

## Phenolic composition and antioxidant capacity of integrated and conventionally grown strawberry (*Fragaria × ananassa* Duch.)

M. PEŠAKOVIĆ<sup>1</sup>, S. MILENKOVIĆ<sup>2</sup>, D. ĐUKIĆ<sup>3</sup>, L. MANDIĆ<sup>3</sup>,  
Ž. KARAKLAJIĆ-STAJIĆ<sup>1</sup>, J. TOMIĆ<sup>1</sup>, N. MILETIĆ<sup>4</sup>

<sup>1</sup>Fruit Research Institute Čačak, Čačak, Republic of Serbia

<sup>2</sup>Department of Fruit Protection, Faculty of Biofarming, Megatrend University  
Bačka Topola, Republic of Serbia

<sup>3</sup>Department of Microbiology, Faculty of Agronomy, University of Kragujevac Čačak,  
Republic of Serbia

<sup>4</sup>Department of Food Science and Technology, University of the Basque Country,  
Bilbao Area, Spain

### Abstract

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Evaluation was performed of the effect made by integrated and conventional production systems on phenolic composition and antioxidant capacity of cv. Senga Sengana strawberry grown under greenhouse conditions over two consecutive years. The experiment also involved three different types of fertilizer. The integrated production system provides significantly larger quantities of polyphenols as well as a higher antioxidant capacity, compared to values obtained using the conventional system. The investigation into total phenolics content and total antioxidant capacity, as well as individual flavonols and phenolic acids (except gallic acid) content reveals a pronounced effect of the applied biofertilizers (PGPR 1 and PGPR 2). On the other hand, applied chemical fertilizer (Multi KMg) gives the best results in terms of anthocyanins content. These results indicate that there were significant differences in phenols content among integrated and conventional production systems, as well as between biofertilizers and a chemical fertilizer.

**Keywords:** garden strawberry; cultivation techniques; phenolic compounds

Flavonols, phenolic acids and other polyphenols possess anti-inflammatory, antioxidative, antiproliferative, anticarcinogenic, antimutagenic, antibacterial or antiviral activities to greater or lesser

extent (LIU et al. 2002; RATNAM et al. 2006). Cultivated strawberry (*Fragaria × ananassa* Duch.) has been known to have a high content of phenolic compounds and natural antioxidants (AABY et al.

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2007). Numerous studies were done to evaluate the mechanisms for increased synthesis of polyphenol compounds in strawberry fruits. Their content varies among species and cultivars (MILIVOJEVIĆ et al. 2011), but it can also be affected by growth conditions including environmental factors and cultivations techniques. Exogenous factors such as cultivation system or fertilization can affect the yield and yield-related characteristics of strawberry (PEŠAKOVIĆ et al. 2013), but also the concentration of anthocyanins and antioxidant activity (WANG, LIN 2002; CRESPO et al. 2010). Organically cultivated cv. Jonsok had a 12% higher concentration of total phenolics compared to those cultivated conventionally (HÄKKINEN, TÖRRÖNEN 2000). REGANOLD et al. (2010) also reported significantly higher total phenolics as well as total antioxidant capacity in organically cultivated strawberries than in conventional berries.

Accordingly, the objectives of this investigation were to evaluate the effect of production systems (integrated, conventional) and the fertilizer type (biofertilizers, chemical fertilizer) on the total phenolic content and total antioxidant capacity, as well as on the content of individual phenolic compounds of cv. Senga Sengana strawberry fruits.

## MATERIALS AND METHODS

### Plant materials and experimental design.

Strawberry plants of cv. Senga Sengana were grown in the greenhouse of the Fruit Research Institute, Čačak, Republic of Serbia (43°53'N, 20°20'E, 225 m a.s.l.) during the two consecutive seasons. The experiment was set up at a randomized block design in three replications. Runners were planted in 3 dm<sup>3</sup> plastic pots filled with Klasmann substrate (TS1, 0.7 g/l nutrient content), a mixture of fine white sphagnum peat (0–10 mm) and perlite (25%). The substrate is slightly acidic (pH = 6), enriched with water-soluble nutrients, microelements and wetting agents. Prior to planting, runners were dipped in warm water (46°C) for 10 min to prevent infestation with strawberry tarsonemid mite (*Phytonemus pallidus*). The treatments contained two production systems, i.e. integrated (biological insecticides) and conventional (chemical insecticides) and three fertilizer variants i.e. PGPR 1 (biofertilizer), PGPR 2 (biofertilizer) and Multi-KMg (chemical fertilizer).

**Treatments.** Integrated system involved the application of biological insecticides, i.e. neem (NeemAzal 0.4%) and pyrethrin (Pyros 0.2%), whereas the conventional one included chemical insecticides, i.e. endosulphan (Thiosulphan 0.2%) and gusathion (Gusathion 0.15%). Both insecticide categories were applied in two replications.

PGPR 1 (biofertilizer) consists of pure culture of Gram-negative diazotrophic nitrogen-fixing bacteria *Klebsiella planticola* obtained from the microorganisms collection of Microbiology Laboratory, Faculty of Agronomy, Čačak. PGPR 2 (biofertilizer) combines nitrogen-fixing and phospho-mineralizing bacteria (*Azotobacter chroococcum*, *A. vinelandii*, *Bacillus megatherium*, *B. licheniformis*, *B. subtilis*). Multi KMg (12:0:43 + 2MgO) is a chemical fertilizer applied at a concentration of 0.16% (0.8 g fertilizer/500 cm<sup>3</sup> water). PGPR 1 and PGPR 2 were applied during plant establishment by dipping roots in liquid inoculum of bacteria for 30 minutes. Bacteria titer in the inoculum ranged from 20–40 × 10<sup>6</sup> CFU/cm<sup>3</sup>. Planted plants were periodically watered (every 7 days) with 100 cm<sup>3</sup> inoculum in the course of the two-year growing period. Multi KMg was also applied in an identical manner.

**Determination of total phenolic content (TPC).** TPC was determined using a modified Folin-Ciocalteu colorimetric method (SINGLETON et al. 1999; LIU et al. 2002). Results were expressed as milligrams of gallic acid equivalents (GAE)/100 g fresh weight.

**Determination of the total antioxidant capacity (TAC).** TAC was determined using the DPPH method reported by BRAND-WILLIAMS et al. (1995) with modifications (SANCHEZ-MORENO et al. 1998). Results were expressed as Trolox equivalent antioxidant capacity (μmol TE/100g FW).

**Determination of the individual phenolic compounds.** Phenolic compounds were identified and quantified using an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, USA) linked to a ChemStation data handling system, using a ZORBAX Eclipse Plus C18 column (4.6 × 150 mm, 3.5 μm particles). Samples were prepared according to the method of HERTOOG et al. (1992) before they were analysed. Injection volume was 5 μl and the temperature was set at 30°C. Solvent A was 1% formic acid and solvent B was acetonitrile. The gradient used was as follows: 0–10 min, 10% of B in A; 10–25 min, 15–50% of B in A; 25–30 min, 50–80% of B in A; 30–32 min, 10% of B in A. By us-

ing this gradient (flow rate 0.5 ml/min), a good level of purity and separation was achieved in fruit samples. The HPLC equipment was used with a diode array detector (DAD). Phenolic compounds were detected at 260 nm (rhamnetin, protocatechuic acid, ellagic acid), 280 nm (gallic acid, *p*-coumaric acid), 360 nm (quercetin, kaempferol), and 520 nm (cyanidin, pelargonidin). Phenolic compounds were identified according to peak retention time (RT) and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg/100 g FW.

**Statistical analysis.** The data were subjected to analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, USA). The Least Significance Difference (LSD) was used to compare treatment means and treatments declared different at  $P = 0.05$  level of significance. Correlations between TPC and TAC were calculated separately according to the Pearson's test at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Total phenolic content and total antioxidant capacity

The production systems showed no significant effect on total phenolic content, but it significantly affected total antioxidant capacity (Table 1). A significant influence of the interaction effect of the

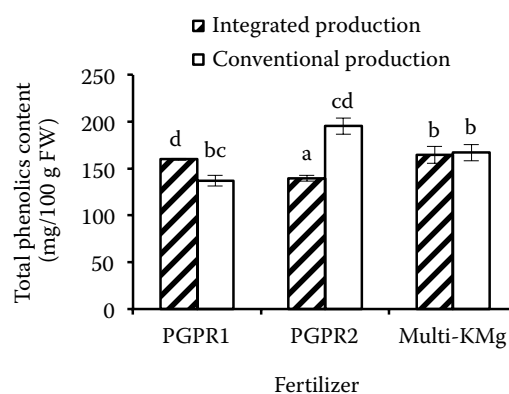


Fig. 1. Interaction effect of the production system and fertilizer type on the total phenolics content in cv. Senga Sengana strawberry fruits

production system and fertilizer applied on the total phenolic content was also evident (Fig. 1). The results of our study inferred greater values of total antioxidant capacity in integrated than in conventional production system. According to the literature reports (REGANOLD et al. 2010) strawberries from organic farms had significantly higher total antioxidant capacity (8.5% more) than those from conventional treatment. TÖNUTARE et al. (2009) also reported significantly higher total antioxidant capacity of organically grown strawberries, although the content of the major antioxidants did not show any increase.

The total phenolic content and total antioxidant capacity were significantly affected by the fertilizer type (Table 1). From the present study, PGPR 2 and Multi K-Mg treatments gave the highest total phe-

Table 1. The influence of production system and fertilizer type on the total phenolic content and total antioxidant capacity in cv. Senga Sengana strawberry fruits

Factor		Total phenolic content (mg/100 g FW)	Total antioxidant capacity (Trolox, mmol/100 g FW)
Production system (A)	integrated	166.41 ± 8.83 <sup>a</sup>	317.85 ± 6.90 <sup>a</sup>
	conventional	154.61 ± 5.44 <sup>a</sup>	300.36 ± 5.29 <sup>b</sup>
Fertilizer type (B)	PGPR 1	148.55 ± 5.71 <sup>b</sup>	321.46 ± 7.14 <sup>a</sup>
	PGPR 2	167.42 ± 13.09 <sup>a</sup>	312.70 ± 6.36 <sup>ab</sup>
	Multi-KMg	165.65 ± 5.60 <sup>a</sup>	293.16 ± 7.22 <sup>b</sup>
ANOVA			
A		ns	*
B		*	*
A × B		*	ns

means of three replicates ± standard error; values within each column followed by different lower-case letters indicate significant differences at  $P \leq 0.05$  by LSD test; ns – non significant differences; FW – fresh weight

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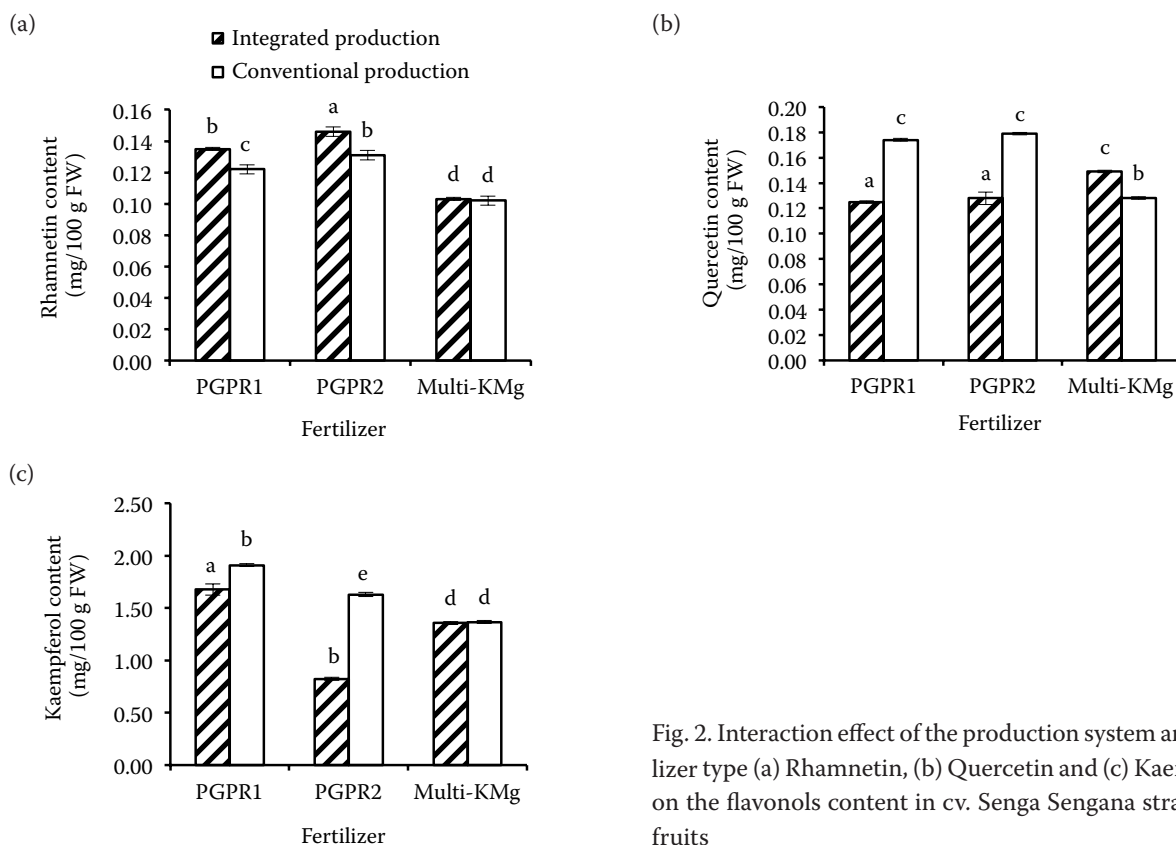


Fig. 2. Interaction effect of the production system and fertilizer type (a) Rhamnetin, (b) Quercetin and (c) Kaempferol on the flavonols content in cv. Senga Sengana strawberry fruits

nolic content, whereas the application of PGPR 1 gave the highest total antioxidant capacity. Our previous comparative study of bio- and chemical fertilization in strawberry production also showed greater TPC and TAC in fruits of cvs Clery, Joly and Dely cultivars, compared to the biofertilizer application treatment (PEŠAKOVIĆ, MILIVOJEVIĆ 2014). According to KIVIJÄRVI (1999) synthesis

of phenolic compounds might be accelerated in organic berry production where herbicides, pesticides, insecticides and fertilizers are not used. The TPC and TAC increase in our study resulting from the biofertilizer used could be explained by similar reasons.

According to the results of our study, no correlation ( $r = -0.17$ ) between TPC and TAC was ob-

Table 2. The influence of production system and fertilizer type on the flavonols content in cv. Senga Sengana strawberry fruits

Factor		Flavonols content (mg/100 g FW)		
		Rhamnetin	Quercetin	Kaempferol
Production system (A)	integrated	0.13 ± 0.01 <sup>a</sup>	0.16 ± 0.01 <sup>a</sup>	1.64 ± 0.08 <sup>a</sup>
	conventional	0.12 ± 0.00 <sup>b</sup>	0.13 ± 0.00 <sup>b</sup>	1.29 ± 0.13 <sup>b</sup>
Fertilizer type (B)	PGPR 1	0.13 ± 0.00 <sup>b</sup>	0.15 ± 0.01 <sup>a</sup>	1.79 ± 0.06 <sup>a</sup>
	PGPR 2	0.14 ± 0.00 <sup>a</sup>	0.15 ± 0.01 <sup>a</sup>	1.23 ± 0.18 <sup>c</sup>
	Multi-KMg	0.10 ± 0.00 <sup>c</sup>	0.14 ± 0.01 <sup>b</sup>	1.37 ± 0.00 <sup>b</sup>
ANOVA				
A		*	*	*
B		*	*	*
A × B		*	*	*

for explanation see Table 1

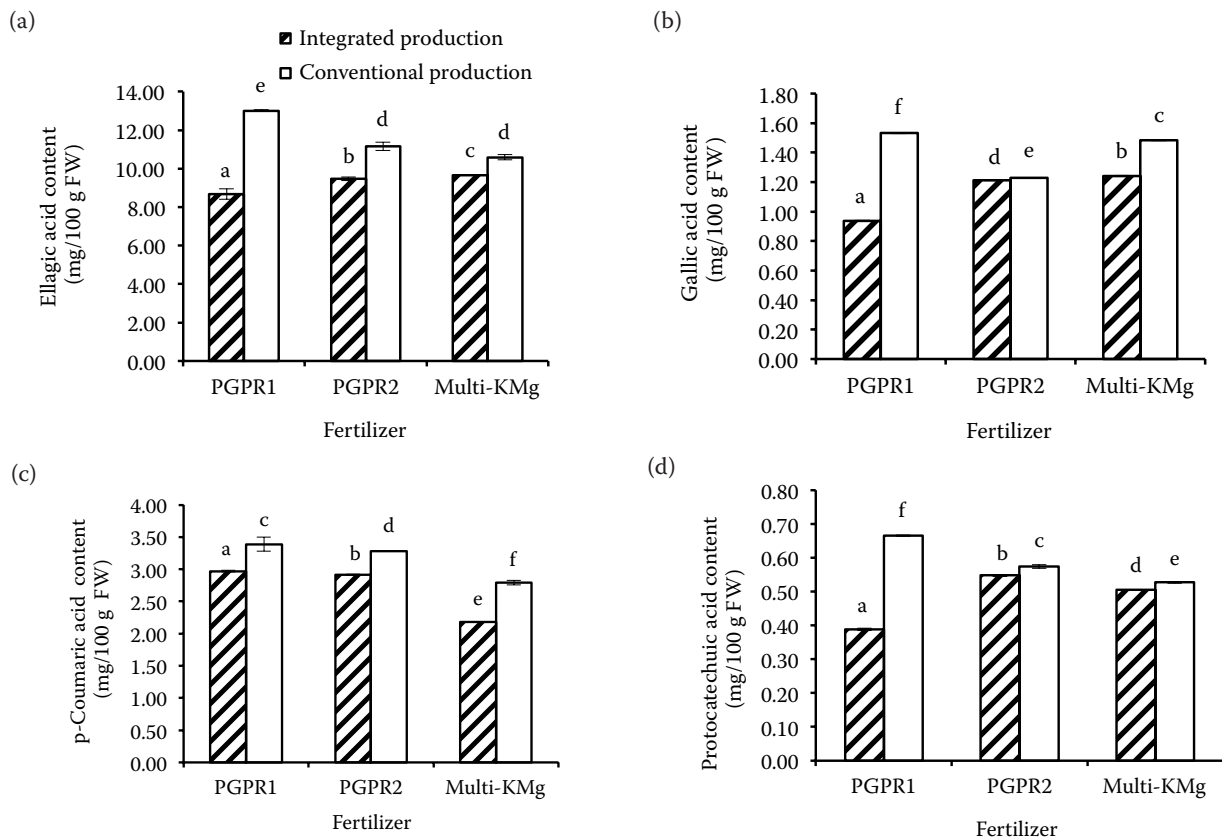


Fig. 3. Interaction effect of the production system and fertilizer type (a) Ellagic, (b) Gallic, (c) *p*-Coumaric, and (d) Protocatechuic on the phenolic acids content in cv. Senga Sengana strawberry fruits

served, which is in accordance with the results of DA SILVA PINTO et al. (2008). These results suggested that the free radical-linked antioxidant capacity of these samples could be associated not only with the TPC, but with other compounds as well. Authors reported that the cv. Sweet Charlie had one of

the lowest TPC, but had the highest TAC among all cultivars. HASSIMOTTO et al. (2005) also recorded no correlation between TPC and TAC. This study suggested that the TAC is a result of the combination of different compounds having synergistic and antagonistic effect. The presence of vitamin C in

Table 3. Influence of production system and fertilizer type on the phenolic acids content in cv. Senga Sengana strawberry fruits

Factor		Phenolic acids (mg/100g FW)			
		Ellagic	Gallic	<i>p</i> -Coumaric	Protocatechuic
Production system (A)	integrated	11.58 ± 0.38 <sup>a</sup>	1.42 ± 0.05 <sup>a</sup>	3.15 ± 0.09 <sup>a</sup>	0.59 ± 0.02 <sup>a</sup>
	conventional	9.27 ± 0.17 <sup>b</sup>	1.13 ± 0.05 <sup>b</sup>	2.69 ± 0.13 <sup>b</sup>	0.48 ± 0.02 <sup>b</sup>
Fertilizer type (B)	PGPR 1	10.85 ± 0.98 <sup>a</sup>	1.24 ± 0.13 <sup>b</sup>	3.18 ± 0.09 <sup>a</sup>	0.53 ± 0.06 <sup>b</sup>
	PGPR 2	10.31 ± 0.39 <sup>b</sup>	1.21 ± 0.00 <sup>c</sup>	3.09 ± 0.08 <sup>b</sup>	0.56 ± 0.01 <sup>a</sup>
	Multi-KMg	10.13 ± 0.21 <sup>b</sup>	1.36 ± 0.05 <sup>a</sup>	2.49 ± 0.14 <sup>c</sup>	0.52 ± 0.01 <sup>c</sup>
ANOVA					
A		*	*	*	*
B		*	*	*	*
A × B		*	*	*	*

for explanation see Table 1

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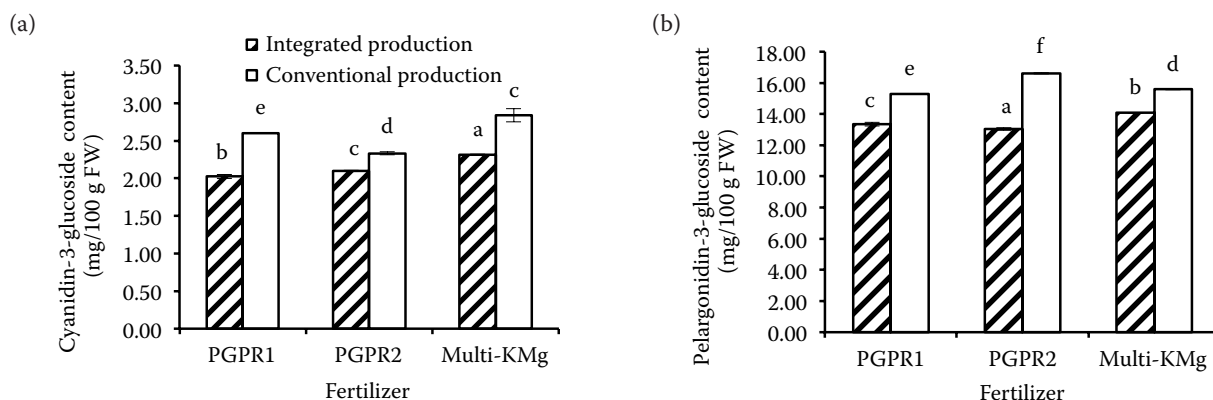


Fig. 4. Interaction effect of the production system and fertilizer type (a) Cyanidin-3-glucoside and (b) Pelargonidin-3-glucoside on the anthocyanins content in cv. Senga Sengana strawberry fruits

some strawberry fruits as a powerful antioxidant that works independently or in combination with different phenolic components, significantly contributes (30–35%) to the expression of antioxidant activity (TULIPANI et al. 2008).

### Individual phenolic compounds

#### Flavonols content

The flavonols content was significantly affected by the production system and the fertilizer type, as well as by their interaction (Table 2, Fig. 2). The results of our study inferred that levels of all the examined flavonols (rhamnetin, quercetin and kaempferol) were higher in the integrated production system. Similarly to our study, DIXON and PAIVA (1995) reported higher contents of kaempferol

in organically cultivated strawberries than in those cultivated conventionally.

Among the different fertilizer treatments, PGPR 2 gave the highest rhamnetin and quercetin content, whereas the application of PGPR 1 resulted in the highest kaempferol content. It is possible that biofertilizers cause changes in chemical, physical and biological characteristics of soil, increasing beneficial microorganisms, as well as the nutrient availability and uptake, thus favouring fruit growth and synthesis of these compounds in fruits. According to WOESE et al. (1997) the optimal conditions for plant growth generally result in the highest levels of antioxidants. By analysing the influence of the different fertilizer technology, WANG and LIN (2003) also reported differences in kaempferol 3-glucoside-succinate content. They showed that strawberry plants cultivated with addition of compost

Table 4. The influence of production system and fertilizer type on the anthocyanins content in cv. Senga Sengana strawberry fruits

Factor		Anthocyanins content (mg/100g FW)	
		Cyanidin-3-glucoside	Pelargonidin-3-glucoside
Production system (A)	integrated	2.59 ± 0.07 <sup>a</sup>	15.82 ± 0.21 <sup>a</sup>
	conventional	2.15 ± 0.04 <sup>b</sup>	13.49 ± 0.16 <sup>b</sup>
Fertilizer type (B)	PGPR 1	2.31 ± 0.13 <sup>b</sup>	14.31 ± 0.43 <sup>b</sup>
	PGPR 2	2.22 ± 0.05 <sup>c</sup>	14.82 ± 0.80 <sup>a</sup>
	Multi-KMg	2.58 ± 0.12 <sup>a</sup>	14.83 ± 0.34 <sup>a</sup>
ANOVA			
A		*	*
B		*	*
A × B		*	*

for explanation see Table 1

and fertilizer had significantly increased the content of kaempferol 3-glucoside-succinate.

#### **Phenolic acids content**

The content of phenolic acids analysed in this study showed a significant impact of the production system, fertilizer type, as well as the interaction effect of production system and fertilizers type (Table 3, Fig. 3). The dominant phenolic acid identified in our study was ellagic acid. JAKOBEK et al. (2007) and MILIVOJEVIĆ et al. (2011) also found ellagic acid to be the major phenolic acid in strawberry fruits.

Compared to different production system, the higher contents of ellagic, gallic, *p*-coumaric and protochatecuic acids were recorded in integrated production system. OLSSON et al. (2006), also revealed that the production system significantly influenced the ellagic and *p*-coumaric acids content in cvs Polka and Jonskok cultivated by organic techniques. Contrary to this, HÄKKINEN and TÖRRÖNEN (2000) found that the levels of phenolic acids were similar in the cvs Polka and Honeoye cultivated by conventional or organic technique and that the only cv. Jonskok cultivated by organic culture had a higher content of ellagic acid.

In our study, the highest concentrations of ellagic and *p*-coumaric acids were recorded in PGPR 1 nutrient application treatment, whereas the application of PGPR 2 gave the highest concentration of protochatecuic acid. The highest concentrations of gallic acid were observed in Multi-KMg nutrient application treatment. Analysing the impact made by different fertilizer types, PEŠAKOVIĆ and MILIVOJEVIĆ (2014) also reported that the phenolic acids content is greatly influenced by fertilizer type.

#### **Anthocyanins content**

With regard to the anthocyanins content, the results suggested that the contents of both cyanidin-3-glucoside and pelargonidin-3-glucoside were higher in the integrated production systems (Table 4). In order to investigate the effect of fruit quality, antioxidant capacity of organically and conventionally grown blueberries, WANG and LIN (2002) reported significantly greater anthocyanins content in organic than in conventional farms. Comparing the phenolic composition and antioxidant properties from integrated pest management and organic farming FERNANDES et al. (2012) reported higher levels of anthocyanins of organically grown strawberries.

In our study Multi KMg has proved dominant in the cyanidin-3-glucoside and pelargonidin-3-glu-

coside content compared to PGPR 1, and to a lesser extent compared to PGPR 2. WANG and LIN (2002), also revealed that fertilizer type significantly influenced anthocyanin content in strawberry fruits. They concluded that increased anthocyanins content (in fertilizer and compost treatment) was associated with increased antioxidant capacities which may allow for quenching of the excited state of active oxygen species.

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*Corresponding author:*

ŽAKLINA M. KARAKLAJIĆ STAJIĆ, Fruit Research Institute Čačak, Čačak, Republic of Serbia, Kralja Petra I No 9, Republic of Serbia; e-mail: zaklinaks@yahoo.com