a purity of 99.98%.

Three independent replicates of each leafy vegetable were analyzed. The results are presented as mean per 100 g of fresh weight (f.w.) ± standard deviation (SD). The data were subjected to a multi-factor analysis of variance ANOVA, followed by the parametric post-hoc Tukey's test ($\alpha = 0.05$), using Statgraphics 5.1. software (StatPoint Technologies, Inc, Warrenton, Virginia, USA). The factors were: the production method (organic and conventional) and plant species. The *p*-values are given in the tables. If the result of the analysis was not statistically significant, it was designated as n.s.

3. Results and discussion

No significant differences ($p>0.05$) were observed between organic and conventional leafy vegetables (on average) in terms of dry matter content. Considering the individual species, the statistically highest content of dry matter was found in coriander from organic production (Figure 1). According to previously published studies higher dry matter content was observed in organic green leafy herbs in comparison to conventional ones [18, 19, 20]. Similar relationship has been confirmed in many studies of fruits and vegetables from both growing systems [21, 22]. As explained by Brandt and Mølgard [23], the use of mineral nitrogen fertilizers in conventional production causes the increase in yields, as well as the increase in water content in plant cells, lowering at the same time the dry matter content in the conventional plants. Analysis of the literature done by Stefanelli et al. [7] confirms that high doses of mineral fertilizers often used in conventional agriculture lead to excessive vegetative growth and reduce dry matter content in crops.



* average ± standard deviation

** means followed by the same letter are not significantly different at the 5% level of probability (Tukey's test, p< 0.05)

Source: own work / *Źródło:* opracowanie własne

Fig. 1. Dry matter content in selected species of leafy vegetables from organic (ORG) and conventional (CONV) production (g 100 g^{-1} f.w.) (average ± s.d.)*

*Rys. 1. Zawartość suchej masy w wybranych gatunkach warzyw liściowych z produkcji ekologicznej i konwencjonalnej (mg 100 g^{-1} ś.m.) (średnia ± s.d.)**

All vegetable species tested in the study were abundant in polyphenols, in that mainly in phenolic acids. Higher concentrations of these compounds were found on average in organically produced vegetables when compared to their conventional counterparts. In contrast, more flavonoids were found in plants from conventional production (Table 2). Total polyphenols as well as total flavonoids and phenolic acids content differed also significantly (p<0.0001) between tested vegetable species. The highest concentrations of these substances were found in coriander leaves. The lowest levels of total polyphenols and phenolic acids were observed in leaf mustard and watercress, respectively (Table 1-2). On average, organically produced vegetables contained significantly more of each of the identified phenolic acids (gallic, chlorogenic, caffeic and ferulic acid) than conventional ones (Table 2).

Table 1. Polyphenols and flavonoids content in selected species of leafy vegetables from organic (ORG) and conventional (CONV) production (mg 100 g^{-1} f.w.) (average ± s.d.)*

*Tab. 1. Zawartość polifenoli ogółem i flawonoidów w wybranych gatunkach warzyw liściowych z produkcji ekologicznej i konwencjonalnej (mg 100 g^{-1} ś.m.) (średnia ± s.d.)**

Species		Total polyphenols	Total flavonoids	Quercetin-3-*O*-glucoside	Quercetin	Kaempferol
ORG	leaf mustard	38.39±0.71 e**	8.61±0.80 c	8.03±0.79 e	0.47±0.04 c	0.11±0.01 a
	watercress	30.52±1.41 c	10.83±0.22 d	10.10±0.18 f	0.51±0.05 cd	0.22±0.02 a
	rocket salad	34.33±2.43 d	1.64±0.03 a	0.55±0.06 a	0.29±0.02 b	0.80±0.01 c
	coriander	114.85±0.32 g	3.33±0.12 b	2.08±0.09 b	0.78±0.01 e	0.47±0.17 b
CONV	leaf mustard	7.43±0.24 a	1.16±0.03 a	0.51±0.02 a	0.54±0.01 d	0.11±0.01 a
	watercress	26.00±0.22 b	12.77±0.27 e	2.65±0.03 c	0.19±0.01 a	9.93±0.24 d
	rocket salad	44.52±0.27 f	8.59±0.29 c	7.24±0.12 d	0.75±0.01 e	0.60±0.17 b
	coriander	42.88±1.94 f	27.90±0.61 f	14.36±0.42 g	0.77±0.04 e	12.77±0.18 e
Average						
ORG		54.52±34.97 B	6.10±3.76 A	5.19±3.99 A	0.51±0.18 A	0.40±0.28 A
CONV		30.21±15.04 A	12.61± 9.77 B	6.19±5.31 B	0.56±0.23 B	5.85±5.59 B
Average						
leaf mustard		22.91±15.49 a	4.89± 3.76 a	4.27±3.79 b	0.50±0.04 b	0.11±0.01 a
watercress		28.26±2.42 b	11.80± 1.00 b	6.37±3.72 c	0.35±0.16 a	5.08±4.86 c
rocket salad		39.43±5.31 c	5.12± 3.48 a	3.90±3.34 a	0.52±0.23 b	0.70±0.15 b
coriander		78.87±36.01 d	15.62± 12.29 c	8.22±6.15 d	0.77±0.03 c	6.62±6.16 d
***p*-value**						
A – production system		<0.0001	<0.0001	<0.0001	0.0001	<0.0001
B - species		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction AB		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* average ± standard deviation

** means in a column followed by the same letter are not significantly different at the 5% level of probability (Tukey's test, p< 0.05)

Source: own work / *Źródło:* opracowanie własne

Table 2. Phenolic acids contents in selected species of leafy vegetables (mg 100 g^{-1} f.w.) (average ± s.d.)*
*Tab. 2. Zawartość kwasów fenolowych, w wybranych gatunkach warzyw liściowych (mg 100 g^{-1} ś.m.) (średnia ± s.d.)**

Species		Total phenolic acids	Gallic acid	Chlorogenic acid	Caffeic acid	Ferulic acid
ORG	leaf mustard	29.78±0.92 d**	19.72±0.66 d	5.77±0.14 c	2.87±0.07 c	1.41±0.05 d
	watercress	19.69±1.22 c	18.98±1.18 d	0.25±0.02 a	0.11±0.01 a	0.35±0.04 a
	rocket salad	32.69±2.40 e	12.07±1.35 c	13.44±0.67 d	6.70±0.34 d	0.47±0.08 b
	coriander	111.52±0.32 g	21.66±0.86 e	56.66±0.74 f	28.31±0.37 f	4.89±0.04 f
CONV	leaf mustard	6.27±0.21 a	4.92±0.09 a	0.59±0.09 a	0.28±0.05 a	0.48±0.01 b
	watercress	13.23±0.21 b	9.85±0.32 b	1.84±0.04 b	0.91±0.02 b	0.63±0.06 c
	rocket salad	35.94±0.54 f	12.11±0.86 c	14.35±0.16 e	7.16±0.08 e	2.32±0.09 e
	coriander	14.982.14 b	6.48±2.10 a	5.40±0.34 c	2.68±0.17 e	0.42±0.08 ab
Average						
ORG		48.42±36.77 B	18.11±3.73 B	19.03±22.23 B	9.50±11.11 B	1.78±1.84 B
CONV		17.60±11.12 A	8.34±2.98 A	5.54±5.38 A	2.76±2.69 A	0.96±0.79 A
Average						
leaf mustard		18.02±11.77 b	12.32±7.41 a	3.18±2.59 b	1.57±1.30 b	0.95±0.47 b
watercress		16.46±3.32 a	14.41±4.63 b	1.05±0.80 a	0.51±0.40 a	0.49±0.15 a
rocket salad		34.31±2.22 c	12.09±0.98 a	13.89±0.62 c	6.93±0.31 c	1.40±0.93 c
coriander		63.25±48.29 d	14.07±7.72 b	31.03±25.64 d	15.49±12.82 d	2.65±2.24 d
***p*-value**						
A – production sytem		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
B - species		<0.0001	0.0003	<0.0001	<0.0001	<0.0001
Interaction AB		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* average ± standard deviation
** means in a column followed by the same letter are not significantly different at the 5% level of probability (Tukey's test, p< 0.05)

Source: own work / *Źródło:* opracowanie własne

At the same time conventional vegetables, on average, were richer in all individual flavonoids, in that quercetin-3-O-glucoside, quercetin and kaempferol (Table 1). The quality and nutritional value (i.e. content of important phytochemicals) of agricultural products such as vegetables is affected by genetic (species, cultivars) and environmental factors as well as production conditions (cultivation system and post-harvest management) [6]. Thus the production of certain compounds, like e.g. polyphenols, can be enhanced by providing certain conditions of plant growth. This is mostly related to the supply of mineral nitrogen in the soil. Many researchers explained that in growing environments poor in readily assimilable nitrogen, such as organic systems, the metabolism of plants changes in the direction of intensive production of carbon-containing compounds (i.e. phenolic acids, flavonoids, sugars, vitamin C). In contrast, high doses of easily assimilable mineral nitrogen fertilizers commonly used in conventional production system cause a decrease in the content of phenolic compounds in fruits and vegetables [5, 6, 7, 23, 24, 25]. Recent studies suggest that plant products from organic production can have positive sensory attributes, and are richer in polyphenols as valuable health-promoting bioactive compounds compared to conventional products [21, 22, 26, 27, 28, 29, 30]. This was also confirmed by the results of recently published meta-analysis based on 343 carefully selected publications [31] concluding that plants from organic system contain nearly 60 percent more polyphenolic compounds in comparison to conventional ones.

Total carotenoids, in that beta-carotene contents, were found to be dependent on both the way of vegetable cultivation and plant species (Table 3). Conventionally produced plant materials contained higher levels of total carotenoids and beta-carotene compared to organic ones. At the same time organic vegetables did not differ from their conventional counterparts in terms of lutein, zeaxanthin and chlorophyll contents (Table 3). Considering individual leafy vegetable species, the highest concentrations of total carotenoids, including beta-carotene, luteolin and zeaxantin, as well as total chlorophylls, were found in coriander leafs. The other three species of plants contained comparable amounts of these compounds. Similar tendency in terms of carotenoids content in organic and conventional plants was described by Kazimierczak et al. [20] – medicinal plants from conventional production were found to contain significantly more carotenoids, including lutein and beta-carotene, than the organic ones. Dean et al. [32] studied the effect of different doses of nitrogen on the production of carotenoids by watercress and showed a positive linear relationship between the concentration of nitrogen in the substrate and the concentration of beta-carotene, zeaxanthin and lutein in plants. Also Kaack et al. [34] have shown, that the more mineral nitrogen was readily available for carrots at the beginning of the cultivation, the more beta-carotene has been synthesized in the carrot roots before the harvest time. As well known, soil in organic farming contains nitrogen in less assimilable form than in conventional cultivation, therefore its availability to plants is lower, what could explain lower contents of carotenoids in organic plants. However, it should be underlined that the content of carotenoids in plants strongly depends not only on the production system (organic/conventional, availability of minerals in the soil), but also on many non-agricultural factors and stressful conditions faced by plants (e.g. insolation intensity, temperature during vegetation season) [33].

To summarize, the above results point to the need for further research concerning the content of carotenoids and other bioactive compounds in organic vs. conventional plants. It is worth underlying that other factors, not considered in the presented study, such as e.g. storage and transport conditions, are known to affect the quality of plant material and should be further investigated.

Table 3. The carotenoids and chlorophyll contents in selected species of leafy vegetables from organic (ORG) and conventional (CONV) production (mg 100 g^{-1} f.w.) (average ± s.d.)*

*Tab. 3. Zawartość karotenoidów i chlorofilu w wybranych gatunkach warzyw liściowych z produkcji ekologicznej i konwencjonalnej (mg 100 g^{-1} ś.m.) (średnia ± s.d.)**

Species		Total carotenoids	Lutein	Zeaxantin	Beta-carotene	Total chlorophylls
ORG	leaf mustard	6.09±0.27 a**	0.96±0.05 a	0.56±0.02 a	4.57±0.20 a	24.56±0.72 a
	watercress	9.15±1.81 b	1.23±0.01 b	0.71±0.01 b	7.21±1.81 b	39.70±0.43 b
	rocket salad	8.40±0.49 b	0.99±0.05 a	0.68±0.06a b	6.73±0.42 b	26.14±1.80 a
	coriander	22.33±1.48 f	3.13±0.26 e	2.18±0.14 e	17.02±1.08 f	103.84±6.54 e
CONV	leaf mustard	14.58±0.79 e	1.56±0.10 c	1.07±0.14 d	11.95±0.69 e	53.77±9.06 d
	watercress	11.38±0.66 c	1.47±0.08 c	0.90±0.05 c	9.01±0.52 c	44.51±4.51 bc
	rocket salad	12.66±0.80 cd	1.50±0.09 c	1.11±0.04 d	10.06±0.68 cd	36.91±2.54 b
	coriander	14.18±0.78 de	1.94±0.14 d	1.04±0.07 cd	11.20±0.58 de	50.06±3.85 cd
Average						
ORG		11.49±6.43 A	1.58±0.91 A	1.03±0.67 A	8.88±4.88 A	48.56±32.57 A
CONV		13.20±1.42 B	1.61±0.21 A	1.03±0.11 A	10.55±1.23 B	46.31±7.81 A
Average						
leaf mustard		10.33±4.27 a	1.26±0.31 a	0.82±0.27 a	8.26±3.71 a	39.17±15.52 b
watercress		10.26±1.57 a	1.35±0.13 a	0.80±0.10 a	8.11±1.41 a	42.10±3.56 b
rocket salad		10.53±2.20 a	1.24±0.26 a	0.89±0.22 a	8.40±1.72 a	31.52±5.67 a
coriander		18.26±4.19 b	2.53±0.62 b	1.61±0.58 b	14.11±3.00 b	76.95±27.25 c
***p*-value**						
A – production system		0.0007	n.s.	n.s.	0.0003	n.s.
B - species		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction AB		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* average ± standard deviation

** means in a column followed by the same letter are not significantly different at the 5% level of probability (Tukey‘s test, p< 0.05); ns, not statistically significant

Source: own work / *Źródło:* opracowanie własne

4. Conclusions

1. The results confirm differences described in the literature, showing the higher content of polyphenolic compounds in organic when compared to conventional plants. This is especially pronounced for the phenolic acids, which dominate among the polyphenols in the examined leafy vegetables.
2. Organic production positively influenced the concentrations of all identified individual phenolic acids (gallic, chlorogenic, caffeic and ferulic) in the examined vegetable species; in contrast, vegetables from conventional production were more abundant in flavonoids (quercetin-3-O-glucoside and quercetin, kaempferol) and carotenoids (beta-carotene); there were no differences in luteolin and zeaxantin content in plants from both agricultural production systems.
3. The study indicated no differences in dry matter and chlorophylls content between plants from organic and conventional production.
4. Among the examined species, coriander, especially the organically produced one, was the richest in total polyphenols, in that phenolic acids and flavonoids, as well as carotenoids and chlorophylls.
5. The obtained results provide a strong confirmation that the leafy vegetables, and especially those from organic farming, are an important source of valuable compounds, i.e. antioxidants with beneficial effects on the human body. Thus, they should be recommended in a preventive healthcare.

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