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# Effects of Weather Conditions on Phenolic Content and Antioxidant Capacity in Juice of Chokeberries (*Aronia melanocarpa* L.)

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Chokeberries are a subject of numerous studies due to a high phenolic compound content, antioxidant properties and potential positive influence on the health. Effects of weather conditions on fruit quality attributes, phenolic compounds and antioxidant capacity of chokeberry (*Aronia melanocarpa*) juice over three consecutive years were investigated. Total phenolic content and total flavonoids range were from 8834 to 11093 mg/L and from 6993 to 9710 mg/L, respectively. High variations and discrepancy during different growing seasons are due to the different air temperature, sunlight and rainfall rate. The highest concentrations of anthocyanins and phenolics were observed in fruits harvested in 2012, which is most likely due to the favorable weather conditions (temperature and bright sunshine hours). All chokeberry juices possess high antioxidant activity (12.9–14.6 mmol/L; 128–167 mmol/L). Strong correlation implies that flavonoids and non-flavonoids were the major contributors to the antioxidant capacity. This study indicates that although the examined properties vary considerably through the growing seasons ($p \leq 0.05$), chokeberry juices can serve as a good source of bioactive phytochemicals in a human diet.

## INTRODUCTION

The Aronia berries (Family-*Rosaceae*, subfamily-*Maloideae*) are a deciduous shrub native to eastern parts of North America and Canada and they were introduced to Europe about a century ago [Jeppsson, 2000; Kulling & Rawel, 2008]. Two species of Aronia berries can be distinguished: *Aronia melanocarpa* [Michx.] Elliot (black chokeberry) and *Aronia arbutifolia* [L.] Elliot (red chokeberry). A third controversial entity is intermediate *Aronia prunifolia* (purple chokeberry) generally considered as a hybrid between *Aronia melanocarpa* and *Aronia arbutifolia* [Kulling & Rawel, 2008]. Some cultivars are bred of true black chokeberry (*Aronia melanocarpa*) and some are hybrid cultivars (*e.g. Aronia x Sorbus)*. There are numerous known Aronia genotypes and the most important in Europe are: Aron (Denmark), Nero (Czech Republic), Viking (Finland), Rubin (Russia through Finland), Kurkumäcki (Finland), Hugin (Sweden), and Fertödi (Hungary) [Jeppsson, 2000]. Chokeberries show high resistance to frost, mechanized harvesting, damage during transportation and cold storage. Due to these advantages, popularity of chokeberry has raised recently but they are not popularly consumed in fresh and frozen forms. Usually they are used in processed and derived products including juices, wines, jams, jellies and tea [Kulling & Rawel, 2008; Ochmian *et al*., 2012; Wilkes *et al*., 2014]. Juice consumers prefer mixture products with other kinds of fruits, such as apple, pear and black currant. Currently, most European juice manufacturers include black chokeberry juice among their products and the demand for black chokeberry concentrate is increasing. Chokeberries can be also used in the processing food industry as a raw material for the production of natural food colorants [Jeppsson, 2000].

In Croatian diet, berries like red raspberries, blackberries, blueberries and strawberries are commonly used while chokeberries are slowly introduced [Jakobek *et al*., 2007]. Among all common fruits and vegetables in the diet, berries, especially those with dark blue or red colors, have the highest antioxidant capacities [Wu *et al*., 2004]. Black chokeberries accumulate extremely high amounts of anthocyanins and other polyphenolic substances [Oszmianski & Wojdylo, 2005]. It was reported that they accumulate higher amounts of anthocyanins compared to black currants, blackberries and elderberries [Jakobek *et al*., 2007]. Also it has been demonstrated that chokeberry extract or juice possesses antioxidative, antiviral, antimutagenic, anticancer hepatoprotective, anti-inflammatory, gastroprotective or antidiabetic activities [Kulling & Rawel, 2008]. The onset of symptoms and the develop-

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ment of degenerative diseases may be affected by the phenolic compounds that are known to have a high antioxidant activity [Seeram, 2008]. The high content of polyphenols, especially anthocyanins, is responsible for the strong antioxidant properties of chokeberry products and their health-promoting effects [Denev *et al*., 2012].

Similarly to other agricultural products, chemical composition of berries depends on many factors: climate conditions, soil composition, berry maturity, harvest methods and storage conditions. These factors make it very difficult to identify specific one which has the greatest impact on the quality of berries. Different compounds naturally present in fruit, like carbohydrates, proteins, vitamins, minerals and acids, can react with polyphenols in many ways [Jeppsson, 2000].

Although phenolic compounds have been widely investigated in chokeberries there is limited information concerning the effects of individual weather parameters, such as temperature, rainfall, relative humidity and bright sunshine hours, on their composition. A better knowledge of these factors would rise up the possibility to cultivate fruits with high levels of bioactive compounds beneficial for human health, which was addressed in the present study.

## MATERIALS AND METHODS

### Samples

Chokeberry samples were collected at the end of harvest season during August 2012, August 2013 and August 2014 from experimental orchard of the Institute of Pomology of the Croatian Centre for Agriculture, Food and Rural Affairs in Donja Zelina, Croatia. Location of drip-irrigated experimental orchard is on 180 m above sea level with open southwest exposition. The soil in orchard is described as albic stagnosol. Based on several parameters including maturity, color, percentage and location of berries on the bush and quality parameters appropriate berry samples were collected. Berries were harvested from all parts of the bush and frozen in the next 4 h. Damaged or over-matured chokeberries were excluded from the sample. Frozen samples were stored at –20°C for less than 2 weeks prior to analysis. The day before analysis chokeberries were thawed at room temperature. 200 g of randomly selected chokeberries were individually weighed in triplicate and mixed in a house blender (Mixy, Zepter International). Juice was separated from the mash by subsequent pressing, bottled and stored at 4°C. All analyses were carried out in triplicate. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

### Determination of physicochemical parameters

The content of dry matter (TSC) was determined by drying at 105±2°C. Soluble solids content (SSC) was determined with a digital refractometer (Atago PAL-3, Tokyo, Japan) and expressed as °Brix. Sample pH was determined at room temperature using a MA 5740 pH meter (ISKRA, Kranj, Slovenia). The amount of acids (titrable acidity) was determined in an aqueous extract with a potentiometer by titration of sodium hydroxide (0.1 mol/L) at the pH=8.1±0.2. The total titratable acidity (TTA) was expressed as per cent of citric acid using a conversion factor of 0.070 [AOAC, 2005].

### Determination of total phenolic content

The Folin-Ciocalteu method [Waterhouse, 2002] was used to determine the total phenolic content (TPC). An aliquot (20 $\mu$L) of diluted chokeberry juices or standard solutions of gallic acid (25–500 mg/L) was mixed with 1580 $\mu$L of distilled water and 100 $\mu$L of Folin-Ciocalteu reagent. A volume of 300 $\mu$L of sodium carbonate solution (200 g/L) was added to the mixture which was then shaken. After incubation at room temperature for 2 h, the resulting absorbance was measured by a Pye Unicam SP6–500 spectrophotometer (Pye Ltd. Philips, Cambridge, UK) at the wavelength of 765 nm against the blind sample, which was used as reference. TPC was expressed in mg of gallic acid equivalents (GAE) per L.

### Determination of total nonflavonoid and flavonoid contents

Formaldehyde precipitation was used to determine total flavonoids (TF) as described by Ough & Amerine [1998]. A mixture of 3 mL diluted chokeberry juices, 1.5 mL of an aqueous solution of hydrochloric acid (1:4, by volume) and 3 mL of formaldehyde was prepared in a 25-mL flask. In order to remove air, nitrogen gas was injected and the stoppered flask was left in the dark for 24 h at 22°C. The next day it was filtered; and the clear liquid was used in the same procedure as the one used to prepare samples for TPC determination [Waterhouse, 2002]. The amount of total flavonoids (TF) was calculated as a difference between total phenolic content (TPC) and total nonflavonoids (TN). The results were expressed as mg GAE per L.

### Determination of total anthocyanins

The total anthocyanin content (TA) in chokeberry juices was determined using pH differential method described by Giusti & Wrolstad [2002]. Two dilutions of each chokeberry juice were prepared, one with potassium chloride buffer (pH=1.0), and the other with sodium acetate buffer (pH=4.5). After 15 min of incubation at room temperature, the absorbance was measured simultaneously at the wavelengths of 510 and 700 nm. The content of TA was expressed as mg of cyanidin-3-*O*-glucoside equivalents (CGE) per L using a molar extinction coefficient (ε) of cyanidin-3-*O*-glucoside of 26.900 L/mol/cm and molar weight (MW) (449.2 g/mol).

### Determination of total antioxidant capacity by DPPH$^{\bullet}$ method

The free radical scavenging capacity of chokeberry juices was determined using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [Brand-Williams *et al*., 1995]. The method was based on the reduction of stable DPPH radicals in the presence of antioxidants. A volume of 2 mL of diluted chokeberry juice or a methanol solution of Trolox (25–200 $\mu$mol/L) was mixed with 2 mL of methanol and 1 mL of 0.5 mmol/L DPPH methanolic solution. The mixture was vortexed and kept in the dark for 20 min. After incubation, the absorbance was measured at the wavelengths of 517 nm against a blank of methanol without DPPH. The Trolox calibration curve was used to calculate the total antioxidant capacity (TAC) of each diluted fruit juice and to express the antioxidant activity in mmol of Trolox equivalent (TE) per L.

### Determination of ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was done according to Benzie & Strain [1996]. The method was based on the reduction of the $Fe^{3+}$ by 2,4,6-tripyridyls-triazine (TPTZ) complex to the ferrous form at low pH. This reduction is monitored by measuring the absorbance change at 595 nm. The FRAP reagent was prepared from 5 mL of a TPTZ solution (10 mmol/L) in hydrochloric acid (40 mmol/L) and 5 mL of an $FeCl_3$ solution (20 mmol/L) mixed with 50 mL of acetate buffer (0.3 mol/L, pH=3.6). For the determination of the antioxidant capacity, the FRAP reagent (2.08 mL) was mixed with 240 $\mu$L of water and 80 $\mu$L of the appropriately diluted sample or standard solution of $FeSO_4 \times 7H_2O$ (0.125––2.000 mmol/L). The mixture was incubated at 37°C for 5 min and the absorbance was measured at 595 nm. The reducing power values (RP) were calculated according to the calibration curve for $FeSO_4 \times 7H_2O$ and expressed as mmol of $Fe^{2+}$ equivalents (FE) per L.

### Statistical analysis

In all the experiments, three samples were analyzed and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) to examine differences among harvest years, using Statistica v. 12.0 (StatSoft, Inc., Tulsa, OK, USA). For comparison of the phenolic contents and DPPH or FRAP assays and also for comparison of weather parameters, phenolic contents and fruit quality attributes the coefficients of correlation were determined for each combination. Differences were considered significant at $p \leq 0.05$.

## RESULTS AND DISCUSSION

### Physicochemical parameters

Physicochemical properties of juices from three different growing seasons are presented in Table 1. The total solids content (TSC) ranged from 19.22% (season 2014) to 26.94% (season 2012) which is in agreement with results of Mayer-Miebach *et al*. [2012] (berries: 17.90–26.00%; juices: 11.1––17.4%; pomace: 44.6–50%). Similar values were obtained for soluble solid content (SSC) which ranged from 18.15 to 25.61 °Brix. SSC depends upon numerous factors: weather, environmental conditions, crop period and variety [Ochmian *et al*., 2012]. Juice SSC should be as low as possible since it determines the limit for the gain in volume decrease when producing fruit concentrates [Jeppsson, 2000]. SSC is also a good indicator of berry maturity and also very important in the food industry and critical in comparative studies where variations by cultivar and environment are high [Clark & Finn, 2011]. Chokeberry products had a mean pH value of 3.89 that ranged from 3.77 (season 2013) to 3.96 (season 2012). The mean total titratable acidity (TTA) of all juices was 0.99% citric acid and ranged from 0.89% (season 2012) to 1.06% (season 2013). Ochmian *et al*. [2012] reported similar values for titratable acidity in range from 0.75 to 1.05 g citric acid per 100 g in berries. The °Brix/TTA ratio is a quality attribute used by the fruit industry to indicate the tartness of fruits and fruit juices. This ratio increases with maturity of the fruit and is used to identify the optimum maturity for harvesting to produce maximum product quality. The mean °Brix/TTA ratio was 22.48 and ranged from 19.35 in the juice from 2014 to 28.718 in the juice from 2012 (Table 1).

### Total phenolics, flavonoids, nonflavonoids and anthocyanins

The content of total phenolics (TPC), total flavonoids (TF) and total nonflavonoids (TN) in chokeberry juices is given in Table 2. There were large variations in TPC among growing seasons; the highest TPC was found in growing season 2012 (11093 mg GAE/L) and the lowest in growing season 2014 (8834 mg GAE/L) which agrees with previously reported values [Oszmianski & Wojdylo, 2005; Kulling & Rawel, 2008; Denev *et al*., 2012; Jakobek *et al*., 2012]. Some authors reported lower values of TPC [Mayer-Miebach *et al*., 2012; Kapci *et al*., 2013] and some higher [Zheng & Wang, 2003; Rop *et al*., 2010; Jakobek *et al*., 2012]. Jurgoński *et al*. [2008] reported much higher values of total phenolics in commercial chokeberry extract. The range of values reported in the literature might be due to differences in sample preparation methods, analytical procedures used, storage conditions but also due to different chokeberry varieties [Denev *et al*., 2012]. While comparing chokeberry juices to other chokeberry products it must be taken in consideration that lower phenolics might be related with the differences in their moisture content [Shin *et al*., 2008]. A wide range (8563.8–12055.7 mg GAE/kg of FW) of TPC was observed in the analysis of different cultivars of chokeberries from two growing seasons [Jakobek *et al*., 2012].

The content of total flavonoids (TF) varied form 6994 mg GAE/L (season 2014) to 9710 mg GAE/L season 2012). Flavonoids are the most abundant phenolics in chokeberries; 79.2 and 87.5% of TPC are TF (Table 2). It is known that chokeberries are a rich source of anthocyanins, proanthocyanidins and hydroxycinnamic acids [Denev *et al*., 2012] where polymeric proanthocyanins are the major class of polyphenolic compounds and represent 66% of fruits polyphenols

TABLE 1. Physicochemical properties of chokeberry juices from different growing seasons.

| Growing season | pH | TTA | TSC | SSC | °Brix/TTA |
|---|---|---|---|---|---|
| 2012 | 3.96±0.01[a] | 0.89±0.06 | 26.94±0.04 | 25.61±0.03 | 28.72±0.03 |
| 2013 | 3.77±0.02 | 1.06±0.06 | 21.65±0.05 | 20.54±0.03 | 19.37±0.04[b] |
| 2014 | 3.94±0.02[a] | 0.94±0.07 | 19.22±0.03 | 18.15±0.02 | 19.35±0.04[b] |

Data are expressed as mean values ± standard deviations (n=3). Values with the same superscript letters within a column are not significantly different ($p<0.05$). TTA – total titratable acidity (%) as citric acid; TSC – total solids content (%); SSC – soluble solids content (°Brix).

TABLE 2. Total phenolics (TPC), total nonflavonoids (TN), total flavonoids (TF) and total anthocyanins (TA) of chokeberry juices from different growing seasons.

| Growing season | TPC | TN | TF | TA |
|---|---|---|---|---|
| 2012 | 11093±249 | 1383±45 | 9710±268 | 2768±85 |
| 2013 | 9339±311 | 1519±50 | 7820±343 | 1829±120 |
| 2014 | 8834±175 | 1840±84 | 6994±255 | 2532±82 |

Data are expressed as mean values ± standard deviations (n=3). Values within a column are significantly different ($p<0.05$). Total phenol content (TPC), total nonflavonoids (TN), total flavonoids (TF) and total anthocyanin content (TA) are expressed per L. TPC, TN and TF are expressed as mg of gallic acid equivalent (GAE) while TA are expressed as mg of cyanidin-3-glucoside equivalents (CGE).

TABLE 3. The total antioxidant capacity (TAC) and reducing power (RP) of chokeberry juices from different growing seasons.

| Growing season | TAC | RP |
|---|---|---|
| 2012 | 14.6±0.1 | 166.7±5.9 |
| 2013 | 13.4±0.1 | 179.5±3.7 |
| 2014 | 12.9±0.2 | 128.2±6.6 |

Data are expressed as mean values ± standard deviations (n=3). Values within a column are significantly different ($p<0.05$). Total antioxidant capacity (TAC) and reducing power (RP) are expressed per L. TAC are expressed as mmol of Trolox equivalent (TE) while RP are expressed as mmol of $Fe^{2+}$ equivalents (FE).

[Oszmianski & Wojdylo, 2005]. Based on literature data, the main contributor of total flavonoid content is quercetin [Denev *et al*., 2012; Wilkes *et al*., 2014]. Mikulic-Petkovsek *et al*. [2012] reported that galactoside was the predominant quercetin derivative followed by glucoside, rutinoside, vicianoside, robinobioside, and dihexosides. Quercetin and several quercetin glycosides were detected in chokeberries in relatively low concentrations of about 26–71 mg per 100 g FW [Denev *et al*., 2012; Mikulic-Petkovsek *et al*., 2012; Wilkes *et al*., 2014].

Much lower contents of TN and TA were found in all chokeberry juices. Major non-flavonoid polyphenol compounds in chokeberries are chlorogenic and neochlorogenic acids [Oszmianski & Wojdylo, 2005; Jurgoński *et al*., 2008; Mayer-Miebach *et al*., 2012; Wilkes *et al*., 2014] and according to Oszmianski & Wojdylo [2005] they represent about 7.5% of chokeberry fruit polyphenols. In the 2012 and 2014, relatively high contents of TA were observed, while lower values for total anthocyanins content were noted for juices form 2013 growing season. Earlier results reported higher content of TA [Oszmianski & Wojdylo, 2005; Jakobek *et al*., 2007; Denev *et al*., 2012; Mayer-Miebach *et al*., 2012; Kapci *et al*., 2013]. Compared to other fruits such as blueberry, blackberry, raspberry, grape and cherry, known as rich sources of anthocyanins, chokeberries have relatively higher amounts [Denev *et al*., 2012]. Jakobek *et al*. [2007] found that the portion of anthocyanins in chokeberry was almost twice as high in relation to the red raspberry and strawberry. Chokeberry anthocyanin profile is very simple consisting almost exclusively of cyanidin glycosides, namely cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-xyloside. The content of cyanidin-3-*O*-arabinoside in chokeberries was reported to be 1424 mg/kg of FW [Zheng & Wang, 2003] and 941–1553 mg/kg of FW [Rop *et al*., 2010]. The second major anthocyanin in chokeberries, cyanidin-3-*O*-galactoside was found in the content of 1256 mg/kg of FW [Zheng & Wang, 2003] or 1010–1204 mg/kg of FW [Rop *et al*., 2010]. Content of TA is a very important quality attribute, influencing notably berry taste. Factors such as pH, chemical composition, temperature, light and oxygen might influence the levels of total anthocyanins and decrease them. This often happens during fruit processing namely during pressing, clarification and pasteurization in juice processing [Walkowiak-Tomczak, 2007; Kapci *et al*., 2013; Wilkes *et al*., 2014]. The anthocyanin losses in chokeberries, observed in response to thermal treatments, were much higher than those previously reported for blueberry, blackberry, and black raspberry juices processed under similar conditions [Wilkes *et al*., 2014]. Jeppsson [2000] reported that application of fertilizer affects the anthocyanin content which is very important to the producers. Total acidity of berries and juices should be high since anthocyanins are more stable in acid environments and the stability of anthocyanins during processing is strongly influenced by the pH of the media [Jakobek *et al*., 2007].

**The total antioxidant capacity and reducing power**

Reactive Oxygen Species (ROS) are a number of reactive molecules and free radicals derived from molecular oxygen. These molecules have the potential to cause a number of deleterious events like disruption of membrane fluidity, protein denaturation, lipid peroxidation and alteration of platelet functions. Damage to cells caused by free radicals is believed to play a central role in the development of various diseases such as cancers, inflammation, aging and atherosclerosis [Williams & Jeffrey, 2000]. Since antioxidants prevent the oxidation of other molecules they may have beneficial effects in the prevention of degenerative diseases. Due to the above features, it is important to evaluate antioxidant activity of different foods. In our research two methods were applied for determination of antioxidant activity of chokeberry juices, *i.e.* DPPH and FRAP method. The results showed (Table 3) that the antioxidant activity was not constant over a period of three years. The decrease in the absorbance at 517 nm is taken as a measure of the extent of radical scavenging. Juices from all three studied years showed high TAC values. The highest radical scavenging activity was in 2012 followed by 2013 and 2014 growing season, respectively. There are a number of reports on the antioxidant activity determined by several methods indicating that chokeberries possess strong antiradical activities [Zheng & Wang, 2003; Oszmianski & Wojdylo, 2005; Jakobek *et al*., 2007; Kulling & Rawel, 2008; Denev *et al*., 2012]. TAC values of chokeberry were higher than the antioxidant activity of black currant, elderberry, red currant, strawberry, red raspberry and cherry concentrate [Jakobek *et al*., 2007]. The reducing power in this study was determined as the $Fe^{3+}$ to $Fe^{2+}$ transformation. RP is generally linked with the presence of reducing substances, which have

TABLE 4. Correlation coefficient (r) between phenolics and antioxidant capacity (TAC) or reducing power (RP).

| Phenolics | 2012 | | 2013 | | 2014 | |
|---|---|---|---|---|---|---|
| | TAC | RP | TAC | RP | TAC | RP |
| TPC | -0.25 | -0.67 | 0.43 | 0.98* | 0.63 | 0.98* |
| TN | 0.99* | 0.92* | 0.47 | -0.75* | -0.87* | -0.84* |
| TF | -0.40 | -0.78* | 0.32 | 0,99* | 0.72* | 0.94* |
| TA | 0.09 | -0.38 | 0.22 | -0.89* | 0.56 | -0.49 |

* designates significance at $p \leq 0.05$.

TABLE 5. Comparison of seasonal weather parameters during period from May to September.

| Weather parameter* | 2012 | 2013 | 2014 |
|---|---|---|---|
| STMX (°C) | 34.33 | 34.05 | 31.45 |
| STMN (°C) | 5.85 | 8.20[a] | 8.05[a] |
| STMEAN (°C) | 21.08 | 19.73 | 18.88 |
| SRH (%) | 63.75 | 70.50 | 74.00 |
| SRAIN (mm) | 48.95 | 88.08 | 109.20 |
| ARAIN (mm) | 195.80 | 352.30 | 436.80 |
| SBSS (h) | 301.95 | 274.60 | 243.35 |

Values with the same superscript letters within a row are not significantly different ($p<0.05$).
*STMX – seasonal mean maximum temperature; STMN – seasonal mean minimum temperature; STMEAN – seasonal mean temperature; SRH – seasonal mean relative humidity; SRAIN – seasonal mean rainfall; ARAIN – accumulated seasonal rainfall; SBSS – seasonal mean bright sunshine.

been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. It is known that antioxidant activity of chokeberry juices is influenced by pasteurization and storage [Walkowiak-Tomczak, 2007].

The total antioxidant activity is effected differently by different groups of polyphenolic compounds therefore it is necessary to observe the existence of a correlation between antioxidant activity and individual groups of polyphenolic compounds. Correlation between RP and TPC and between RP and TA was higher than correlation between TAC and TPC and TAC and TA (Table 4). In our study, no significant correlation was evidenced between TAC and TA. TAC is more closely related to the total phenolics content ($r$=0.43 and 0.63 for 2013 and 2014, respectively; unexpectedly, $r$=-0.25 was obtained for 2012, most likely due to the different climate conditions over the observed harvest year). Strong correlation suggests that phenolic components of the chokeberry juices contributed significantly to the antioxidant activity, in particular flavonoids and nonflavonoids (Table 4). Authors have studied the correlation between bioactive compounds and antioxidant capacity in various fruits [Tlili *et al*., 2011]. In data presented by other authors [Wu *et al*., 2004; Jakobek *et al*., 2007], TPC of various small fruits correlates better with the antioxidant activity than TA does. These correlations confirm that the phenolic compounds are the main micro constituents contributing to the antioxidant activities of these fruits.

**Weather parameters and content of phenolic compounds**

Data about weather conditions recorded at the weather station Sveti Ivan Zelina were provided by the Croatian Meteorological and Hydrological Service. Table 5 presents a comparison of seasonal mean maximum temperature (STMX), seasonal mean minimum temperature (STMN), seasonal mean temperature (STMEAN), seasonal mean relative humidity (SRH), seasonal mean rainfall (SRAIN), accumulated seasonal rainfall (ARAIN) and seasonal mean bright sunshine (SBSS) during period from May to September. These parameters were used for a crop weather interaction study during three years (2012, 2013 and 2014). During growing season 2012, the climate was warm and dry; ARAIN was lowest compared to 2013 and 2014 growing season. During 2013 the climate was cold with the highest ARAIN and low SBSS; however, during 2014 the weather was warmer with the highest humidity and intermediate ARAIN and SBSS (Figure 1).

In order to visualize the impact of weather parameters on the physicochemical properties and phenolic profile of chokeberry juices correlations among physicochemical properties, TPC, TN, TF, TA and weather parameters were carried out (Table 6). During all three growing seasons TPC and TN exhibited a positive correlation with mean bright sunshine hours, maximum and mean monthly temperature; however TPC and TN content had negative correlation with minimum monthly temperature, relative humidity and rainfall. Positive correlations were observed between TF content and minimum monthly temperature, relative humidity and rainfall, which is opposite to the content of TN. Positive correlation was also reported between TSC and SSC content and between those physicochemical properties with mean bright sunshine hours and maximum and mean monthly temperature.

It was of interest to note that whereas relative humidity exhibited a significant negative correlation, bright sunshine hours had significant positive correlation with phenolic contents and physicochemical properties of chokeberry juice. Lack of any correlation of weather parameters with polyphenolic contents in the present study could be due to the natural interdependency among weather parameters and their distinct differences during the three cropping years. In research on Australian teas, seasonal variations of phenolic compounds have been attributed to day length, sunlight and temperature

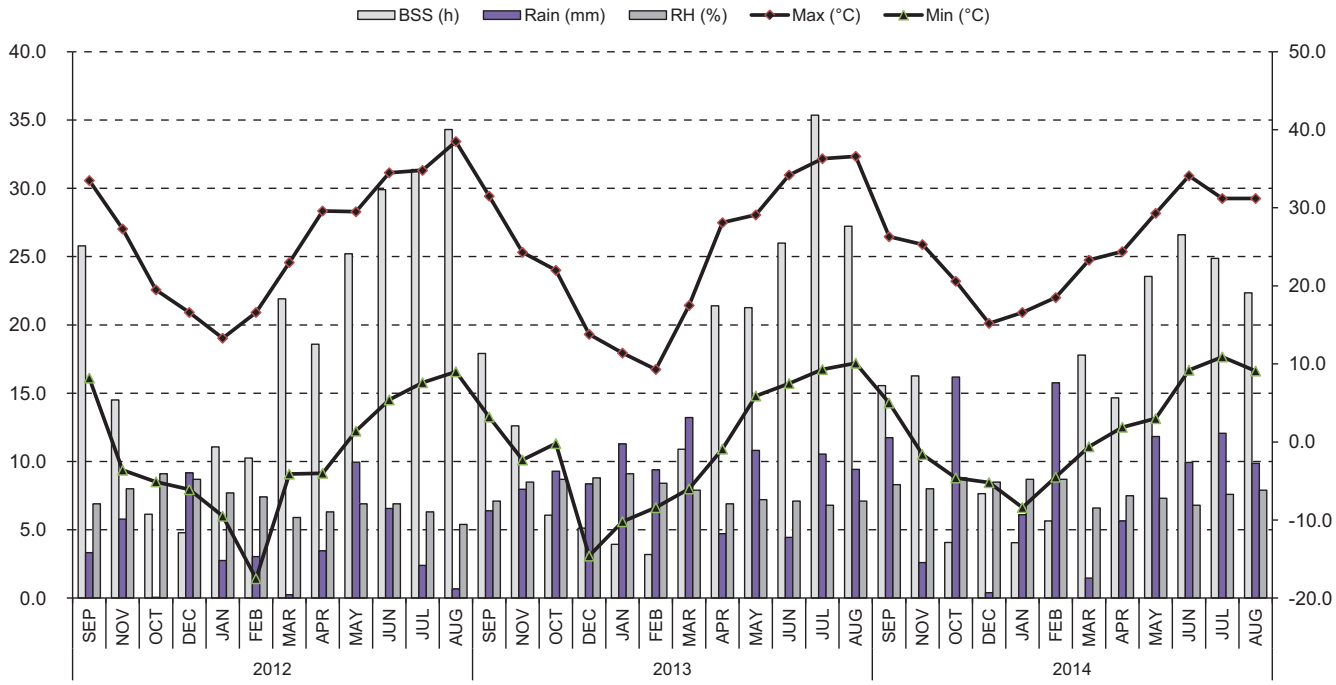


FIGURE. 1. Monthly variations in weather parameters: maximum temperature (Max), minimum temperature (Min), relative humidity (RH), rainfall (Rain) and bright sunshine hours (BSS) during 2012, 2013 and 2014 growing seasons.

[Yao *et al*., 2005]. Previously it was reported that loss of TA in blackberry fruits was accelerated with increasing pre-harvest temperature [Milošević *et al*., 2012].

Other studies showed high variations and discrepancy among cultivars and years in other berry species, like blackberry [Sellappan *et al*., 2002; Reyes-Carmona *et al*., 2005]. It could be connected with genetic differences, maturity at harvest, cultural practices, different extraction and laboratory methods applied. Differences in phenolic components during different growing seasons are most likely owing to the different outside air temperature and rainfall rate, given the well-known inverse correlation between phenolic concentrations and air temperature [Xu *et al*., 2011]. Plants grown in cold climate very often have higher antioxidant properties, because it is one of strategies to counter the oxidative stress. The dependence between the content of antioxidant-active compounds present in the fruits and vegetables and location of origin and harvest years might be due to variability in light levels and ambient temperature [Kalt, 2005]. Among small fruits, the most significant influence of environment conditions on phytonutrient content and antioxidant capacity was observed in strawberry and grape [Li *et al*., 2003; Mori *et al*., 2007]. The activity of phenolic pathway enzymes [Li *et al*., 2003] and events which control their synthesis [Mori *et al*., 2007] are known to be specifically influenced by light exposure and temperature.

## CONCLUSION

Although quality parameters and phenolic composition vary over growing seasons, chokeberry juices from all three seasons have very high contents of phenolic substances and high values of antioxidant properties. The results of this

TABLE 6. Correlation coefficient (r) between weather parameters, physicochemical properties and phenolic profile.

| | STMX | STMN | STMEAN | SRH | SRAIN | ARAIN | SBSS |
|---|---|---|---|---|---|---|---|
| pH | -0.349 | -0.626 | 0.224 | -0.273 | -0.264 | -0.264 | 0.057 |
| TTA | 0.168 | 0.751 | -0.387 | 0.433 | 0.423 | 0.423 | -0.226 |
| TSC | 0.798 | -0.932 | 0.997 | -0.999* | -0.999* | -0.999* | 0.969 |
| SSC | 0.801 | -0.930 | 0.997* | -0.999* | -0.999* | -0.999* | 0.971 |
| TPC | 0.734 | -0.963 | 0.984 | -0.992 | -0.990 | -0.990 | 0.940 |
| TF | -0.979 | 0.688 | -0.935 | 0.916 | 0.920 | 0.920 | -0.982 |
| TN | 0.791 | -0.936 | 0.996 | -0.999* | -0.999* | -0.999* | 0.966 |
| TA | -0.197 | -0.730 | 0.360 | -0.407 | -0.398 | -0.398 | 0.199 |

* designates significance at $p \leq 0.05$.

study confirm that weather conditions affect the concentration of antioxidative compounds. Juices from growing season 2012, in which climate was warm and dry and with low accumulated rainfall compared to 2013 and 2014 growing seasons, have higher amount of total phenolics, total nonflavonoids and total anthocyanins compared to juices from the two other seasons. Statistical analysis showed that mean monthly temperature and bright sunshine hours in the period from May to September have the positive impact on the concentration of phenolic substances and therefore their antioxidative activity, while negative correlation was observed for minimum monthly temperature, relative humidity and rainfall. It was also shown that chokeberry juices with higher phenolic compound values had a higher antioxidant activity. Chokeberries can become a valuable source of nutritionally important substances in human nutrition. Due to the high proportion of natural antioxidants their consumption could bring health benefits.

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## CONFLICT OF INTEREST

None declared.

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