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### Fruit Quality of New Early Ripening Strawberry Cultivars in Croatia

Sandra Voća<sup>1\*</sup>, Nadica Dobričević<sup>1</sup>, Verica Dragović-Uzelac<sup>2</sup>, Boris Duralija<sup>1</sup>, Jasmina Družić<sup>1</sup>, Zlatko Čmelik<sup>1</sup> and Martina Skendrović Babojelić<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, University of Zagreb, Svetošimunska 25, HR-10000 Zagreb, Croatia <sup>2</sup>Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, HR-10000 Zagreb, Croatia

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#### Summary

In this research fruit quality of seven early ripening strawberry cultivars (Clerry, Maya, Alba, Miss, Camarosa, Queen Elisa and Elsanta) during successive harvesting periods has been investigated. The following quality parameters were determined in the harvested fruits: dry matter, total soluble solids (TSS), pH, total acidity (TA), TSS/TA ratio, reducing sugars, sucrose, vitamin C, total anthocyanins, total phenolics (TP), nonflavonoids, flavonoids, antioxidant activity, and colour. Investigated cultivars differ significantly according to the basic chemical composition. The highest, statistically significant values of dry matter, soluble solids, TSS/TA ratio, vitamin C, total phenolics, nonflavonoids and antioxidant activity were determined for cultivar Elsanta. Among the other early ripening cultivars involved in the research, Alba and Maya had the lowest contents of dry matter, total soluble solids, total phenolics and nonflavonoids, reducing sugars and sucrose. The anthocyanin content was the highest in cultivars Camarosa, Miss and Maya; consequently, the most intensive colour was noted in the same cultivars. Parameters that determine fruit quality had lower values in all the investigated early ripening cultivars than in cultivar Elsanta; however, their quality was satisfactory.

Key words: strawberry, vitamin C, polyphenols, antioxidant capacity, colour

### Introduction

Strawberry production in the Republic of Croatia is not developed to its full potential. There is a grater demand on the market for this fruit than there is production, for this reason there is still a big import of strawberries to Croatia. Together with the researchers, producers are trying to solve this problem and to enhance the production and broaden the growth of early ripening cultivars, in order to have domestic strawberries on the market before the imported ones. To achieve this, it is of vital importance to select the assortment which is of satisfactory fruit quality and appealing to the consumers. Categorization of a modern strawberry cultivar depends on many different criteria (selection of mulch, air temperature and humidity at maturation, irrigation and plant protection). The producer wants cultivars to be high-yielding, to provide continuous supply over a long season, and to bear large fruits. Consumers prefer strawberries with a wide range of sensory features. The evidence-based health benefits of berry consumption provide a means of promoting strawberries (1,2).

The quality components can be sensory and nutritional. It is implicit in the use of single and multiple physical or chemical characteristics for determination of optimum maturity that changes in the selected parame-

<sup>\*</sup>Corresponding author; Fax: ++385 1 2393 919; E-mail: svoca@agr.hr

ters correlate with the attainment of the general composition of quality characteristics of the product (3). Parameters like total acids (TA), total soluble solids (TSS) and their ratio (TSS/TA) are very important in determining strawberry fruit quality (3-6). Total soluble solids to total acids ratio is a very important parameter in evaluating fruit quality, because it determines fruit flavour harmony. Hence, along with fruit colour, it is a major factor in determining strawberry fruit quality (3). According to the research of Sturm et al. (3), the TSS/TA ratio, depending on harvest time, is considered to be very high. Saied et al. (7) obtained somewhat different values for the TSS/TA ratio in cultivar Elsanta. Strawberries are a delicious, low-energy food and a well-known source of vitamin C (2). Besides ascorbic acid, polyphenols are often discussed for their positive effect on human health (8). Strawberries contain several bioactive phytochemicals, including anthocyanins, flavonols, flavan-3-ols and phenolic acids (9). Anthocyanins are natural pigments, responsible for a wide range of red fruits (10). Antioxidant capacity in berries seems to be mostly due to the activity of phenolic compounds, such as anthocyanins, and antioxidative vitamins, mainly vitamin C. According to Lopes da Silva et al. (10), antioxidant capacity of fruits results mainly from phenolics, particularly flavonoids. Flavonoids have been demonstrated to have anti--inflammatory, antiallergenic, antiviral, anti-aging and anticarcinogenic activity (10).

Due to the fact that strawberry fruits are very popular in Croatia, we have conducted this research with a goal to determine the quality of selected early ripening strawberry cultivars and to compare them with the usually grown cultivars. This is the first research of this type in Croatia and it should give us a novel result for specific ecological conditions and also, a valuable answer for further practical use and application.

This study has been designed to investigate the quality of strawberry fruits with earlier ripening time by using physicochemical parameters (dry matter, total soluble solids (TSS), pH, total acidity (TA), TSS/TA ratio, reducing sugars, sucrose, vitamin C, total phenolics (TP), flavonoids, non-flavonoids and anthocyanins, as well as antioxidant capacity.

Furthermore, the aim is to compare different cultivars and to determine which one contains higher amounts of biologically active compounds, especially anthocyanins and polyphenols.

### Materials and Methods

### Materials

Seven strawberry cultivars *Fragaria* × *ananassa* (Clery, Maya, Alba, Miss, Camarosa, Queen Elisa, Elsanta), grown in an open field near Zagreb, were used in investigations. The experiment was set up according to the randomized block design with three replications, each comprising 50 plants. Fruits were harvested at full maturity (full red colour) at the beginning of the strawberry harvest season (end of May 2005). A mass of 500 g of fruits was taken by random choice for each replication. Fruit samples were analyzed immediately after harvesting. The fruits were mashed in a homogeniser (Mixy, Zepter International) and prepared for further analysis. Three replicates were used per analysis.

The following quality parameters of harvested fruits were determined: dry matter (total and soluble), total acids (TA), pH value, reducing sugars, vitamin C, total soluble solids/total acids ratio (TSS/TA), total phenolics, total anthocyanins and antioxidant capacity.

For determining fruit colour, 50 fruits were taken for each repetition, and measurements for each fruit were repeated three times. Fruit colour was represented by the hue angle (h), chroma (C), lightness (L), a (redgreen) and b (blue-yellow) values, according to the CIE-Lab colour space on a colorimeter (ColorTec-PCM, USA). External skin colour (opposite sides) and internal flesh colour (each side of a longitudinally sliced fruit) were measured using a ColorTec colorimeter. Fruit colour was expressed as  $L^*a^*b^*$  colour values (Minolta, Japan).  $L^*$ defines the lightness, and  $a^*$  and  $b^*$  define the colour between red and green, and blue and yellow, respectively. Hue angle (h) was calculated as h=arctan  $b^*/a^*$  (deg).

Total dry matter (DM) was obtained by drying homogenised berries at 105 °C until constant mass (11). Total soluble solids (TSS), expressed in °Brix, were measured using an Abbe refractometer (A. Krüss Optronic, Germany) calibrated against sucrose. Total acidity (TA) was measured according to the AOAC method (11) and expressed in g per L of citric acid. pH value was measured with a pH meter (Mettler Toledo, Switzerland). Ascorbic acid (AA) was determined by the 2,6-dichloroindophenol titrimetric method according to the AOAC (12).

Reducing sugars were determined by Luff-Schoorl method (11). Extraction was done from 5 g of fresh fruit using 200 mL of H<sub>2</sub>O. For elimination of ballast matter from the samples, Carrez clarification was done by adding 5 mL of Carrez I and 5 mL of Carrez II to the sample. The content was then mixed, pipetted into a 250-mL volumetric flask, and filled to the mark with double distilled water (ddH<sub>2</sub>O), then mixed again and filtered. From the obtained filtrate, 25 mL were pipetted into a 100-mL volumetric flask and diluted. After that, 25 mL of Luff-Schoorl solution and 25 mL of diluted filtrate were put into an Erlenmeyer flask and heated for 2 min on direct flame. Boiling then continued on reverse cooler for 10 min. To a cooled content, 10 mL of 30 % potassium iodide and 25 mL of 6 mol/L  $H_2SO_4$  were added. The obtained mixture was titrated with 0.1 mol/L  $Na_2S_2O_3$ until the yellow colour appeared, then a few drops of starch solution were added and titration continued until the blue colour disappeared. Procedure for the blind trial was conducted parallely. Unreduced sugars were first transformed by acid hydrolysis into reduced monosaccharides, after that the procedure was continued by using Luff-Schoorl reagent. The sucrose mass fraction was determined from the difference between total and reduced sugars.

### Determination of total phenolics, flavonoids and nonflavonoids in strawberry cultivars

Total phenolics (TP), flavonoids (TF), and non-flavonoids (TNF) were determined using the Folin-Ciocalteu colorimetric method described by Ough and Amerine (13) with some modifications. Fruit phenolics were extracted from 10 g of fresh samples using 40 mL of 80 % (by volume) aqueous ethanol. The mixture was extracted (in water bath at 80 °C), kept for 20 min in inert atmosphere, and filtered through a Whatman filter paper using a Büchner funnel. Extraction of the residue was repeated under the same conditions. The filtrates were combined and diluted to 100 mL in volumetric flask with 80 % aqueous ethanol, and the obtained extract was used for determination of TP, TF and TNF. The formaldehyde precipitation was used to determine flavonoids in fruit samples (14). The content of TP and TNF was measured as follows: 0.5 mL of diluted extract or standard solutions of gallic acid (20–500 mg/L) was added to a 50-mL volumetric flask containing 30 mL of ddH<sub>2</sub>O, then 2.5 mL of Folin-Ciocalteu reagent were added to the mixture and shaken. After 5 min, 7.5 mL of 7 % Na<sub>2</sub>CO<sub>3</sub> solution were added with mixing and the solution was immediately diluted to 50 mL with ddH<sub>2</sub>O. After incubation at room temperature for 2 h the absorbance of the solution was measured at 760 nm. The flavonoid mass fraction was calculated as the difference between total phenolic and non-flavonoid mass fraction. TP, TF and TNF were expressed as mg of gallic acid equivalents (GAE) per kg of fresh mass of edible part of fruits. The extract of total phenolics was also used for DPPH assay.

## Determination of anthocyanins in strawberry cultivars

The total anthocyanin mass fraction in the extract from selected fruits was determined using bisulphite bleaching method (15). Fruit anthocyanins were extracted from 2 g of fresh samples using 2 mL of 0.1 % HCl (by volume) in 96 % ethanol and 40 mL of 2 % aqueous HCl (by volume). The mixture was centrifugated at 5500 rpm for 10 min. The obtained supernatant was used for the determination of total anthocyanins. The mass fraction of total anthocyanins was measured as follows: 10 mL of extract were put into two test tubes, then 4 mL of 15 % sodium bisulphite were added to one tube and 4 mL of ddH<sub>2</sub>O to the other. After 15 min of incubation at room temperature, the absorbance of each mixture was measured at 520 nm. The molar absorption coefficient for cyanidin-3,5-diglucoside was used as a standard value. Results were expressed as mg of cyanidin-3,5-diglucoside equivalents per kg of fresh mass of edible part of fruits.

# Determination of antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The free radical scavenging capacity of fruit extracts was determined according to the previously reported procedure using the stable DPPH<sup>-</sup> radical (*16*). The method is based on reduction of stable DPPH<sup>-</sup> nitrogen radicals in the presence of antioxidants.

The antioxidant capacity of fruit extracts was measured spectrophotometrically with a UV-Vis spectrophotometer (Shimadzu UV 1650 PC, Japan). In the DPPH method, five dilutions of fruit extract were analyzed. A volume of 50  $\mu$ L of diluted fruit extract was mixed with 300  $\mu$ L of methanolic DPPH solution (1 mmol/L) and brought to 3 mL with methanol. The solution was kept in dark at room temperature for 15 min. The absorbance  $(A_{\text{extract}})$  was measured against the prepared blank (50  $\mu$ L of diluted fruit extract, 2950  $\mu L$  of methanol) at 517 nm. A DPPH blank solution was prepared freshly each day (300 µL of 1 mmol/L DPPH, 2.7 mL of methanol) and its absorbance  $(A_{\text{DPPH}})$  was measured daily. Trolox standards of the final concentration of 0-2500 µmol/L were prepared in methanol and assayed under the same conditions. A volume of 50 µL of Trolox was mixed with 300 µL of methanolic DPPH solution (1 mmol/L) and brought to 3 mL with methanol. After 15 min, the absorbance  $(A_{\text{Trolox}})$  was measured against the prepared blank at 517 nm. Calibration curve of Trolox was calculated by linear regression of the absorbance value  $(A_{\text{Trolox}})$  vs. concentration. Trolox calibration curve was used to calculate antioxidant capacity of each diluted fruit extract and to express the antioxidant activity in mmol of Trolox equivalent (TE) per kg of fruits. Using the obtained curve, final results were expressed as mmol of TE per kg of fruits needed to reduce DPPH<sup>-</sup> radical by 50 %.

The ability of the fruit extract to act as a free radical scavenger against DPPH<sup>-</sup> radical was tested by measuring the disappearance of the absorbance at 517 nm after the addition of fruit extract and by comparing it with the disappearance of the absorbance produced by the addition of the known amounts of Trolox, a water-soluble vitamin E analogue, under the same conditions.

The results were expressed as mmol of Trolox equivalents per kg of fresh mass of the edible part of fruits.

### Statistical analysis

Data were analyzed by the Windows SAS program (v. 8.0) and expressed as mean $\pm$ SE using ANOVA, and if justified by the statistical probability (p<0.05), they were subjected to Duncan's new multiple range test. The relationship among all parameters in strawberry fruit was described as the Pearson product-moment correlation coefficient (r). Differences were considered statistically significant if p<0.05 (17).

### **Results and Discussion**

Cultivar quality is defined by different parameters, which united give an integral picture of selected fruit. Different studies report that data on the colour, fruit size and mass are not sufficient to consumers; they want to know more about the fruits they consume (3). The hypothesis underlying this research was that early ripening cultivars have satisfying quality and make an enjoyable eating experience, which is demanded by consumers. The following quality parameters were determined in this investigation: total dry matter, total acids (TA), total soluble solids (TSS), TSS/TA ratio, pH value, reducing sugars, sucrose, vitamin C, total anthocyanins, total phenolics (TP), nonflavonoids, total flavonoids, antioxidant capacity and fruit colour. The mentioned parameters were investigated with the aim to monitor fruit quality of early ripening strawberry cultivars. In Table 1, parameters of the basic chemical composition of fruits are given: total dry matter, total acids, total soluble solids, TSS/TA ratio, pH value, reducing sugars and sucrose.

Cultivar	w(DM)/%	TA γ(citric acid)/(g/L)	TSS/°Brix	TSS/TA	рН	w(reducing sugars)/%	w(sucrose)/%
Clery	$(9.98 \pm 0.01)^{b}$	$(6.4\pm0.01)^{c}$	(8.10±0.10) <sup>c</sup>	(1.27±0.31) <sup>b</sup>	(3.66±0.01) <sup>c</sup>	$(6.48 \pm 0.01)^{b}$	$(0.72 \pm 0.01)^{a}$
Maya	(7.11±0.01) <sup>g</sup>	$(6.2\pm0.02)^{c}$	$(6.00\pm0.01)^{\rm f}$	$(0.97 \pm 0.22)^{d}$	(3.57±0.02) <sup>d</sup>	$(3.84 \pm 0.01)^{e}$	$(0.22\pm0.03)^{\rm e}$
Alba	$(8.22 \pm 0.01)^{e}$	$(8.4\pm0.01)^{a}$	(7.01±0.02) <sup>e</sup>	(0.83±0.12) <sup>e</sup>	(3.44±0.01) <sup>e</sup>	(4.81±0.01) <sup>d</sup>	$(0.26 \pm 0.01)^{d}$
Miss	$(7.81 \pm 0.04)^{\rm f}$	(4.9±0.01) <sup>d</sup>	(7.01±0.03) <sup>e</sup>	$(1.23\pm0.30)^{b}$	$(3.80\pm0.02)^{b}$	$(6.24 \pm 0.04)^{c}$	(0.62±0.03) <sup>b</sup>
Camarosa	(9.93±0.02) <sup>c</sup>	$(8.1\pm0.01)^{\rm b}$	(9.01±0.02) <sup>b</sup>	(1.11±0.12) <sup>c</sup>	$(3.66 \pm 0.01)^{c}$	$(4.81 \pm 0.02)^{d}$	$(0.29 \pm 0.02)^{d}$
Queen Elisa	(9.75±0.01) <sup>d</sup>	$(6.4\pm0.03)^{c}$	$(8.01 \pm 0.02)^{d}$	$(1.25\pm0.61)^{b}$	(3.91±0.03) <sup>a</sup>	(6.96±0.02) <sup>a</sup>	$(0.64 \pm 0.04)^{b}$
Elsanta	(10.76±0.02) <sup>a</sup>	$(6.5\pm0.02)^{c}$	$(10.01 \pm 0.01)^{a}$	$(1.54\pm0.48)^{a}$	$(3.80 \pm 0.01)^{b}$	(6.24±0.01) <sup>c</sup>	$(0.51 \pm 0.01)^{c}$
Pr>F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.3998	< 0.0001	< 0.0001

Table 1. Chemical composition of the investigated strawberry cultivars

Data are expressed as average value±standard deviation of three replicates

Different letters within a column indicate significant differences at the 5 % level by Duncan's test

Cultivar Elsanta had the highest statistically significant content of dry matter, total soluble solids and TSS/ TA ratio compared to other researched cultivars. This cultivar is widely grown in Croatia and is favoured by Croatian strawberry producers, because its production technology is not very demanding and it is very well accepted on the market. Chemical composition of Elsanta justifies its leading position in the Croatian strawberry production. The conducted research has shown that other investigated cultivars are also of satisfactory quality. Cultivars Camarosa, Clery and Queen Elisa had slightly lower values of dry matter and total soluble solids, and their TSS/TA ratio values were very similar to Elsanta's. Cultivars Maya and Miss had somewhat lower values of dry matter and total soluble solids. In our research, total soluble solids ranged from 6.00 to 10.01 °Brix. The obtained data are in accordance with the investigations of other authors. The difference exists due to different cultivars, cultivation system, climatic conditions, etc. According to Rutkowski et al. (4), the quantity of total soluble solids in the investigated cultivars ranged from 5.2 to 10.4 %, while Laugale and Bite (5) reported values for total soluble solids from 8.4 to 11.6 %, depending on the cultivar. In the research of Testoni et al. (6), the quantity of total soluble solids for cultivar Marmolada ranged from 5.8 to 7.5 %, and for cultivar Miss from 8.6 to 9.6 %.

Total acidity values for cultivar Miss were somewhat lower, which caused its also lower TSS/TA ratio compared to cultivar Elsanta. Cultivar Miss is known for its harmonious TSS/TA ratio, which was also confirmed in this research. The relationship between total soluble solids and total acidity is very important in determining fruit quality. In numerous researches conducted on different strawberry cultivars, the total soluble TSS/TA ratio was found to be very important, because it provides information on the balance of sugars and acids in the fruit. For this reason, along with fruit colour, this was until recently considered to be the main parameter for determining fruit quality (3). The mass fraction of sucrose ranged from 0.22 to 0.72 %, which is characteristic of strawberry cultivars according to Sturm et al. (3), who reported values of 2.1 to 2.2 g/kg sucrose for cultivar Miss, and high values from 4.4 to 4.9 g/kg fresh mass for cultivar Elsanta.

Vitamin C, polyphenols and antioxidant capacity in fruits are shown in Table 2. Vitamin C ranged from 44.88 mg per 100 g of fresh mass in cultivar Miss to 65.86 mg per 100 g of fresh mass in cultivar Elsanta. This data are in agreement with the work of other researchers. In their research on the vitamin C mass fraction in five different strawberry cultivars, Cordenunsi *et al.* (2) obtained values ranging from 44 to 62 mg per 100 g of fresh mass. Later,

Table 2. Mass fraction of vitamin C and polyphenols, and antioxidant capacity of strawberry cultivars

Cultivar	w(AA)/	w(total anthocyanins)/	TP TF		TNF	Antioxidant	
	(mg/100 g)	(mg/kg)	w(	capacity/(mmol/kg)			
Clery	(54.18±0.06) <sup>d</sup>	(181.85±6.64) <sup>d</sup>	(157.23±0.02) <sup>d</sup>	(65.58±0.01) <sup>a</sup>	(91.65±0.02) <sup>e</sup>	$(3.53\pm0.03)^{\rm e}$	
Maya	(53.05±2.22) <sup>e</sup>	(253.93±0.02) <sup>b</sup>	(190.75±0.02) <sup>b</sup>	$(34.84 \pm 0.04)^{\rm f}$	(155.91±0.02) <sup>b</sup>	$(3.63\pm0.03)^{c}$	
Alba	(57.08±0.07) <sup>b</sup>	(170.89±2.98) <sup>e</sup>	(135.60±0.02) <sup>f</sup>	(64.43±0.01) <sup>b</sup>	(71.17±0.02) <sup>f</sup>	(3.70±0.01) <sup>b</sup>	
Miss	(44.88±0.06) <sup>g</sup>	(207.80±1.26) <sup>c</sup>	(96.23±0.02) <sup>g</sup>	(31.45±0.03) <sup>g</sup>	(64.78±0.01) <sup>g</sup>	$(3.10\pm0.01)^{\rm f}$	
Camarosa	$(52.44 \pm 0.04)^{f}$	(327.39±2.48) <sup>a</sup>	(176.09±0.01) <sup>c</sup>	(45.11±0.02) <sup>c</sup>	(130.98±0.01) <sup>c</sup>	(3.60±0.01) <sup>d</sup>	
Queen Elisa	(55.92±0.07) <sup>c</sup>	(152.64±1.15) <sup>f</sup>	(146.06±0.02) <sup>e</sup>	$(44.27 \pm 0.01)^{d}$	(101.79±0.01) <sup>d</sup>	(3.61±0.01) <sup>dc</sup>	
Elsanta	(65.86±0.11) <sup>a</sup>	(114.76±4.05) <sup>g</sup>	(208.97±0.02) <sup>a</sup>	(40.60±0.01) <sup>e</sup>	(168.37±0.02) <sup>a</sup>	$(4.42\pm0.02)^{a}$	
Pr>F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

Data are expressed as average value±standard deviation of three replicates

Different letters within a column indicate significant differences at the 5 % level by Duncan's test

Cordenunsi *et al.* (9) reported values of vitamin C ranging from 47 to 80 mg per 100 g of fresh mass, depending on the strawberry cultivar.

In recent research of other authors, similar values are reported for vitamin C, from 52.25 to 53.21 mg (18), and from 38.4 to 72.1 mg per 100 g of fresh mass, depending on the cultivar (5). Besides vitamin C, different polyphenol groups (anthocyanins, flavonols, flavanols, phenolic acids, etc.) are present in strawberry fruits. In our study, the quantity of total anthocyanins was the highest in cultivar Camarosa (327.39 mg/kg), and the lowest in cultivar Elsanta (114.76 mg/kg). In cultivars Maya and Miss remarkable mass fractions of anthocyanins were observed too. Furthermore, in Clery, Alba and Queen Elise anthocyanins were determined in lower mass fractions compared to the previously described cultivars, except cv. Elsanta. In general, these anthocyanin mass fractions are roughly similar to those reported by other authors. In their study carried out on eight different strawberry cultivars, Meyers et al. (19) reported an average anthocyanin mass fraction of 414 mg/kg, with great differences between cultivars; cultivar Earliglow had twice the mass fraction of cultivar Allstar. Garcia-Viguera et al. (20) report values from 185.0 mg/kg for cultivar Oso Grande to 840.2 mg/kg for cultivar Camarosa. They maintain that high levels of anthocyanins found in cultivar Camarosa are a consequence of the more intense pigmentation of the fruit inner tissues than found in other cultivars. Cultivar Camarosa was also studied by other researchers, but they obtained somewhat lower values (482.0 mg/kg) (21). Differences in mass fraction found for the same cultivar by different authors could be a consequence of the use of different extraction solvents, climate conditions, geographical region in which cultivars were grown, cultivation system, etc. Data in Table 3 show a correlation between the anthocyanin content and colour determined in the inner part of strawberry fruit. The darkest fruit colour (L) had cultivar Camarosa (Table 3), whose colour intensity (C) was lower than in other researched cultivars. This cultivar also had the highest anthocyanin content. Maya and Miss followed, also with low colour intensity (C), dark fruit colour (L) and high content of anthocyanins. When compared to Elsanta as a standard cultivar, the above cultivars had higher content of anthocyanins and darker red colour. Values for hue (h) were significantly different among cultivars. It is reported that fruit colour is also a cultivar characteristic, so the differences among them are to be expected.

Pelayo *et al.* (22) reported that distribution of anthocyanin pigments in the fruit tissue of different strawberry cultivars is not uniform; the internal colour of Aromas and Diamante strawberries is mostly white, whereas in Selva it is light red. Data obtained in our research are mainly in agreement with the work of other researchers (8,23).

Total phenols ranged from 96.23 mg/kg in cultivar Miss to 208.97 mg per kg of fresh mass in cultivar Elsanta. Among total phenolics, nonflavonoids had the major share, in quantities from 71.17 (Alba) to 168.37 mg/kg (Elsanta). Levels of nonflavonoids were significantly higher than of flavonoids in all investigated cultivars, except for cultivar Alba, where the obtained values for flavonoids and nonflavonoids were similar (64.43 and 71.17 mg per kg of fresh mass, respectively). The greatest difference between nonflavonoid (TNF) and flavonoid (TF) quantities was determined in cultivars Elsanta (127.77 mg per kg of fresh mass) and Maya (121.07 mg per kg of fresh mass) (Table 2). Numerous authors studied total phenols in different strawberry cultivars and the obtained values were very ununiform and different, depending on the cultivars, climatic conditions, cultivation systems and harvest time (9,24,25). Törrönen and Määttä (26) report values of total phenols ranging from 96 mg/ 100 g to 133 mg/100 g of fresh mass. A significant correlation between the antioxidant capacity and total phenolics or nonflavonoids was obtained, whereas no correlation between antioxidant capacity and flavonoids was observed (Table 4).

Antioxidant capacity was uniform in all cultivars except Elsanta, where it was somewhat higher (4.42 mmol TE/kg). Values of antioxidant capacity in other cultivars ranged from 3.10 in cultivar Miss to 3.70 mmol TE/kg in cultivar Alba. Also, antioxidant capacity values were quite uniform for cultivars Camarosa, Queen Elisa and Maya (3.60, 3.61 and 3.63 mmol TE/g, respectively). Since in this research antioxidant capacity was not determined by other methods, we cannot claim with certainty that the quantity of total phenols is directly

Table 3. Colour of the investigated strawberry cultivars

Cultivar	L (external)	L (internal)	C (external)	C (internal)	h (external)	h (internal)
Clery	(36.04±3.53) <sup>ab</sup>	(50.89±4.03) <sup>cb</sup>	(30.60±4.65) <sup>a</sup>	(32.19±4.18) <sup>ab</sup>	(49.75±6.07) <sup>e</sup>	(49.32±4.27) <sup>e</sup>
Maya	(34.67±3.86) <sup>b</sup>	(47.26±4.70) <sup>c</sup>	(23.54±4.66) <sup>b</sup>	(30.44±5.04) <sup>bc</sup>	(58.38±8.12) <sup>ab</sup>	(51.46±3.89) <sup>ed</sup>
Alba	(35.88±2.56) <sup>ab</sup>	(51.79±5.83) <sup>b</sup>	(22.23±3.64) <sup>cb</sup>	(27.95±6.15) <sup>dc</sup>	(53.52±5.12) <sup>dc</sup>	(54.63±4.16) <sup>abc</sup>
Miss	(34.56±2.63) <sup>b</sup>	(55.66±4.77) <sup>a</sup>	(19.16±4.71) <sup>d</sup>	(23.53±4.60) <sup>e</sup>	(56.38±8.76) <sup>bc</sup>	(52.08±3.42) <sup>cde</sup>
Camarosa	(34.40±1.96) <sup>b</sup>	(48.01±4.47) <sup>cb</sup>	(20.15±3.73) <sup>cd</sup>	(34.90±3.11) <sup>a</sup>	(61.53±6.28) <sup>a</sup>	(55.78±2.80) <sup>ab</sup>
Queen Elisa	(36.96±1.71) <sup>a</sup>	(56.26±5.54) <sup>a</sup>	(24.27±3.44) <sup>b</sup>	(25.50±4.59) <sup>de</sup>	(47.75±6.00) <sup>e</sup>	(53.48±4.37) <sup>bcd</sup>
Elsanta	(36.43±4.21) <sup>ab</sup>	(59.09±7.31) <sup>a</sup>	(23.93±3.64)	(27.41±2.74) <sup>dc</sup>	(61.27±4.14) <sup>a</sup>	(56.55±4.63) <sup>a</sup>
Pr>F	0.1403	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Data are expressed as average value±standard deviation of three replicates

Different letters within a column indicate significant differences at the 5 % level by Duncan's test

	DM	TA	TSS	TSS/TA	pН	AA	Total anthocyanins	TPC	TNF	TF	Reducing sugars	Sucrose	Antioxidant capacity
TA	0.216 ns	_											
TSS	0.953***	0.352 ns	_										
TSS/TA	0.713***	-0.487*	0.641**	-									
pН	0.456*	-0.604**	0.321 ns	0.785***	-								
AA	0.604 **	0.374 ns	0.720***	0.378 ns	0.017 ns	-							
Total anthocyanins	-0.303 ns	0.250 ns	–0.216 ns	-0.467*	–0.319 ns	-0.573**	-						
TPC	0.410 ns	0.284 ns	0.595**	0.268 ns	–0.075 ns	0.717***	0.030 ns	-					
TNF	0.285 ns	0.070 ns	0.484*	0.336 ns	0.0950 ns	0.575**	0.096 ns	0.943***	-				
TF	0.285 ns	0.589**	0.100 ns	–0.264 ns	-0.496*	0.265 ns	-0.207 ns	–0.056 ns	–0.384 ns	-			
Reducing sugars	0.586**	-0.437*	0.356 ns	0.7243***	0.777***	0.113 ns	-0.628**	-0.306 ns	–0.311 ns	0.084 ns	-		
Sucrose	0.542*	–0.268 ns	0.520*	0.692***	0.877***	0.132 ns	-0.093 ns	0.096 ns	0.274 ns	-0.557**	0.541*	-	
Antioxidant capacity	0.565**	0.323 ns	0.728***	0.417 ns	0.021 ns	0.969***	-0.452*	0.807***	0.717***	0.089 ns	0.003 ns	0.189 ns	-
L(external)	0.567**	–0.040 ns	0.475*	0.483*	0.434*	0.449*	-0.570**	0.101 ns	0.029 ns	0.193 ns	0.621**	0.344 ns	0.356 ns
L(internal)	0.388 ns	–0.378 ns	0.342 ns	0.686***	0.635**	0.355 ns	-0.694***	-0.095 ns	-0.007 ns	–0.244 ns	0.661**	0.575**	0.345 ns
C(external)	0.290 ns	–0.026 ns	0.153 ns	0.152 ns	–0.081 ns	0.243 ns	-0.319 ns	0.192 ns	–0.019 ns	0.593**	0.276 ns	-0.363 ns	0.120 ns
C(internal)	0.241 ns	0.598**	0.329 ns	–0.240 ns	-0.443*	0.173 ns	0.549**	0.574**	0.403 ns	0.387 ns	-0.470*	–0.269 ns	0.196 ns
h(external)	-0.003 ns	0.184 ns	0.252 ns	0.064 ns	–0.197 ns	0.164 ns	0.420 ns	0.511*	0.624**	-0.453*	-0.550**	0.150 ns	0.376 ns
h(internal)	0.246 ns	0.503*	0.424 ns	0.009 ns	0.048 ns	0.366 ns	0.053 ns	0.202 ns	0.248 ns	–0.182 ns	–0.157 ns	0.444*	0.412 ns

Table 4. Correlation coefficients (R) among different characteristics of strawberry cultivars

\* Correlation coefficients (*R*) significant at  $p \le 0.05$ 

\*\* Correlation coefficients (*R*) significant at  $p \le 0.01$ 

\*\*\* Correlation coefficients (R) significant at  $p \le 0.001$  ns=not significant

connected with antioxidant capacity. This was also confirmed in other studies conducted on different fruit species (strawberry, sour cherry, cornelian cherry, black thorn), where the determined differences were in correlation with the methods applied (9,19,27–29).

According to the Roemer-Orphal correlation distribution (Table 4), low correlation is when coefficient is 0.4 or lower, which can also be seen by the fact that even the coefficient alone is not significant among these parameters. With higher values of the coefficient, the strength of correlation increases, as well as the significance among the investigated parameters (30).

Statistical analysis showed that early ripening cultivars are of satisfying quality and the obtained parameters are within the range of previous values obtained by other researchers (5–7).

### Conclusions

The obtained results show that selected early cultivars grown in climate conditions of the Republic of Croatia, when compared with cultivar Elsanta as a standard cultivar, have a satisfying quality. The investigated cultivars contained significant mass fractions of biologically active compounds such as vitamin C, anthocyanins, total phenols, nonflavonoids and flavonoids. The mass fraction of anthocyanins was higher in all cultivars compared to cultivar Elsanta, which is very important, because anthocyanins are very strong antioxidants. Generally, we can conclude that the investigated strawberry cultivars have been extensively evaluated as potential sources of natural antioxidants. This is very valuable information for future selection of cultivars to be grown in ecological conditions of the Republic of Croatia.

### References

- E. Schöpplein, E. Krüger, A Rechner, E. Hoberg, Analytical and sensory qualities of strawberry cultivars, *Acta Hortic*. 567 (2002) 805–808.
- B.R. Cordenunsi, J.R.O. Nascimento, F.M. Lajolo, Physicochemical changes related to quality of five strawberry fruit cultivars during cool-storage, *Food Chem.* 83 (2003) 167–173.
- 3. K. Sturm, D. Koron, F. Stampar, The composition of fruit of different strawberry varieties depending on maturity stage, *Food Chem.* 83 (2003) 417–422.
- P.K. Rutkowski, D.E. Kruczynska, E. Zurawicz, Quality and shelf life of strawberry cultivars in Poland, *Acta Hortic.* 708 (2006) 329–332.
- V. Laugale, A. Bite, Fresh and processing quality of different strawberry cultivars for Latvia, *Acta Hortic*. 708 (2006) 333–336.
- A. Testoni, F. Lovati, M. Nuzzi, Evaluation of postharvest quality of strawberries in Italy, *Acta Hortic.* 708 (2006) 355– 358.
- A.S. Saied, A.J. Keutgen, G. Noga, The influence of NaCl salinity on growth, yield and fruit quality of strawberry cvs. Elsanta and Korona, *Sci. Hort.* 103 (2005) 289–303.
- M.I. Gil, D.M. Holcroft, A.A. Kader, Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments, *J. Agric. Food Chem.* 45 (1997) 1662–1667.

- B.R. Cordenunsi, M.I. Genovese, J.R.O. do Nascimento, N.M.A. Hassimotto, R.J. dos Santos, F. M. Lajolo, Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars, *Food Chem.* 91 (2005) 113–121.
- F. Lopes da Silva, M.T. Escribano-Bailón, J.J. Perez Alonso, J.C. Rivas-Gonzalo, C. Santos-Buelga, Anthocyanin pigments in strawberry, *LWT-Food Sci. Technol.* 40 (2007) 374–382.
- Official Methods of Analysis of AOAC International, AOAC International, Washington, USA (1995) Secs. 942.15.
- Official Methods of Analysis of AOAC International, AOAC International, Washington, USA (2002) Secs. 967.21.
- C.S. Ough, M.A. Amerine: Methods for Analysis of Musts and Wines, John Wiley and Sons, New York, USA (1988) pp. 187–188,192–194.
- T.E. Kramling, V.L. Singleton, An estimate of the nonflavonoid phenols in wines, Am. J. Enol. Vitic. 20 (1969) 86–92.
- N. Pellegrini, M. Serafini, B. Colombi, D. Del Rio, S. Salvatore, M. Bianchi, P. Riberéau-Gayon, E. Stonestreet, The amount of anthocyanins in red wines, *Bull. Soc. Chem. France*, 9 (1965) 2642–2649.
- W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, *LWT-Food Sci. Technol.* 28 (1995) 25–30.
- SAS/STAT User's Guide, v. 8, Vol. 2, SAS Institute, Cary, NC, USA (1997).
- C. Hansawasdi, S. Rithiudom, P. Chaiprasart, Quality and antioxidant activity changes during low-temperature storage of strawberry fruits, *Acta Hortic.* 708 (2006) 301–306.
- K.J. Meyers, C.B. Watkins, M.P. Pritts, R.H. Liu, Antioxidant and antiproliferative activities of strawberries, *J. Agric. Food Chem.* 51 (2003) 6887–6892.
- C. Garcia-Viguera, P. Zafrilla, F.A. Tomás-Barberán, The use of acetone as an extraction solvent for anthocyanins from strawberry fruit, *Phythochem. Anal.* 9 (1998) 274–277.
- I. Castro, O. Gonçalves, J.A. Teixiera, A.A. Vicente, Comparative study of a Selva and Camarosa strawberries for the commercial market, J. Food Sci. 67 (2002) 2132–2137.
- C. Pelayo, S.E. Ebeler, A.A. Kader, Postharvest life and flavor quality of three strawberry cultivars kept at 5 °C in air or air+20 kPa CO<sub>2</sub>, *Postharvest Biol. Technol.* 27 (2003) 171–183.
- J.F. Ayala-Zavala, S.Y. Wang, C.Y. Wang, G.A. González-Aguilar, Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit, *LWT-Food Sci. Technol.* 37 (2004) 687–695.
- J. Scalzo, A. Politi, N. Pelligrini, B. Mezzetti, M. Battino, Plant genotype affects total antioxidant capacity and phenolic contents in fruit, *Nutrition*, 21 (2005) 207–213.
- Y. Zheng, S.Y Wang, C.Y. Wang, W. Zheng, Changes in strawberry phenolics, anthocyanins, and anitoxidant capacity in response to high oxygen treatments, *LWT-Food Sci. Technol.* 40 (2007) 49–57.
- A.R. Törrönen, K. Määttä, Bioactive substances and health benefits of strawberries, *Acta Hortic.* 567 (2002) 797–803.
- V. Dragović-Uzelac, B. Levaj, D. Bursać, S. Pedisić, I. Radojčić, A. Biško, Total phenolics and antioxidant capacity assays of selected fruits, *Agric. Conspec. Sci.* 72 (2007) 279–284.
- L. Jakobek, M. Šeruga, M. Medvidović-Kosanović, I. Novak, Anthocyanin content and antioxidant activity of various red fruit juices, *Deut. Lebensm-Rundsch.* 103 (2007) 58–64.
- L. Jakobek, M. Šeruga, I. Novak, M. Medvidović-Kosanović, Flavonols, phenolic acids and antioxidant activity of some red fruits, *Deut. Lebensm-Rundsch.* 103 (2007) 369–378.
- D. Vasilj: Biometrics and Experimenting in Plant Production, Croatian Society of Agronomists, Zagreb, Croatia (2000) pp. 102–119.