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Multi-elemental composition and antioxidant properties of strawberry tree (*Arbutus unedo* L.) honey from the coastal region of Croatia: Risk-benefit analysis

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ABSTRACT

The concentration of 23 major and trace elements, total phenolic content (TPC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were determined in nine samples of strawberry tree honey and compared to other types of unifloral honeys. The most abundant elements in strawberry tree honey were potassium, calcium, magnesium and sodium, ranging between 1276 and 2367, 95.2–154, 14.4–74.4 and 13.4–64.3 mg/kg, respectively. Strawberry tree honey had generally higher TPC (range: 0.314–0.522 g GA/kg) and DPPH (1.94–4.45 mM TE/kg) compared to other analysed unifloral honeys. A strong positive relationship was found between TPC and DPPH, TPC and concentration of homogentisic acid (HGA), chemical marker of strawberry tree honey, and between DPPH and HGA. Regarding daily intake of essential elements, strawberry tree honey can be considered nutritionally richer than the majority of unifloral honeys available in Croatia, while contribution to tolerable intake set for potentially toxic elements was very low, corresponding to pristine areas.

1. Introduction

Strawberry tree (*Arbutus unedo* L.) honey is a product of Mediterranean origin, typical for certain regions of Italy, Portugal, Spain and Croatia. The low production and reputed biological properties of this typically bitter honey [1] are the reason why its market price is several times higher than that of other unifloral honeys, which makes its economic importance worthy of attention.

Regardless of its great value, strawberry tree honey has rarely been studied, with only few papers present in the literature mainly concerning its botanical classification [2,3], physicochemical characterization [4] and antioxidant properties [1,4,5]. Strawberry tree honey has the highest phenolic content and shows the strongest antioxidant activity when compared to honeys of other botanical origin [1,4,6–8]. Its antioxidant capacity has been attributed to high amounts of phenolic content, with homogentisic acid (HGA) being the most abundant phenolic compound and the most reliable marker of botanical origin [3].

Besides the polyphenolic health-promoting properties of honey, an additional positive dietary feature is attributed to the natural presence of certain essential elements. With the exception of the studies on Portuguese honey [6,7] that report the concentration of few major elements in only one sample of strawberry tree honey, to the best of authors' knowledge, there have been no published data about the multi-elemental composition of strawberry tree honey. Particular interest in the analysis of essential elements in honeys has recently increased in order to demonstrate their nutritive role as well as to correlate the content of essential elements with antioxidant parameters. The beneficial effects of consumption of strawberry tree honey on the antioxidative status, haematological indices, enzyme levels and concentration of iron in serum have recently been suggested in a study by Brčić Karačonji et al. [9] while an *in vitro* study carried out by da Silva et al. [10] showed that treatment with strawberry tree honey resulted in significant inhibition of MRSA strains and demonstrated anti-biofilm formation and anti-inflammatory activities.

Except for the beneficial properties of honey itself, some honey types can contain potentially hazardous pyrrolizidine alkaloids [11,12].

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Table 1
Limits of detection (LOD) and quantification (LOQ), results of the analyses of standard reference material (NBS SRM 1571 Orchard leaves) with respect to certified values, and recovery of spiked honey samples.

ELEMENT	LOD	LOQ	Certified value NBS SRM 1571 Orchard leaves (mean (range))	Measured value (mean ± SD)	Spike concentration[a]	Recovery
	μg/kg		mg/kg		mg/kg	(%)
^{27}Al	0.025	0.082	–	–	0.230	134
^{75}As	0.005	0.015	14 (12–16)	12.3 ± 0.1	0.025	102
^{11}B	0.054	0.179	–	–	0.070	125
^{43}Ca	1.74	5.78	–	–	71.5	115
^{114}Cd	0.008	0.027	0.11 (0.09–0.13)	0.111 ± 0.003	0.025	100
^{59}Co	0.006	0.020	0.2	0.161 ± 0.002	0.025	109
^{52}Cr	0.011	0.037	2.3	2.47 ± 0.11	0.093	106
^{65}Cu	0.380	1.27	12 (11–13)	11.9 ± 0.2	1.20	101
^{56}Fe	0.001	0.002	300 (280–320)	294 ± 4	0.064	111
^{202}Hg	0.003	0.011	0.155 (0.14–0.17)	0.141 ± 0.002	0.015	100
^{39}K	1.10	3.67	14700 (14400–15000)	14683 ± 360	81.0	133
^{7}Li	0.009	0.029	–	–	2.00	102
^{24}Mg	0.050	0.167	6200 (6000–6400)	6229 ± 96	71.5	112
^{55}Mn	0.011	0.036	91 (87–95)	92.0 ± 1.3	0.114	116
^{95}Mo	0.018	0.060	–	–	0.093	104
^{23}Na	3.11	10.4	82 (76–88)	61.2 ± 0.6	71.5	111
^{60}Ni	0.011	0.037	1.3 (1.1–1.5)	1.26 ± 0.02	0.093	107
^{208}Pb	0.020	0.065	–	–	0.460	99
^{121}Sb	0.005	0.018	–	–	0.088	105
^{78}Se	0.044	0.147	0.08 (0.07–0.09)	0.095 ± 0.015	0.025	99
^{118}Sn	0.009	0.030	–	–	0.010	100
^{51}V	0.001	0.003	–	–	0.093	109
^{68}Zn	0.189	0.629	25 (22–28)	26.8 ± 1.6	1.20	94

[a] One sample of strawberry tree honey spiked in triplicates.

However, available literature to date did not report strawberry tree honey or plant to contain these compounds. Of unifloral honey types investigated here, only sunflower (*Helianthus annuus*) is a member of risk genus *Heliotropium* although there are no reports on the occurrence of pyrrolizidine alkaloids in sunflower honey.

In the present work, the concentration of 23 major and trace elements and the total phenolic content of strawberry tree honey from the coastal region of Croatia were investigated and correlated to its antiradical activity. Honey samples were collected from different locations and the measured parameters were compared to other types of unifloral honey. The study also characterised both health benefits regarding the intake of essential elements and the risks associated with the intake of toxic elements according to the latest European Food Safety Agency (EFSA) [13] recommendations and safety limits.

2. Experimental

2.1. Honey sampling and the authenticity of the samples

Representative honey sampling in this study was carried out at the filling facilities of primary producers (beekeepers). Upon collection, all honey samples were placed into clean glass jars, labelled, transferred to the laboratory and kept in a dark at room temperature until analysis. Honey sampling consisted of nine representative strawberry tree honey samples originating from locations of the southern Croatian Adriatic coast. In addition, samples of fourteen genuine honeys of different unifloral origin collected from locations in the northern Adriatic coast and the continental part of Croatia were also analysed in order to make a distinctive analytical comparison with strawberry tree honey samples. All honey samples were collected during the 2013, 2014 and 2015 beekeeping seasons.

All honey samples underwent a thorough assessment of their botanical origin. With the intention of reaching confirmation of the botanical origin of honey, a comprehensive melissopalynological and sensory assessment of honey samples was performed. With respect to the main scope of this study, the confirmation of the botanical origin of strawberry tree honey was also obtained by the analysis of homogentisic acid (HGA) [14].

2.2. Sample digestion

For multi-element analysis, honey samples (0.7 g) were digested with 5 mL of HNO_3 (65% v/v) in quartz glass vessels with an UltraCLAVE IV (Milestone Srl, Sorisole, Italy). Samples were digested according to the following programme: (1) 3.5 min at 70 °C, 700 W and 100 bar, (2) 12 min at 140 °C, 700 W and 120 bar, (3) 7 min at 210 °C, 1000 W and 160 bar, (4) 8 min at 250 °C, 1000 W and 160 bar, (5) 15 min at 250 °C, 800 W and 160 bar. After cooling, the digested solution was diluted up to 10 mL with ultrapure water. Prior to the analysis of elements, an aliquot of 300 mL was further diluted 1 + 9 with 1% v/v HNO_3. Blanks, reference materials and calibration standards were prepared in the same way as the samples and were analysed accordingly. Particular attention was paid to avoid external contamination. All equipment and laboratory glassware were soaked in 10% v/v HNO_3 for 24 h, then rinsed with ultrapure water and dried in a clean box until use.

2.3. Multi-element analysis

The analysis was carried out using an Agilent 7500cx ICP-MS (Agilent Technologies, Waldbronn, Germany) with an Octopole Reaction System (ORS) collision/reaction cell. The ORS was operated in one of 2 different modes: no-gas for measurement of Al, B, Hg and Li, and helium mode for As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, V and Zn. Optimized instrumental conditions are presented in appendix, Table A1.

Individual standard solutions of the measured elements (1000 ± 7 mg/L in 4–10% HNO_3 or 20% HCl) were obtained from SCP SCIENCE (SCP Science, Quebec, Canada). Detection limits were determined as the concentration corresponding to three times the standard deviation of 10 blank samples. Since no suitable reference material for honey was available, standard reference material NBS SRM 1571 Orchard leaves was used. The obtained recoveries ranged from 74% for Na to 125% for Se. In addition, to calculate the recovery percentage, we processed one strawberry tree honey sample that had been spiked in triplicates with known amounts of analysed elements. The obtained

Table 2
Concentration (mean ± SD, n = 3) of elements in strawberry tree honey and in other unifloral honeys.

SAMPLE	Al	B	Ca	Fe	K	Mg	Mn	Na	Zn	As	Cd	Co
	mg/kg									µg/kg		
Strawberry tree honey												
Sample 1	0.938 ± 0.007	5.86 ± 0.01	123 ± 2	0.870 ± 0.001	1276 ± 6	14.4 ± 0.1	1.07 ± 0.00	22.3 ± 0.1	0.515 ± 0.004	0.166 ± 0.047	0.551 ± 0.037	11.2 ± 0.1
Sample 2	1.14 ± 0.01	10.3 ± 0.1	123 ± 1	3.82 ± 0.02	2110 ± 25	74.4 ± ± 0.4	0.636 ± 0.004	64.3 ± 0.4	9.12 ± 0.07	0.988 ± 0.135	0.990 ± 0.056	2.16 ± 0.00
Sample 3	0.896 ± 0.004	9.43 ± 0.05	105 ± 2	2.94 ± 0.01	1815 ± 5	59.0 ± 0.3	0.414 ± 0.003	38.1 ± 0.2	1.39 ± 0.01	1.16 ± 0.11	0.535 ± 0.076	3.21 ± 0.01
Sample 4	1.51 ± 0.02	9.34 ± 0.08	134 ± 2	2.38 ± 0.01	1903 ± 6	60.1 ± 0.5	0.350 ± 0.004	54.1 ± 0.5	1.30 ± 0.09	1.20 ± 0.33	0.486 ± 0.065	5.99 ± 0.11
Sample 5	1.51 ± 0.00	8.08 ± 0.01	128 ± 1	2.38 ± 0.01	1626 ± 1	54.1 ± 0.1	0.336 ± 0.005	48.6 ± 0.2	1.90 ± 0.12	1.77 ± 0.47	0.508 ± 0.006	4.35 ± 0.02
Sample 6	1.11 ± 0.00	10.0 ± 0.0	154 ± 1	2.90 ± 0.00	2367 ± 6	72.9 ± 0.2	0.509 ± 0.002	43.4 ± 0.1	0.564 ± 0.018	1.39 ± 0.29	1.06 ± 0.13	1.36 ± 0.09
Sample 7	1.19 ± 0.02	9.61 ± 0.02	141 ± 2	1.37 ± 0.00	2021 ± 9	49.6 ± 0.2	0.435 ± 0.001	28.6 ± 0.2	0.710 ± 0.001	1.17 ± 0.03	1.72 ± 0.04	2.27 ± 0.10
Sample 8	1.64 ± 0.01	5.60 ± 0.00	128 ± 1	1.32 ± 0.00	1656 ± 11	26.2 ± 0.1	0.523 ± 0.001	13.4 ± 0.7	1.84 ± 0.01	1.92 ± 0.07	1.06 ± 0.01	4.04 ± 0.25
Sample 9	10.2 ± 0.0	6.99 ± 0.02	95.2 ± 0.2	7.55 ± 0.09	2162 ± 24	70.8 ± 0.4	0.869 ± 0.002	46.5 ± 0.3	2.49 ± 0.01	3.95 ± 0.60	1.21 ± 0.01	10.0 ± 0.3
All samples	2.23 ± 2.99	8.36 ± 1.80	126 ± 18	2.84 ± 2.00	1882 ± 331	53.5 ± 20.9	0.572 ± 0.250	39.9 ± 16.1	2.20 ± 2.68	1.52 ± 1.04	0.903 ± 0.420	4.96 ± 3.50
Various unifloral honeys												
Acacia	0.742 ± 0.009	3.14 ± 0.03	16.4 ± 0.6	0.363 ± 0.001	241 ± 2	5.87 ± 0.03	0.153 ± 0.002	4.46 ± 0.08	0.627 ± 0.012	0.890 ± 0.452	0.609 ± 0.025	26.6 ± 0.2
Chestnut	1.15 ± 0.01	3.92 ± 0.02	189 ± 0	0.699 ± 0.001	3432 ± 14	52.9 ± 0.2	27.2 ± 0.1	7.73 ± 0.11	1.52 ± 0.04	2.75 ± 0.19	0.930 ± 0.149	8.14 ± 0.01
Fir tree (honeydew)	27.6 ± 0.2	3.54 ± 0.01	31.9 ± 1.9	2.41 ± 0.02	3066 ± 14	65.4 ± 0.4	2.90 ± 0.01	5.07 ± 0.07	1.46 ± 0.02	2.91 ± 0.22	7.15 ± 0.11	39.7 ± 0.1
Indigo bush	1.09 ± 0.01	4.64 ± 0.02	66.2 ± 0.4	1.17 ± 0.03	642 ± 4	22.1 ± 0.2	0.677 ± 0.005	21.9 ± 0.3	1.15 ± 0.11	1.17 ± 0.27	1.20 ± 0.11	13.6 ± 0.2
Ivy	1.16 ± 0.01	9.29 ± 0.08	107 ± 0	0.502 ± 0.003	899 ± 5	35.2 ± 0.1	0.174 ± 0.000	8.08 ± 0.02	1.13 ± 0.02	0.958 ± 0.000	2.53 ± 0.03	2.64 ± 0.17
Jerusalem thorn	1.34 ± 0.01	4.02 ± 0.02	49.5 ± 0.2	0.910 ± 0.004	1671 ± 9	6.55 ± 0.01	0.263 ± 0.003	8.76 ± 0.10	1.61 ± 0.01	1.04 ± 0.30	0.857 ± 0.208	2.04 ± 0.03
Lime tree	0.741 ± 0.012	2.82 ± 0.00	123 ± 1	0.476 ± 0.004	2307 ± 2	30.8 ± 0.2	1.05 ± 0.01	6.87 ± 0.02	0.815 ± 0.003	1.24 ± 0.09	1.70 ± 0.14	2.14 ± 0.03
Mint	0.924 ± 0.003	4.96 ± 0.02	53.5 ± 0.4	0.841 ± 0.002	1251 ± 5	15.6 ± 0.0	2.04 ± 0.00	59.7 ± 0.2	1.86 ± 0.00	1.11 ± 0.06	0.239 ± 0.012	9.71 ± 0.05
Oak (honeydew)	2.89 ± 0.01	4.47 ± 0.01	60.3 ± 1.4	2.81 ± 0.04	4159 ± 33	77.4 ± 0.4	0.747 ± 0.009	40.7 ± 0.2	1.59 ± 0.02	2.38 ± 0.18	1.47 ± 0.08	7.14 ± 0.15
Rapeseed	2.92 ± 0.00	11.5 ± 0.1	88.4 ± 0.1	3.14 ± 0.02	579 ± 2	26.6 ± 0.1	0.867 ± 0.004	22.3 ± 0.0	1.30 ± 0.02	1.68 ± 0.27	1.01 ± 0.06	12.1 ± 0.2
Sage	1.01 ± 0.00	5.94 ± 0.02	25.8 ± 0.0	0.365 ± 0.002	615 ± 1	7.15 ± 0.00	0.131 ± 0.003	8.83 ± 0.02	0.683 ± 0.014	1.15 ± 0.31	0.539 ± 0.039	1.18 ± 0.01
Sunflower	1.25 ± 0.02	8.05 ± 0.06	190 ± 1	1.25 ± 0.00	1375 ± 8	47.5 ± 0.4	6.83 ± 0.04	7.55 ± 0.06	1.33 ± 0.00	1.25 ± 0.46	1.39 ± 0.08	3.55 ± 0.06
Thyme	2.82 ± 0.03	3.08 ± 0.02	73.7 ± 0.4	1.37 ± 0.00	612 ± 7	18.5 ± 0.1	0.175 ± 0.003	51.5 ± 0.1	1.04 ± 0.00	1.59 ± 0.13	1.88 ± 0.03	4.95 ± 0.03
Winter savory	3.44 ± 0.02	5.05 ± 0.03	85.5 ± 1.7	2.40 ± 0.06	1397 ± 10	28.8 ± 0.2	0.942 ± 0.006	24.9 ± 0.1	14.2 ± 0.1	2.27 ± 0.23	1.55 ± 0.06	13.1 ± 0.0

SAMPLE	Cr	Cu	Hg	Li	Mo	Ni	Pb	Sb	Se	Sn	V	Total elemental content%
	µg/kg											
Strawberry tree honey												
Sample 1	10.4 ± 0.1	146 ± 2	0.877 ± 0.109	2.51 ± 0.01	2.93 ± 0.00	22.6 ± 0.2	4.03 ± 0.22	0.283 ± 0.068	8.98 ± 2.17	11.3 ± 0.2	0.906 ± 0.030	0.144
Sample 2	9.84 ± 0.08	176 ± 7	0.939 ± 0.032	3.89 ± 0.19	4.13 ± 0.29	21.0 ± 0.9	10.6 ± 1.0	10.1 ± 0.1	3.35 ± 0.37	26.0 ± 0.2	1.70 ± 0.01	0.239
Sample 3	11.2 ± 0.3	153 ± 1	0.930 ± 0.083	3.34 ± 0.04	2.52 ± 0.08	9.08 ± 0.53	12.8 ± 0.2	0.312 ± 0.022	3.19 ± 0.89	16.2 ± 0.1	1.36 ± 0.02	0.202
Sample 4	8.43 ± 0.43	226 ± 6	0.645 ± 0.081	5.46 ± 0.44	5.40 ± 0.33	16.8 ± 0.1	7.55 ± 0.21	1.14 ± 0.10	5.35 ± 0.15	2.65 ± 0.31	3.06 ± 0.10	0.215
Sample 5	12.8 ± 0.1	247 ± 1	0.527 ± 0.103	5.62 ± 0.11	5.44 ± 0.36	19.1 ± 0.1	8.14 ± 0.56	1.09 ± 0.12	9.53 ± 0.98	2.95 ± 0.12	2.64 ± 0.06	0.186
Sample 6	25.8 ± 0.1	158 ± 2	0.988 ± 0.010	4.25 ± 0.34	3.60 ± 0.22	28.5 ± 0.7	3.84 ± 0.69	0.271 ± 0.049	7.30 ± 1.73	35.5 ± 0.0	1.26 ± 0.00	0.264
Sample 7	13.5 ± 1.4	212 ± 1	0.437 ± 0.024	1.61 ± 0.03	2.98 ± 0.26	15.9 ± 0.1	3.95 ± 0.27	0.248 ± 0.014	5.54 ± 1.00	25.5 ± 0.4	1.67 ± 0.05	0.224
Sample 8	3.84 ± 0.09	247 ± 4	0.364 ± 0.081	2.08 ± 0.12	5.96 ± 0.17	28.4 ± 0.0	4.77 ± 0.22	0.368 ± 0.036	10.7 ± 0.1	14.8 ± 0.1	2.63 ± 0.16	0.183
Sample 9	20.8 ± 0.0	588 ± 8	1.24 ± 0.06	14.7 ± 0.6	7.08 ± 0.16	121 ± 0	27.1 ± 0.1	1.76 ± 0.12	12.6 ± 0.2	14.0 ± 0.4	25.9 ± 0.1	0.239
All samples	13.0 ± 6.6	239 ± 137	0.772 ± 0.292	4.82 ± 3.94	4.45 ± 1.59	31.4 ± 34.1	9.20 ± 7.42	1.73 ± 3.17	7.39 ± 3.29	16.6 ± 10.9	4.57 ± 8.03	–
Various unifloral honeys												
Acacia	11.0 ± 0.1	56.2 ± 0.3	0.344 ± 0.068	0.767 ± 0.172	1.67 ± 1.04	44.5 ± 0.9	5.24 ± 0.08	0.293 ± 0.024	11.2 ± 2.1	17.8 ± 0.3	0.664 ± 0.030	0.027
Chestnut	6.22 ± 0.02	205 ± 1	0.290 ± 0.050	5.28 ± 0.20	1.63 ± 0.26	65.1 ± 0.3	8.73 ± 0.05	0.213 ± 0.055	11.9 ± 2.2	20.0 ± 1.4	1.05 ± 0.05	0.368
Fir tree (honeydew)	6.65 ± 0.50	1591 ± 1	1.38 ± 0.12	1.74 ± 0.04	13.1 ± 0.9	674 ± 4	8.98 ± 0.48	0.597 ± 0.006	10.6 ± 3.0	2.33 ± 0.01	2.68 ± 0.24	0.317
Indigo bush	14.8 ± 0.8	251 ± 1	0.356 ± 0.086	2.89 ± 0.39	3.35 ± 0.09	33.7 ± 1.6	7.84 ± 2.99	0.366 ± 0.116	11.3 ± 2.7	8.70 ± 5.05	1.69 ± 0.07	0.075

(continued on next page)

Table 2 (*continued*)

SAMPLE	Cr	Cu	Hg	Li	Mo	Ni	Pb	Sb	Se	Sn	V	Total elemental content%
	μg/kg											
Ivy	24.0 ± 0.3	160 ± 1	0.447 ± 0.136	1.19 ± 0.01	4.10 ± 0.17	22.6 ± 0.2	3.18 ± 0.10	0.251 ± 0.073	11.7 ± 0.5	3.30 ± 0.03	1.47 ± 0.02	0.105
Jerusalem thorn	6.01 ± 0.08	193 ± 4	0.660 ± 0.200	1.52 ± 0.18	3.01 ± 0.30	16.8 ± 0.2	3.58 ± 0.06	0.244 ± 0.021	8.49 ± 2.91	29.9 ± 0.5	1.88 ± 0.07	0.174
Lime tree	4.76 ± 0.37	251 ± 2	0.333 ± 0.067	0.528 ± 0.146	3.12 ± 0.09	18.9 ± 0.2	5.74 ± 0.06	0.330 ± 0.067	12.0 ± 1.5	51.7 ± 0.3	1.24 ± 0.02	0.247
Mint	15.5 ± 0.0	145 ± 0	0.440 ± 0.019	1.79 ± 0.05	3.80 ± 0.24	186 ± 0	10.2 ± 0.1	0.201 ± 0.036	18.4 ± 1.4	4.21 ± 0.25	1.12 ± 0.09	0.138
Oak (honeydew)	6.54 ± 0.13	1302 ± 7	0.935 ± 0.081	3.46 ± 0.24	5.69 ± 0.13	56.8 ± 0.4	13.1 ± 0.4	1.06 ± 0.15	13.1 ± 0.3	11.0 ± 0.0	15.0 ± 0.4	0.434
Rapeseed	25.5 ± 0.2	96.9 ± 0.3	0.210 ± 0.287	3.18 ± 0.09	2.91 ± 0.17	33.3 ± 0.8	13.2 ± 0.2	0.620 ± 0.102	17.4 ± 1.7	3.49 ± 0.36	4.32 ± 0.07	0.072
Sage	10.7 ± 0.2	412 ± 5	0.327 ± 0.084	1.27 ± 0.09	0.902 ± 0.013	19.3 ± 0.7	2.45 ± 0.35	0.184 ± 0.022	14.1 ± 2.5	8.57 ± 0.20	1.01 ± 0.01	0.066
Sunflower	5.17 ± 0.13	221 ± 3	0.426 ± 0.022	1.16 ± 0.08	5.22 ± 0.30	94.2 ± 0.3	6.20 ± 0.27	0.230 ± 0.057	7.32 ± 2.44	11.2 ± 0.1	1.30 ± 0.14	0.162
Thyme	10.7 ± 0.4	109 ± 0	0.383 ± 0.077	4.12 ± 0.19	2.93 ± 0.33	22.2 ± 0.7	8.85 ± 0.06	1.49 ± 0.01	13.5 ± 0.4	45.9 ± 0.0	4.08 ± 0.14	0.076
Winter savory	10.9 ± 0.2	482 ± 4	0.387 ± 0.046	10.3 ± 0.1	7.90 ± 0.69	54.8 ± 0.6	8.79 ± 0.03	0.493 ± 0.041	9.17 ± 0.19	1.98 ± 0.08	3.67 ± 0.03	0.155

recoveries ranged from 94% for Zn to 134% for Al (Table 1).

2.4. Total phenolic content

The total phenolic content was quantified by the Folin-Ciocalteau method according to Beretta et al. [8]. An aliquot (0.1 mL) of 20% (w/v) honey solution was vortexed for 2 min with 1 mL of 10% Folin-Ciocalteau reagent (Sigma-Aldrich, Germany) and was measured after 20 min at 750 nm against a sugar analogue. A water solution containing 40% fructose, 30% glucose, 8% maltose and 2% sucrose was made to mimic honey with its main sugar components and was used as the sugar analogue to control for interferences. Gallic acid (Sigma-Aldrich, Germany) solutions (0–150 mg/L) were used to construct the calibration curve and quantify samples. Results were expressed as g gallic acid (GA)/kg honey.

2.5. DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined by a modified method proposed by Tuberoso et al. [5]. An aliquot (0.1 mL) of 20% (w/v) aqueous honey solution was mixed with 0.9 mL of methanol (Merck, Germany). Then, 1.5 mL of DPPH (Sigma-Aldrich, Germany) methanolic solution (0.18 mmol/L) was added to the honey sample solution and vortexed vigorously. The mixture was incubated in the dark for 30 min at 25 °C. The absorbance was measured at 517 nm. A calibration curve in the range 0.01–0.1 mmol/L was used for the Trolox (Fluka, Germany) and results were expressed as mmol of the Trolox equivalent antioxidant capacity per kg of honey (mmol TE/kg).

2.6. Statistics

Statistical analyses were performed using Statistica 12 for Windows (StatSoft Inc., Tulsa, OK, USA). The assays were carried out in triplicate and the results were expressed as the mean values and standard deviation (SD). Normality of data distribution was tested and confirmed with a Shapiro-Wilk test. To normalize the distribution of the HGA concentration data, a logarithmic transformation was applied. Correlation analysis between the total phenolic content, DPPH and logHGA was performed by Pearson correlation, while the analysis including major and trace elements was performed by Spearman correlation. The level of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Characterization of honey samples

The confirmation of the botanical origin of honey was conducted using the melissopalynological and sensory analyses. The physico-chemical parameters (moisture content, electrical conductivity, free acidity, diastase activity, hydroxymethylfurfural content, sugar content (fructose, glucose, sucrose)) determined in all honey samples were in accordance with literature data and with the requirements of the Council Directive 2001/110/EC and EU Directive 2014/63/EU [15]. Since strawberry tree pollen is commonly underrepresented in the honey, the frequent incidence of contaminating pollens causes a wide variation in the absolute number of pollen grains in the sediment and in the percentages of strawberry tree pollen [2]. In order to achieve greater reliability in confirming the strawberry tree honey's botanical origin, HGA as a specific chemical marker was determined by gas chromatography-mass spectrometry. The level of HGA in samples of strawberry tree honey ranged from 245.1 mg/kg to 485.9 mg/kg [14].

3.2. Multi-elemental composition

The amount of different elements in honey is largely dependent on the soil composition and floral type of honey plants. Elements are transported to plants and flowers through the root system, pass to nectar and then to honey produced from it. The presence of various elements in honey is also influenced by the anthropogenic factors such as beekeeping practices, environmental pollution and honey processing [16].

The mean concentration of each of the 23 elements measured in nine samples of strawberry tree honey and 14 samples of other various unifloral honeys is shown in Table 2. The total elemental content of each honey was determined by summarising the mean concentration of each element analysed in that honey. The total elemental content of strawberry tree honey samples ranged from 0.144 to 0.264% (1437–2642 mg/kg). As for other unifloral honeys, the total elemental content of honeydew (oak: 0.434%, fir tree: 0.317%) and chestnut honey (0.368%) was higher than that of strawberry tree honey, while for the remaining honeys, the total elemental content was lower and ranged from 0.027 to 0.174%, with the exception of lime tree honey where the total elemental content was 0.247%. This is in accordance with previous reports confirming that darker honeys are customarily more robust and contain more elements [17].

3.2.1. Major elements

The most abundant elements found in all honey samples analysed in this study were Ca, K, Mg and Na (Table 2). The mean concentration of these elements in strawberry tree honey was generally higher than the concentration found in other unifloral honeys. A higher concentration of Ca, compared to the samples of strawberry tree honey, was found in chestnut and in sunflower honey, while a higher concentration of K was measured in chestnut and both samples of honeydew honey. Honeydew honeys also contained the highest concentration of Mg.

The mean concentration of Ca (126 mg/kg), K (1882 mg/kg) and Mg (53.5 mg/kg) in Croatian strawberry tree honey was higher than the concentration measured in samples of Portuguese strawberry tree honey as reported by Aazza et al. [6] (Ca 24.9 mg/kg, K 1736 mg/kg, Mg 50.0 mg/kg) and Alves et al. [7] (Ca 57.9 mg/kg, K 712 mg/kg, Mg 21.4 mg/kg). The concentration of Na measured in strawberry tree honey from Croatia (39.9 mg/kg) was higher than the concentration reported by Alves et al. [7] (19.6 mg/kg) but lower than the concentration found by Aazza et al. [6] (161 mg/kg) in Portuguese honey. Ca, K, Mg and Na were also the most abundant elements found in various unifloral honeys from Spain [17], New Zealand [18] and Italy [19].

3.2.2. Essential trace elements

Major and trace elements such as Co, Cu, Fe, Mn, Mo, Se and Zn are essential for a wide range of physiological processes and have certain nutritional benefits. However, these elements are safe and adequate for the body only within a specific range of intake whereas excessive exposure may lead to both acute and chronic toxicity [20].

The concentration of Fe, Mo and Zn measured in samples of strawberry tree honey was similar to the concentrations found in other unifloral honeys analysed in this study (Table 2). The mean concentration of Fe in Croatian strawberry tree honey was also similar to the concentration reported by Alves et al. [7] determined in one sample of Portuguese strawberry tree honey.

A notably higher concentration of Mn was found in chestnut honey (27.2 μg/kg), while honeydew honeys contained more than two times higher concentration of Cu (both oak and fir tree) and more than three times higher concentration of Co (fir tree), compared to the maximum concentration measured in samples of strawberry tree honey. Similarly high concentrations of Co and Cu were measured in Spanish and Turkish honeys of different botanical origin [17,21,22], while the concentration of Mn up to 43 mg/kg was found in uni- and multi-floral honey sold in France [23].

The mean concentration of Se measured in strawberry tree honey was generally lower than the concentration measured in 14 unifloral honey types studied here. Considering previously published studies where authors reported a concentration of Se in a range from 20 to 972 μg/kg in Turkish and Spanish honey of different botanical origin [21,22], it appears that strawberry tree honey is a rather poor source of Se. On the other hand, the concentration of Se in uni- and multi-floral honeys from Italy [24] was generally in the same range as in our strawberry tree honey samples.

The concentration of B, Li and V measured in samples of strawberry tree honey was similar to the concentrations found in 14 other unifloral honey types (Table 2). Similar concentrations of B were found in unifloral honey samples from Turkey and Poland [21,25], while the concentration of V measured in this study corresponds to the concentrations found in uni- and multi-floral honey samples from Croatia and Italy [24,26]. The concentration of Li measured in this study was similar to the concentration reported by Devillers [23] for French acacia honeys but much lower than the concentration measured in uni- and multi-floral honey from Spain where the concentration of Li ranged from 12 to 28 mg/kg [22].

3.2.3. Non-essential trace elements

Trace elements such as Al, As, Cd, Cr, Hg, Ni, Pb, Sb and Sn are not considered to be essential for humans. They have the ability to accumulate in an organism and interfere with normal biological functions and can be toxic even at low concentrations. These elements are mainly of anthropogenic origin, i.e., industrial pollution, factory emissions, pollution from busy highways, pesticides and fertilizers containing As or Cd as well as improper procedures during honey processing and conservation [27].

In samples of strawberry tree honey, the concentrations of As, Cd and Hg were below 4 μg/kg, while the highest measured concentrations of Sb, Cr, Pb, Sn and Ni were 10.1 μg/kg, 25.8 μg/kg, 27.0 μg/kg, 35.5 μg/kg and 121 μg/kg, respectively. The concentration of Al was below 2 mg/kg in all except one sample of strawberry tree honey where the measured concentration was 10.2 mg/kg. Regarding other unifloral honey types, the concentrations of potentially toxic elements were generally similar to strawberry tree honey. However, notably higher concentrations of Al and Ni were measured in both samples of honeydew honey (Table 2). Similarly high concentrations of Al and Ni were found in Spanish avocado honey [22] and in unifloral honeys from New Zealand [18]. In previous reports from Croatia [28,29], the concentration of Pb measured in multi-floral honey was up to 50 times higher than in this study, which may be due to the position of hives in zones near highways and railways. Concentrations of up to 447 μg Sn/kg of honey were found in Brazilian honey of different botanical origin [30], while Yücel and Sultanoglu [21] reported the concentration as high as 7.208 mg Sn/kg in citrus honey from Turkey. The authors explain that the high concentration of Sn measured in Turkish honey could be due to the location of apiaries not far from large iron and steel factories.

The European Union Directive 2014/63/EU [15] does not contain any part referring to the contaminants such as potentially toxic elements, while the Codex Alimentarius [31] states in general that honey should not contain toxic elements in such amounts that may pose a threat to human health. Only recently, the European Commission has issued a regulation [32] that introduces the maximum admitted level for Pb content in honey of 100 μg/kg. All samples of strawberry tree honey, as well as other unifloral honeys analysed in this study, had concentrations of Pb below the specified values, pointing to very low level of contamination in the environment.

3.3. Exposure assessment

We made nutritional and risk estimation of honey consumption regarding the mean element concentrations in nine strawberry tree honeys from Croatia, relying on the most recent EFSA data on Dietary Reference Values (DRV) for essential elements, and Tolerable Intake

Table 3
Nutritional and risk estimation of daily strawberry tree honey consumption regarding elements and EFSA's dietary recommendations (DRV for essential and TI for non-essential elements) for an adult weighing 60 kg.

Element	Mean conc. in honey (mg/kg)	EDI (mg/daily portion of 15.1g[*])	DRV (> 18 years) (mg/day)	% DRV
Mg	53.5	0.810	300–**350**[a]	0.2
K	1882	28.5	**3500**[a]	0.8
Ca	126	1.90	750[a]–**1000**[b]	0.2
Mn	5.72×10^{-4}	8.66×10^{-6}	**3**[a]	0.0003
Fe	2.84	0.043	6[c]–**16**[b]	0.3
Cu	239	3.62	1.3–**1.6**[a]	0.2
Zn	2.20×10^{-3}	3.33×10^{-5}	7.5–**16.3**[a]	0.0002
Se	7.39×10^{-3}	1.12×10^{-4}	**0.07**[a]	0.2
Mo	4.45×10^{-3}	6.74×10^{-5}	**0.065**[a]	0.1
	Mean conc. in honey (μg/kg)	EDI (ng/daily portion/kg b.w.)	TI (μg/kg b.w.)	% TI
Al	2.23	0.564	**1000**[d]	0.0004
Cr	13.0	3.27	**300**[e]	0.001
Ni	31.4	7.91	**2.8**[e]	0.3
As	1.55	0.391	**0.3**–8[f]	0.1
Cd	0.903	0.228	**2.5**[d]	0.06
Hg	0.772	0.195	**4**[d]	0.03
Pb	9.20	2.32	**0.5**[f]	0.5

EDI-Estimated Daily Intake, DRV-Dietary Reference Values, TI-Tolerable Intake; bold values are taken in calculation of % DRV/TI.

[*] Average daily portion of honey consumed by adult population in Croatia (CFA, 2015).

[a] AI-Adequate Intake (Mg: EFSA Journal 2015;13(7):4186; K: EFSA Journal 2016;14(10):4592; Ca: EFSA Journal 2015;13(5):4101; Mn: EFSA Journal 2013;11(11):3419; Cu: EFSA Journal 2015;13(10):4253; Zn: EFSA Journal 2014;12(10):3844; Se: EFSA Journal 2014;12(10):3846; Mo: EFSA Journal 2013;11(8):3333).

[b] PRI-Population Reference Intakes (Ca: EFSA Journal 2015;13(5):4101; Fe: EFSA Journal 2015;13(10):4254).

[c] AR-Average Requirement (Fe: EFSA Journal 2015;13(10):4254).

[d] TWI-Tolerable Weekly Intake (Al: EFSA Journal 2008;754,1-34; Cd: EFSA Journal 2011;9(2):1975; Hg: EFSA Journal 2012;10(12):2985).

[e] TDI-Tolerable Daily Intake (Cr: EFSA Journal 2014;12(3):3595; Ni: EFSA Journal 2015;13(2):4002).

[f] $BMDL_{01}$-Benchmark Dose lower confidence limit at 1% extra risk: $BMDL_{01}$ for As for an increased risk of cancer of the lung, skin and bladder, skin lesions (As: EFSA Journal 2014;12(3):3597); developmental neurotoxicity $BMDL_{01}$ for Pb (EFSA Journal 2010; 8(4):1570).

(TI) data and Benchmark Doses (BMD) for non-essential elements (Table 3). Calculation is based on the average daily honey consumption data for Croatian population (15.1 g/day) published by Croatian Food Agency [33]. Apparent annual consumption (calculated as production (data from FAOSTAT) + import − export (data from Croatian Bureau of Statistics [34]) in Croatia has grown from 0.43 to 2.14 kg per capita in the period 2010–2014, thus pointing to the Croatian honey market as one of the fastest growing in the European Union. For comparison sake, apparent consumption in European countries with traditionally high honey consumption like Germany, Austria, etc. ranged 1–1.8 kg per capita [35].

The nutritional contribution of elements from strawberry tree honey is generally low and ranges from 0.0002% of DRV for Zn to 0.8% of DRV for K (Zn < Mn < Mo < Se < Ca < Cu < Mg < Fe < K) (Table 3). Other authors concluded that the intake of elements from various honeys could be considered marginal [35,36]. However, among all other studied honey types here, strawberry tree honey is in the upper half of the highest contributors to the essential element pool gained through food (Table 2). Contribution to the recommended DRV through consumption of e.g. acacia honey, the most frequently consumed honey types in Croatia, is for some elements (e.g. Mg, K, Ca or Fe) almost 10 times lower than through the consumption of strawberry tree honey.

The EFSA Panel on contaminants in the food chain proposed a set of non-essential (toxic or potentially toxic) element intake levels expressed as kg of body weight and defined as tolerable weekly intake (TWI), tolerable daily intake (TDI) or benchmark dose (BMD), which are considered safe i.e. free of risk of adverse health effects. Regarding the exposure assessment to non-essential elements, regular consumption of strawberry tree honey will contribute negligibly (Cr, Al, Cd, Hg) or very little (Pb, Ni, As) to the dietary intake amount of respective elements proposed to still be safe for human health (Table 3). Lead as a ubiquitous contaminant showed the highest contribution (0.5% $BDML_{01}$).

3.4. Total phenolic content and radical scavenging activity

The antioxidant activity of honey is a characteristic generally associated with its phenolic content. Phenolic compounds found in honey are mainly flavonoids, phenolic acids and phenolic acid derivatives [35]. Besides the antioxidant activity, these compounds display a wide range of health-related properties, such as anti-allergenic, anti-inflammatory, anti-microbial, cardio protective and vasodilatory effects [37]. Strawberry tree honey analysed in this study shows high antioxidant activity with a mean phenolic content of 0.415 g GA/kg (range: 0.314–0.522 g GA/kg). The total phenolic content of other unifloral honeys analysed in this study was lower than that of strawberry tree honey, with the exception of oak honeydew honey and mint honey whose total phenolic content was 0.424 g GA/kg and 0.356 g GA/kg, respectively (Fig. 1). A few other authors found strawberry tree honey as the richest in phenol compounds compared to honeys from different floral sources [1,4,8]. According to literature, analysis of strawberry tree honey showed that HGA is the most abundant phenolic compound, representing approximately 50–60% of the total phenolic content with an average amount of 414 mg/kg [1,2], which makes an important contribution to high antioxidant and antiradical properties of strawberry tree honey. The average HGA content in strawberry tree honey analysed in our study was 311 mg/kg which is in accordance with those reported in other studies with mean values ranging from 326 mg/kg to 425 mg/kg [1,2,14].

In humans, HGA is an intermediate in the metabolism of tyrosine [38]. It was found to scavenge intracellular reactive oxygen species and DPPH radicals and protect human fibroblasts against H_2O_2 damage by enhancing the intracellular antioxidative activity [39].

Strawberry tree honey also showed higher DPPH activity (mean and range: 3.34 (1.94–4.45) mmol TE/kg) compared to other unifloral honeys analysed in this study (Fig. 1). Our results are in accordance with previous reports for strawberry tree honey, showing it to be the most active in the capacity for scavenging free radicals in contrast with different unifloral honeys [1,2,8].

The phenolic compounds appeared to be the main contributor to the antioxidant properties of honey, as indicated by high significant correlation coefficients between the antioxidant activity and total phenol content. The Pearson correlation coefficients (r) showed a strong relationship between the total phenolic content and DPPH (r = 0.979), total phenolic content and logHGA (r = 0.913) and between DPPH and logHGA (r = 0.928) in samples of strawberry tree honey. The sample of strawberry tree honey with the highest HGA concentration was also the one with the highest DPPH and total phenolic content. A significant correlation was also found between the total elemental content and total phenolic content (r = 0.574) as well as between the total elemental content and DPPH (r = 0.537) when all honey samples were considered. Major and trace elements show considerable synergism in the antioxidant capacity when complexed with phenolic compounds since most metals can work as electron donors and their charges are stabilized by the phenolic structures [40]. Also, some elements (e.g. Se, Fe, Cu, Zn, Mn) are known for their role as co-factors in antioxidant enzymes which cleave free radicals and reactive oxygen species.

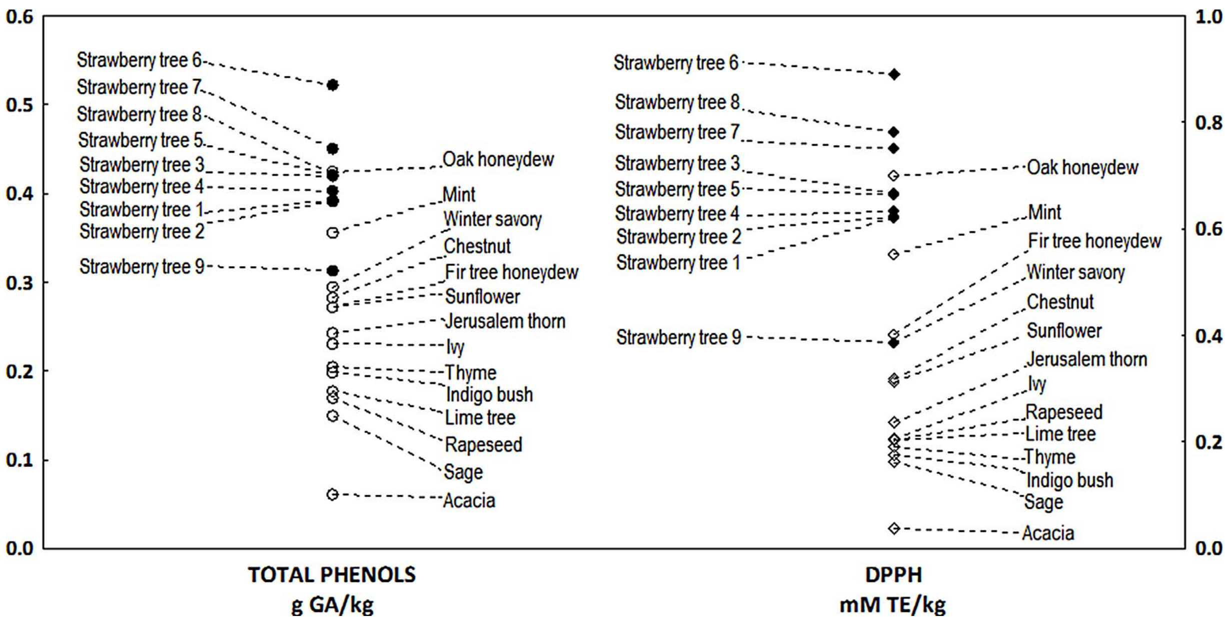

Fig. 1. Total phenolic content and radical scavenging activity (DPPH) in strawberry tree honey and in other unifloral honey types. The results are presented as means (n = 3).

4. Conclusions

This is the first study that reports the concentration of a larger number of elements in strawberry tree honey collected from various beekeepers. When considering the majority of Croatian unifloral honeys studied here, strawberry tree honey seems to be a dietary item of particular value, especially because of its rich phenolic content, presence of essential elements and very low risk of adverse health effects due to the intake of potentially toxic elements. Measured level of potentially toxic elements corresponds to pristine areas. Being able to scavenge free radicals, compared to other honeys analysed in this study, strawberry tree honey pointed to respectable beneficial characteristics considering human health.

Appendix A

Table A1
Optimized instrumental conditions used in different gas modes (ICP-MS Agilent 7500cx).

Gas mode	No-gas	Helium
Parameter	Value	
RF power/W	1500	1500
Rf matching/V	1.7	1.7
Smpl depth/mm	7.8	7.8
Torch-H/mm	0.8	0.8
Torch-V/mm	0.3	0.3
Carrier gas/L min^{-1}	1.03	1.05
Makeup gas/L min^{-1}	0.1	0.1
Nebulizer pump/rps	0.08	0.08
S/C temp/°C	2	2
Extract lens 1/V	1	1.8
Extract lens 2/V	−160	−166
Omega bias/V	−26	−30
Omega lens/V	0.4	−1.4
Cell entrance/V	−28	−40
Cell exit/V	−38	−60
Quadrupole bias/V	−3	−16
Octopole bias/V	−6	−19
Gas flow/mL min^{-1}	Not used	3.6

Conflicts of interest

None.

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