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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF TWO STRAWBERRY CULTIVARS

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The macro- and micro-chemical composition, as well as antioxidant activity of two strawberry cultivars, Marmolada and Clery, were studied. Results showed a noticeable difference in the sugar, protein and pectin contents. Clery had 6.92% and Marmolada 4.93% of total sugar. Also, protein and pectin contents were higher in the Clery cultivar. No significant difference was observed in acidity, as well as in ash and cellulose content. Marmolada had a higher content of total phenolics and flavonoids (228.04 mg GAE /100 g FW and 136.01 mg RE/100 g FW, respectively). The anthocyanins content in Marmolada (32.0 mg CGE/100 g FW) was slightly lower than in Clery (36.0 mg CGE/100 g FW). The antioxidant activity was evaluated spectrophotometrically, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay. The DPPH free radical scavenging activity, expressed as EC_{50} value, of Marmolada (0.77 mg/ml)was higher than of Clery (0.83 mg/ml). There was a significant positive correlation (R^2>0.90) between the concentration of phenolics/flavonoids/anthocyanins and DPPH radical scavenging activity of both strawberry cultivars. These results also showed that the antioxidant value of 100 g FW Marmolada and Clery is equivalent to 237.91 mg and 219.01 mg of vitamin C, respectively.

KEY WORDS: strawberry, macro- and micro-chemical composition, antioxidant activity, phenolic compounds

INTRODUCTION

Strawberries are fruit with high visual appeal, desirable flavor and nutritional value. There is an increasing interest of consumers towards berry fruit, as they have been proved to have potential health benefits. Numerous studies revealed that consumption of berry fruit can lower the risk to many diseases, due to the presence of vitamins, minerals, fibers and potentionally bioactive compounds (1,2). Strawberries have been show to possess high antioxidant activity which can influence some health-protecting activities (e.g.

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decreased the risk of cardiovascular deseases, increased the plaque stability, etc.) (3-5). They also may exhibit antiproliferative activity towards certain cancer cell lines. Shin et al. (5) reported that strawberry phytochemicals showed an inhibitory activity against human liver cancer cell proliferation.

Antioxidant capacity of berry fruits is affected by phenolics, anthocyanins and other flavonoids (6). Strawberries are a good source of phenolic compounds such as phenolic acids (ellagic, coumaric and *p*-hydroxybenzoic acid), flavonoids (quercetin, kaempferol, myricetin, catehins), and anthocyanins (5). The total phenolic content varies from 0.4 up to 3 mg/g (7-10). Total anthocyanin content in strawberry fruits can vary from 150 to 600 mg/kg fresh weight (11, 12). Pelargonidin 3-glucoside, pelargonidin 3-rutinoside and cyanidin-3-glucoside are the most present compounds in strawberries (3, 9, 11, 13). The content of these phytochemicals is proved to be affected by many factors, such as edaphic-climatic conditions, maturity degree, growing conditions and the cultivar as the most important (3, 5, 11, 14). A positive correlation between antioxidant activity and total anthocyanin and total phenolic content (1, 6) of strawberries was found, and consequently, with their potential health benefits (7).

Strawberries are also a very good source of blood sugar-regulating dietary fibers (pectins, celluloses, etc.) and thyroid health-promoting iodine (15). Strawberry fruits are rich in sugars (mainly glucose and fructose, with smaller amounts of sucrose) and acids (citric acid being the dominant) (2). Sugars and acids are very important compounds that contribute to the fruit flavor and sweetness. Their content can vary (4.66-8.43% of total sugars, 0.56-1.6% of total acids) (16). Sugars are also considered as main nonvolatile flavor components (17). Among vitamins, vitamin C is the dominant. It has been proved that vitamin C and phenolic compounds contribute to antioxidant capacity of fruits, as they act as oxygen radical scavengers and may exhibit beneficial health effects (2, 6, 7, 11). Strawberries are rich in potassium (the most abundant mineral), calcium and magnesium (14). They are also a good source of folate, omega-3 fatty acids, vitamin B6, and vitamin K, as well as energy-promoting vitamins B2 and B5 (18).

Although there are many data on chemical composition and antioxidant capacities of different strawberry cultivars, comparison of the research results is not simple, as they are affected by genetic background (species and variety), ripeness, maturity, as well as by growing conditions (environmental factors, cultivation techniques, irrigation, fertilization, etc.) (3, 9) and different methods of analyses.

Considering its increasing importance, cultivating areas of this fruit in our country are increasing every year. Growing of strawberry has been developed in the surroundings of large consumer centers or processing capacities. Moreover, Serbia has extraordinary natural preconditions for berry fruit growing (16). Geographic altitude, climate, relief and soil characteristics, classify Serbia among growing regions capable of continuous provision the market with quality goods during the whole season.

Despite of plenty of research on strawberries, the data on chemical composition and antioxidant capacities of Marmolada and Clery cultivars, examined in this work, are poor, especially concerning the growing region (2, 19). For these reasons, the object of this research was the examination of the macro-chemical composition, total phenolics, flavonids and anthocyanins content, as well as of antioxidant activity of two strawberry cultivars (Marmolada and Clery) grown in the region of northern Serbia.

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EXPERIMENTAL

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, chlorogenic acid and rutin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). These chemicals were of analytical reagent grade. Other chemicals and solvents used were of the highest analytical grade, obtained from ‘‘Zorka” Šabac (Serbia).

Plant material

Two strawberry cultivars, Marmolada and Clery, were grown at the same plantation located in the Subotica region (northern Serbia) and harvested at commercial maturity stage in the season 2011. The collected fruits were washed in water, frozen, and stored at -35°C until the analysis.

Macro-chemical composition

Total solids, total ash, sugar content (total sugars, reducing sugars, sucrose) were asseyed according to the Regulation on methods of sampling and chemical and physical analyses of fruit and vegetable products, 29/83 (20). Total solids were determined by drying the samples at 105°C to constant weight; soluble solids using an Abbe refractometer (Carl Zeiss, Jena, Germany), at 20°C; total ash gravimetrically by incenerating the samples at 525°C to constant weight; sugar content by the method of Luff-Schoorl. The cellulose was measured using a Kirschner-Ganakova's method; pectin content colorimetrically, by carbasole method; acidity by titration with NaOH standard solution, and protein content by Kjeldahl's method (21).

Anthocyanin content

Anthocyanin content (total monomeric anthocyanin content) in strawberries was determined according to the Giusti and Wrolstad method (22) based on the pH-differential method previously described by Fuleki and Francis (23). Anthocyanin content was expressed as mg of cyanidin 3-glucoside equivalents per 100 g of fresh weight of strawberry (mg CGE/100 g FW).

Extraction for measurement of phenolics, flavonoids and antioxidant activity

Sample of strawberry mash (20 g) was extracted with different solvents (80% methanol; 0.05% acetic acid in 80% methanol; 60% methanol; 0.05% acetic acid in 60% methanol) at room temperature using an homogenizer, Ultraturax DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed three times with different amounts of solvent: 160 ml in 60 min, 80 ml in 60 min, 80 ml in 30 min. The total extraction time was 150 min. The obtained extracts were combined and evaporated to dryness under reduced pressure. The yields (g) of extracts are shown in Table 1.

Table 1. The yields of strawberry cultivar extracts obtained using different solvents

Solvent	Yield of strawberry cultivar extract (g)	
	Marmolada	Clery
80% methanol	1.68	1.97
0.05% acetic acid in 80% methanol	1.22	1.77
60% methanol	0.79	1.14
0.05% acetic acid in 60% methanol	1.30	1.52

The highest yield of phytochemicals extract from both strawberry cultivars was obtained with 80% methanol. These strawberry exstracts were used for the measurement of total phenolic and flavonoid content as well as antioxidant activity.

Total phenolic content

The amount of total phenolics in the strawberry extracts was determined spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan) by the Folin-Ciocalteu method (24). The total phenolic content was determined from the regression equation of the gallic acid calibration curve, and expressed as mg of gallic acid equivalents per 100 g fresh weight of strawberry (mg GAE/100 g FW).

Total flavonoid content

Total flavonoids in the strawberry extracts were measured by the aluminum chloride spectrophotometric assay (25). Total flavonoid content was determined from the regression equation of the rutin calibration curve, and expressed as mg of rutin equivalents per 100 g fresh weight of strawberry (mg RE/100 g FW).

DPPH radical scavenging activity

The DPPH radical scavenging activity of strawberries was determined spectrophotometrically using the modified DPPH method of Chen et al. (26). Briefly, 1 ml of extract solution in distilled water (sample) or 1 ml of distilled water (blank) was mixed with 2 ml of DPPH solution (2 mg of DPPH was dessolved in 50 ml of methanol). The range of the investigated extract concentrations was 0.02 - 166.67 mg/ml. The mixture was shaken vigorously and left at room temperature for 30 min, then the absorbance was read at 517 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The capability to scavenge the DPPH radicals (DPPH radical scavenging activity, SA) was calculated using the following equation:

$$SA\ (\%) = 100 \times (A_{blank} - A_{sample})/A_{blank}$$

where A_{blank} is the absorbance of the blank and A_{sample} is the absorbance of the sample. Ascorbic acid was used as a reference compound.

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The effective concentration (EC_{50}), defined as the concentration of sample required for 50% scavenging of DPPH radicals under experimental conditions used to measure the free radical scavenging activity.

Statistical analysis

All measurements were carried out in triplicate, and presented as mean ±SD. Correlation and linear regression analyses were performed using Microsoft Office Excel 2003.

RESULTS AND DISCUSSION

As can be seen from the results of the macro-chemical composition (Table 2), Clery cultivar had a noticeably higher content of total solids than Marmolada (9.47 and 7.75, respectively). As sugars represent most of the solids in fruits, the total solids content can be related to the sugar content. The Sugar content in the examined strawberries is in agreement with the results published by Sánchez-Moreno et al. (27), who reported 6.2% of total sugar. According to total and soluble solids, it was expected for Clery to have a higher sugar content, which was confirmed by the analysis. As total sugar content is dependent on many factors (variety, growing conditions, harvesting, maturity stage, transport and handling conditions), and in view of the fact that the samples were grown in the same conditions (on the same location and in the same season), it can be assumed that the difference is either a cultivar feature or a consenquence of the maturity stage at the moment of harvest. As the above a mentioned, sugars and acids contribute to sweetness/tartness of the fruit. Some authors reported that strawberry fruits contain 0.7-1.8% organic acids (16), depending on the cultivar, with citric and malic acids being the dominant. These acids are present partly bound, but mostly as free acids. Free acids and sugars, besides aroma compounds, are very important sensory compounds, giving the characteristic flavor to strawberry and its products, and the very important sweetness index. Titrable acidities in strawberries examined in this research were 0.57% and 0.58% (for Marmolada and Clery, respectively), and are in agreement with the results published by Milivojević et al. (28), who reported 0.54% and 0.59% of titrable acidities for Marmolada and Clery, respectively. The result could indicate the maturity level at the harvest moment, i.e. a higher acidity level corresponds to a lower ripeness degree and vice versa. Also, as the difference in acidity of samples was small, and considering the sugar content, it can be stated that Clery had a higher sweetness index, which makes it more attractive to consumers from the sensory point of view. Clery had a slightly higher pectin content, while the differences in pectic acid and protopectin contents are not noticeable. It is well known that pectic substances are heteropolysaccharides, contributing to the cohesion and stablity of fruit tissues, so the fruit texture is strongly affected by the pectin content. The examined strawberry samples had a slightly lower pectin content than reported by Šulc (29), and considerably lower than reported by Šoškić (16). Knowing that pectic substances are considered as water-soluble dietary fibers, whose health benefitial effect is well known, the importance of their intake from this point of view should not be neglected. The differences in the cellulose content in examined strawberry cultivars were small, but noticeably lower than the average cellulose content in fruit, which can vary from 0.5 to 5.5% (29). It can be concluded that the contents of dietary fiber, cellulose and pectins are probably a cultivar characteristic.

Table 2. Macro-chemical composition of Marmolada and Clery strawberry cultivars

Compound (g/100g FW)	Marmolada	Clery
Total solids	7.75 ± 0.08	9.47 ± 0.13
Soluble solids	7.25 ± 0.09	9.05 ± 0.05
Ash	0.21 ± 0.01	0.27 ± 0.01
Cellulose	0.26 ± 0.001	0.30 ± 0.01
Pectin	0.14 ± 0.04	0.25 ± 0.02
Pectic acid	0.29 ± 0.02	0.27 ± 0.03
Protopectin	0.09 ± 0.04	0.07 ± 0.05
Acidity	0.57 ± 0.01	0.55 ± 0.02
Total sugars	4.93 ± 0.13	6.92 ± 0.22
Reducing sugars	4.04 ± 0.03	6.26 ± 0.10
Sucrose	0.85 ± 0.12	0.63 ± 0.28
Proteins	0.45 ± 0.02	0.75 ± 0.02

The typical color of strawberry fruits is due to the presence of water-soluble color compounds, i.e. anthocyanins and flavonoids. Some liposoluble dyes, as carotenes and xanthophylls, are present in minor amounts (30). In our study, the Clery strawberry cultivar had a higher content of anthocyanins than Marmolada (Table 3). Although other researchers reported higher anthocyanins in some other red fruits (22), these values are in accordance with the results of Timberlake (31), who reported strawberries to have 15-35 mg of anthocyanins per 100 g fresh weight.

It is observed that the contents of total phenolics and flavonoids in Marmolada are higher than in Clery (Table 3). The content of total phenolics in both strawberry cultivars is slightly higher than that in the Marmolada strawberry cultivar grown in western Serbia (1.56 mg GAE/g FW) (2).

Table 3. Total phenolics, flavonoids and anthocyanins content in strawberry cultivars

Strawberry cultivar	Total phenolics (mg GAE/100 g FW)	Total flavonoids (mg RE/100 g FW)	Anthocyanins content (mg CGE/100 g FW)
Marmolada	228.04 ± 10.21	136.01 ± 6.12	32.0 ± 1.54
Clery	170.75 ± 7.58	122.31 ± 5.93	36.0 ± 2.98

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities due to its simple, rapid, sensitive, and reproducible procedure (32). The antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e., 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in the absorbance at 517 nm (Soares, 1997). Figure 1 shows the dose response for the DPPH radical scavenging activity (SA) of Marmolada and Clery extracts. The DPPH free radical scavenging activity of the extracts increased with increasing concentration.

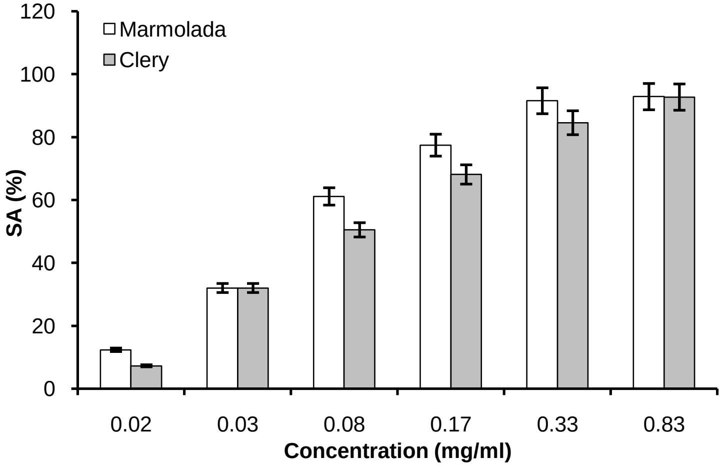

Figure 1. DPPH radical scavenging activity (SA) of the strawberry cultivar extracts

The EC_{50} value, defined as the concentration of the extract required for 50% scavenging of DPPH radicals under experimental conditions employed, is a parameter widely used to measure the free radical scavenging activity (33); a smaller EC_{50} value corresponds to a higher antioxidant activity. Marmolada showed a higher DPPH free radical scavenging activity, expressed as EC_{50} value in mg of fresh weight of strawberry per ml of the reaction mixture (0.7652 ± 0.0310 mg/ml), than the Clery (0.8311 ± 0.0335 mg/ml). These results are in agreement with the results of Milivojević et al. (28), who reported that the higher total antioxidant capacity (determined according to the ABTS method) had Maromalada than Clery. Ascorbic acid (vitamin C), because of its antioxidant activity (EC_{50} value = 1.82 ± 0.07 μg/ml) was used as a reference compound. These results showed that the antioxidant value of 100g Marmolada and Clery is equivalent to 237.91 mg and 219.01 mg of vitamin C, respectively.

The high correlation coefficients (R^2>0.90), calculated from the logarithmic regression analysis, indicates that there is a significant positive correlation between the concentration of phenolics/flavonoids/anthocyanins and DPPH radical scavenging activity of both strawberry cultivars (Figure 2).

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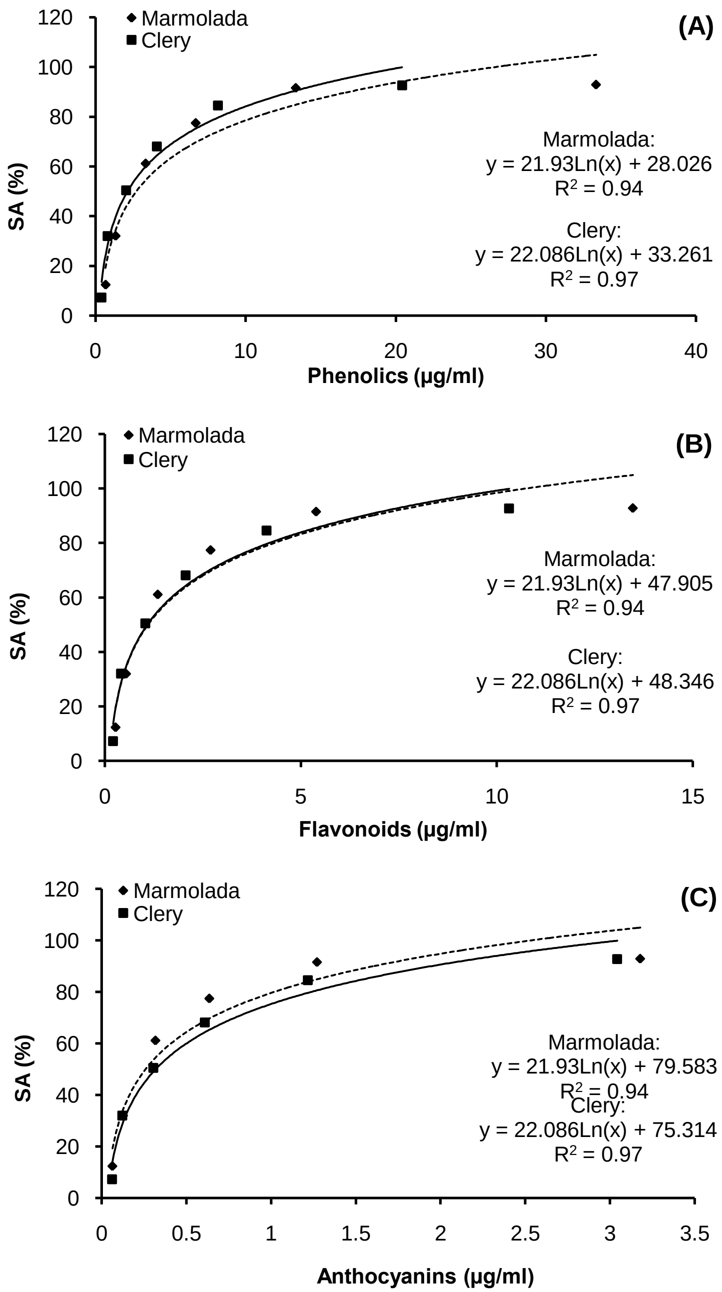

Figure 2. Correlation between strawberry phenolics (A), flavonoids (B) and anthocyanins (C) and DPPH radical scavenging activity in the reaction mixtures

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CONCLUSION

The results obtained in this study could be of interest in confirming the importance of the genetic background of strawberries for the availability of specific compounds, phytonutrients and phytochemicals with high biological effect. Clery cultivar is richer in many chemical compounds, owing to its higher total solids content. The two cultivars did not differ much in the anthocyanins content, in contrast to the phenolics and flavonoids contents. The contents of total phenolics and flavonoids in Marmolada is higher than in Clery. A higher DPPH free radical scavenging activity showed the Marmolada. The antioxidant value of 100 g Marmolada and Clery is equivalent to 237.91 mg and 219.01 mg of vitamin C, respectively. The high antioxidant activity and significant positive correlation between the concentration of phenolics/flavonoids/anthocyanins and DPPH radical scavenging activity indicate that both strawberry cultivars could be considered as a good source of natural antioxidants that may have potential health effects.

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ХЕМИЈСКИ САСТАВ И АНТИОКСИДАТИВНА АКТИВНОСТ ДВЕ СОРТЕ ЈАГОДЕ

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У овом раду испитани су макро- и микро-хемијски састав, као и антиоксидативна активност две сорте јагоде, Marmolada и Clery. Утврђена је значајна разлика у садржају шећера, протеина и пектина. Садржај укупног шећера у сорти Clery је износио 6,92%, а у Marmoladi 4,93%. Такође, садржај протеина и пектина је био већи у сорти Clery. Није уочена значајнија разлика у киселости, садржају пепела и целулозе. Садржаји укупних фенолних једињења и флавоноида у сорти Marmolada (228,04 mg GAE /100 g свеже јагоде и 136,01 mg RE/100 g свеже јагоде, редом) су били већи него у сорти Clery (170,75 mg GAE/100 g свеже јагоде и 122,31 mg RE/100 g свеже јагоде, редом). Marmolada је имала нешто мањи садржај антоцијана (32,0 mg CGE/100 g свеже јагоде) од Clery (36,0 mg CGE/100 g свеже јагоде). Антиоксидативна активност јагода на стабилне 2,2-дифенил-1-пикрилхидразил (DPPH) радикале испитана је спектрофотометријском методом. Сорта Marmolada показала је већу антиоксидативну активност од сорте Clery. Скевинџер активност на DPPH радикале сорти Marmolada и Clery, изражена као EC_{50} (mg свеже јагоде/ml реакционе смеше) износи 0,765 mg/ml, односно 0,831 mg/ml. Утврђена је висока линеарна корелација (R^2>0.90) између концентрације фенолних једињења, флавоноида, односно антоцијанина и скевинџер активности обе сорте јагода. Такође, утврђено је да је антиоксидативна вредност 100 g свежих јагода Marmolada, односно Clery једнака антиоксидативној вредности 237,91 mg, односно 219,01 mg витамина Ц.

Кључне речи: јагода, макро- и микро-хемијски састав, антиоксидативна активност, фенолна једињења

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