Polyphenol content of Marasca sour cherry ecotypes (Prunus cerasus Marasca) and its stability during freezing storage

Abstract
The present research was undertaken to evaluate the polyphenol content of two Marasca sour cherry ecotypes (Recta and Brač 2) grown in two areas in Croatia (Zadar and Split), their stability during freezing storage at -18 °C for six months and contribution to antioxidant capacity (AOC), respectively. Polyphenolic content was assessed by high performance liquid chromatography (HPLC UV/VIS PDA) and anthocyanins (ANT), hydroxycinnamic acids (HCA), flavonol glycosides (FG) and flavonols (FLAV) were determined. ANT dominated in fresh and frozen cherry ecotypes (321.90 – 774.61 mg/100 g dm) and cyanidin-3-glucosylrutinoside (CYGR) was present in the highest concentration (223.22 – 501.04 mg/100 g dm). The contents of other phenolics were considerably lower compared to ANT: HCA=40.72 – 86.28 mg/100 g dm, FG=22.34 – 41.03 mg/100 g dm and FLAV=0.16 – 0.59 mg/100 g dm, where major compounds presented in HCA were p-coumaric and neochlorogenic acids, in FG quercetin-3-glucoside and in FLAV catechin. Furthermore, statistical analysis showed that all sour cherry samples significantly differed according to the ANT, HCA, FG and FLAV content (p<0.01). Generally, ecotype Recta and Zadar growing area had higher polyphenolic content. Freezing storage caused decrease of almost all polyphenolic groups, particularly ANT (16 – 30 %) and HCA (26 – 36 %) while better stability of FG (10.5 – 20 %) and higher variation of FLAV content was observed. For determination of AOC three methods (DPPH, FRAP, ABTS) were used and the AOC was as follows: DPPH=3.284 – 6.38 mmol TE/100 g dm, FRAP=7.18 – 18 mmol TE/100 g dm and ABTS=73.16 – 119.93 mmol TE/100 g dm. The results indicate higher AOC in samples of Brač 2 ecotype from Zadar growing area.

Keywords: sour cherry Marasca, polyphenolics, antioxidant capacity, growing area, freezing storage

Introduction
The cherry fruits are considered as high nutrient food with relatively low energy value and significant amount of powerful bioactive compounds (Kelley et al., 2018). Marasca sour cherry (Prunus cerasus var. Marasca) (MSC) is indigenous variety and important fruit crop in Croatia. Due to favourable climatic and soil conditions it is mostly cultivated on the north and central part of Dalmatia and on the part of the islands, where it achieves the best fruit quality, i.e. high content of dry matter and sugars, agreeable aroma and intense color. Because of its quality, MSC also received geographical indication status. Several MSC ecotypes are known, but the most cultivated ecotypes are Recta, Brač 2, Sokoluša and Brač 6. Regarding their phytochemical content, sour cherries are rich sources of hormone melatonin and phenolic compounds. They represent sources of several group of phenolic compounds, anthocyanins (ANT), hydroxycinnamic acids (HCA), flavonols (FG), flavanols (FLAV) and procyanidins, which all demonstrated considerable antioxidant properties (Chaovalanikit and Wrolstad, 2004, Šimunić et al., 2005, Kim et al., 2005, Dragović-Uzelac, 2007, Chon et al., 2009, Kirakosyan et al., 2009, Pedisić et al., 2010, Levaj et al., 2010, Ferretti et al., 2010). Several studies have been associated with sour cherries with many positive effects on human health, especially in reducing oxidative stress and inflammation.
(Kelley et al., 2018). According to the literature data, total phenolic content (TPC) of different varieties of sour cherries varied from 74–754 mg/100 g fresh weight (fw) and total anthocyanin content (TAC) from 21–490 mg/100 g fw, respectively (Kim et al., 2005, Pedisić et al., 2007, Pedisić et al., 2010, Dragović-Uzelac, 2007, Veres et al., 2008, Khoo et al., 2011, Mitić et al., 2012). The differences in the phenolic and anthocyanin composition and its mass fraction in cherries and consequently in antioxidant capacity (AOC) are affected by the cultivar type, genetic differences, growing location, ripening stage and harvest time (Goncalves et al., 2004, Kim et al., 2005, Pedisić et al., 2007, Pedisić et al., 2010, Kirakosyan et al., 2009, Mitić et al., 2012, Ferretti et al., 2010). As highly perishable fruits only a small percentage of cherries reach the fresh market and most cherries end up as frozen or processed (canned, jams, purees, jellies, alcoholic beverages, juices, etc.). The freezing is the simplest way to preserve and prevent spoilage of sour cherries and greatly extends its shelf life. Typically used standard storage temperature to reduce the chemical and biological spoilage of foods is at −18 °C and it is the most common way of storage in households (Khattab et al., 2015). It is important to preserve the nutritional and biological value of fruits during storage, thus postharvest storage and processing require great attention since polyphenolic content and AOC could also be changed (de Ancos et al., 2000, Hakkinen et al., 2000). Effects of storage temperature and duration demonstrated that phenolic and anthocyanin content as well as AOC varied among cherry cultivars and across storage conditions (Goncalves et al., 2004). For example, significant losses of phenolics and decrease of AOC were reported in frozen cherries after 3 and 6 months of storage at −23 °C, while storage at −70 °C caused less phenolic degradation and increase of AOC (Chaovalanakit and Wrolstad, 2004). In the available literature on MSC, the influences of cultivar, ripening, growing area, extraction, drying and industrial processing on polyphenol content and AOC have been investigated (Pedisić et al., 2007, Dragović-Uzelac, 2007, Dragović-Uzelac et al., 2009, Pedisić et al., 2010, Levaj et al., 2010, Elez Garofulić et al., 2013, Zorić et al., 2014, Repajić et al., 2015, Elez Garofulić et al., 2016, Garofulić et al., 2017, Zorić et al., 2017, Repajić et al., 2018, Repajić et al., 2019). It seems to be a lack of information focused on polyphenol content variation and AOC of different ecotypes of MSC grown at different locations and about stability of their polyphenols as well as AOC during fruit freezing at −18 °C. Therefore, the objectives of this study were to evaluate the polyphenol content of two MSC ecotypes (Recta and Brač 2) grown in two areas in Croatia (Zadar and Split), their stability during freezing storage at −18 °C for six months and contribution to AOC, respectively.

Materials and Methods

Plant material

Two ecotypes of MSC (Recta and Brač 2) were hand-picked in two regions in Dalmatia (orchard Zadar and orchard Split) at commercial technological maturity stage. Fruits were harvested randomly from both the outer and internal canopy of selected trees in order to obtain a homogeneous sample. Immediately after harvesting, fresh MSC fruits were transported at 4 °C to the Laboratory. The half quantities of MSC samples were analysed on the next day, while other MSC were frozen in liquid nitrogen, packed in PE/PA bags (300 g) and stored at −18 °C for six months. Prior to extraction of polyphenols, frozen fruits were allowed to completely thaw at room temperature (25±2 °C) for 12 h, depitted and homogenised in house blender (Mixy, Zep- ter International).

Chemicals

All chemicals and solvents were reagent or HPLC-grade (Merck, Darmstad, Germany). The standards of p-coumaric acid (p-COA), caffeic acid (CA), chlorogenic acid (CHA), ferulic (FERA),
quercetin-3-ß-D-glucoside (Q3G), kaempferol (K), catechin hydrate (CAT), epicatechin (EP) and cyanidin (kuromanin chloride) (CY3G) were obtained from Sigma (Deisenhofen, Germany).

**Analysis of anthocyanin and phenolic compounds**

**Extraction and purification method of phenolics and anthocyanins**

Phenolic compounds were extracted and purified to obtain anthocyanin and non-ant-hocyanin phenolic fractions according to the method as previously described by Pedisić et al. (2010). For each ecotype two replicates of phenolic cherry extracts were prepared (n=2). The purification process of phenolic cherry extracts was performed using C18 Sep-Pac cartridge (Supelco, Bellefonte, PA). The elution of phenolics and anthocyanins was carried out with ethyl acetate and acidified methanol (0.01 % HCl in MeOH, v/v), respectively. The eluents were evaporated at 40 °C and before injection into HPLC apparatus redissolved in acidified water (0.01 % aqueous HCl, v/v).

**HPLC analysis of polyphenolic compounds**

Solvent composition and the gradient conditions for anthocyanin and phenolic separation were used as described previously by Chaovanalikit and Wrolstad (2004). The chromatographic analysis was performed on a Varian ProStar system equipped with a ProStar Solvent Delivery Module 230, Injector Rheodyne 7125, ProStar 330 UV/VIS Photodiode Array detector. The column was a Pinnacle II C-18 (250 × 4.6 mm i.d., 5µm) protected with Pinnacle C-18 guard column (10× 4 mm i.d., 5µm) (Restek, Bellefonte, U.S.A.). The ANT were analysed at 520 nm, HCA and FLAV at 280 nm and FG at 320 nm. The identification of phenolic compounds was based on the comparison of spectral properties and retention times of peaks in cherry extracts with those of authentic standards and additionally confirmed using characteristic UV/VIS spectra, polarity and previous literature reports (Šimunić et al., 2005, Pedisić et al., 2010, Mitić et al., 2012). Quantification was made by the external standard method using calibration of standards as a reference. Standard solutions of CY3G were prepared in acidified water in a range from 15 to 400 mg/L. The HCA, flavonol and flavanol standards were prepared in methanol in the following concentrations: FERA 1–114 mg/L; CA, p-COA, CHA, Q3G 1–100 mg/L; K, 1–90 mg/L, EP and CAT 24–60 mg/L. In case when commercial standard was not available, quantification was performed using the calibration curve of standards from the same phenolic group. All HPLC determinations were performed in triplicate (n=3) and results were expressed in mg per 100 g of dry matter (mg/100 g dm) as mean values ± standard error. Dry matter was determined by drying at 105 °C to constant mass.

**Antioxidant capacity determinations**

The fruit extracts for determination of total AOC were prepared according to the procedure described in section 2.3.1. AOC was estimated by three standard procedures, i.e. DPPH, FRAP and ABTS assays. The free radical scavenging capacity DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was performed according to the method of Brand-Williams et al. (1995). The ferric reducing antioxidant power (FRAP) assay was performed according to the method of Benzie and Strain (1996). The ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay was based on previously reported protocol of Miller and Rice-Evans (1997).

**Statistical analysis**

Statistical analysis was done using the Statsoft STATISTICA v. 10. Differences between means were analysed by multivariate analysis of variances (MANOVA) and post-hoc Tukey’s HSD test, where differences were considered significant at level of p≤0.01.
**Results and Discussion**

Previous research carried out on MSC have been confirmed that MSC variety is a rich source of various groups of phenolic compounds. The accumulation of phenolic compounds during vegetation is influenced by a number of environmental factors such as temperature, harvesting time, growing area, etc. As a contribution to previously mentioned investigations conducted on MSC, the objectives of this study were to evaluate the polyphenol content of two MSC ecotypes (Recta and Brač 2) grown in two areas in Croatia (Zadar and Split), their stability during freezing storage at 18 °C for six months and contribution to AOC. The phenolic content in fresh and frozen MSC after freezing at 18 °C during the 6 months of storage are shown in Table 1. Statistical analysis showed significant differences between samples according to the content of ANT, HCA, FG, FLAV as well as AOC (p≤0.01).

**Table 1.** The content of polyphenolic compounds and antioxidant capacity in Marasca sour cherries influenced by ecotype, growing area and sample type

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Ecotype</th>
<th>Growing area</th>
<th>Polyphenolic compounds (mg/100 g dm)†</th>
<th>Antioxidant capacity (mmol TE/100 g dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ANT</td>
<td>HCA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.01*</td>
<td>p&lt;0.01*</td>
</tr>
<tr>
<td>Fresh</td>
<td>Recta</td>
<td>Zadar</td>
<td>774.61±0.42*</td>
<td>76.59±2.49*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Split</td>
<td>650.96±0.42*</td>
<td>86.23±2.49*</td>
</tr>
<tr>
<td></td>
<td>Brač 2</td>
<td>Zadar</td>
<td>617.06±0.42*</td>
<td>65.53±2.49*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Split</td>
<td>459.92±0.42*</td>
<td>54.93±2.49*</td>
</tr>
<tr>
<td>Frozen</td>
<td>Recta</td>
<td>Zadar</td>
<td>635.00±0.42*</td>
<td>52.02±2.49*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Split</td>
<td>506.30±0.42*</td>
<td>86.28±2.49*</td>
</tr>
<tr>
<td></td>
<td>Brač 2</td>
<td>Zadar</td>
<td>469.48±0.42*</td>
<td>41.98±2.49*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Split</td>
<td>321.90±0.42*</td>
<td>40.72±2.49*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± standard error. *Statistically significant at 99 % confidence level. Means with the same letter within each column are not significantly different at p≤0.01.

†Sum of individually polyphenolic compounds quantified HPLC

ANT- anthocyanins, HCA- hydroxycinnamic acids, FG- flavonol glycosides, FLAV- flavanols

Identified phenolic compounds of Marasca ecotypes were ANT, HCA, FG and FLAV. The same phenolic compounds were present in each ecotype, but there were differences in relative levels. Major phenolics among all Marasca ecotypes were anthocyanins, while other determined phenolics were in considerably lower contents.

Four different anthocyanins were identified in MSC ecotypes: cyanidin-3-glucosylrutinoside (CYGR), cyanidin-3-rutinoside (CY3R), cyanidin-3-glucoside (CY3G) and cyanidin-3-sophoroside (CY3S) (Fig. 1&2).

Anthocyanins (ANT): CY3GR - cyanidin-3-glucosylrutinoside; CY3R - cyanidin-3-rutinoside; CY3SOF - cyanidin-3-sophoroside; CY3G - cyanidin-3-glucoside. Hydroxycinnamic acids (HCA): p-COAU - p-coumaric acid; CHA- chlorogenic acid; NeoCHA- neochlorogenic acid; CA - caffeic acid; FERA - ferulic acid. Flavonol glycosides (FG): K3G - kaempferol-3-glucoside; K3R - kaempferol-3-rutinoside; Q3G - quercetin-3-glucoside; Q3R - quercetin-3-rutinoside. Flavanols (FLAV): CAT - catechin; EP - epicatechin
Figure 1. Polyphenolic compounds in fresh Marasca cherry ecotypes determined by HPLC UV/Vis DAD

Slika 1. Polifenolni spojevi u svježim ekotipovima višnje Maraske određenim pomoću HPLC UV/Vis DAD

Anthocyanins (ANT): CY3GR - cyanidin-3-glucosylrutinoside; CY3R - cyanidin-3-rutinoside; CY3SOF - cyanidin-3-sophoroside; CY3G - cyanidin-3-glucoside. Hydroxycinnamic acids (HCA): p-COUA – p-coumaric acid; CHA - chlorogenic acid; NeoCHA - neochlorogenic acid; CA - caffic acid; FERA –ferulic acid. Flavonol glycosides (FG): K3G - kaempferol 3-glucoside; K3R - kaempferol-3-rutinoside; Q3G - quercetin 3-glucoside; Q3R - quercetin 3-rutinoside. Flavanols (FLAV): CAT-catechin; EP - epicatechin

Figure 2. Polyphenolic compounds in frozen Marasca cherry ecotypes determined by HPLC UV/Vis DAD

Slika 2. Polifenolni spojevi u smrznutim ekotipovima višnje Maraske određenim pomoću HPLC UV/Vis DAD
The content of predominant anthocyanin CY3GR in fresh cherries ranged from 223.22–501.04 mg/100 g dm (42.36 % to 64.72 % in total ANT content) followed by CY3R in range from 141.01–202.36 mg/100 g dm (Fig. 1), while in frozen cherries CY3GR ranged from 105.58–381.2 mg/100 g dm and CY3R from 134.57–160.28 mg/100 g dm (Fig. 2). The mean values of ANT (sum of individual identified anthocyanins) in fresh cherries ranged from 459.72 (Brač 2/Split) to 774.09 mg/100 g of dm (Recta/Zadar), where ecotypes from Zadar (ZD) had higher ANT content compared to same ecotypes from Split (ST). Obtained results are generally in accordance with previous studies on MSC ecotypes (Pedisić et al., 2010, Repajić et al., 2015), but higher in comparison with results on other Croatian sour cherry cultivars (Simunić et al., 2005, Levaj et al., 2010). Anthocyanin content in fruits varies due to nutritional, environmental and seasonal factors as well as due to different cultivar types (Seeram et al., 2001). Furthermore, higher ANT content in MSC is mostly due to their distribution in both fruit skin and flesh (Pedisić et al., 2010), but temperature, solar radiation, water stress and soil are also considered as the major elements affecting anthocyanin content in fruit (Spinardi et al., 2019). In frozen MSC samples ANT content ranged from 321.90 (Brač 2/ST) to 635 mg/100 g dm (Recta/ZD). Freezing storage decreased ANT content from 16 to 30 %, where higher ANT decrease was observed in ecotype Brač 2. In frozen products the enzymatic reactions are slow, but not completely blocked due to the presence of non-frozen water (Poiana et al., 2010). According to the Poiana et al. (2010), ANT contents decreased in frozen berries by 9–20 % showing that period of 6 months of storage did not caused significant loss of anthocyanins. Furthermore, Chaovanalikit and Wrolstad, (2004) found that more than 75 % of ANT in frozen cherries were destroyed after six months of storage at −23 °C.

The sour cherries are also rich source of colorless polyphenols with hydroxycinnamates being the major class. Among HCA in fresh cherries, predominant \( p \)-COU comprised 56.2–73.82 % of the total HCA and ranged from 34.07–67.54 mg/100 g dm. The second abundant was neo-chlorogenic acid (NeoCHA) (8.58–16.69 mg/100 g of dm) and other HCA determined in MSC were CHA, CA and FERA (1.02–11.68 mg/100 g dm) (Fig. 1). Compared to the results of Mitić et al., (2012) in Serbian cherry cultivars NeoCHA composed 73–79% among the HCA. According to Kim et al. (2005) CHA derivatives were the major HCA in various sour cherries (75.5–92.5%) and their contents were higher compared to the results of this study (18.13–37.33%). In frozen MSC samples \( p \)-COU ranged from 18.86–50.04 mg/100 g of dm, NeoCHA from 8.05–14.77 mg/100 g of dm and CHA, CA and FERA ranged from 0.23–10.85 mg/100 g of dm, respectively (Fig. 2). The total HCA (sum of individual identified acids) in fresh MSC was in range from 54.93 (Brač 2/ST) to 86.23 mg/100 g of dm (Recta/ST) while lower HCA contents were determined in Brač 2 ecotype. In frozen Marasca cherries HCA contents ranged from 40.72 (Brač 2/ST) to 86.28 mg/100 g dm (Recta/ST) (Table 1). Compared to the results of Mitić et al., (2012), Serbian cherry cultivars had lower total HCA contents. Freezing storage decreased HCA contents from 26–36 % in all samples except in Recta/ST and higher decrease was present in cherries from ZD. The \( p \)-COU ranged in frozen cherries from 18.86 (Brač 2/ZD) to 50.04 mg/100 g dm (Recta/ST) and it decrease amounted from 25.91–58.6 %, respectively (Fig. 2).

The following FG were identified in Marasca ecotypes studied here: Q3G, quercetin 3-rutinoside (Q3R), kaempferol 3-glucoside (K3G) and kaempferol 3-rutinoside (K3R) (Fig. 1&2). The Q3G dominated in all fresh MSC and its content ranged from 14.56 (Brač 2/ST) to 16.26 mg/100 dm (Recta/ST). Kaempferol glycosides ranged from 7.36–12.85 mg/100 dm and comprised 50–62 % of the total FG (Fig. 1). Results of this study showed lower values for Q3R and K3R and higher values for Q3G as compared with results obtained by Kim et al. (2005). As for frozen cherries Q3G ranged from 9.23 (Brač 2/ZD) to 18.86 mg/100 dm (Recta/ST) and kaempferol
glycosides were in range from 3.54–14.89 mg/100 dm (Fig. 2). The total FG (sum of individual identified FG) in fresh MSC was in range from 31.06 (Brač 2/ST) to 41.03 mg/100 g dm (Recta/ST) and in frozen from 22.34 (Brač 2/ZD) to 36.72 mg/100 g dm (Recta/ST) (Table 1). Decrease of total FG in MSC after freezing was from 10.5–20 %, while in Brač 2/ST total FG remained almost same (Table 1). K3R showed the highest decrease, which content ranged from 3.54 to 5.23 mg/100 g dm. Better stability of Q3G during freezing was observed in ecotype Recta regardless growing area, where it content slightly increased (Fig. 2). In all fresh and frozen MSC ecotypes (+) catechin (CAT) and (-) epicatehin (EP) were identified as FLAV (Fig. 1 & 2). Fresh cherries contained CAT in range from 0.17 (Brač 2/ZD) to 0.47 mg/100 g dm (Brač2/ST) and frozen ones from 0.12 (Recta/ZD) to 0.55 mg/100 g dm (Recta/ST), respectively. In fresh and frozen cherry ecotypes EP was present in considerably lower amount (0.02–0.09 mg/100 g dm). Total FLAV ranged from 0.28 to 0.49 mg/100 g dm and cherries grown in ST had higher concentrations of FLAV (Table 1). Based on Chaovanalikit and Wrolstad (2004) study, Montmorency cherry had higher content of FLAV compared with our results. Freezing caused variation in FLAV content of MSC. Decrease of FLAV was observed in Recta/ST (67 %) and Brač 2/ZD (21.4 %), while Recta/ ZD and Brač 2/ST showed increase for 38 and 22 %, respectively. Several methods have been developed to determine the AOC of fruit extracts since the single antioxidant method cannot give complete profiles of the AOC of compounds due to huge varieties of antioxidants present in fruits. In our study, all MSC extracts exhibited significant AOC which were in range from 4.24–6.38 mmol TE/100 g dm determined using DPPH, from 7.18–11.19 mmol TE/100 g dm using FRAP and from 73.26 – 99.53 mmol TE/100 g dm by ABTS method, respectively (Table 1). Generally, results of all three methods indicate higher AOC in samples of Brač 2/ZD. At the end of storage period the losses of AOC of MSC determined by DPPH method were in range from 22.64–39.27 %. On the contrary the AOC as determined by FRAP and ABTS increased considerably. It can be concluded that polyphenolic losses in cherries after freezing storage were not in relation with AOC. According to the Chaovanalikit and Wrolstad (2004) results of AOC of cherries decreased during 6 month of storage at −23 °C for 28–58 %, but increased in cherries stored at −70 °C (46–81 %). Native enzymes cause degradation and changes of anthocyanin and polyphenolic composition and the structure is associated with the antioxidant activity. Possible explanation is that anthocyanin and polyphenolic degradation products retain antioxidant activities.

**Conclusion**

With this study more information regarding the polyphenolic contents and antioxidant capacity of two Marasca sour cherry ecotypes and their stability after freezing storage was gathered. The polyphenolic compounds are among the primary antioxidants in Marasca cherries. The anthocyanins dominated in Marasca sour cherry samples and main anthocyanin was cyanidin-3-glucosylrutinoside. The polyphenolic content and antioxidant capacity varied within cultivar and growing area. The main compound of hydroxycinnamic acids was found to be p-coumaric acid, among flavonol glycosides was quercetin 3-glucoside and catechin was major flavanol. Freezing storage affected cherries polyphenolic content and antioxidant capacity but their contents were still satisfactory. Generally, higher decrease was of anthocyanins and hydroxycinnamic acids while better stability was of flavonol glycosides and flavanols. The antioxidant capacity did not change uniformly due to changes in the polyphenolic compounds. Cultivation approaches and postharvest storage surely could enable the improvement of fruit quality and AOC of preferred cherry cultivars.
Literature


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Izvorni znanstveni rad

Polifenolni sastav ekotipova višnje maraske (Prunus cerasus Marasca) i njihova stabilnost tijekom skladištenja zamrzavanjem

Sažetak

Ovo istraživanje provedeno je radi utvrđivanja razlike u masenom udjelu polifenola dva ekotipa višnje Maraske (Recta i Brač 2) uzgojenih na dva područja u Hrvatskoj (Zadar i Split), utvrđivanja stabilnosti polifenola tijekom skladištenja na -18 °C tijekom šest mjeseci i doprinosu polifenolnih spojeva antioksidacijskom kapacitetu (AOC). Primjenom tekućinske kromatografije visoke djelatnosti (HPLC UV/Vis PDA) identificirani su fenoli spojevi iz skupine antocijana (ANT), hidroksicimetnih kiselina (HCA), flavonol glukozida (FG) i flavonola (FLAV). ANT su dominirali u svim uzorcima višanja, svježim i zamrznutim (321,90 - 774,61 mg/100 g st), a u najvećim masenim udjelima određen je cijanidin-3-glukozilitiuzoizid (CYGR) (223,22-501,04 mg/100 g st). Moseni udjeli drugih polifenolnih spojeva: HCA (40,72-86,28 mg/100 g st), FG (22,34-41,03 mg/100 g st) i FLAV (0,16-0,59 mg/100 g st) bili su znatno niži u odnosu na ANT. Glavni spojevi iz skupine HCA bili su p-kumarinska i neoklorgenska kiselina, iz skupine FG kверcetin-3-glukozid te iz skupine FLAV katechin. Statistička analiza pokazala je da su se svi uzori višanja značajno razlikovali prema masenom udjelu ANT, HCA, FG, FLAV i AOC (p ≤ 0,01). Ekotip Recta i zadarsko uzgojno područje su općenito imali veći maseni udio fenolnih spojeva. Skladištenje zamrznutih plodova višnje pri temperaturi od -18 °C tijekom 6 mjeseci uzrokovalo je smanjenje gotovo svih skupina fenolnih spojeva, naročito ANT (16 - 30 %) i HCA (26 - 36 %) dok je uočena bolja stabilnost FG (10.5 - 20 %) te veće varijacije u masenom udjelu FLAV. U određivanju AOC korištene su tri metode (DPPH, FRAP, ABTS), a AOC je iznosila kako slijedi: DPPH (3,28 - 6,38 mmol TE/100 g st); FRAP (7,18 - 18 mmol TE/100 g st) te ABTS (73,16 - 119,93 mmol TE/100 g st). Viši AOC određen je u uzorcima ekotipa Brač 2 / Zadar.

Ključne riječi: višnja maraska, polifenoli, antioksidativna aktivnost, uzgojno područje, zamrzavanje