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## The phenolic profile of strawberry tree (*Arbutus unedo* L.) honey

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**Abstract:** Despite of the many beneficial health effects of strawberry tree (*Arbutus unedo* L.) honey due to its strong antioxidant activity derived mostly from polyphenols, a detailed phenolic profile has not been previously studied. The aims of this study were to identify the phenolic compounds, determine the total phenolic content (TPC) and evaluate the radical scavenging activity (RSA) of strawberry tree honey from south Croatia. Fifty-two polyphenolics (twenty-seven phenolic acids and twenty-five flavonoids) were identified using ultra-high-performance liquid chromatograph (UHPLC) coupled to a hybrid mass spectrometer (LTQ Orbitrap MS). Our overall results point to higher TPC (1038 mg gallic acid equivalents per kg of honey) and stronger RSA (3.32 mmol Trolox equivalents per kg of honey) compared to other monofloral honeys. Due to the presence of large quantities of polyphenolic compounds, strawberry tree honey may have great potential as a health promoting food.

**Keywords:** Polyphenolics; UHPLC-LTQ Orbitrap MS; TPC; RSA

### INTRODUCTION

Strawberry tree (*Arbutus unedo* L.) is a wild evergreen shrub that typically grows in the Mediterranean area. All of its plant parts (leaves, fruits, bark, and root) have been used in folk medicine as an antiseptic, diuretic, and laxative, as well as for treating cardiovascular, urological and gastrointestinal diseases.<sup>1</sup> This plant has also shown significant antiproliferative properties and its health benefits are mainly attributed to phenolic compounds such as flavonoids, phenolic acids, and tannins.<sup>1–3</sup>

In addition, it is the floral source of strawberry tree honey, known as “bitter honey”, and produced in Sardinia, Corsica, some parts of Spain, Portugal, and Croatia.<sup>4,5</sup> The characterization of this rare honey is a very challenging task

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because of the low pollen content. *Arbutus* pollen is under-represented due to the upside-down position of the flowers and, therefore, melissopalynological analysis should be carefully performed and combined with sensory and physicochemical characteristics.<sup>6,7</sup> Although strawberry tree honey is not described in the descriptive sheets of the main European unifloral honeys, its characteristic physicochemical parameters are given.<sup>8</sup> This unifloral honey is very dark, shows high values of water and acidity and a low value of diastase activity. The European Directive concerning honey (2001/110/CE) includes *Arbutus* honey in a group whose electrical conductivity may go beyond the 0.8 mS cm<sup>-1</sup> limit.<sup>8</sup>

Our previous study has suggested that strawberry tree honey consumption improved antioxidative status, increased serum iron level, decreased activity of liver enzymes, and increased leukocyte and platelet counts.<sup>9</sup> A significant decrease in DNA damage in leukocytes of almost all participants who consumed strawberry tree honey was observed after an *ex vivo* challenge with H<sub>2</sub>O<sub>2</sub> compared to the control group with no honey supplementation.<sup>10</sup> Therefore, it is recognised as a health-promoting food due to its strong antioxidant activity mainly attributed to high polyphenol contents. The limited production and respected biological properties make this honey particularly appreciated.<sup>11</sup>

Although honeys of different botanical origin, namely polyfloral,<sup>12</sup> lime,<sup>13</sup> sage,<sup>14</sup> acacia, sunflower, linden, basil, buckwheat, oilseed rape, and goldenrod<sup>15</sup> have previously been characterized on the basis of their phenolic fraction, to the best of the authors' knowledge no previous studies regarding the detailed polyphenolic profile of strawberry tree honey has been published.

The aim of this study was to determine, for the first time, a characteristic polyphenolic profile of strawberry tree honey by the UHPLC-LTQ Orbitrap MS technique. In addition, the TPC and RSA of honey sample were determined.

## EXPERIMENTAL

### *Chemicals*

Acetonitrile and acetic acid (both MS grade), gallic acid, phenolic standards, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (Steinheim, Germany), while 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) was purchased from Fluka AG (Buch, Switzerland). Folin-Ciocalteu reagent, hydrochloric acid, methanol (HPLC grade), and sodium carbonate were obtained from Merck (Darmstadt, Germany). Standard solutions and dilutions were prepared using ultrapure water (TKA Germany MicroPure water purification system, 0.055 µS cm<sup>-1</sup>). Syringe filters (25 mm, nylon membrane 0.45 µm) were purchased from Supelco (Bellefonte, PA, USA). The cartridges for solid-phase extraction (SPE) were Strata C18-E (500 mg / 3 mL) obtained from Phenomenex (Torrance, CA, USA).

### *Sample*

Honey was collected in Dalmatia, Pelješac peninsula (Croatia) in 2014. Detailed melissopalynological and sensory assessments of the honey sample were performed. Apart from strawberry tree pollen found at 7 % in the analysed sample, the rest of the identified

pollen originated from species belonging to the families Ericaceae, Cistaceae, Fagaceae, Lamiaceae, Oleaceae, and Amaryllidaceae. The strawberry tree honey's botanical origin was additionally confirmed by determining the specific chemical marker homogentisic acid (HGA). The mass fraction of HGA in this honey sample determined by gas chromatography-mass spectrometry was 280.6 mg kg<sup>-1</sup>.<sup>16</sup>

#### *Total phenolic content (TPC)*

The TPC determined by a modified method reported by Gašić *et al.*<sup>12</sup> Fifty µL of honey diluted with ultrapure water (1:10, w/v) was mixed with 1.4 mL of ultrapure water and 100 µL of 2 M Folin-Ciocalteu reagent. The reaction mixture was incubated at room temperature for 5 min and mixed with 1.5 mL of sodium carbonate solution (6 % w/v). Absorbance was measured at 765 nm after 30 min at 40 °C on Cary 50 UV-Vis spectrophotometer (Varian, Mulgrave, Australia) and the results were expressed as mg of gallic acid equivalents (GAE) per kg of honey.

#### *DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging activity (RSA)*

RSA was determined by a method proposed by Tariba Lovaković *et al.*<sup>11</sup> One hundred µL of honey diluted with ultrapure water (1:10, w/v) was mixed with 1.9 mL of methanol. Then, 1.5 mL of DPPH methanolic solution (0.18 mM) was added and vortexed vigorously. The mixture was incubated in the dark for 30 min at 25 °C. The absorbance was measured at 517 nm on a Cary 50 UV-Vis spectrophotometer (Varian, Mulgrave, Australia) and the results were expressed as mmol of the Trolox equivalents (TE) per kg of honey.

#### *Honey sample preparation for the analysis of polyphenolic compounds*

The honey sample (5 g) was mixed with 5 mL of ultrapure water, adjusted to pH 2 with 0.1 % hydrochloric acid and homogenised in an ultrasonic bath (30 min at room temperature). The sample was filtered through filter paper. An SPE cartridge was conditioned (3 mL of acetonitrile and 9 mL of ultrapure water). The filtrate was passed through a cartridge, which was then washed with 6 mL of acidified water to remove all sugars and other polar constituents of honey. The adsorbed compounds were eluted with acetonitrile (1.5 mL). The extracts were filtered through a 0.45 µm PTFE membrane filter before analysis.

#### *Analysis of polyphenolic compounds*

Analyses were carried out using Accela UHPLC system connected to a hybrid mass spectrometer (LTQ Orbitrap MS) with a HESI (heated electrospray ionization) probe (Thermo Fisher Scientific, Bremen, Germany). The analytical column used for separation was Syncronis C18 (100 × 2.1 mm, 1.7 µm particle size). The mobile phase consisted of (A) water containing 0.01 % acetic acid and (B) acetonitrile. The gradient program was as follows: 0.0 - 1.0 min 5 % B, 1.0–16.0 min from 5 to 95 % (B), 16.0–16.1 min from 95 to 5 % (B), then 5 % (B) for 4 min. Flow rate was set to 0.300 mL min<sup>-1</sup> and the injection volume 5 µL.<sup>17</sup>

The mass spectrometer was operated in negative ionisation mode covering a range from 100 to 1000 *m/z*. Ion source parameters were determined as previously described by Gašić *et al.*<sup>14</sup> The ions of interest were isolated in the ion trap and activated with 35 % collision energy levels (CEL). Full scan analysis was employed to calculate the monoisotopic mass of unknown compounds, while the fragmentation pathway was obtained by MS<sup>n</sup>. Phenolics were identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation and characteristic retention time.<sup>17</sup>

## RESULTS AND DISCUSSION

*Polyphenolic profile*

Fifty-two polyphenolics (twenty-seven derivatives of phenolic acids and twenty-five flavonoid glycosides and aglycones) were identified according to their  $[M-H]^-$  exact masses (Table I) and fragmentation pattern (Table S-I). The base peak chromatogram of *A. unedo* honey polyphenolics is shown in Figure 1.

TABLE I. Phytochemical fingerprint of strawberry tree (*Arbutus unedo* L.) honey from Croatia using UHPLC-LTQ Orbitrap MS.

No	Compound name	$t_R$ / min	Molecular formula, $[M-H]^-$	Calculated mass, $[M-H]^-$	Exact mass, $[M-H]^-$	$\Delta$ / ppm
<i>Phenolic acids and their derivatives</i>						
1	Gallic acid <sup>a</sup>	2.75	C <sub>7</sub> H <sub>5</sub> O <sub>5</sub> <sup>-</sup>	169.0143	169.0134	4.9
2	Protocatechuic acid hexoside	4.10	C <sub>13</sub> H <sub>15</sub> O <sub>9</sub> <sup>-</sup>	315.0722	315.0708	4.4
3	Vanillic acid hexoside	4.44	C <sub>14</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	329.0878	329.0862	4.8
4	Protocatechuic acid <sup>a</sup>	4.46	C <sub>7</sub> H <sub>5</sub> O <sub>4</sub> <sup>-</sup>	153.0193	153.0187	4.3
5	Chlorogenic acid hexoside	4.54	C <sub>22</sub> H <sub>27</sub> O <sub>14</sub> <sup>-</sup>	515.1401	515.1392	1.8
6	Hydroxybenzoic acid hexoside	4.69	C <sub>13</sub> H <sub>15</sub> O <sub>8</sub> <sup>-</sup>	299.0772	299.0760	4.3
7	Caffeic acid hexoside	5.01	C <sub>15</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	341.0878	341.0862	4.8
8	Aesculin <sup>a</sup>	5.02	C <sub>15</sub> H <sub>15</sub> O <sub>9</sub> <sup>-</sup>	339.0722	339.0711	3.0
9	<i>p</i> -Hydroxybenzoic acid <sup>a</sup>	5.32	C <sub>7</sub> H <sub>5</sub> O <sub>3</sub> <sup>-</sup>	137.0244	137.0239	3.8
10	5- <i>O</i> -Caffeoylquinic acid <sup>a</sup>	5.38	C <sub>16</sub> H <sub>17</sub> O <sub>9</sub> <sup>-</sup>	353.0878	353.0864	4.0
11	Coumaric acid hexoside	5.50	C <sub>15</sub> H <sub>17</sub> O <sub>8</sub> <sup>-</sup>	325.0929	325.0913	5.0
12	Caffeic acid <sup>a</sup>	5.71	C <sub>9</sub> H <sub>7</sub> O <sub>4</sub> <sup>-</sup>	179.0350	179.0343	4.0
13	Vanillic acid <sup>a</sup>	5.74	C <sub>8</sub> H <sub>7</sub> O <sub>4</sub> <sup>-</sup>	167.0350	167.0342	4.6
14	Ferulic acid hexoside	5.76	C <sub>16</sub> H <sub>19</sub> O <sub>9</sub> <sup>-</sup>	355.1035	355.1024	2.9
15	Syringic acid <sup>a</sup>	5.83	C <sub>9</sub> H <sub>9</sub> O <sub>5</sub> <sup>-</sup>	197.0456	197.0446	4.7
16	Caffeoylshikimic acid	5.99	C <sub>16</sub> H <sub>15</sub> O <sub>8</sub> <sup>-</sup>	335.0772	335.0762	3.1
17	5- <i>O-p</i> -Coumaroylquinic acid	6.01	C <sub>16</sub> H <sub>17</sub> O <sub>8</sub> <sup>-</sup>	337.0929	337.0916	4.0
18	<i>p</i> -Coumaric acid <sup>a</sup>	6.51	C <sub>9</sub> H <sub>7</sub> O <sub>3</sub> <sup>-</sup>	163.0401	163.0393	4.7
19	Vanillin <sup>a</sup>	6.83	C <sub>8</sub> H <sub>7</sub> O <sub>3</sub> <sup>-</sup>	151.0401	151.0395	4.0
20	Sinapic acid <sup>a</sup>	6.85	C <sub>11</sub> H <sub>11</sub> O <sub>5</sub> <sup>-</sup>	223.0612	223.0601	4.9
21	Ferulic acid <sup>a</sup>	6.86	C <sub>10</sub> H <sub>9</sub> O <sub>4</sub> <sup>-</sup>	193.0506	193.0497	4.6
22	Dicaffeoylquinic acid	7.33	C <sub>25</sub> H <sub>23</sub> O <sub>12</sub> <sup>-</sup>	515.1195	515.1179	3.1
23	Coniferyl aldehyde <sup>a</sup>	7.80	C <sub>10</sub> H <sub>9</sub> O <sub>3</sub> <sup>-</sup>	177.0557	177.0549	4.9
24	Caffeoylcoumaroylquinic acid	7.94	C <sub>25</sub> H <sub>23</sub> O <sub>11</sub> <sup>-</sup>	499.1240	499.1233	1.4
25	Methyl dicaffeoylquinic acid	8.03	C <sub>26</sub> H <sub>25</sub> O <sub>12</sub> <sup>-</sup>	529.1352	529.1339	2.5
26	Cinnamic acid <sup>a</sup>	8.84	C <sub>9</sub> H <sub>7</sub> O <sub>2</sub> <sup>-</sup>	147.0452	147.0446	3.5
27	<i>p</i> -Coumaric acid methyl ester	9.12	C <sub>10</sub> H <sub>9</sub> O <sub>3</sub> <sup>-</sup>	177.0557	177.0550	3.8
<i>Flavonoids and their derivatives</i>						
28	Prodelphinidin dimer B type	4.35	C <sub>30</sub> H <sub>25</sub> O <sub>13</sub> <sup>-</sup>	593.1301	593.1288	2.2
29	Catechin <sup>a</sup>	5.45	C <sub>15</sub> H <sub>13</sub> O <sub>6</sub> <sup>-</sup>	289.0718	289.0706	4.2
30	Procyanidin dimer B type	5.63	C <sub>30</sub> H <sub>25</sub> O <sub>12</sub> <sup>-</sup>	577.1352	577.1334	3.1
31	Epicatechin <sup>a</sup>	5.86	C <sub>15</sub> H <sub>13</sub> O <sub>6</sub> <sup>-</sup>	289.0718	289.0704	4.8
32	Isorhamnetin	6.19	C <sub>28</sub> H <sub>31</sub> O <sub>17</sub> <sup>-</sup>	639.1567	639.1545	3.4
	3- <i>O</i> -(2"-hexosyl)-hexoside					

No	Compound name	$t_R$ / min	Molecular formula, [M-H] <sup>-</sup>	Calculated mass, [M-H] <sup>-</sup>	Exact mass, [M-H] <sup>-</sup>	$\Delta$ / ppm
33	Kaempferol 3- <i>O</i> -(2"-hexosyl)-hexoside	6.26	C <sub>27</sub> H <sub>29</sub> O <sub>16</sub> <sup>-</sup>	609.1461	609.1443	3.0
34	Quercetin 3- <i>O</i> -(6"-rhamnosyl)-glucoside (Rutin) <sup>a</sup>	6.45	C <sub>27</sub> H <sub>29</sub> O <sub>16</sub> <sup>-</sup>	609.1461	609.1431	4.9
35	Apigenin 8- <i>C</i> -glucoside (Vitexin) <sup>a</sup>	6.56	C <sub>21</sub> H <sub>19</sub> O <sub>10</sub> <sup>-</sup>	431.0984	431.0965	4.4
36	Quercetin 3- <i>O</i> -galactoside (Hyperoside) <sup>a</sup>	6.70	C <sub>21</sub> H <sub>19</sub> O <sub>12</sub> <sup>-</sup>	463.0882	463.0869	2.9
37	Kaempferol 7- <i>O</i> -(6"-rhamnosyl)-hexoside	6.82	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub> <sup>-</sup>	593.1512	593.1497	2.5
38	Isorhamnetin 3- <i>O</i> -(6"-rhamnosyl)-hexoside	6.92	C <sub>28</sub> H <sub>31</sub> O <sub>16</sub> <sup>-</sup>	623.1618	623.1594	3.9
39	Quercetin 3- <i>O</i> -pentoside	6.97	C <sub>20</sub> H <sub>17</sub> O <sub>11</sub> <sup>-</sup>	433.0776	433.0755	4.8
40	Naringenin 7- <i>O</i> -(2"-rhamnosyl)-glucoside (Naringin) <sup>a</sup>	7.01	C <sub>27</sub> H <sub>31</sub> O <sub>14</sub> <sup>-</sup>	579.1719	579.1692	4.7
41	Isorhamnetin 3- <i>O</i> -hexoside	7.02	C <sub>22</sub> H <sub>21</sub> O <sub>12</sub> <sup>-</sup>	477.1039	477.1025	2.8
42	Kaempferol 3- <i>O</i> -glucoside (Astragalin) <sup>a</sup>	7.08	C <sub>21</sub> H <sub>19</sub> O <sub>11</sub> <sup>-</sup>	447.0933	447.0922	2.4
43	Kaempferol 3- <i>O</i> -acetylhexoside	7.98	C <sub>23</sub> H <sub>21</sub> O <sub>12</sub> <sup>-</sup>	489.1039	489.1030	1.7
44	Luteolin <sup>a</sup>	8.52	C <sub>15</sub> H <sub>9</sub> O <sub>6</sub> <sup>-</sup>	285.0405	285.0392	4.5
45	Quercetin <sup>a</sup>	8.62	C <sub>15</sub> H <sub>9</sub> O <sub>7</sub> <sup>-</sup>	301.0354	301.0339	4.9
46	Naringenin <sup>a</sup>	9.31	C <sub>15</sub> H <sub>11</sub> O <sub>5</sub> <sup>-</sup>	271.0612	271.0599	4.7
47	Kaempferol <sup>a</sup>	9.49	C <sub>15</sub> H <sub>9</sub> O <sub>6</sub> <sup>-</sup>	285.0405	285.0392	4.4
48	Isorhamnetin	9.52	C <sub>16</sub> H <sub>11</sub> O <sub>7</sub> <sup>-</sup>	315.0510	315.0498	4.0
49	Rhamnetin	9.69	C <sub>16</sub> H <sub>11</sub> O <sub>7</sub> <sup>-</sup>	315.0510	315.0499	3.8
50	Chrysin <sup>a</sup>	11.3	C <sub>15</sub> H <sub>9</sub> O <sub>4</sub> <sup>-</sup>	253.0506	253.0494	4.9
51	Pinocembrin <sup>a</sup>	11.44	C <sub>15</sub> H <sub>11</sub> O <sub>4</sub> <sup>-</sup>	255.0663	255.0651	4.7
52	Galangin <sup>a</sup>	11.60	C <sub>15</sub> H <sub>9</sub> O <sub>5</sub> <sup>-</sup>	269.0455	269.0443	4.7

<sup>a</sup>Confirmed using reference standards;  $t_R$  / min – retention time;  $\Delta$  / ppm – mean mass accuracy; Major MS<sup>2</sup>, MS<sup>3</sup> and MS<sup>4</sup> fragment ions are summarized in Table S-I.

The presence of twenty-eight compounds was confirmed by comparison with commercial analytical standards, while the other twenty-four compounds were identified using high resolution mass spectrometry (HRMS) in combination with MS<sup>4</sup> fragmentation. Phenolic acids were represented as hydroxy derivatives of benzoic and cinnamic acids. In addition to free phenolic acids, some of their derivatives were identified in the form of hexosides (loss of 162 Da) and esters with quinic acid and shikimic acid. In addition to twenty-three identified flavonoids (twelve of them were glycosides and eleven were aglycones), two B type proanthocyanidins were also identified. It is interesting to note that only one flavonoid C-glycoside was identified in this honey sample, namely vitexin (apigenin 8-*C*-glucoside), and its presence was confirmed by an appropriate

standard. The other flavonoid glycosides were identified as *O*-glycosides, mainly with the glycosidic unit at 3-*O* position. Two compounds (**37** and **40**) were identified as 7-*O* glycosides, showing specific fragmentation to support this claim.<sup>18</sup>

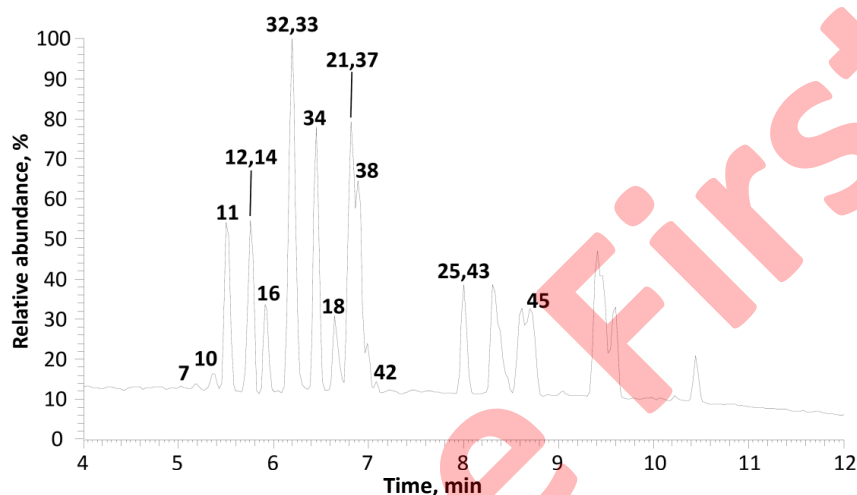
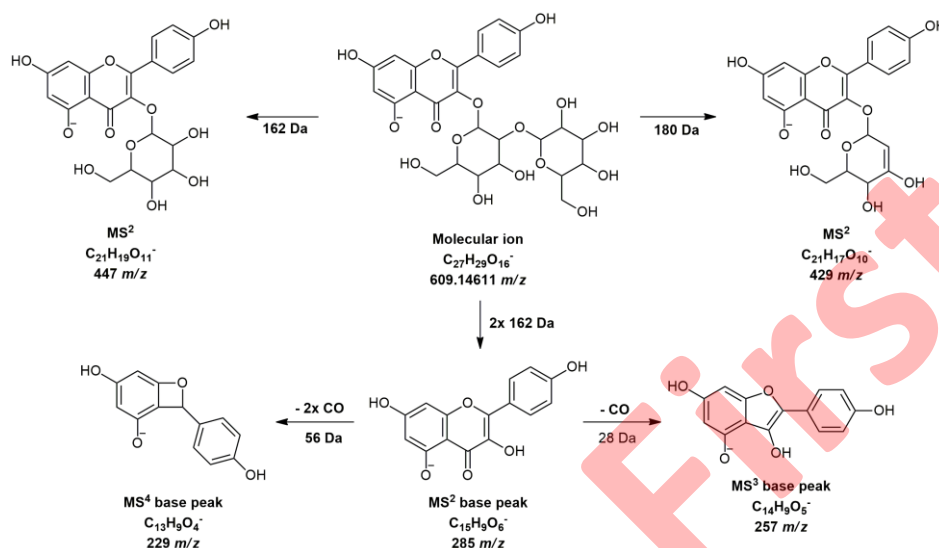


Figure 1. Base peak chromatogram of polyphenolics identified in strawberry tree (*Arbutus unedo* L.) honey (peak number corresponding to Table I).

The base peak chromatogram (Figure 1) shows the peaks of the most represented phenolic compounds found in investigated honey. Thus, compound **32** (639  $m/z$  and 6.19 min) showing an  $MS^2$  base peak at 315  $m/z$  and  $MS^3$  base peak at 300  $m/z$  (Table S-I) was identified as methoxy kaempferol 3-*O*-(2''-hexosyl)-hexoside. The fragmentation of this compound has already been described in the literature, as it has been identified in honeydew honey from Croatia.<sup>17</sup> The second most abundant compound (**33**) found in investigated honey at a retention time 6.26 min and molecular ion 609  $m/z$ , was identified as kaempferol 3-*O*-(2''-hexosyl)-hexoside. It gave an  $MS^2$  base peak at 285  $m/z$  (deprotonated kaempferol) and a secondary  $MS^2$  peak at 447  $m/z$  ( $[M-H-162]^-$ ) and 429  $m/z$  ( $[M-H-180]^-$ ). The presence of fragment ion at 429  $m/z$  ( $[M-H-162-18]^-$ ) indicated that the interglycosidic linkage between the two sugars in this glycoside is type 1→2.<sup>19</sup> The detailed fragmentation pathway of compound **33** is depicted in Figure 2.





**Figure 2.** Proposed fragmentation pathway of compound 33 (kaempferol 3-*O*-(2''-hexosyl)-hexoside).

Phenolic compounds in strawberry tree honey have previously been identified only in hydrolysed honey extracts by high performance liquid chromatography with a diode array detector (HPLV-DAD) that revealed two phenolic acids and seven flavonoids.<sup>20</sup> The identified phenolic compounds that correspond to those previously described and HGA content higher than 200 mg kg<sup>-1</sup>, as proposed by Cabras *et al.*<sup>6</sup> and confirmed by Brčić Karačonji and Jurica,<sup>16</sup> contributed to the confirmation of the botanical origin of strawberry tree honey despite its low pollen content.

#### Total phenolic content and radical scavenging activity

Strawberry tree honey showed high total phenolic content (1038 mg GAE kg<sup>-1</sup>) and strong DPPH scavenging activity (3.32 mmol TE kg<sup>-1</sup>) in accordance with previous studies.<sup>4,5,21–27</sup> Moreover, strawberry tree honey was the richest in total phenols when compared to other honeys (*e.g.* eucalyptus, sunflower, lavender, thyme, rosemary, orange, lime, acacia, black locust, coriander, chestnut, asphodel, thistle).<sup>21,23–25,27,28</sup>

#### CONCLUSION

Hyphenated techniques that combine chromatographic with high resolution and high mass accuracy spectral methods, such as UHPLC-LTQ OrbiTrap MS<sup>4</sup>, are very useful in getting information about phenolic structures with high reliability. Using this technique, large numbers of phenolic acids and their derivatives as well as flavonoid aglycones and flavonoid glycosides in *A. unedo* honey were determined. The obtained phenolic profile can be used for further

analysis of the content of the particular phenolic substances in strawberry tree honey for the purpose of its complete characterization.

Our results indicate that strawberry tree honey has great potential as a health promoting food due to the presence of a large number of phenolic compounds.

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#### SUPPLEMENTARY MATERIAL

Supplementary material is available from <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

#### ИЗВОД

#### ПОЛИФЕНОЛНИ ПРОФИЛ МЕДА ОД ОБИЧНЕ ПЛАНИКЕ (*Arbutus unedo* L.)

АНДРЕЈА ЈУРИЧ, УРОШ ГАШИЋ<sup>1</sup>, ИРЕНА БРКИЋ КАРАЧОЊИ, КАРЛО ЈУРИЦА<sup>2</sup> и  
ДУШАНКА МИЛОЈКОВИЋ ОПСЕНИЦА<sup>3</sup>

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Упркос многим благотворним здравственим ефектима меда од обичне планике (*Arbutus unedo* L.) због снажног антиоксидативног деловања које потиче највећим делом од полифенола, детаљни полифенолни профил овог меда није претходно проучен. Циљеви овог рада били су идентификација полифенолних једињења, одређивање садржаја укупних фенола и процена антиоксидативне активности меда од обичне планике из јужног дела Хрватске. Педесет два полифенола (двадесет и седам фенолних киселина и двадесет пет флавоноида) идентификовано је ултра-високоефикасном течном хроматографијом (UHPLC) повезаном са хибридном масеним спектрометром (LTQ Orbitrap MS). Наши резултати указују на већи садржај укупних фенола (1038 mg еквивалента галне киселине по kg меда), као и знатну антиоксидативну активност (3,32 mmol Trolox еквивалента по kg меда) у поређењу са другим монофлоралним медовима. Због присуства велике количине полифенолних једињења, мед од обичне планике може имати велики потенцијал као храна благотворна за здравље.

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On Line First

SUPPLEMENTARY MATERIAL TO  
**The phenolic profile of strawberry tree (*Arbutus unedo* L.) honey**

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Table S-I Negative ion MS<sup>4</sup> fragmentation data for the phenolics identified in *A. unedo* honey.

No	Molecular ion, m/z	MS <sup>2</sup> Fragments, m/z (% Base Peak)	MS <sup>3</sup> Fragments, m/z (% Base Peak)	MS <sup>4</sup> Fragments, m/z (% Base Peak)
<i>Phenolic acids and their derivatives</i>				
1	169	125(100)	107(100)	ND
2	315	153(100), 152(50), 109(15), 108(10)	109(100)	84(100), 81(60)
3	329	167(100)	152(100), 123(70), 108(20)	124(5), 108(100)
4	153	109(100), 95(75), 79(20), 59(10)	81(100), 68(25), 65(15)	ND
5	515	353(100), 341(5), 323(10), 191(90), 179(5)	191(100), 179(10)	173(65), 127(80), 111(30), 85(100)
6	299	137(100)	93(10)	ND
7	341	179(100), 161(35), 135(10)	135(100)	135(10), 117(15), 107(100), 91(35)
8	339	177(100)	177(5), 149(10), 133(100), 105(10), 89(5)	89(100)
9	137	109(10), 93(100)	93(100)	ND
10	353	191(100), 179(5)	173(75), 127(100), 111(40), 93(60), 85(90)	109(30), 99(60), 85(100)
11	325	163(80), 145(100), 119(10)	117(100)	ND
12	179	135(100), 117(10), 91(20), 59(15)	107(100), 59(50)	ND
13	167	153(10), 152(80), 124(10), 123(100), 108(20)	108(100)	123(30), 80(35), 78(100)

No	Molecular ion, <i>m/z</i>	MS <sup>2</sup> Fragments, <i>m/z</i> (% Base Peak)	MS <sup>3</sup> Fragments, <i>m/z</i> (% Base Peak)	MS <sup>4</sup> Fragments, <i>m/z</i> (% Base Peak)
14	355	217(60), 193(100), 175(40), 134(10)	178(20), 149(40), 134(100)	134(50), 106(100)
15	197	182(100), 153(50), 138(10)	167(100), 138(10), 123(5)	123(100)
16	335	179(100), 135(25)	135(100)	107(100)
17	337	191(100), 179(5), 163(10)	173(75), 127(100), 111(40), 93(60), 85(90)	109(30), 99(40), 85(100)
18	163	119(100)	119(60), 101(20), 93(25), 91(100), 72(10)	ND
19	151	136(100)	108(25), 92(100)	108(100)
20	223	208(100), 179(30), 164(20)	193(10), 164(100), 149(15), 135(5)	149(100), 135(35)
21	193	178(70), 149(100), 134(50)	134(100)	106(100)
22	515	353(100)	191(100), 179(40), 135(10)	173(100), 127(50), 111(40), 85(70)
23	177	163(10), 162(100)	134(100), 133(40), 120(20), 106(30)	106(100), 65(80)
24	499	361(5), 337(100), 163(10)	191(10), 173(60), 163(100), 119(10)	119(100)
25	529	367(100), 349(10), 179(10), 161(10)	193(10), 191(25), 179(100), 161(80), 135(60)	135(100)
26	147	104(10), 103(100), 87(10)	119(100)	ND
27	177	177(10), 162(40), 145(100), 118(50)	177(100)	ND
<i>Flavonoids and their derivatives</i>				
28	593	467(15), 425(100), 407(30), 289(20)	407(100), 281(5), 273(10)	389(30), 297(30), 285(100), 243(70)
29	289	271(5), 245(100), 205(40), 179(15), 125(5)	227(30), 203(100), 187(25), 175(10), 161(20)	188(70), 185(20), 175(100), 161(40), 157(10)
30	577	451(15), 425(100), 407(40), 289(20), 287(10)	407(100), 281(5), 273(10)	389(30), 297(30), 285(100), 243(70)
31	289	271(5), 245(100), 205(40), 179(15), 125(5)	227(35), 203(100), 187(30), 175(15), 161(25)	188(60), 185(20), 175(100), 161(35), 157(15)
32	639	459(65), 315(100), 314(70), 300(60), 299(50)	300(100)	271(100), 255(55), 165(15)
33	609	447(10), 429(80), 285(100), 284(70), 255(20)	257(100), 241(50), 229(40), 213(30), 151(70)	255(10), 239(30), 229(100), 163(40)
34	609	343(5), 301(100), 300(30), 271(10), 255(5)	273(25), 257(20), 179(100), 151(75)	151(100)

No	Molecular ion, $m/z$	MS <sup>2</sup> Fragments, $m/z$ (% Base Peak)	MS <sup>3</sup> Fragments, $m/z$ (% Base Peak)	MS <sup>4</sup> Fragments, $m/z$ (% Base Peak)
35	431	341(20), 311(100)	283(100)	283(80), 239(100), 183(70)
36	463	301(100), 300(30)	273(25), 257(20), 179(100), 151(75)	151(100)
37	593	285(100)	267(40), 257(100), 241(30), 229(40), 213(30)	255(10), 239(30), 229(100), 163(40)
38	623	315(100), 300(20), 271(10), 255(5)	300(100), 287(5), 272(5)	271(100), 255(50), 151(5)
39	433	343(5), 301(80), 300(1000)	271(100), 255(60), 179(10), 151(10)	243(100), 227(80), 215(20), 199(20)
40	579	459(100), 357(5), 313(25), 271(45), 235(10)	441(30), 357(100), 339(30), 271(55), 235(85)	339(100), 169(20), 151(50), 125(20)
41	477	462(40), 315(100), 314(30), 300(25), 299(25)	300(100), 299(5)	272(100), 271(60), 255(80)
42	447	327(20), 285(80), 284(100), 255(10)	255(100), 227(10)	227(100), 211(60)
43	489	285(50), 284(100), 255(20), 227(10)	257(100), 241(50), 229(40), 213(30), 151(70)	255(10), 239(30), 229(100), 163(40)
44	285	257(40), 241(100), 217(50), 199(70), 175(70)	255(50), 227(100), 211(75), 197(35), 183(85)	ND
45	301	271(50), 255(20), 179(100), 151(80), 107(5)	151(100)	107(100), 83(10)
46	271	225(5), 177(10), 151(100)	107(100)	65(100)
47	285	255(100), 227(10)	211(100), 195(5), 167(15)	211(40), 137(100)
48	315	301(20), 300(100)	283(40), 271(80), 255(30), 227(30), 151(100)	107(100), 83(15)
49	315	301(20), 300(100)	283(10), 271(50), 255(100), 227(25), 165(30)	227(100), 200(15), 183(10)
50	253	253(30), 209(100), 181(20), 165(15), 151(15)	181(100), 165(30), 153(20), 141(10)	171(10), 153(100), 152(10), 139(50), 185(10), 157(15),
51	255	213(100), 187(15), 151(30), 145(10), 107(5)	185(100), 169(20), 145(20)	143(100), 141(50), 117(15)
52	269	241(40), 227(80), 213(100), 197(90), 169(50)	211(10), 198(20), 185(40), 169(100), 143(25)	ND

ND – not detected.