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Antioxidant activity in selected Slovenian organic and conventional crops

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ABSTRACT

The demand for organically produced food is increasing. There is widespread belief that organic food is substantially healthier and safer than conventional food. According to literature organic food is free of phytopharmaceutical residues, contain less nitrates and more antioxidants. The aim of the present study was to verify if there are any differences in the antioxidant activity between selected Slovenian organic and conventional crops. Method of DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to determine the antioxidant activity of 16 samples from organic and conventional farms. The same varieties of crops were analysed. DPPH method was employed to measure the antioxidant activity of polar antioxidants (AA_p) and antioxidant activity of fraction in ethyl acetate soluble antioxidants (EA AA). Descriptive statistics and variance analysis were used to describe differences between farming systems. Estimated differences between interactions for the same crop and different farming practice were mostly not statistically significant except for the AA_p for basil and beetroot. Higher statistically significant values were estimated for conventional crops. For the EA AA in broccoli, cucumber, rocket and cherry statistically significant higher values were estimated for organic production.

Key words: antioxidant activity, organic farming, conventional farming, fruits, herbs, vegetables

IZVLEČEK

ANTIOKSIDATIVNA UČINKOVITOST V PRIDELKIH IZ SLOVENSKE EKOLOŠKE IN KONVENCIONALNE PRIDELAVE

Povpraševanje po ekološko pridelanih živilih se povečuje. Ekološki proizvodi veljajo za bolj zdrave v primerjavi s konvencionalnimi. Po navajanju drugih virov ne vsebujejo fitofarmaceutskih sredstev imajo manjšo vsebnost nitratov in vsebujejo več antioksidantov. Namen študije je bil preveriti ali obstajajo razlike v antioksidativni učinkovitosti med slovenskimi pridelki pridelanimi na ekološki in konvencionalni način. Za določevanje antioksidativne učinkovitosti smo uporabili metodo DPPH (2,2-difenil-1-pikrilhidrazil). Analiza je bila opravljena na 16 vrstah iste sorte pridelkov iz ekološke in konvencionalne pridelave. Izmerili smo polarno antioksidativno učinkovitost (AA_p) in antioksidativno učinkovitost v etil-acetatu topnih antioksidantov (EA AA). Izračunali smo osnovne statistične parameter po vrstah pridelkov in načinu kmetovanja in z analizo variance ocenili razlike med načinoma kmetovanja za posamezne pridelke. Ocenjene razlike med interakcijami pridelka in načina kmetovanja večinoma niso statistično značilne. Izjema za AA_p sta bazilika in rdeča pesa, kjer so bile večje vrednosti za konvencionalne pridelke. Za vrednost EA AA so bile statistično značilno večje vrednosti ocenjene za ekološki brokoli, kumaro, rukolo in češnjo.

Ključne besede: antioksidativna učinkovitost, ekološko kmetijstvo, konvencionalno kmetijstvo, sadje, zelišča, zelenjava

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1 INTRODUCTION

For a variety of reasons all crop species vary in their composition of antioxidants, and other nutritional relevant substances. Fruits, herbs and vegetables are very important for human nutrition. Besides providing energy, food of plant origin, is a rich source of dietary fibres and minerals. Food of plant origin is thus the most important source of antioxidants (Hertog and Hollman, 1996; Pietta, 2000; Chandrasekara and Shahidi, 2011).

Antioxidant activity depends on different chemical attributes and may be specific to variety. It depends mainly on phenolics, which are considered more potent antioxidants as compared to vitamins (Koleva et al., 2002; Usenik et al., 2008). In foods of plant origin, there are numerous compounds that contribute to antioxidative activity and function in different ways. The most important antioxidants include ascorbic acid, carotenoids and phenolic compounds. Group of phenolic compounds include monophenols with a single benzene ring, hydroxycinnamic acids, flavonoids and their glycosides which include catechins, proanthocyanidins, anthocyanins and flavonols (Gulcin, 2012). The most complex and poorly defined are high molecular weight tannins regularly present in fruit. In the above mentioned groups there are altogether few thousand of active substances which in different ways contribute to the total antioxidant activity (Hribar and Simčič, 2000).

Phenols are considered polar compounds and are soluble in polar solvents like water, methanol, etc. However some of them predominantly phenols with more aromatic rings, less OH groups or deglycosylated phenols are partially soluble in nonpolar solvents. Phenolic compounds also show partial solubility in less polar solvents like n-butanol and ethyl acetate (Dhingra et al. 2014).

Higher EA AA means more above mentioned phenol compounds are present in ethyl acetate fraction (Rice-Evans et al., 1996).

Soil, climate, variety, degree of ripeness and also the freshness, storage conditions can all affect the content of biologically active compounds. Type of farming system may also affect the chemical composition of foodstuffs, especially on those that originate from the use of chemical fertilizer and pesticides (Dangour et al., 2009). Organic farming represents a production system, looking for harmony between the environment and the agriculture production (Casado and de Molina, 2009; Bavec et al., 2010). It excludes the use of synthetic fertilizers and pesticides, plant growth regulators and genetically modified organisms (Singh et al., 2009). The use of pesticides was perceived to be associated with effects on health, but it was also associated with benefits like cheaper foods and higher yield (Huang, 1996; Miles and Frewer, 2001; Torjusen et al., 2001).

It is widely believed that mineral fertilizers reduce antioxidant levels in plants, while organic fertilizers enhance the antioxidant levels (Dumas et al., 2003; Aldrich et al., 2010; Oliveira et al., 2013). However literature show mixed data regarding the phytochemical status of organic and conventional vegetables (Faller and Fialho, 2010; Sinkovič et al., 2015).

In this study the antioxidant activity in selected organic and conventionally produced fruits, herbs and vegetables in Slovenia was measured. The aim of the study was to check if there are differences in antioxidant activity between analyzed organic and conventional crops.

2 MATERIALS AND METHODS

2.1 Plant material

Herbs (basil, parsley, celery) and vegetables (broccoli, beetroot, carrot, cherry tomato, cucumber, eggplant, tomato and rocket) were grown in experimental field, where mineral fertilizers had not been used for more than 30

years. The same varieties of crops were used in both farming systems. Crop varieties used in this study are presented in Table 1 and Table 2. Basic soil cultivation, sowing, and harvesting dates and methods were identical for organic and conventional experimental plot. Organic crops

were only irrigated while conventional crops were fertilized with Plantella extra plus NPK (15:15:15), according to instructions given by the manufacturer, on the 14th, 21st, 28th, 35th and 42nd day during growth period. The climatic conditions, variety, irrigation, ripening time, and storage conditions were the same for the crops grown organically and conventionally.

Samples of same variety of fruits (apple, cherry, pear and raspberry) were obtained from known organic and conventional farms in Slovenia. Organic fruits derived from certified organic productions possessed certification for organic farming, according to the Institute of Certification (KON-CERT Maribor, Slovenia). Conventional fruit samples were from conventional farms. Samples were harvested in the year 2012 when they were in commercial maturity stage. Samples were cleaned, each plot stored separately in a cooling room at +4 °C and 95% relative humidity until the analyses in laboratory were performed.

2.2 Sample preparation

Samples were prepared in three repetitions no later than 12 h after harvest. They were washed, dried out in air and cut into small pieces. Ten gram of each sample were homogenised with 20 g of 2% methaphosphoric acid, using an Ultra-turrax T 25 (IKA, Germany). Methaphosphoric acid was used to get low pH which stabilises ascorbic acid in samples (Osborn-Barnes and Akoh, 2003). The obtained homogenates were immediately frozen at -20 °C till further use.

Samples were thawed before use and centrifuged at 1700 ×g for 5 minutes (Rotanta 460R; Hittich, Germany). The supernatants were transferred into micro centrifuge tubes and centrifuged again at 16 ×g for 5 minutes (Centrifuge 5415c; Eppendorf, Germany). Finally, supernatants were filtered through 0.45 µm filter and used as a sample.

2.3 Determination of antioxidant activity

The antioxidant activity of fruits, herbs and vegetables from organic and conventional farming was measured by means of free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) as previously reported by Brand-Williams et al. (1995) and Shyu and

Hwang (2002) with some modifications. This method is based on the reduction of the stable DPPH radical (2,2-diphenyl-1-picrylhydrazyl) in to DPPH₂. A solution of 4 mg DPPH /100 ml of ethyl acetate was used to determine antioxidant activity of fraction in ethyl acetate soluble antioxidants (EA AA) and a solution of 4 mg DPPH /100 ml of methanol was used for polar antioxidant activity (AA_p).

For the determination of EA AA 5 ml of supernatant and 5 ml of the ethyl acetate were thoroughly mixed and upper layer (ethyl acetate fraction) was used for the essay. For AA_p 60 or 100 µl of supernatant was mixed with 1.5 ml freshly prepared DPPH. After stirring, the tubes were left in the dark for 30 minutes. The absorbance of the samples was measured at 517 nm after the reaction time. All samples were analysed as triplicate. For EA AA ethyl acetate was used as a blank and for AA_p methanol was used as a blank. The antioxidant activity was expressed as millimol DPPH equivalents per 100 g of fresh weight (mmol DPPH/100 g FW).

2.4 Statistical analysis

The data were analysed using SAS/STAT statistical software (SAS, 2012). Analysis of variance was performed using the GLM (General Linear Models) procedure. Statistical model included farming system, crop species and interaction between farming system and crop species as fixed effects (statistical model (1)). In statistical model y_{ijk} is dependent variable (AA_p or AE AA), μ is estimated overall mean, F_i is farming system fixed effect $i=1,2$ (organic farming, conventional farming), C_j is crop fixed effect $j=1,2,3,\dots,16$ (basil, apple, ...), FC_{ij} is interaction between F_i and C_j and e_{ijk} is residual.

Differences between LSMs (Least Square Means) for the same crop in different farming system were estimated using t-test and significance level was set at $p < 0.05$.

$$y_{ijk} = \mu + F_i + C_j + FC_{ij} + e_{ijk} \quad \dots(1)$$

3 RESULTS AND DISCUSSION

3.1 Descriptive statistic

The descriptive statistic for antioxidant activities of fraction in ethyl acetate soluble antioxidants and antioxidant activities of polar antioxidants of crops

grown according to organic farming system are shown in the Table 1 and according to conventional farming system in the Table 2.

Table 1: Descriptive statistics for antioxidant activities of fraction in ethyl acetate soluble antioxidants (EA AA) and polar antioxidants' activities (AA_p) of crops produced in organic farming

Farming	Crop	Variety	EA AA (mmol/100 g FW)				AA _p (mmol/100 g FW)			
			M	SD	Min	Max	M	SD	Min	Max
Organic crops	Apple	<i>Malus domestica</i> 'Idared'	0.33	0.018	0.31	0.35	0.35	0.008	0.34	0.36
	Basil	<i>Ocimum basilicum</i>	0.33	0.024	0.30	0.34	0.93	0.007	0.92	0.93
	Beetroot	<i>Beta vulgaris</i> 'Rote Kugel'	0.06	0.032	0.04	0.10	0.82	0.492	0.26	1.17
	Broccoli	<i>Brassica oleracea</i> 'Corvet'	0.14	0.020	0.12	0.16	0.86	0.046	0.83	0.92
	Carrot	<i>Daucus carota</i> 'Kuroda'	0.14	0.040	0.11	0.19	0.18	0.003	0.18	0.18
	Cherry	<i>Prunus avium</i> 'Burlat'	0.29	0.024	0.26	0.31	0.44	0.015	0.43	0.46
	Cherry tomato	<i>Lycopersicon esculentum</i> var. <i>cerasiforme</i> 'Gardener's Delight'	0.13	0.013	0.12	0.14	0.31	0.008	0.31	0.32
	Cucumber	<i>Cucumis sativus</i> 'Darina'	0.10	0.011	0.09	0.11	0.13	0.004	0.12	0.13
	Eggplant	<i>Solanum melongena</i> 'Halflange Violette'	0.19	0.020	0.17	0.21	0.80	0.018	0.78	0.81
	Parsley	<i>Petroselinum crispum</i> 'Italian Parsley'	0.11	0.010	0.10	0.12	0.58	0.044	0.53	0.62
	Pear	<i>Pyrus communis</i> 'Conference'	0.14	0.044	0.11	0.17	0.23	0.008	0.22	0.23
	Pepper	<i>Capsicum anuum</i> 'California Wonder'	0.11	0.051	0.05	0.15	0.38	0.017	0.36	0.40
	Raspberry	<i>Rubus idaeus</i> 'Willamette'	0.30	0.023	0.27	0.31	0.80	0.009	0.79	0.81
	Rocket	<i>Eruca sativa</i>	0.13	0.009	0.12	0.13	0.73	0.055	0.67	0.76
	Tomato	<i>Lycopersicon esculentum</i> 'Volovsko srce'	0.08	0.027	0.05	0.10	0.34	0.015	0.32	0.35
	Subtotal			0.18	0.092	0.04	0.35	0.56	0.314	0.12

AA_p – polar antioxidants' activity; EA AA – antioxidant activity of fraction in ethyl-acetate soluble antioxidants; FW – fresh weight; M – average value; SD – standard deviation; Min – minimum; Max - maximum

The AA_p of the organic samples varied from the lowest at 0.13 mmol DPPH/100 g FW (cucumber) to 0.93 mmol DPPH/100 g FW (basil), while the

EA AA ranged from 0.08 mmol DPPH/100 g FW (tomato) to 0.33 mmol DPPH/100g FW (basil and apple).

Table 2: Descriptive statistics for antioxidant activities of fraction in ethyl acetate soluble antioxidants (EA AA) and polar antioxidants' activities (AA_p) of crops produced according to conventional farming

Farming	Crop	Species/Variety	EA AA (mmol/100 g FW)				AA _p (mmol/100 g FW)			
			M	SD	Min	Max	M	SD	Min	Max
Conventional crops	Apple	<i>Malus domestica</i> 'Idared'	0.30	0.023	0.28	0.32	0.51	0.039	0.47	0.55
	Basil	<i>Ocimum basilicum</i>	0.36	0.009	0.35	0.37	1.68	0.072	1.60	1.73
	Beetroot	<i>Beta vulgaris</i> 'Rote Kugel'	0.07	0.009	0.06	0.08	1.25	1.326	0.10	2.70
	Broccoli	<i>Brassica oleracea</i> 'Corvet'	0.09	0.011	0.08	0.10	0.74	0.020	0.71	0.75
	Carrot	<i>Daucus carota</i> 'Kuroda'	0.11	0.022	0.09	0.13	0.15	0.011	0.14	0.16
	Cherry	<i>Prunus avium</i> 'Burlat'	0.20	0.018	0.18	0.22	0.45	0.002	0.45	0.45
	Cherry tomato	<i>Lycopersicon esculentum</i> var. <i>cerasiforme</i> 'Gardener's Delight"	0.12	0.021	0.11	0.15	0.42	0.034	0.38	0.45
	Cucumber	<i>Cucumis sativus</i> 'Darina'	0.05	0.035	0.02	0.08	0.08	0.003	0.07	0.08
	Eggplant	<i>Solanum melongena</i> 'Halflange Violette'	0.24	0.006	0.23	0.24	0.55	0.005	0.55	0.56
	Parsley	<i>Petroselinum crispum</i> 'Italian Parsley'	0.10	0.032	0.06	0.12	0.45	0.017	0.43	0.46
	Pear	<i>Pyrus communis</i> 'Conferense'	0.14	0.037	0.10	0.17	0.23	0.021	0.21	0.25
	Pepper	<i>Capsicum anuum</i> 'California Wonder'	0.14	0.026	0.12	0.16	0.34	0.018	0.32	0.35
	Raspberry	<i>Rubus idaeus</i> 'Willamette"	0.26	0.029	0.24	0.30	0.68	0.014	0.66	0.69
	Rocket	<i>Eruca sativa</i>	0.06	0.022	0.04	0.08	0.50	0.009	0.49	0.51
	Tomato	<i>Lycopersicon esculentum</i> 'Volovsko sree'	0.08	0.016	0.07	0.10	0.17	0.014	0.16	0.18
		Subtotal		0.16	0.094	0.02	0.37	0.56	0.489	0.07

AA_p – polar antioxidant activity; EA AA – antioxidant activity of fraction in ethyl-acetate soluble antioxidants; FW – fresh weight; M – average value; SD – standard deviation; Min – minimum; Max - maximum

The AA_p of the conventional samples varied from the lowest value of 0.07 mmol DPPH/100 g FW (cucumber) to 2.70 mmol DPPH/100 g FW (beetroot), while the EA AA ranged from

0.02 mmol DPPH/100 g FW (cucumber) to 0.37 mmol DPPH/100g FW for basil.

The mean AA_p for cherry was very similar between the farming systems (organic, 0.44 mmol

DPPH/100 g FW; conventional, 0.45 mmol DPPH/100 g FW). The mean AA_p for pear were equal (0.23 mmol DPPH/100 g FW).

The mean EA AA for pear (0.14 mmol DPPH/100 g FW) and tomato (0.08 mmol DPPH/100 g FW) were unchanged between the farming system.

There are a number of reviews that compare nutritional quality of organically versus conventionally grown foods (Weibel et al., 2000; Brandt and Molgaard, 2001; Bourn and Prescott, 2002). Weibel et al. (2000) showed that fruit quality of organic apples were either similar or slightly better than that of conventional ones. According to literature data, the organic crops presented higher antioxidant activity than conventional ones (Worthington, 2001; Wang et al., 2008; Aldrich et al., 2010; Arbos et al., 2010; Crinnion, 2010; Lairon, 2010; Borguini et al., 2013). This can be attributed to the fact that

organic farming uses less phytopharmaceuticals and plant therefore develop several defense mechanisms (Koleva et al., 2002; Borguini et al., 2013). On the other hand, Lamperi et al. (2008) and Cardoso et al. (2011) argue that there is no difference in the antioxidant activity between products from different farming systems.

3.2 Analysis of variance

In AA_p 75 % and for EA AA 94 % of total variability were explained with statistical model (1). Effect of farming system was not statistically significant in AA_p but it was in EA AA. In both antioxidant activity measurements crop was highly statistical significant. Interaction between farming system and crop was statistically significant for EA AA, but in AA_p interaction has a trend that approached significance (Table 3). This shows that variability between crops was higher than variability between farming systems.

Table 3: *F*-values and *p*-values for effects included in statistical model (1) for AA_p and EA AA

Effect	AA _p		EA AA	
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Farming system	0.00	0.949	7.65	0.008
Crop	10.74	<0.001	60.84	<0.001
Farming system and crop interaction	1.76	0.061	2.54	0.005

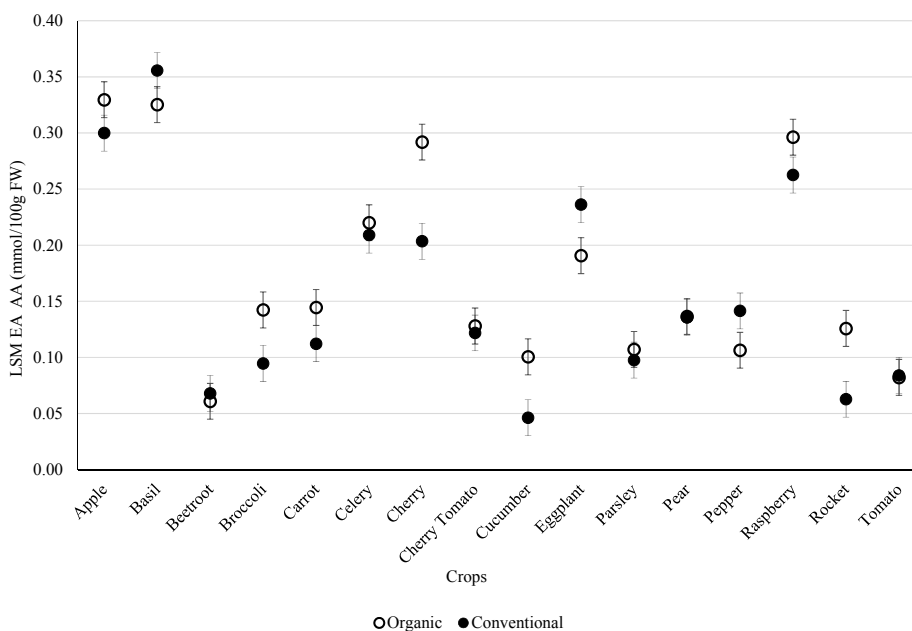


Figure 1: LSM (least square means) values for antioxidant activity of fraction in ethyl acetate soluble antioxidants (EA AA)

Estimated LSMs for interaction between farming system and crop in EA AA show that all observed types of fruits from organically farming (cherries, apples, raspberries and pears) had higher estimated EA AA compared to conventional ones (Figure 1) but only for cherry difference was statistically significant ($p < 0.001$). Higher values of antioxidant activity in organic fruit were also reported by Wang et al. (2008) and Vrčec et al. (2011).

Bavec et al. (2010) and Borguini et al. (2013) found higher antioxidant activity in organic vegetables as compared to conventional. In our study statistically significant differences in the EA AA of LSMs between farming systems for vegetables were estimated only for broccoli ($p < 0.05$), cucumber ($p < 0.05$) and rocket ($p < 0.01$) (Figure 1).

Estimated LSMs for interaction between farming system and crop for AA_p were higher for conventionally produced basil, cherries, apples,

cherry tomatoes and beetroots had higher AA_p compared to organic (Figure 2). Other crops had higher estimated LSMs for AA_p from organic production compared to conventional. But statistically significant differences in AA_p LSMs between farming practices were estimated only for basil ($p < 0.001$) and beetroot ($p < 0.05$).

In the study of Faller and Failho (2010) broccoli stems also demonstrated higher antioxidant activity from conventional production. In our study broccoli had higher estimated LSMs for AA_p in samples from organic production but differences were not statistically significant.

Cardoso et al. (2011) and Lamperi et al. (2008) also found no statistically significant differences for antioxidant activity between organic and conventional crops. Nevertheless some researchers found higher antioxidant activity values in organic compared to conventional crops (Aldrich et al., 2010; Arbos et al., 2010; Borguini et al., 2013).

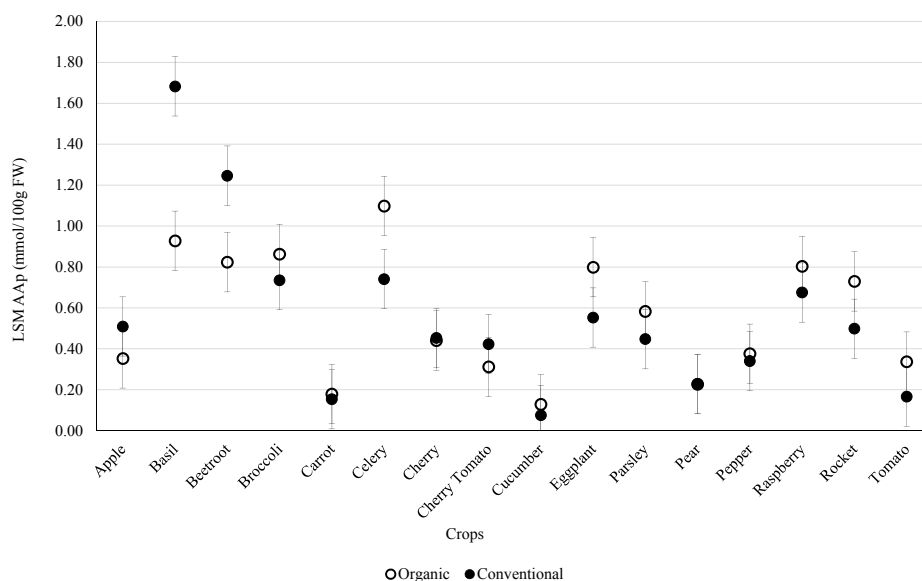


Figure 2: LSM values for polar antioxidants' activity (AAP)

We expected that the highest antioxidant activity, among fruits, will be measured in raspberries. As reported by Kahkonen et al. (1999) berries, had clearly higher antioxidant activities compared to other fruits predominantly due to high content of anthocyanins which is reflected in profound red

colour. In our study AA_p of raspberry was the highest among fruits, but when comparing fruits EA AA, just pear had lower values than raspberry. Obviously no antioxidants soluble in ethyl acetate were present in pear and raspberry. As reported by Kahkonen et al. (1999) herbs possess strong

antioxidant activity which is in accordance with our study. In this study the highest antioxidant activity (AA_p and EA AA) was measured for basil.

Analysis of variance showed no statistically significant differences between farming practise in general, while estimated differences between crop species were very high (till 0.3 for EA AA and 1.6 for AA_p).

4 CONCLUSIONS

Although some literature report that the exposure of crop plant to stress conditions during growth could modulate the synthesis of defence substances such as antioxidants. Often reported benefits of organic agriculture are an increased concentration of antioxidants due to stress provoked by nitrogen deficiency. That was not clearly demonstrated in this study. Our results showed that antioxidant activity of water extract of plant material varied among organic and conventional crops with no prevalence from either production type. Estimated differences between interactions for the same crop

and different farming practice were mostly not statistically significant except for the AA_p for basil and beetroot. Higher statistically significant values were estimated for conventional crops. For the EA AA in broccoli, cucumber, rocket and cherry statistically significantly higher values were estimated for organic production. We can conclude that, except for some crops, there are no statistically significant differences in antioxidant activity between organic and conventional fruits, herbs and vegetables, which were included in this study.

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