

Organic versus conventional ‘Willamette’ raspberry: yield, bioactive compounds and antioxidant properties

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Abstract: This paper presents the results of a two-year trial on the yield, total phenolic and flavonoid contents and total antioxidant capacity of the ‘Willamette’ red raspberry under organic and conventional farming. A trial was conducted in a commercial plantation of ‘Willamette’ raspberries located in the southwestern part of Serbia. The total phenolics and flavonoids in the raspberry fruits were determined by the Folin-Ciocalteu reagent and aluminium chloride assay, respectively. The ferric reducing antioxidant power assay was used to evaluate the total antioxidant capacity of the raspberry fruits. The results of this study showed that the raspberry yields were significantly affected by the different farming systems. The raspberry yield in conventional farming was higher than that of organic farming. Contrastingly, the raspberries from the organic farming contained higher levels of the total phenolics and flavonoids and also had a higher total antioxidant activity compared to those from the conventional farming in both 2021 and 2022. This study also showed that the total phenolic and flavonoid contents of the ‘Willamette’ red raspberry have a significant and positive correlation with their total antioxidant capacity, indicating that the phenolic compounds play an important role in the antioxidant activity of the raspberry.

Keywords: environment; fertilisation; fruits; health; plant protection

The raspberry is one of the most popular fruit crops having a large agricultural potential. It is a highly appreciated fruit due to its unique taste and high content of bioactive compounds that provide beneficial effects to human health and helps to reduce the risk of diseases (Burton-Freeman et al. 2016; Čanadanović-Brunet et al. 2017). As a result, raspberry consumer demand has continuously risen in both

Europe and the world, especially in the past 20 years (Wróblewska et al. 2019; Bojkovska et al. 2021).

Serbia is one of the world’s leading producers and exporters of raspberries. In 2020 alone, Serbia produced more than 118 000 tonnes of this fruit. Currently, the total production area of raspberries in Serbia is estimated at more than 25 000 hectares. The raspberry production is mostly concentrated

in West Serbia, and the most represented variety is ‘Willamette’ (Parašić, Simeunović 2016; Kljajić et al. 2022).

‘Willamette’ is a mid-season raspberry variety having an excellent flavour and good disease resistance. It produces delicious dark red coloured medium-sized fruits that ripen in mid-summer to autumn (Milinković et al. 2021). The largest percentage of the ‘Willamette’ raspberry production in Serbia is from conventional production. However, in recent decades, organic food has gained in popularity, and ‘Willamette’ raspberry fruits have followed this trend (Eynade et al. 2021; Kotuła et al. 2022).

There is plenty of confusion surrounding organic and conventional raspberries as well as foods in general. Consumers often assume that foods not classified as organic are less nutritious and may even contain substances that can cause adverse health effects (Mie et al. 2017; Gundala, Singh 2021). The reality is that the differences between organic and conventional farming do not actually refer to food health, but rather are an indication of how farmers grow and process the food. The conventional farming system is the traditional practice of agriculture which includes the use of synthetic pesticides and fertilisers to maximise crop yield. Organic farming instead used organic pesticides and natural organic fertilisers such as animal manure, compost and green manures (Schimmenti et al. 2014; Souček et al. 2017). Organic pesticides and fertilisers are typically viewed as safer, mainly because they are more environmentally benign than their synthetic counterparts.

Numerous studies have found that organic fruits contain more bioactive compounds than conventional fruits (Hamouz et al. 2013; Jurica, Petříková 2014; Šrednicka-Tober et al. 2020; Breza-Boruta et al. 2022; Czech et al. 2022). This is expected, considering the fact that the use of synthetic pesticides in organic agriculture is restricted and, therefore, plants tend to produce more or higher levels of bioactive compounds protecting them against pests, diseases and oxidative damage (Dragišić Maksimović et al. 2013; George et al. 2021). Among the bioactive compounds in plants, the most abundant are phenolics and flavonoids (Tungmunthum et al. 2018). However, several studies have come to contrasting conclusions in establishing the causalities between the synthesis of bioactive components in plants and the organic farming system (Rossi et al. 2008; Cardoso et al. 2011). The farming system is also one of the main factors that affects the raspberry yields. In general, organic

raspberry farming has been found to be characterised by lower yields (Bodiroga, Sredojević 2017; Frias-Moreno et al. 2018).

Indeed, inconsistent findings concerning the differences between the organic and conventional farming suggest that more studies are needed to better understand the plants’ behaviour in both organic and conventional farming, especially in terms of the bioactive compound contents. From an agricultural point of view, it is a very important topic and one that needs to be debated more. Thus, the main goal of this two-year study was to evaluate the effect of farming practices, organic vs. conventional, on the yield, antioxidant activity and bioactive compound contents in raspberries. The study subject was the ‘Willamette’ raspberry variety, particularly because it is one of the most widely planted varieties in Serbia and, therefore, any attempt to improve its production is of great interest for both the producers and end consumers.

MATERIAL AND METHODS

Study area and experimental design. The field experiment was carried out in two consecutive years (2021 and 2022) in a commercial plantation of ‘Willamette’ raspberries situated in the southwestern part of Serbia in the village of Osoje, the municipality of Prijepolje (43°20'50"N, 19°24'14"E, 480 metres above sea level). The site has been used in ‘Willamette’ raspberry cultivation since 2015. According to the Köppen and Geiger classification (Kottek et al. 2006), the climate in the studied area is classified as Dfb (warm-summer humid continental climate) typified by cold and not so humid winters and warm and humid summers. The mean annual air temperature in that area is 8.1 °C and the mean annual precipitation is 1 031 mm.

The experiment with two farming practices/treatments (organic vs. conventional) that differ only in the fertilisation and plant protection regimes was conducted on a typical soil in Western Serbia, classified as ranker or a humus-silicate soil (FAO 1998). Each treatment was set up in three replications where the experimental plot of each repetition consisted of a 10 m row, separated from adjacent plots by guard plants. The distance between the raspberry plants in a row was 0.25 m with 3 m between the rows.

The organic farming included only organic-based fertilisers, whereas the conventional farming was based on the application of mineral fertilisers.

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The fertiliser recommendation rates were given on the basis of the soil chemical analysis, plot size and expected raspberry yield. At 0–30 cm depth of the studied soil, the pH was 5.6, the total nitrogen was 0.7 g/kg, the organic matter was 3.1%, and the available macronutrients (P and K) were 4.1 and 32.1 mg/100 g. Soil samples from experimental raspberry plantation were collected in February 2021 and analysed using standard soil testing methods. All the data related to the fertiliser management system, i.e., the type, dose and time of the fertiliser application are presented in Table 1.

The conventional protection of raspberry against fungal diseases and pests was performed using Cuprablau (fungicide/bactericide), Calypso 480 SC (insecticide), Confidor 70 WG (insecticide) and Abas-tate (acaricide). In the organic raspberry farming, the following pesticides were used to control the pests and fungal pathogens; Cuprablau (fungicide/bactericide), Fitomite (acaricide) and Laser (insecticide). All the pesticides used in this study were applied in accordance with the manufacturer's recommendations and raspberry protection programme.

Fruit yield estimation. 'Willamette' raspberry fruits were collected at the stage of commercial maturity (July). In 2021 and 2022, harvest occurred from 3 to 25 July and from 2 to 27 July, respectively. In both years, the fruits were manually harvested three times per week, and the weight of the fruits were recorded with a RADWAG WPX 4 500 electronic scale (0.01 g accuracy). The total yield per plant across the whole harvest season was calculated by summing the single fruit weights from the same plant.

Antioxidant properties evaluation. To assess the antioxidant properties (total phenolic and flavonoid contents and total antioxidant capacity), 100 healthy fruits from each experimental unit were randomly harvested at the full ripe stage. All the collected fruits were placed in sealed plastic bags, immediately transported under refrigerated conditions to the Laboratory of Plant Physiology at the Faculty

of Agriculture and Food Sciences University of Sarajevo, and then stored at -20°C (no more than 1 month) until extraction. Prior to the extraction, the raspberry fruits were dried in an oven at 40°C for 48 hours, and then ground into powder using an electric blender. After that, the fruit powder samples (1 g) were extracted with 30 mL of a 60% ethanol aqueous solution at room temperature for 24 hours. Subsequently, the mixture was filtered through Whatman filter paper (11 μm pore size) into a 50 mL flask and diluted to the mark with the extract solution. The extract thus obtained was used for the estimation of the total phenolic contents, total flavonoid contents and total antioxidant activity.

The total phenolic content of all the extracts was evaluated with the Folin-Ciocalteu assay (Ough, Amerine 1988). Briefly, 0.1 mL of the extract was mixed with 6 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent (previously diluted with distilled water 1:2 v/v). The solution was kept at room temperature for 10 min before adding 1.5 mL of a sodium carbonate solution 20% (w/v) and adjusting the volume to 10 mL with distilled water.

After two hours, the absorbance of the resulting solution was measured at 725 nm against a reagent blank. The calibration curve of gallic acid (concentration range of 0–500 mg/L) was used as the standard for quantitatively estimating the content of the phenolic compounds in the sample, and the results were expressed as milligrams of gallic acid equivalents per gram dry mass of the sample (mg GAE/g).

The total flavonoid content of all the extracts was evaluated with an aluminium chloride assay (Zhishen et al. 1999). Briefly, 1 mL of the extract was mixed with 4 mL of distilled water and 0.3 mL of 5% NaNO_2 . The solution was kept at room temperature for five minutes before adding 0.3 mL of 10% AlCl_3 , 2 mL of 1 M NaOH and adjusting the volume to 10 mL with distilled water. After 1 h, the absorbance of the resulting solution was measured at 510 nm against a reagent blank. The cali-

Table 1. Fertilisation regime used for conventional and organic farming of the raspberries

Farming system	Fertilizer management
Organic farming	Cow manure – 12 t/ha (one-time application in February)
	Bioazot Top – 2 kg/ha (fertilizer was applied every four weeks during growing season until mid of harvest)
Conventional farming	NPK 7:14:21 – 400 kg/ha (one-time application in February)
	KAN (27% N) – 120 kg/ha (one-time application in March)
	NPK 8:11:36 – 180 kg/ha (fertilizer was applied in three split doses, first was applied at full flowering, second when the fruit was of pea grain size, and third before the beginning of ripening)

bration curve of catechin (concentration range of 0–100 mg/L) was used as the standard for quantitatively estimating the flavonoid contents in the sample, and the results were expressed as milligrams of catechin equivalents per gram dry mass of the sample (mg C/g).

The total antioxidant activity of all the extracts was evaluated with a ferric reducing antioxidant power (FRAP) assay (Benzie, train 1996). Briefly, 80 µL of the extract was mixed with 240 µL of distilled water and 2 080 µL of the fresh FRAP reagent (the FRAP reagent was prepared immediately before use by mixing acetate buffer (300 mM, pH = 3.6), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and 20 mM FeCl₃ in a volume ratio of 10:1:1). The solution was incubated at 37 °C for 15 minutes in a water bath. Thereafter, the absorbance of the resulting solution was measured at 595 nm against a reagent blank. The calibration curve of FeSO₄ × 7 H₂O (concentration range of 0–2 000 µmol /L) was used as the standard for estimating the total antioxidant activity of the sample, and the results were expressed as µmol Fe²⁺ per gram dry mass of the sample (µmol Fe²⁺/g).

All the spectrophotometric measurements were carried out with an Amersham ultrospec 2 100 pro spectrophotometer (Biochrom, USA).

Statistical analysis. All the experimental measurements were performed in triplicate and the measurement data were expressed as the mean ± standard deviation. The obtained data were subjected to a one-way analysis of variance in the Microsoft Excel 2010 package program (Office 2010, Redmond, WA, USA) and the significant differences were determined using the least-significant-difference test (LSD test). Statistical significance was considered at $P < 0.05$. The data obtained were also evaluated to identify the relationship between the total phenolics/flavonoids and total antioxidant activity using Pearson's

correlation coefficients. The Pearson correlation coefficient (r) values from 0.1 to 0.3 indicate a weak, values from 0.4 to 0.6 indicate a moderate, and values from 0.7 to 1.0 indicate a strong linear relationship between two variables (Dancey, Reidy 2006).

RESULTS AND DISCUSSION

The obtained results showed that the raspberry yields were significantly affected by the different farming systems (Table 2). The raspberry yield in the conventional farming was significantly higher than that of the organic farming in both 2021 and 2022. These findings are in line with some other previous studies, which analysed the raspberry yield from different farming systems (Sangiorgio et al. 2021; Çelik, Çolak 2022).

The lower yields often observed in organic farming can be largely attributed to the untimely nutrient availability to plants after fertilising (Timsina 2018). Namely, the essential plant nutrients, primarily nitrogen and phosphorus, are held in the organic fertiliser until soil microorganisms release them for plant use (Järvan et al. 2014). This means that organic fertilisers release the nutrients more slowly than conventional, i.e., chemical fertilisers, and often does not keep up with the high plant nutrient demand in the growing period (Shang et al. 2020). Besides, organic fertilisers typically have a lower macronutrient, i.e., nitrogen, potassium and phosphorus, content compared to chemical fertilisers, causing a substantial decline in plant productivity (Allam et al. 2022). On the other hand, organic food is potentially more beneficial for human health because it is produced without the use of synthetic fertilisers and pesticides and can therefore fetch higher market prices than their conventional counterparts, compensating for the

Table 2. Yield, total phenolic content, total flavonoid content and total antioxidant capacity of the 'Willamette' raspberry from two different cultivation systems

Treatment	Yield (kg/cane)	TPC (mg/g dry mass)	TFC (mg/g dry mass)	FRAP (µmol Fe ²⁺ /g dry mass)
Conventional farming 2021	0.46 ± 0.05 ^{a*}	19.11 ± 1.51 ^b	7.14 ± 0.24 ^b	169.88 ± 2.76 ^b
Organic farming 2021	0.40 ± 0.04 ^b	20.68 ± 2.11 ^a	7.59 ± 0.34 ^a	181.64 ± 3.55 ^a
Conventional farming 2022	0.45 ± 0.07 ^a	19.71 ± 1.73 ^a	7.18 ± 0.43 ^b	170.04 ± 3.83 ^b
Organic farming 2022	0.41 ± 0.04 ^b	20.53 ± 2.01 ^a	7.55 ± 0.41 ^a	180.47 ± 2.11 ^a
LSD _{0.05}	0.032	1.04	0.28	2.74

^{a,b*} Means followed by different letters indicate significant differences at $P < 0.05$

TPC – total phenolic content; TFC – total flavonoid content; FRAP – total antioxidant capacity

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economic loss due to the lower yields (Meemken, Qaim 2018; Reddy et al. 2022).

Recent studies have proven the effectiveness of raspberries as a good source of antioxidants (Milivojević et al. 2011; Alibabić et al. 2018). Antioxidants have become scientifically interesting compounds mainly due to their association with the beneficial effects against ageing and degenerative diseases (Shahidi, Ambigaimpalan 2015). Furthermore, antioxidants have an excellent ability to neutralise harmful free radicals in both plants and humans, and therefore many farmers have applied different agriculture techniques in order to increase the levels of the antioxidant compounds in the plants. Numerous studies have shown that farming practices play a significant role in improving the nutritional composition and antioxidant properties of fruits and vegetables (Johansson et al. 2014; Dragišić Maksimović et al. 2017; Prasad 2021). Hallmann et al. (2020) reported that organic raspberries contained more antioxidants, i.e., phenolics and flavonoids than conventionally grown raspberries. These findings are in line with the results of our study. Namely, in this study, the total phenolic and flavonoid contents in the fruits of the ‘Willamette’ raspberry from organic farming were significantly higher than that of conventional farming in both 2021 and 2022 (Table 2).

Different hypotheses have been put forward to explain why organic raspberries and organic food in general, have more antioxidants than their conventional counterparts. The two main hypotheses are: (1) the plant stress hypothesis and (2) the growth-differentiation balance hypothesis. The plant stress hypothesis suggests that plants exposed to stressful conditions produce more antioxidants to cope with elevated levels of reactive oxygen species (Orsák et al. 2020). On the other hand, the growth-differentiation balance hypothesis is based on the assumption that there is a physiological trade-off between growth and the production of secondary metabolites. In nutrient-rich soils, i.e., conventionally managed soils, plant resources are used for building new tissues and organs rather than secondary metabolites. Contrastingly, in soils with intermediate levels of nutrients, i.e., organically managed soils, plants invest more in the defence system, including, among other things, a greater synthesis of antioxidants (Massad et al. 2012). The results obtained in our study support this hypothesis.

There is no doubt that health benefits are one of the main reasons for consumers to buy organic foods. Another important reason why organic food has gained popularity among consumers is due to the environmental benefits that organic farming offers. The benefits of organic farming on the environment have been well-documented from reducing soil erosion to improving the biodiversity and soil health (Arcand, Congreves 2018; Cortrufo et al. 2019; Hargreaves et al. 2019; Gonzalez et al. 2020).

Certainly, the raising awareness of environmental issues coupled with the consumer’s desire to buy healthy foods fostered the growth of organic farming systems during the past decades. Despite the above, there are some inconsistencies among the conclusions of studies on the environmental impact of farming systems. Most of the studies that compared organic and conventional farming demonstrated lower environmental impacts from organic farming (Gomiero et al. 2011; Reganold, Wachter 2016). On the other hand, some scientists believe that organic farming is worse for the environment due to the larger land use for the same yield (Redlichová et al. 2021). In this regard, the increase in the area under organic farming leads to more environmentally damaging deforestation and land-clearing, which, in turn, results in more carbon dioxide emissions (Raihan et al. 2022). Vásquez-Ibarra et al. (2021) have reported that the environmental performance of raspberry orchards for both conventional and organic farming systems can be improved through regular soil testing, timing fertiliser applications to minimise emissions, and the use of enhanced efficiency fertilisers and pesticides. In this sense, future agriculture practices must find the way to provide universal access to healthy foods as well as to provide the overall sustainability of food production without creating negative environmental impacts.

The results of this study also showed that organic farming significantly enhanced the antioxidant capacity of raspberry fruits. In this study, the total antioxidant capacity of the raspberry fruits in the organic farming was 6.5% (2021) and 5.8% (2022) higher compared to conventional farming. This leads to the conclusion that the total phenolics/flavonoids content found in the raspberry fruits corresponded positively to their ferric reducing antioxidant power (FRAP) values, regardless of the chosen farming system (Figure 1). Numerous studies have also shown a close positive relationship between the total phenolics/flavonoids content in the plants and their antioxidant

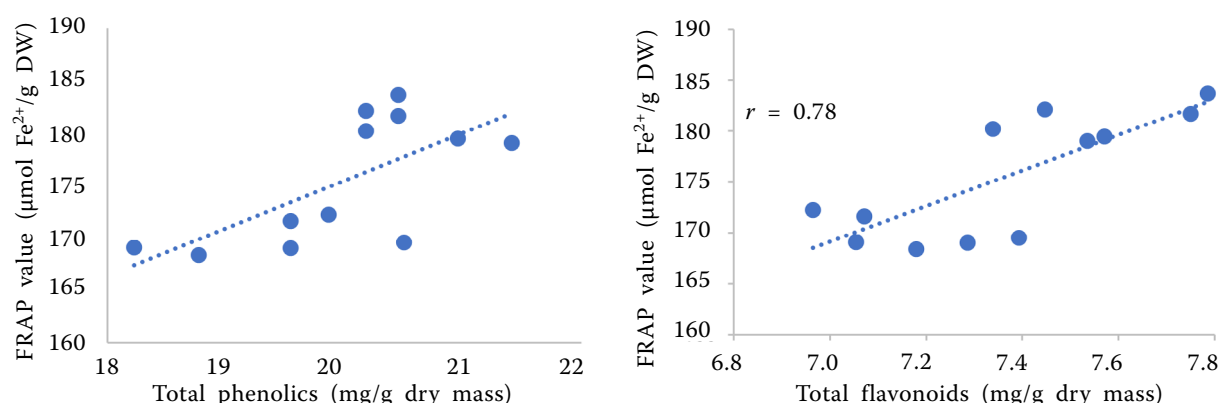


Figure 1. Relationship between the antioxidant capacity (FRAP) and the total phenolics/flavonoids of the raspberry fruits

activity, indicating that phenolic compounds represent good sources of natural antioxidants (Aryal et al. 2019; Butkevičiūtė et al. 2022; Lebedev et al. 2022).

CONCLUSION

The results of this study showed that fruits of the 'Willamette' red raspberry from organic farming contained higher levels of total phenolics and flavonoids and also had a higher total antioxidant activity compared to those from conventional farming. Contrastingly, the yield of the 'Willamette' red raspberries under organic farming were lower than the conventional one. This study also showed that the total phenolics and flavonoids in the fruits of the 'Willamette' red raspberry have a significant and positive correlation with their total antioxidant capacity, indicating that phenolic compounds are one of the main contributors to the antioxidant activity of raspberry fruits.

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