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Nutrients, antioxidants, and antioxidant activity of organically and conventionally grown raspberries

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Summary

The fruits of 'Polka', 'Polana' and 'Pokusa' raspberries grown either under organic practice (ORG) or conventional system (CON) and originating from different locations were evaluated for the content of dry weight, soluble solids, acidity, total sugar, vitamin C, phenolics, and antioxidant activity towards DPPH radicals. Soluble solids varied from 8.3% (CON-'Pokusa') to 10.9% (CON-'Polana'2). Total sugar for organic raspberries was 4.78-6.31 g/100 g, while for conventional group 4.60-7.58 g/100 g. Titratable acidity ranged from 1.67 (CON-'Polana'2) to 2.36 g citric acid/100 g (ECO-'Polana'1). For majority of raspberries vitamin C amounted 20.5-25.6 mg/100 g and only ORG-'Polana'1 showed 17.6 mg/100 g. The scope of total phenol (GAE) in each group was quite similar and overall range was 186.9-249.2 mg/100 g. Antioxidant activity towards DPPH radical was 28.8-45.1% of inhibition for conventionally grown raspberries and 29.0-41.1% for organic fruits. Total anthocyanin content amounted 28.1-48.1 mg/100 g in ORG-group and 26.7-43.6 for conventional fruits. Sum of quercetin derivatives was higher in ORG-group (1.5-3.4 mg/100 g) compared to 1.3-2.4 mg/100 g for conventionally grown raspberries. The values did not show an unequivocal prevalence of any method, because the response of cultivars within specific site conditions was often more pronounced than the system of cultivation.

Introduction

The demand for organic food products has increased rapidly during recent years, partially due to the notion that health benefits are linked with the consumption of organic products. Organic food is perceived to be more nutritious, better tasting, and environmentally friendlier compared to conventionally grown crops (Wang et al., 2008).

The agricultural production system and cultivation practices are critical factors in determining yield and food nutritional quality. Controversy remains regarding whether or not organic foods have a nutritional advantage when compared with their conventionally produced counterparts (Riahi et al., 2009).

Factors such as resources availability, soil quality, climate, and insect and animal herbivory pressures are known to affect levels of nutrients in plants (Asami et al., 2003). The goal of conventional and organic agriculture differs greatly with respect to crop yield, land and pesticide use, and environmental impact. Conventional agriculture practices utilize high-yield crop cultivars, chemical fertilizers and pesticides, irrigation and mechanization. Although conventional practices result in reliable high-yield crops, there is concern regarding the negative biological and environmental consequences and long-term sustainability associated with these practices. Organic crops production is controlled by the entitled organizations and producers must observe strict rules of conformance. Organic crops cannot be genetically engineered, irradiated, or fertilized with sewage sludge. Additionally, farmland used to grow organic crops is prohibited from being treated with synthetic pesticides and herbicides for at least 3 years prior to harvest. Disease-resistant cultivars are often used, and plant nutrients are supplied through crop rotation, cover crops, and animal manure (Asami et al., 2003).

However, there is very little information on the impact various cultural practices have on the production of secondary metabolites in plants. Given that increasing evidence indicates a role for plant phenolics in human health, efforts need to be directed in understanding relationships between cultural practices and phenolic levels in crops (Asami et al., 2003). Epidemiological studies have shown that diets rich in fruits and vegetables are associated with longer life expectancy, and these beneficial effects may be due to rich antioxidants contained in these produce. Fruits and vegetables have shown remarkably high scavenging activity toward chemically generated radicals (Wang et al., 2008). However, the prevalence of organic foods over conventional counterparts regarding higher level of phenolics some researchers consider "anecdotal" (Asami et al., 2003).

To our knowledge, there was no research done to compare chemical composition of fruits of selected raspberry cultivars grown under organic or conventional system in West Pomerania region of Poland. The objective of the study was to evaluate the effect of cultivation method on dry weight, soluble solids, total sugar, acidity, vitamin C, total phenol and phenolics profile, and antioxidant activity against DPPH radicals of raspberries.

Materials and methods

The raspberry fruits (*Rubus idaeus* L.) originating either from organic (ORG) or conventional plantations (CON) were grown in different locations of West Pomerania (Poland) listed in the table below (Tab. 1). The fruits were fully ripe of excellent consumer value regarding their taste and flavor. For each cultivar 500-1000 g samples were collected.

The dry weight content in raspberry fruits was determined with a gravimetric method. The soluble solids content was determined by means of the Abbé refractometer (PN-90/A-75101/02). The titratable acidity was determined by titration of water extracts of raspberry fruit with 0.1 N NaOH to an end point of pH 8.1 (measured with an Orion 720 A pH meter; Orion Research Incorporated, USA) according to PN-90/A-75101/04. Total sugar content was determined according to the Luff-Schoorl method based on the reduction of Cu(II) in an alkaline milieu by reducing sugars present in tested sample. Then, KJ is added and the excess of copper ions liberates J_2 from KJ in an acid milieu. The developing iodine is finally determined by titration with $Na_2S_2O_3$. The quantity of $Na_2S_2O_3$ ml used serves for reading of reducing sugars content. Vitamin C content was measured in oxalic acid extracts of fruits with the Tillmans method prior to the absorption of the pigments on Seppack cartridge C18. Total polyphenol content was estimated spectrophotometrically (at 760 nm) in 70% methanol extracts of fruit with the Folin-Ciocalteau reagent. The data is expressed as gallic acid equivalents (GAE) g/100 g. The DPPH$^{\cdot}$ was obtained from Sigma-Aldrich Co. (USA). Scavenging

Tab. 1: The origin and date of harvests of raspberry fruits analyzed in this assay.

Cultivar	Origin	Date of harvest
	Organic farming (ORG)	
Polana 1	Wolin	13.08.2009
Polka 1	Wołczkowo	12.08.2009
Pokusa*	Rajkowo	26.08.2009
Polana 2	Połczyn-Zdrój	04.10.2010
Polka 2	Połczyn-Zdrój	04.10.2009
	Conventional farming (CON)	
Polana 1	Stargard Szczeciński	11.08.2009
Polana 2	Połczyn-Zdrój	17.08.2009
Polka 1	Stobno	11.08.2009
Polka 2	Połczyn-Zdrój	17.08.2009
Pokusa	Stobno	11.08.2009

Pokusa* plants were cultivated for the last 2 years without chemical protection and fertilization – "transitional" period

effect of raspberry fruit on DPPH-radical was determined according to the spectrophotometric method of Yen and Chen (1995). The DPPH· percent inhibition was calculated according to Rossi et al. (2003) from the formula: Percent inhibition = 100 – [(A_t/A_r) x 100], where A_t – absorbance of test solution and A_r – absorbance of reference solution.

For the HPLC analyses 2 g aliquots of fruit were extracted three times with approx. 8 mL of 80% MeOH acidified with a glacial acetic acid (1 ml of 100% acetic acid L^{-1} 80% MeOH) in an ultrasonic bath for 15 min. The samples were filtered and transferred to the flasks and made up to the final volume 25 mL. Further, the extracts were centrifuged twice at 12,000 × g and 20 µL of supernatants were injected into the HPLC system. The HPLC apparatus consisted of a Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). The separation was performed on a Cadenza CD C18 (75 × 4.6 mm, 5µm) column (Imtakt, Japan). Column oven temperature was set at 30 °C. The mobile phase was composed of solvent A (4.5% formic acid, pH 2.2) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 21% B (0-30 min), followed by washing and reconditioning the column. The flow rate was 1 ml · min^{-1} and the runs were monitored at the following wavelengths: quercetin glycosides, kaempferol and ellagic acid derivatives at 360 nm, and anthocyanin glycosides at 520 nm. The Photo Diode Array spectra were measured over the wavelength range 200-600 nm in steps of 2 nm. Retention times and spectra were compared to those of pure standards within 200-600 nm. Standards of cyanidin and pelargonidin glycosides were obtained from Polyphenols Laboratories (Norway), while, kaempferol 3-glucuronide, quercetin glycosides, and ellagic acid from Extrasynthese (France).

The analyses were made on fruits packed in polyethylene bags and kept frozen (-25 °C) for 27-30 days. Whereas, the detection of phenolic compounds was performed by a high performance liquid chromatography (HPLC) on berries kept frozen for 10 months. Dry weight content, soluble solids, vitamin C, phenolics (GAE), and antiradical activity (DPPH·) was determined in three replicates, whereas total sugar, titratable acidity, and phenolics (HPLC) were determined in two replicates. The results were subjected to one-way analysis of variance for completely randomized design. The data was evaluated by the Tukey test and the differences between the means at $p<0.05$ were considered significant.

Results and discussion

Chemical composition of fruits is conditioned by several factors and among them genetic factors (species, cultivar), agronomic practices (soil management, fertilization, plant protection etc.) and environmental factors (region, soil, climate, weather conditions in the season etc.) exert the most profound effects. The ripening stage of fruits and post-harvest procedures also contribute to maintenance and/or losses of phytochemicals.

The majority of raspberries grown under conventional practice (13.47-14.49%) showed higher content of dry weight (except for CON-'Pokusa' (11.64%)) compared to those of organic farming (Tab. 2). For organically produced berries originating from different locations dry weight content was very similar (12.81-12.99%). Data indicates that correlation is more complex and it covers not only agronomic procedures but also a genotype response within the conditions of the site of growing as well as the method of cultivation. Thus, the response for other species may be quite different. Cayuela et al. (1997) observed an opposite effect. Organically grown strawberries 'Chandler' showed higher dry weight content (8.9%) then berries grown conventionally (8.1%).

Soluble solids content in raspberries ranging from 10.9% (CON-'Polana'2) to 8.3% (CON-'Pokusa') was not clearly affected by agronomic procedures (Tab. 2). 'Polana' raspberries grown conventionally showed higher soluble solids content compared to that of organic farming while, for 'Pokusa' berries the opposite effect was observed. On the other hand, for 'Polka' raspberries neither the system of production, nor the location affected soluble solids content. Whereas, Do Amarante et al. (2008) found higher soluble solids content for 'Royal Gala' and 'Fuji' apples from organic orchard than that of conventional management.

The total sugar content for berries ranged from 4.60 to 7.58 g/100 g (Tab. 2). Conventionally grown 'Polana' and 'Polka' raspberries showed higher total sugar content than the same cultivars grown under organic practice. However, 'Pokusa'* berries (belonging to the ORG-group) had higher level of total sugar than CON-'Pokusa' fruits. On the other hand, for strawberries 'Chandler' Cayuela et al. (1997) observed an opposite effect as berries from ecological management showed, on average, higher total sugar content (8.4%) compared to their conventional counterparts (7.5%).

Total acid content in raspberries was also cultivar, location and agronomic practice related (Tab. 2). The greatest titratable acicidity was determined for ORG-'Polana'1 and ORG-'Pokusa'* raspberries (2.36 and 2.04 g citric acid/100 g, respectively) and the lowest for 'Polana'2 and 'Polka'2 berries originating from conventional practice (1.67 and 1.69 g citric acid/100 g, respectively). Nevertheless the method of growing, for 'Polka' (Δ=0.08 g citric acid/100 g) and 'Pokusa' cultivars (Δ=0.05 g citric acid/100 g) the variation in total acid content was more narrow than that for 'Polana' cv. (Δ=0.69 g citric acid/100 g). Literature data confirm substantial effect of genetic, climatic and production factors on acidity of raspberries. Orhan et al. (2006) determined for 'Heritage' raspberries under organic practice total acid content 1.32-1.40% citric acid, whereas Haffner et al. (2002) found the scope 2.24-2.52% citric acid within 5 raspberry cultivars grown conventionally.

According to the data on food nutritional value (Elmadfa and Muskat, 2001; Kunachowicz et al., 1998) vitamin C content in fresh raspberries varies from 25 to 31.4 mg/100 g. For majority of raspberries tested in this research the content of ascorbic acid was similar ranging from 20.48 mg/100 g (CON-'Pokusa') to 25.56 mg/100 g (CON-'Polka'2) (Tab. 3). The lowest content of ascorbic acid was found for ORG-'Polana'1 berries (17.62 mg/100 g). Vitamin C is a labile component and its content is conditioned by many factors including cultivar, agronomic practices, growing area, maturity stage and post-harvest handling (temperature, light exposure, presence

Tab. 2: Average chemical composition of raspberry fruits collected from the plants grown under ecological and conventional practice.

Raspberry cultivar	Item			
	Dry weight (%)	Soluble solids (%)	Total sugar (g/100 g)	Titratable acidity (g citric acid/100 g)
Organic farming (ORG)				
Polana 1	12.99 cd	9.1 bc	4.78 e	2.36 a
Polka 1	12.81 d	9.8 ab	5.82 d	1.77 e
Pokusa*	12.95 cd	10.3 ab	6.31 c	2.04 b
Conventional farming (CON)				
Polana 1	14.37 ab	10.1 ab	7.58 a	1.79 e
Polana 2	14.81 a	10.9 a	7.13 b	1.67 f
Polka 1	13.74 bc	9.9 ab	6.38 c	1.87 d
Polka 2	14.49 ab	10.2 ab	7.15 b	1.69 f
Pokusa	11,64 e	8,3 c	4,60 f	1,99 c

'Pokusa'* plants were cultivated for the last 2 years without chemical protection and fertilization -"transitional" period

Tab. 3: Antioxidants and antiradical activity (DPPH˙) of raspberry fruits originating from the plants grown under ecological and conventional practice.

Raspberry cultivar	Item			
	Vitamin C (mg/100 g)	Total phenol (mg gallic acid/100 g)	Total phenol HPLC (mg 100/g)	Percent inhibition of DPPH radical (%)
Organic farming (ORG)				
Polana 1	17.62 d	186.9 d	33.70 bc	29.0 c
Polka 1	21.90 b	215.8 c	51.45 a	32.8 bc
Pokusa*	22.88 b	244.7 ab	-	41.1 ab
Conventional farming (CON)				
Polana 1	22.64 b	249.2 ab	46.31 ab	45.1 a
Polana 2	25.38 a	238.3 c	36.64 abc	28.8 c
Polka 1	20.71 c	211.8 c	36.15 abc	36.2 abc
Polka 2	25.56 a	254.6 a	36.84 abc	32.5 bc
Pokusa	20.48 c	195.8 d	29.80 c	34.5 bc

'Pokusa'* plants were cultivated for the last 2 years without chemical protection and fertilization -"transitional" period

of oxygen, pathogen pressure etc.). In this finding, the variation occurred within a cultivar, depending on the site and system of growing. HAKALA et al. (2003) observed somewhat lower values for organically produced strawberries compared to the CON-ones however, the effect of cultivar and season factor were relevant, too.

Regarding total phenol content determined as GAE, the variation noted for particular genotypes occurred both within and between the sites of growing, and due to the ORG- or CON-procedures (Tab. 3). Interestingly, the divergence in phenolics content was similar between the cultivars of ORG-group (Δ=57.8 mg GAE/100 g) and CON-group (Δ=58.8 mg GAE/100 g). Higher values of total phenol were determined for CON-'Polka'2 (254.6 mg GAE/100 g) and CON-'Polana'1 (249.2 mg GAE/100 g) while, the lowest for ORG-'Polana'1 (186.9 mg GAE/100 g) and CON-'Pokusa' (195.8 mg GAE/100 g). The amounts of phenolic compounds detected by the HPLC method were a few folds lower compared to that of spectrophotometric method and the differences between the means were less distinct in statistic terms (Tab. 3). The greatest value was determined for ORG-'Polka'1 (51.45 mg/100 g) whereas, the lowest for CON-'Pokusa' (29.80 mg/100 g). CABONARO et al. (2002) observed an increase of polyphenol content in organic peach and pear compared to the corresponding conventional fruit. However, according to LOMBARDI-BOCCIA et al. (2004) total polyphenol content was higher in conventional plums.

Antiradical activity of berry extracts was rather cultivar- and location-dependent than affected by the method of cultivation (Tab. 3). Among the berries originating from organic farming the greatest antioxidant activity towards DPPH radicals was observed for 'Pokusa'* (41.1% of inhibition) and the lowest for 'Polana'1 (29.0%). Whereas, within traditional system genotype 'Polana' grown in two different locations showed both the highest (45.1% of inhibition) and the lowest (28.8%) antiradical potential. PECK et al. (2006) found that ORG apples showed 10% to 15% higher total antioxidant activity than CON apples. Similarly, WANG et al. (2008) determined higher antioxidant capacity (ORAC) for organically grown blueberries compared to that of conventional cultivation.

An average amount of total anthocyanins was similar for both groups of agronomic procedure (>34.0 mg/100 g) (Tab. 4). The scope of values within both growing systems was also similar. For the genotypes of conventional farming it amounted 26.66-43.63 mg/100 g and for the raspberries of organic cultivation 28.07-48.13 mg/100 g.

The profile of identified anthocyanins (Fig. 1) included predominant participation of cyanidin 3-sophoroside (42.9-72.4%) followed by cyanidin 3-rutinoside (20.7-33.2%) > pelargonidin 3-glucoside (0.2-18.4%) > cyanidin 3-glucoside (0.1-18.0%). From the practical point of view, for the cultivars and system of cultivation less pronounced differences in pigment content were observed for the major anthocyanins (cy-3-sop and cy-3-rut) than for the minors (cy-3-glu and pg-3-glu). In our study, there was no clear relationship between the amounts of anthocyanins in raspberries and each cultivation system. Unlike, WANG et al. (2008) found that organically grown 'Bluecrop' blueberries showed higher content of delphinidin, cyanidin, petunidin, and malvidin glycosides than corresponding conventional ones.

As regards flavonols, ORG-raspberries showed slightly higher amount of all detected quercetin glycosides (1.54-3.44 mg/100 g) compared to the fruits of traditional cultivation (1.26-2.59 mg/100 g) (Tab. 4). For both management systems quercetin 3-glucuronide was a predominant derivative amounting 65-89% of total quercetin glycosides whereas, quercetin 3-glucoside was the least. The highest content of kaempferol derivative was determined for ORG-'Polana' 1 and CON-'Polka'2 (0.41 and 0.38 mg/100 g, respectively) while, for other cultivars it was either not detected or found in negligible quantities (Tab. 4). WANG et al. (2008) excerpt that for organically cultivated strawberries, the high content of kaempferol could be a response to pathogenic attack because kaempferol can act as an antimicrobial compound in plants. On the other hand,

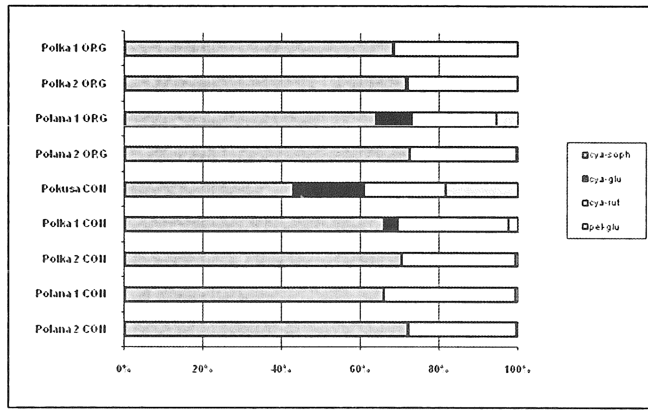

Fig. 1: Percentage participation of anthocyanins in raspberries originating from organic and conventional cultivation.

LOMBARDI-BOCCIA et al. (2004) observed that quercetin was higher in conventional plums, but myricetin and kaempferol were higher in organic plums.

In present study, organically grown raspberries showed 0.16-0.85 mg/100 g of total ellagic acid, whereas for conventionally produced fruits the range was 0.37-0.83 mg/100 g (Tab. 4). Thus, the amounts of ellagic acid found in raspberries were unequivocally related neither to genotype, nor the agronomic procedure. However, on average the content of ellagic acid was slightly higher for the group of conventionally grown raspberries compared to ecological one. Whereas, OLSSON et al. (2006) found that organically grown strawberries had on average, higher ellagic acid content compared to conventionally grown berries. Moreover, within the CON-group a substantial diversification occurred between the cultivars.

In conclusion, our findings did not reveal clear evidence on prevalence of organically produced raspberries over conventional ones. Nutritional value of fruits, antioxidants content, and anti-

Tab. 4: The content of phenolic compounds identified by HPLC in raspberry fruits obtained from the plants grown under ecological and conventional practice.

Raspberry cultivar	Phenolics (mg/100 g)								
	Sum of anthocyanins	Quercetin 3-glucoside	Quercetin 3-glucoronide	Quercetin derivative	Sum of quercetin glycosides	Kaempferol derivative	Ellagic acid derivative[1]	Ellagic acid derivative[2]	Sum of ellagic acid derivatives
Organic farming (ORG)									
Polana 1	29.72 bc	0.15 a	2.22 a	1.07 a	3.44 a	0.41	0.03 c	0.13 a	0.16 b
Polka 1	48.13 a	0.23 a	2.04 a	0.18 bc	2.45 ab	0.02	0.22 b	0.63 a	0.85 a
Pokusa*	-	-	-	-	-	-	-	-	-
Polana 2	30.66 bc	0.21 a	1.25 a	0.09 c	1.54 b	0.00	0.16 ab	0.39 a	0.55 ab
Polka 2	28.07 bc	0.12 a	1.27 a	0.16 bc	1.55 b	0.00	0.14 ab	0.44 a	0.57 ab
Conventional farming (CON)									
Polana 1	43.63 ab	0.00 a	1.66 a	0.21 bc	1.87 ab	0.03	0.20 ab	0.61 a	0.81 a
Polana 2	34.01 abc	0.20 a	1.56 a	0.14 c	1.89 ab	0.05	0.13 a	0.57 a	0.69 ab
Polka 1	33.97 abc	0.00 a	0.90 a	0.37 b	1.26 b	0.09	0.13 ab	0.70 a	0.83 a
Polka 2	33.61 abc	0.21 a	2.15 a	0.23 bc	1.58 ab	0.00	0.14 ab	0.52 a	0.66 ab
Pokusa	26.66 c	0.18 a	1.93 a	0.30 bc	2.4 ab	0.38	0.15 ab	0.22 a	0.37 ab

*'Pokusa' plants were cultivated for the last 2 years without chemical protection and fertilization -"transitional" period

[1] Ellagic acid derivative – retention time 20 min.

[2] Ellagic acid derivative – retention time 21 min.

oxidant activity of raspberries originating from both cultivation systems was also affected by genetic factors, as well as weather and location conditions. The sun-light, temperature, precipitations are the main factors of growing season affecting accumulation of sugars, soluble solids, acidity, vitamin C, and phenolics in fruit (WANG et al., 2002; POLL et al., 2003; ROHLOFF et al., 2004; HOPPULA and KARHU, 2006). Some authors observed an increase of nutritional constituents in organically produced fruits. These results are more evident when derived for the same genotypes grown under both practices in a very close area. However, comparisons for fruits grown in each of systems but in more distant locations indicate that the method of cultivation is only one of the factors affecting chemical composition of berries. In traditional production systems pests and pathogens are controlled by means of synthetic insecticides and fungicides allowed for specific crops. The limits for pesticide remnants are law-regulated. Organic growers must use organically derived and approved measures to remain certified. Thus, a general conclusion on nutritional quality of berries available at the market for consumers is more complex and not unequivocal.

Therefore, further studies should be addressed to elicit the influence of agronomic practice on chemical composition and health-related value of horticultural products.

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