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## Polyphenols and Volatiles in Fruits of Two Sour Cherry Cultivars, Some Berry Fruits and Their Jams

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### Summary

This paper reports about the content of polyphenols and volatiles in fresh fruits of two sour cherry cultivars (Marasca and Oblačinska), some berry fruits (strawberry Maya, raspberry Willamette and wild blueberry) and the corresponding low sugar jams. Phenolic compounds (hydroxybenzoic and hydroxycinnamic acids, flavan-3-ols and flavonols) were determined by high-performance liquid chromatography (HPLC). Those found in the fruits were also found in the jams. Jams contained lower amounts of polyphenols than fresh fruits, but their overall retention in jams was relatively high. Among fruits, sour cherry Marasca had the highest level of polyphenols, while sour cherry Marasca jam and raspberry Willamette jam had the highest level of polyphenols among jams. The major flavonoid in all investigated fruits, except in sour cherry Oblačinska, was (–)-epicatechin. Sour cherry Marasca had the highest level of (–)-epicatechin (95.75 mg/kg), and it also contained very high amounts of flavonols, derivatives of quercetin and kaempferol. Hydroxybenzoic acids (HBAs) were not found in sour cherries Marasca and Oblačinska, but were found in berry fruits and jams. Phenolic compound (+)-gallocatechin was found only in Marasca fruit and jam. Ellagic acid was found in the highest concentration in raspberry Willamette fruit and jam. Hydroxycinnamic acids (HCAs) were found in all the investigated fruits, with the exception of a derivative of ferulic acid, which was not found in strawberry. Derivatives of caffeic, *p*-coumaric and chlorogenic acids were found in all the investigated fruits, with chlorogenic acid being the most abundant, especially in sour cherry Marasca. Volatiles were determined by gas chromatography (GC) and expressed as the peak area of the identified compounds. All investigated volatiles of fresh fruit were also determined in the related jams with relatively high retention. Sour cherries Marasca and Oblačinska contained the same volatile compounds, but Marasca had higher level of total volatiles. The main volatile compound in both sour cherry cultivars was benzaldehyde (characteristic cherry aroma compound), which was followed by hexanal, 2-hexenal, 2-heptanone, linalool, nerol, and  $\alpha$ -terpineol. Our results show that  $\gamma$ -decalactone and linalool were the most abundant volatile compounds in strawberry Maya and raspberry Willamette, respectively. The most abundant group of volatiles in wild blueberry was esters, and they were followed by terpenes, ethyl butanoate and linalool.

Key words: polyphenols, volatiles, sour cherry Marasca, sour cherry Oblačinska, strawberry, raspberry, blueberry, jams, GC, HPLC

## Introduction

Due to the health benefits linked to the consumption of fruit, nutritionists recommend fruits to be part of a daily diet. One of the reasons for such recommendation is the presence of biologically active compounds, among them phenols, in fruits. As fresh fruits are not available throughout the year, jams have become a convenient way to consume fruit ingredients all year round.

In order to keep calorie count in order, many consumers prefer the consumption of low sugar jams. One question that arises is whether the high quality low sugar jams could represent a good source of bioactive compounds as fresh fruit does. In addition to the presence of authentic fruit aroma, which greatly influences consumer acceptability (1,2), the quality of jams is often defined by the amount of bioactive compounds, especially polyphenols. Literature search revealed that volatiles of fruit jams have not been investigated as much as the volatiles of fresh fruits.

Polyphenols are very interesting classes of natural compounds, secondary plant metabolites, known for their colour (anthocyanins) as well as positive influence on human health due to their antioxidant activity (3–5). Several scientific papers describe them as cancer prevention agents (6,7). In sour cherries and red berries very abundant subgroups of polyphenols are procyanidins and anthocyanins, but colourless and pale yellow polyphenols are also present in marked amounts, and they also have marked biological activity (8,9). The goal of this research is to provide more information about colourless and pale yellow polyphenols and volatiles in fresh fruits of sour cherry (*Prunus cerasus*) cv. Marasca and jams in comparison with another sour cherry cv. Oblačinska, strawberry Maya, raspberry Willamette, and wild blueberry, fruits which are known as very aromatic and rich in polyphenols.

Sour cherry Marasca, very popular native fruit in Croatia, is almost unknown in scientific literature with regard to its chemical composition, including polyphenols and volatile content. Recently, a scientific paper dealing with anthocyanins in sour cherry Marasca has been published (10). For over hundred years, botanists have been trying to answer whether Marasca is a variety or cultivar of sour cherry due to its higher dry matter content, more intensive aroma and deeper red colour than other known sour cherries. The best quality sour cherry Marasca can be obtained in the part of Croatia called Dalmatia (11). Furthermore, it is processed into many high quality products. The liqueur Maraschino is the most famous sour cherry Marasca product. Marasca juices as well as jams are high quality products, too.

According to the available literature, sour cherry polyphenols and volatiles have not been investigated much. Polyphenols identified in sour cherry juices, besides anthocyanins, include (–)-epicatechin (flavanol), neochlorogenic, chlorogenic and 3-coumaroylquinic acids (hydroxycinnamic acids), as well as quercetin and kaempferol glycosides (flavonols) (12). Current animal research suggests that sour cherry consumption may confer multiple health benefits (13), which are linked to the anthocyanins and other polyphenols (8,9,14) present in the fruit. The aroma of sour cherries has previously been studied

(15,16). More recently, Poll *et al.* (17) have identified benzaldehyde, benzyl alcohol, eugenol and vanillin as the most important aroma components in sour cherry cv. Stevensbear. Data about volatiles from sour cherry Marasca fruit as well as sour cherry jams are missing from literature. Kirakosyan *et al.* (18) investigated polyphenol composition and antioxidant capacity of some sour cherry products, namely concentrated juice, dried and frozen fruits, but not jam.

Literature data show strawberry as the most investigated fruit among others studied in this work. Many researches have investigated strawberry polyphenols (19–25) and their influence on human health (4,20,26). The mentioned authors identified polyphenols such as hydroxycinnamic acids (*p*-coumaric acid), benzoic acids (ellagic acid), and flavonols (kaempferol, quercetin, and myricetin) in strawberry, but they did not identify any anthocyanins. Aroma compounds of strawberry have been investigated by many authors (1,27–33). Strawberry (*Fragaria×ananassa* Duch.) aroma is characterized by 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (furanol, DHF) (30–32). Pinto *et al.* (34) investigated bioactive compounds of strawberry jams and they found that jams can be a good source of antioxidant compounds, although compared to the fruit important losses were detected.

Raspberry is a very interesting fruit because of a high level of ellagic acid (25,35), which possesses long term health benefits (36). This fruit has a very intense and pleasant aroma, and according to Robertson *et al.* (37) ethyl acetate is the most abundant volatile component in raspberry. Zafrilla *et al.* (38) investigated the effect of raspberry jam processing and storage on the antioxidant ellagic acid derivatives and flavonoids.

Among berry fruits, blueberry has been reported to have the highest antioxidant capacity, which is mainly linked to the anthocyanin content (39,40), as well as some other flavonoids, especially quercetin (35). Schmidt *et al.* (41) studied antiproliferation and antioxidant activity of several blueberry products, as well as jams. The aroma component of blueberry with the highest concentration is linalool (42), although typical blueberry aroma is characterized by 1,8-cineole (43).

The goal of this work is to investigate the stability of polyphenols and volatiles during production of low sugar jams from the above fruits. Special interest has been given to sour cherry Marasca fruit and jam.

## Materials and Methods

### Standards and reagents

#### Polyphenols

Chlorogenic and *p*-coumaric acids were obtained from Fluka (Neu-Ulm, Germany); gallic acid, *p*-hydroxybenzoic acid, (+)-catechin, (–)-epicatechin, quercetin 3-rutinoside, ferulic acid and ellagic acid were obtained from Sigma (Deisenhofen, Germany); caffeic acid was obtained from Merck (Darmstadt, Germany). HPLC grade methanol, acetonitrile, *tert*-butylhydroquinone and acetic acid were also obtained from Merck.

### Volatile compounds

All chemicals except  $\gamma$ -decalactone were purchased from Merck (Darmstadt, Germany).  $\gamma$ -Decalactone was purchased from Roth (Karlsruhe, Germany).

### Samples

Strawberry fruit (*Fragaria×ananassa* Duch.), cultivar Maya, was obtained from commercial orchards in the region of Osijek, Croatia. Raspberry fruit (*Rubus idaeus*), cultivar Willamette, was obtained from commercial orchards in the region of Ludbreg, Croatia. Two sour cherry (*Prunus cerasus*) cultivars, Oblačinska and Marasca, were obtained from commercial orchards in the region of Zadar, Croatia. Wild blueberry fruit (*Vaccinium myrtillus*) was obtained from the region of Delnice, Croatia. The fruits of similar ripening degree were selected in order to have sample uniformity. All the fruits were harvested at the optimum maturity stage for the technology of jam production.

### Jam preparation

All jams were defined as low sugar jams with 45 % of dry matter. Fruit content was 40–60 g per 100 g, depending on the fruit. For the preparation of jams, fruit was puréed and then cooked under atmospheric pressure with the addition of sucrose. After certain dry matter content was reached, commercial low esterified pectin 0.8 % (*m/V*) (Danisco Ingredients, Denmark) was added. Citric acid (Kemika, Zagreb, Croatia) was added towards the end of cooking. The amount of prepared jams was 1000 g. Jams were stored in glass jars at 4 °C and analyzed within one week.

### Polyphenol extraction

The phenolic compounds were extracted using the procedure previously described by Häkkinen *et al.* (44). The sample (10 g of raw fruit or 20 g of jam) was mixed with 80 mg of ascorbic acid previously dissolved in 10 mL of purified water and 25 mL of methanol. A volume of 10 mL of 6 M HCl was added and the solution was sonicated for 2 min using Transsonic T460 (Elma, Germany) sonic bath. The extract was bubbled with nitrogen for 2–5 min and the flask was tightly sealed. Extraction was carried out in a dark room, in a water bath at 35 °C with constant shaking for 12 h. The extract was cooled, filtered through Whatman No. 40 filter (Whatman International Ltd., Kent, UK) and evaporated to dryness under vacuum. The residue was dissolved in 2 mL of methanol and filtered through 0.45- $\mu$ m membrane filter (Nylon membranes, Supelco, Bellefonte, USA) before it was injected (20  $\mu$ L) into the HPLC apparatus.

### HPLC analysis of phenolic compounds

The analytical HPLC system was Varian LC Star system (Palo Alto, CA, USA) equipped with a Star solvent delivery system 9010, Rheodyne 7125 injector, and Polychrom 9065 UV diode-array detector. The HPLC column was Nucleosil C-18 column (250×4.6 mm i.d., 5  $\mu$ m) protected with a Nucleosil C-18 guard column (10×4.6 mm i.d., 5  $\mu$ m) (Supelco, Inc, Bellefonte, PA, USA). The solvents for gradient elution were: (A) 50 mM ammonium

dihydrogen phosphate, pH=2.6; (B) 0.2 mM *o*-phosphoric acid, pH=1.5 and (C) solvent A in 80 % acetonitrile. The following gradient was used: 0–15 min from 100 % A to 96 % A and 4 % C; 15–25 min from 96 % A and 4 % C to 92 % A and 8 % C; 25.01 min 92 % B and 8 % C; 25.01–45 min from 92 % B and 8 % C to 80 % B and 20 % C; 45–50 min from 80 % B and 20 % C to 70 % B and 30 % C; 50–55 min from 70 % B and 30 % C to 60 % B and 40 % C; 55–60 min from 60 % B and 40 % C to 20 % B and 80 % C; 65–70 min 100 % A. The flow rate was 0.5 mL/ min.

Operating conditions were as follows: column temperature was 20 °C and injection volume was 20  $\mu$ L (for standards and samples). Detection was performed with UV diode array detector by scanning from 210 to 360 nm.

Phenolic compounds were identified by comparing retention times and spectral data with those of authentic standards. UV diode-array detection was carried out at 278 nm. Quantification was performed by using the external standard method and was based on peak area. Calibration curves of the standards were made by diluting stock standards in methanol to yield 2–20 mg/L for gallic and *p*-hydroxybenzoic acid, 2–20 mg/L for ferulic acid, 5–50 mg/L for chlorogenic acid and catechins, 5–30 mg/L for caffeic and *p*-coumaric acid, 10–100 mg/L for ellagic acid and 2–20 mg/L for rutin. Derivatives of kaempferol and (+)-gallocatechin were identified by polarity and spectral data from literature and quantified as quercetin 3-rutinoside and (+)-catechin, respectively. The samples were prepared and analyzed in triplicate. Data are presented as mean±standard deviation.

### Headspace-solid phase microextraction (HS/SPME) analysis

The SPME device used was a Supelco (Bellefonte, PA, USA) manual SPME holder 57330-U. Fused silica fibre coated with polydimethylsiloxane (PDMS), 100  $\mu$ m film thicknesses (Supelco), was used for the extraction and concentration of volatile compounds. The fibre was preconditioned at 250 °C for 1 h in the inlet of the GC prior to sampling as instructed by the manufacturer. The homogenized sample of fruit or jam (30 mL) was placed in a 50-mL vial and NaCl p.a. (3 g) was added. The vial was sealed with aluminium cover and Teflon-lined septum, warmed to 50 °C in a water bath and gently mixed. Samples were equilibrated for 10 min prior to insertion of fibre and were kept at 50 °C throughout a 30-minute assay. The fibre was then removed from the headspace and inserted into GC.

### GC/FID and GC/MS analyses

Thermal desorption of the adsorbed volatiles was done by directly exposing the fibre in the injector port of the GC for 5 min at 200 °C. Blank runs were performed regularly prior to the sample analysis to ensure the removal of possible impurities from the GC. The splitless injection mode was used for thermal desorption, the split valve was opened after 3 min. A Varian 3300 gas chromatograph coupled with flame ionization detector was used. Compounds were separated on a DB 624 column (30 m×0.32 mm, i.d. 1.8  $\mu$ m; J&W Scientific, Folsom, CA, USA).

Carrier gas was nitrogen at a flow rate of 5 mL/min. A split/splitless injector was used (ratio 1:5) and maintained at 200 °C. The detector was kept at 250 °C. Temperature programming was as follows: 3 min at 40 °C, then from 40 to 190 °C at 5 °C/min and hold for 10 min at 190 °C (45).

The same conditions were applied for the GC-MS analysis on a Hewlett-Packard 5890 gas chromatograph with a 5970 series mass selective detector. The ionization of samples was achieved at 70 eV using SCAN mode. The mass range studied was from 30 to 250 *m/z*. Carrier gas was helium at a flow rate of 5 mL/min. The constituents were identified by comparing their retention times and MS spectra with the values obtained for standards. The MS spectra were also compared with the data from NBS 75k library spectra.

The results obtained in this investigation are shown as the peak ratio (45). It was calculated by dividing the peak area of the compounds by the peak area of the internal standard (3-decanol). The actual peak area of the internal standard was 14 450 on average, with the coefficient of variation being 3 %.

## Results and Discussion

### Phenolic compounds

Polyphenol compounds found in the investigated fruits and jams can be classified as flavonoids (flavan-3-ols and flavonols) and non-flavonoids (phenolic acids – derivatives of hydroxycinnamic acids (HCA) and hydroxybenzoic acids (HBA)). Obtained results are shown in Table 1.

The same polyphenols that were found in fruits were also found in jams, but in much lower quantities (approx. 2.5 to 3.4 times lower than in fresh fruits). Since jams contained 40–60 g of fruit per 100 g of jam, it was to be expected that polyphenol content would be approximately half the value observed for fresh fruits. The other reason for lower polyphenol content in jams is processing. Literature search revealed limited data on the influence of processing on polyphenol content in jams, and a decrease of polyphenols during jam preparation was observed (25,38).

Analysed samples of sour cherry fruits contained all of the mentioned polyphenol classes except hydroxybenzoic acids. Sour cherry Marasca had the highest level of total polyphenols and was followed by raspberry Willamette, strawberry Maya, wild blueberry and sour cherry Obláčinska. In sour cherry Marasca the polyphenol content was more than twice greater than in Obláčinska. Also, Marasca had the highest levels of individual polyphenols (except derivatives of hydroxybenzoic, *p*-coumaric and ferulic acid) when compared to other investigated fruits. Detailed comparison of polyphenol content in sour cherry Marasca and the other investigated fruits shows the following:

(i) Flavan-3-ol, (+)-gallocatechin, was found only in sour cherry Marasca. The mass fractions of (+)-catechin and (–)-epicatechin were much higher in sour cherry Marasca (29.2 and 95.8 mg/kg) when compared to Obláčinska (11.1 and 13.6 mg/kg). In other investigated fruits, (+)-catechin content ranged from 9.6 mg/kg in rasp-

berry Willamette to 22.2 mg/kg in wild blueberry and (–)-epicatechin content ranged from 13.6 mg/kg in raspberry Willamette to 23.1 mg/kg in wild blueberry.

(ii) Flavonols, derivatives of quercetin and kaempferol, were present at high levels in sour cherry Marasca (53.8 and 29.9 mg/kg). Sour cherry Obláčinska had lower mass fractions of derivatives of quercetin and kaempferol (37.6 and 12.9 mg/kg) when compared to sour cherry Marasca, but their levels were still higher than in the other investigated fruits. Strawberry Maya and wild blueberry fruits had similar mass fractions to quercetin derivative, 6.3 and 6.1 mg/kg, respectively. The results for quercetin derivatives and kaempferol in strawberry Maya fruit were in accordance with those presented in literature (46). Kaempferol derivative was not found in raspberry Willamette. This finding is in accordance with previously published data by Rommel and Wrolstad (47), who reported the main flavonols determined in raspberry juices to be quercetin derivatives.

(iii) Within the group made of hydroxycinnamic acids (HCA), derivatives of chlorogenic and caffeic acid were determined in the highest mass fraction in sour cherry Marasca (45.9 and 15.5 mg/kg). In sour cherry Obláčinska their mass fractions were remarkably lower, 28.3 and 5.4 mg/kg, respectively. Generally, a derivative of chlorogenic acid was the most abundant HCA in all analyzed fruits (especially in wild blueberry), except in sour cherry Marasca, where it was determined at 38.5 mg/kg. The mass fraction of *p*-coumaric acid derivative was similar in both cultivars of sour cherry, Marasca and Obláčinska (11.3 and 12.1 mg/kg, respectively). A derivative of ferulic acid was determined at very low mass fraction in both sour cherries (1.1 mg/kg in Marasca and 1.3 mg/kg in Obláčinska). Among HCAs, derivatives of caffeic, *p*-coumaric and chlorogenic acid were found in all investigated fruits, except the derivative of ferulic acid, which was not found in strawberry Maya. Häkkinen *et al.* (35) also pointed out that ferulic acid was present in blueberry and raspberry, but not in strawberry fruit. The presence of *p*-coumaric and caffeic acids in strawberry and raspberry is known from literature (35). In strawberry Maya, derivative of *p*-coumaric acid was determined in the highest mass fraction in comparison with the other analyzed fruits.

(iv) The main difference between polyphenol compositions in the investigated fruits was the absence of hydroxybenzoic acids (HBA) in sour cherries Marasca and Obláčinska. In contrast, derivatives of ellagic, gallic and *p*-hydroxybenzoic acid were found in raspberry Willamette, strawberry Maya and wild blueberry fruits. According to literature, ellagic acid occurs at around three times higher mass fraction in strawberries and raspberries than in other fruits and nuts (48). Our results indicate that the ellagic acid derivative was determined in the highest mass fraction (163.2 mg/kg) in fresh raspberry Willamette fruit, with its mass fraction being almost 2.4 times higher than in strawberry Maya (69.9 mg/kg). Such high mass fraction of ellagic acid in raspberry fruit is in agreement with earlier reported values (49–51). Wild blueberry fruit contained very low mass fraction of the derivative of ellagic acid.

Derivatives of gallic acid and *p*-hydroxybenzoic acid were found in quantities lower than that of ellagic acid.

Table 1. Concentration of phenolic compounds in fruits and jams

Fruits					
Phenolic compounds	$\gamma$ /(mg/L)				
	Sour cherry Marasca	Sour cherry Oblačinska	Strawberry Maya	Raspberry Willamette	Wild blueberry
(+)-catechin	29.20±1.44	11.10±0.95	9.61±0.45	11.10±0.95	22.20±2.13
(-)-epicatechin	95.80±8.05	13.60±1.11	14.40±1.05	13.60±1.11	23.10±1.97
(+)-gallic acid	17.20±2.11	n.d.	n.d.	n.d.	n.d.
<i>Total flavan-3-ols</i>	142.10	24.60	24.10	24.60	45.30
quercetin 3-rutinoside	53.80±6.40	37.60±2.64	6.25±0.72	1.18±0.15	6.25±0.72
kaempferol derivative	29.90±2.95	12.90±1.54	10.30±1.07	n.d.	10.30±1.07
<i>Total flavonols</i>	83.30	50.40	16.50	1.18	16.30
chlorogenic acid	45.90±3.20	28.30±2.15	27.40±1.26	20.20±2.15	38.51±2.12
caffeic acid	15.50±1.77	5.39±0.95	8.12±0.75	14.20±0.95	12.40±1.25
<i>p</i> -coumaric acid	11.30±1.05	12.10±0.71	15.40±1.95	8.96±0.71	5.12±0.65
ferulic acid	1.140±0.15	1.27±0.12	n.d.	4.19±0.12	6.15±0.96
<i>Total HCA</i>	73.80	47.10	51.00	47.60	62.10
gallic acid	n.d.	n.d.	18.40±1.27	13.10±1.02	4.25±0.26
<i>p</i> -hydroxybenzoic acid	n.d.	n.d.	26.20±1.16	19.10±1.15	2.56±0.11
ellagic acid	n.d.	n.d.	69.90±4.25	163.20±7.25	14.20±1.02
<i>Total HBA</i>	0	0	114.40	195.30	21.00
<i>Total phenolic compounds</i>	299.20	122.00	205.80	268.70	144.90
Jams					
Phenolic compounds	$\gamma$ /(mg/L)				
	Sour cherry Marasca	Sour cherry Oblačinska	Strawberry Maya	Raspberry Willamette	Wild blueberry
(+)-catechin	7.35±1.15	0.55±0.17	3.55±0.45	0.55±0.17	5.07±0.35
(-)-epicatechin	13.00±1.10	5.70±0.25	0.14±0.10	5.70±0.25	6.15±0.70
(+)-gallic acid	4.25±0.92	n.d.	n.d.	n.d.	n.d.
<i>Total flavan-3-ols</i>	24.60	6.25	3.69	6.25	11.20
quercetin 3-rutinoside	20.70±2.76	15.10±1.95	2.55±0.42	n.d.	2.55±0.42
kaempferol derivative	14.60±2.05	4.57±0.75	4.30±0.39	n.d.	4.30±0.39
<i>Total flavonols</i>	35.30	19.60	6.85	0	6.85
chlorogenic acid	13.60±2.97	8.15±0.61	9.60±0.97	8.15±0.61	11.90±1.15
caffeic acid	4.25±1.35	0.25±0.10	0.75±0.15	0.25±0.10	4.19±0.55
<i>p</i> -coumaric acid	3.15±0.27	4.35±0.70	6.45±0.55	4.35±0.70	2.15±0.25
ferulic acid	n.d.	tr	n.d.	tr	2.35±0.24
<i>Total HCA</i>	21.00	12.80	16.80	12.80	20.70
gallic acid	n.d.	n.d.	7.11±1.15	6.08±0.95	1.13±0.16
<i>p</i> -hydroxybenzoic acid	n.d.	n.d.	9.15±1.07	7.85±0.92	0.94±0.17
ellagic acid	n.d.	n.d.	28.40±2.10	73.90±5.15	4.97±0.32
<i>Total HBA</i>	0	0	44.60	87.80	7.94
<i>Total phenolic compounds</i>	80.80	38.60	72.00	106.80	45.80

values are means±S.D. (N=3), n.d. – not detected, tr – traces (<0.1 mg/L)

Precisely, the mass fraction of *p*-hydroxybenzoic acid derivatives in strawberry Maya and raspberry Willamette fruits was 26.2 and 19.1 mg/kg, respectively. Additionally, the mass fractions of the gallic acid derivative were even lower (18.4 mg/kg in strawberry Maya and 13.1 mg/kg in raspberry Willamette).

Even though sour cherry Marasca fruit had the highest mass fraction of total polyphenols among fruits, Ma-

rasca jam had the second highest mass fraction of polyphenols among jams, after raspberry Willamette jam. The reason for such result was mostly the instability of flavan-3-ols, especially (-)-epicatechin, which is present in sour cherry Marasca fruit at very high mass fraction and decreased almost seven times in the jam. In raspberry Willamette jams, the highest decrease was noted in the mass fraction of (+)-catechin and derivative of caffeic acid.

Because of the high mass fraction of ellagic acid derivative in raspberry Willamette fruit as well as in jam, the jam had the highest mass fraction of total polyphenols. In the case of strawberry Maya jam, the most unstable phenols were the derivative of caffeic acid and (–)-epicatechin, which decreased almost 10 times, while the ellagic acid derivative decreased approx. twice (similar to raspberry Willamette). According to literature, the process of making strawberry jam decreased the total ellagic acid content by 20 % and the flavonoid content by 15–20 % (50), which is lower than in our case. In sour cherries, mass fractions of (+)-catechin and caffeic acid derivative were about 20 times lower in jams than in raw fruits. In wild blueberry jam, the decrease was only between 2 and 4 times. Generally, our results showed that the most unstable polyphenols during the processing of the investigated fruits into jams were flavan-3-ols and caffeic acid derivative (with the exception of wild blueberry jam).

The percentage distributions of the identified flavan-3-ols, flavonols, HBA and HCA for investigated fruits are shown in Fig. 1. Sour cherries Marasca and Oblačinska were different from other investigated fruits, because there were no HBAs and flavonoids were the main group of polyphenols. Flavan-3-ols were present in higher level than flavonols in Marasca, but this was opposite in Oblačinska. Additionally, HCAs in sour cherries were present in higher level than flavan-3-ols and in the amount similar to flavonols. Distribution of total polyphenols in strawberry Maya and raspberry Willamette was very similar. HBA with ellagic acid was present in the highest

level, followed by HCA, flavan-3-ols and flavonols. HBA with ellagic acid was present in raspberry Willamette at somewhat higher level (72.7 %) than in strawberry Maya (55.6 %). Distribution of polyphenols in wild blueberry was quite different. HCA was present almost at the same level (42.9 %) as total flavonoids (42.5 %), while HBA were present in much lower level (14.5 %).

Despite the lower mass fractions of polyphenols in jams compared to fresh fruits, the percentage distributions of the mentioned polyphenol groups were the same in jams as in fresh fruit (Fig. 1), which means that the stability of polyphenol groups was similar during the production of jams (with the exception of flavan-3-ols and the derivative of caffeic acid). Only in the case of sour cherry Marasca, polyphenol distribution was different in jams when compared to fresh fruit. Sour cherry Marasca fruit contained mostly flavan-3-ols, while the jam contained mostly flavonols.

### Volatile compounds

The results of gas chromatography analysis of the volatile compounds determined in two varieties of sour cherry, strawberry Maya, raspberry Willamette and wild blueberry fruits and jams are shown in Tables 2–5 and expressed as peak area of the identified compounds.

Both investigated sour cherry cultivars contained the same volatile compounds, but Marasca fruit and jam contained more total volatiles than Oblačinska.

Classes of volatile compounds were alcohols (1-hexanol, 1-butanol, 2-phenylethanol), carbonyls (benzaldehyde, hexanal, 2-hexenal, 2-heptanone), esters (ethyl 2-methylbutanoate, ethyl octanoate) and terpenes (linalool, nerol,  $\alpha$ -terpineol). Benzaldehyde was determined in the highest level, followed by hexanal, 2-hexenal, 2-heptanone, linalool, nerol,  $\alpha$ -terpineol (Table 2). According to literature, benzaldehyde is the volatile compound responsible for a typical cherry aroma (15,17) and could be synthesized from amino acids (aromatic amino acid, phenylalanine) (52). Additionally, in peach fruit, it was found that it could be formed by enzymatic hydrolysis from amygdaline, present in the pit (53,54). The fruity, green and apple-like aroma is given by 2-hexenal (13,55). All compounds identified in fruits were determined in jams as well, but at lower levels. Retention of volatiles was between 23.3 and 54.7 % in sour cherry Marasca jam and between 24.2 and 60.6 % in Oblačinska jam. In Marasca jam, compounds with retention higher than 40 % were 2-hexenal, 2-heptanone, linalool, benzaldehyde, nerol, 2-phenylethanol, ethyl octanoate and in Oblačinska jam they were benzaldehyde, 2-phenylethanol, ethyl 2-methyl butanoate, linalool and ethyl-octanoate, in descending order. Considering that 40 g of fruit pulp is needed per 100 g of jams, the retention of the mentioned compounds was almost 100 %, for some compounds even higher.

In strawberry Maya fruit and jam (Table 3) volatiles included alcohols (1-hexanol, 1-octanol and 2-phenylethanol), carbonyl compounds (benzaldehyde and 2-hexenal), esters (methyl butanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, methyl hexanoate, butyl and hexyl acetate and methyl anthranilate), terpenes (linalool,  $\alpha$ -terpineol) and  $\gamma$ -decalactone. Total

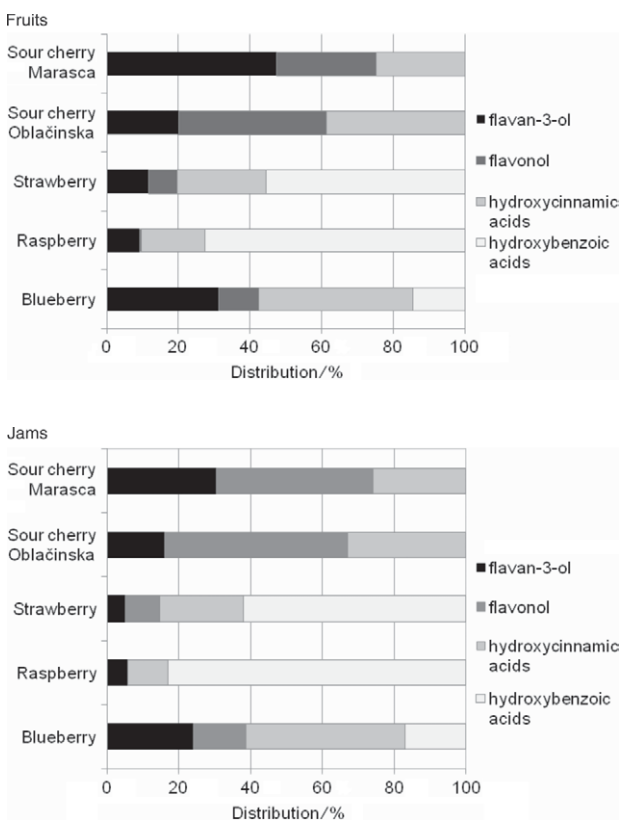


Fig. 1. Distribution of the determined polyphenol classes in the investigated fruits and jams

Table 2. Peak area ratio of identified volatile compounds in fruit and jam of two sour cherry cultivars\*

Compounds	Peak area ratio					
	Sour cherry Marasca			Sour cherry Oblačinska		
	Fruit	Jam	Retention/%	Fruit	Jam	Retention/%
<i>Alcohols</i>						
1-hexanol	0.1469	0.0513	34.90	0.0868	0.0291	33.57
1-butanol	0.0752	0.0175	23.28	0.0633	0.0222	35.12
2-phenylethanol	0.1763	0.0731	41.46	0.1146	0.0633	55.19
<i>Carbonyls</i>						
benzaldehyde	1.6176	0.7502	46.38	1.1174	0.6773	60.61
hexanal	0.4877	0.2665	54.65	0.4254	0.1028	24.16
2-hexenal	0.2529	0.0707	27.93	0.1455	0.0523	35.95
2-heptanone	0.2855	0.1482	51.90	0.2162	0.0779	36.01
<i>Esters</i>						
ethyl 2-methyl butanoate	0.0763	0.0284	37.29	0.0408	0.0198	48.56
ethyl octanoate	0.2113	0.0868	41.07	0.1313	0.0538	41.01
<i>Terpenes</i>						
linalool	1.1056	0.5637	50.99	0.7914	0.3608	45.58
nerol	0.3579	0.1484	41.47	0.2178	0.0795	36.51
$\alpha$ -terpineol	0.4136	0.1392	33.65	0.1740	0.0543	31.18

\*peak area ratio:  $A/IS=(\text{peak area component})/(\text{peak area IS})$ ,  $N=3$ , IS=internal standard used was 3-decanol (0.5 ppm,  $m/V$ )

Table 3. Peak area ratio of identified volatile compounds in strawberry fruit and jam\*

Compounds	Peak area ratio		
	Strawberry Maya		
	Fruit	Jam	Retention/%
<i>Alcohols</i>			
1-hexanol	0.9911	0.1786	18.02
1-octanol	0.7233	0.2557	35.36
2-phenylethanol	0.8876	0.1797	20.25
<i>Carbonyls</i>			
benzaldehyde	0.5852	0.1471	25.13
2-hexenal	0.7829	0.3035	38.77
<i>Esters</i>			
methyl butanoate	0.3837	0.1468	38.26
ethyl butanoate	1.2239	0.5779	47.22
ethyl hexanoate	1.9892	1.1403	57.33
ethyl octanoate	1.4065	0.6520	46.35
ethyl decanoate	1.1246	0.5111	45.45
methyl hexanoate	0.6708	0.2943	43.88
butyl acetate	0.5367	0.2646	49.30
hexyl acetate	1.5245	0.7928	52.00
methyl anthranilate	0.9911	0.4030	40.67
<i>Terpenes</i>			
linalool	1.6513	1.0852	65.72
$\alpha$ -terpineol	1.3509	0.6529	48.33
<i>Lactone</i>			
$\gamma$ -decalactone	2.6615	1.2723	47.80

\*peak area ratio:  $A/IS=(\text{peak area component})/(\text{peak area IS})$ ,  $N=3$ , IS=internal standard used was 3-decanol (0.5 ppm,  $m/V$ )

strawberry Maya aroma is the result of the presence of different compounds such as esters, alcohols and carbonyl compounds. Among them, esters are the most important group because they are responsible for fruity and fresh strawberry flavour. Although over 360 compounds have been identified in the aroma of strawberries (31,56), only a few volatiles (primarily methyl and ethyl esters) appear to be the most important contributors to strawberry aroma (31). It was shown that only about 15 odour-active compounds make an important contribution to strawberry flavour (57). Esters are both quantitatively and qualitatively the most abundant class of aroma compounds and there are 131 different esters identified in strawberry Maya aroma (56). Our results showed that ethyl hexanoate, hexyl acetate and ethyl octanoate were present in the highest levels, which is in accordance with literature data (1). Methyl anthranilate was present in lower level than the mentioned compounds. According to literature, methyl anthranilate is responsible for the typical character of the wood strawberry aroma, characterized by an intensive spicy-aromatic and flowery note (29). Also, the level of methyl anthranilate depends on the cultivar of strawberry (27). Approximately 50 g of strawberry Maya pulp was used to prepare 100 g of jams. Therefore, the jam contained aroma compounds in lower level but the same compounds were determined in the fresh fruit, too. The percentage of retention was between 18.0 % for 1-hexanol and 65.7 % for linalool. Ethyl decanoate (45.5 %), ethyl hexanoate (57.3 %) and hexyl acetate (52.0 %) also had high retention.

Volatile compounds identified in raspberry Willamette fruit and jam were esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate, hexyl acetate and butyl acetate), carbonyls (benzaldehyde) and terpenes (linalool,  $\alpha$ -



-terpineol and eugenol). The presence of the listed terpenes is in accordance with the data from literature (58). The obtained results showed that linalool was present at the highest level (Table 4). Latrasse *et al.* (56) also pointed out the presence of linalool at high concentration (3 ppm) in raspberry. Robertson *et al.* (37) confirmed the presence of linalool in raspberry, but not at the highest concentration, as well as carbonyl compound benzaldehyde. Raspberry Willamette jam contained the same compounds as fresh fruit, but at lower levels. The percentage of retention ranged from 21.0 % (eugenol) to 50.2 % (hexyl acetate). The compounds that had high percentage of retention in strawberry Maya showed the same behaviour in raspberry Willamette, e.g. ethyl hexanoate (44.7 %), hexyl acetate (50.8 %), ethyl decanoate (41.3 %) and linalool (40.6 %).

Table 4. Peak area ratio of identified volatile compounds in raspberry fruit and jam\*

Compounds	Peak area ratio		
	Raspberry Willamette		
	Fruit	Jam	Retention/%
<i>Carbonyl</i>			
benzaldehyde	0.3608	0.0794	22.00
<i>Esters</i>			
ethyl hexanoate	0.7511	0.3358	44.71
ethyl octanoate	0.5943	0.2162	36.38
ethyl decanoate	0.3771	0.1558	41.31
hexyl acetate	0.2127	0.1067	50.18
butyl acetate	0.2151	0.0778	36.17
<i>Terpenes</i>			
linalool	1.9191	0.7791	40.60
$\alpha$ -terpineol	0.4588	0.1758	38.33
eugenol	0.1168	0.0245	20.97

\*peak area ratio:  $A/IS=(\text{peak area component})/(\text{peak area IS})$ ,  $N=3$ , IS=internal standard used was 3-decanol (0.5 ppm,  $m/V$ )

Volatile compounds identified in wild blueberry were alcohols (2-phenylethanol and *cis*-3-hexen-1-ol), esters (ethyl butanoate, ethyl hexanoate and butyl acetate) and terpenes (nerol, geraniol, linalool,  $\alpha$ -terpineol). Forney (59) reported that highbush blueberry (*Vaccinium corymbosum*) aroma is dominated by aromatic hydrocarbons, esters, terpenes and long chain alcohols. Results presented in Table 5 show that esters were quantitatively the most abundant class of volatiles, followed by terpenes. The most abundant compounds were ethyl butanoate and linalool. Di Cesare *et al.* (42) found terpenes to be the most important aroma compounds of blueberry, and linalool to be the most abundant. Differences in the identified volatiles between our study and others may in part be due to the extraction techniques as well as different cultivars (43,60). According to the obtained results, percentage of retention of the determined volatiles was very high, for most volatiles more than 50 % (from 42.80 % for 2-phenylethanol to 88.1 % for butyl acetate). Considering that our recipe required 50 g of wild blue-

Table 5. Peak area ratio of identified volatile compounds in blueberry fruit and jam\*

Compounds	Peak area ratio		
	Wild blueberry		
	Fruit	Jam	Retention/%
<i>Alcohols</i>			
2-phenylethanol	0.3471	0.1486	42.80
<i>cis</i> -3-hexen-1-ol	0.7182	0.3620	50.40
<i>Esters</i>			
ethyl butanoate	1.3904	1.1228	80.75
ethyl hexanoate	1.0112	0.7009	69.31
butyl acetate	0.9012	0.7943	88.14
<i>Terpenes</i>			
nerol	0.0779	0.0474	60.89
geraniol	0.3008	0.1459	48.49
linalool	1.2543	0.6851	54.62
$\alpha$ -terpineol	0.1587	0.0790	49.76

\*peak area ratio:  $A/IS=(\text{peak area component})/(\text{peak area IS})$ ,  $N=3$ , IS=internal standard used was 3-decanol (0.5 ppm,  $m/V$ )

berry pulp to prepare 100 g of jam, such a high retention means that volatiles were very stable during jam production. These results are consistent with previous reports about certain treatments which may cause the increase of some volatiles such as microwave heating (43) and irradiation (61).

## Conclusions

Phenolic acids were the main polyphenol classes in the investigated fruit. Sour cherry cultivars did not contain hydroxybenzoic acids. The three most abundant compounds found in sour cherry cultivars were (–)-epicatechin (especially in sour cherry Marasca), and chlorogenic acid and quercetin derivatives (especially in Oblačinska). Ellagic acid was the main phenolic compound in raspberry Willamette and strawberry Maya. The derivative of chlorogenic acid was the main polyphenolic compound in wild blueberry. (+)-Gallocatechin was found only in sour cherry Marasca.

All determined polyphenol compounds were found in lower concentrations in jams than in fresh fruits, but when the recipe for jam was taken into consideration (50 g of fresh fruit per 100 g of jam), their retention was good.

High level of retention is observed for derivatives of flavonols (quercetin and kaempferol) and some phenolic acids (ellagic acid and *p*-coumaric acid derivative) in all the investigated jams (especially ellagic acid in raspberry Willamette jam and flavonols in sour cherry Marasca jam). Among all the investigated samples, sour cherry Marasca and raspberry Willamette fruits and jams contained the highest level of polyphenol compounds. Since polyphenols are known for their high antioxidant activity, all jams, especially the ones made from raspberry Willamette and sour cherry Marasca, could be a good source of biologically active compounds.

Among volatiles, benzaldehyde was the most abundant in sour cherries,  $\gamma$ -decalactone in strawberry Maya, linalool in raspberry Willamette and butyl acetate in wild blueberry. Based on the retention of volatiles, it could be concluded that all jams were high-quality products. Sour cherry Marasca fruit and jam had higher level of volatiles in comparison with sour cherry Oblačinska.

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