Phenolic composition of Croatian olive leaves and their infusions obtained by hot and cold preparation

Majetić Germek, Valerija; Žurga, Paula; Koprivnjak, Olivera; Grozić, Kristina; Previšić, Iva; Marcelić, Šime; Goreta Ban, Smiljana; Pasković, Igor

Source / Izvornik: **Czech journal of food sciences, 2021, 39, 393 - 341**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.17221/185/2020-CJFS

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:472999

https://doi.org/10.17221/185/2020-CJFS

Phenolic composition of Croatian olive leaves and their infusions obtained by hot and cold preparation

Valerija Majetić Germek[1], Paula Žurga[2], Olivera Koprivnjak[1], Kristina Grozić[3], Iva Previšić[1], Šime Marcelić[4], Smiljana Goreta Ban[3,5], Igor Pasković[3]*

[1]*Department of Food Technology and Control, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*
[2]*Teaching Institute of Public Health of Primorje-Gorski Kotar County, Rijeka, Croatia*
[3]*Department of Agriculture and Nutrition, Institute of Agriculture and Tourism, Poreč, Croatia*
[4]*Department of Ecology, Agronomy and Aquaculture, University of Zadar, Zadar, Croatia*
[5]*Centre of Excellence for Biodiversity and Molecular Plant Breeding, Zagreb, Croatia*

**Corresponding author: valerija.majetic@medri.uniri.hr*

Citation: Majetić Germek V., Žurga P., Koprivnjak O., Grozić K., Previšić I., Marcelić Š., Goreta Ban S., Pasković I. (2021): Phenolic composition of Croatian olive leaves and their infusions obtained by hot and cold preparation. Czech J. Food Sci., 39: 393–401.

Abstract: Leaves and infusions of six Croatian olive cultivars grown in an organic orchard under the same agronomic conditions were characterised by high-performance liquid chromatography-ultraviolet/visible spectrophotometry (HPLC-UV/VIS). The total identified phenols in leaves ranged from 3 818 mg 100 g^{-1} [cultivar Istarska crnica (IC)] to 10 572 mg 100 g^{-1} of dry mass [cultivar Oblica (OB)]. The canonical discriminant analysis (CDA) provided a distinct separation of cultivars based on leaves' phenolic profiles. Hot- and cold-water infusions (200 mL) were prepared from 1 g of dry leaves. The average transfer rate of the total phenols in the cold-water infusions was 40% (25 °C/30 min), while in the hot-water infusions was 63% (75 °C/3 min) and 76% (100 °C/3 min). Although the cold-water infusions had the lowest transfer rate, they contained important levels of hydroxytyrosol derivatives ranging from 16.6 mg 200 mL^{-1} to 36.5 mg 200 mL^{-1} depending on the cultivar. Therefore, both hot and cold preparations are effective in obtaining antioxidant-rich natural beverages.

Keywords: brewing conditions; cultivars; herbal tea; *Olea europaea*; phenols

Olive leaves (OL) are a cheap natural source of phenolic compounds, which can be recovered and used as value-added ingredients in the food and beverage industry (Souilem et al. 2016). Different classes of phenols are present, like secoiridoids, flavonoids, triterpenes and other simple phenols as alcohols and phenolic acids (Abaza et al. 2017). Specific varietal properties of the *Olea europaea* L. contribute to the differences in the phenolic profile of OL (Talhaoui et al. 2015a; Ben Mohamed et al. 2018). This strong influence was confirmed for Spanish (Talhaoui et al. 2015a; Romero et al. 2017), Italian (Nicolì et al. 2019), Turkish

Supported by the Croatian Science Foundation (CSF) (Project No. HRZZ-UIP-2017-05-8464 and Project No. HRZZ--DOK-2018-09-1841).

https://doi.org/10.17221/185/2020-CJFS

(Orak et al. 2019), Tunisian (Ben Mohamed et al. 2018) and Croatian cultivars (Mujić et al. 2011).

A commercially available form of dried OL is used for herbal infusion preparation (Medina et al. 2019; Güzelmeriç et al. 2020). Simple OL steeping in hot water enables an adequate diffusion of phenols to the aqueous phase and a substantial intake of these compounds (Peršurić et al. 2019). Amany and Shaker (2018) proposed OL infusions (OLI) as a great substitute for the green tea due to beneficial health effects, such as serum lipid-lowering effect (Araki et al. 2019) and increased red blood cells count and haemoglobin in healthy females compared to the green tea (Ferdousi et al. 2018). Recent studies have characterised phenols in commercially available OL and their infusions (Medina et al. 2019) and optimised OL mixtures based on the phenolic profile to achieve the desirable biological effects (Peršurić et al. 2019). Considering brewing conditions, infusion and decoction processes were used to evaluate the influence on phenolic compounds (Casazza et al. 2017) and preparation temperature had a pronounced effect on the antioxidants extraction in different teas (Pastoriza et al. 2017). To our knowledge, only Peršurić et al. (2019) have published so far the study on the impact of hot-water temperature on the release of phenolic compounds in OLI.

This study investigates the effect of hot and cold preparation on the transfer of phenols from OL of six autochthonous Croatian cultivars to OLI. We explored cold extraction as a new brewing strategy of OL since it was demonstrated as efficient in antioxidants extraction from white teas, and it contributes to the pleasantness of tea (Castiglioni et al. 2015). Moreover, this study was undertaken to characterise and discriminate six Croatian olive cultivars according to their leaf phenolic profile determined by high-performance liquid chromatography (HPLC).

MATERIAL AND METHODS

OL sampling. Leaves of cultivar Drobnica (DR), Istarska bjelica (IB), Istarska crnica (IC), Lastovka (LA), Levantinka (LE) and Oblica (OB) were manually harvested from the organic olive orchard in Dubrovnik-Neretva County (latitude: 43°3'60"N, longitude: 17°32'9"E), in the first week of November 2017, from 8-year-old trees. From each of three consecutive trees of a single cultivar, 200 OL were sampled, taking 50 OL from the east, west, north and south side of the tree, respectively. OL were collected at the height 1.6–1.8 m from the ground, from the middle part of olive shoots. Each OL sample was washed [successively rinsed with tap water, distilled water with 1% (v/v) acetic acid (VWR International, Leuven, Belgium) and pure distilled water] and air dried at room temperature until a constant mass. OL were kept uncrushed in paper bags at room temperature until analyses and OLI preparation.

Preparation of OL extracts for HPLC analysis. Phenols were extracted according to Marinova et al. (2005) with minor modifications. Finely ground OL (0.5 g; grinder KSW 3306; Clatronic International, Kempen, Germany) were extracted with 20 mL of methanol (HPLC gradient grade, Merck, Darmstadt, Germany) 80% (v/v) in an ultrasonic bath (20 min; Sonorex Digitec; Bandelin electronic, Berlin, Germany). After centrifugation (4000 rpm/7 min; centrifuge EBA 200; Hettich, Tuttlingen, Germany), the extract supernatant was filtered through a 0.45 µm-pore syringe filter of cellulose acetate (Filtres Fioroni, Ingré, France). Extraction was done in triplicate for each cultivar.

Preparation of OLI. OLI were prepared by pouring 200 mL of distilled water of appropriate temperature over 1.0 g of OL ground for 30 s by a household grinder (KSW 3306; Clatronic International, Kempen, Germany). Water was at a room temperature (24 ± 1 °C) for cold preparation while for hot brews, it was heated up to 75 °C and 100 °C, separately. The suspension was vigorously stirred, and a glass beaker was covered with a watch glass. During cold preparation, OL were soaking for 30 min, while for hot brewing glass beakers were immersed in a water bath at 75 °C or 100 °C, respectively, for 3 min (Sub Aqua Pro; Grant Instruments, Shepreth, United Kingdom). The suspension was filtered through a filter paper into another beaker and left to cool down. OLI were centrifuged (4 000 rpm/10 min; centrifuge EBA 200; Hettich, Tuttlingen, Germany), filtered through a syringe filter of cellulose acetate (0.45 µm; Filtres Fioroni, Ingré, France) and prepared in triplicate for each preparation condition. Transfer rates (%) of phenols from OL to OLI were calculated by dividing the content of phenols in OLI (mg L^{-1}) with the content of phenols in 5 g of dry OL (mg) taken for OLI preparation (1 L) and multiplied by 100.

HPLC-UV/VIS analysis. Phenols in OL methanolic extracts and freshly prepared OLI were determined by HPLC Ultimate 3000 System with ultraviolet/visible spectrophotometry (UV/VIS) detector (ThermoFisher Scientific, Waltham, USA) according to the method of Pasković et al. (2020). The analytical column was Lichrospher 100 RP-18 (250 × 4 mm, 5 µm)

https://doi.org/10.17221/185/2020-CJFS

with pre-column Lichrospher 100 (4 × 4 mm, 5 μm), supplied by Agilent Technologies (Santa Clara, USA). The mobile phase consisted of A) 0.2% phosphoric acid (Sigma-Aldrich, St. Louis, USA) and B) methanol : acetonitrile (1 : 1, v/v; Merck, Darmstadt, Germany). The solvent gradient was as follows: 10% B 0–0.5 min; 10–16.5% B 0.5–25 min; 16.5–30% B 25–80 min; 30–100% B 80–95 min; 100% B 95–100 min; 100–10% B 100–102 min; 10% B 102–105 min; equilibration time 10 min, injection volume 10 μL. The flow rate was 0.8 mL min^{-1} at 25 °C. UV/VIS detection was set at 250 nm for 4-hydroxybenzoic acid, luteolin-7-O--glucoside, oleuropein and vanillic acid; 280 nm for apigenin-7-O-glucoside, catechin, hydroxytyrosol, tyrosol and vanillin; 305 nm for apigenin, caffeic acid, ferulic acid and verbascoside; 370 nm for luteolin and rutin. All phenolic standards were purchased from Extrasynthese (Genay, France). The stock solutions of standards were prepared in methanol (80%, v/v). Phenols were identified by comparison of their retention times with those of standards and quantified by using the external standard method. Phenols in OL and OLI were expressed as means of three determinations in mg 100 g^{-1} of OL dry mass (DM) and mg L^{-1}, respectively.

Statistical analysis. The one-way analysis of variance was performed at the 5% significance level in order to test the differences among OL of different cultivars and preparation temperatures of OLI. Multiple comparisons of means were done by Tukey's honest significant difference (HSD) test. The canonical discriminant analysis (CDA) was performed on data expressing the phenol composition of OL (independent variables) in order to discriminate cultivars (grouping variable). Statistical analyses were performed by the software Statistica 13.0 (Stat-Soft, Tulsa, USA).

RESULTS AND DISCUSSION

The total content of identified OL phenols varied from 3 818 mg 100 g^{-1} (IC) to 10 572 mg 100 g^{-1} (OB) DM (Table 1). A similar range (4 479–10 828 mg 100 g^{-1} DM) was found in commercially available OL (Medina et al. 2019). Oleuropein constituted 82–91% of the total phenols in IC and IB, respectively. Other cultivars contained 87% (LE), 88% (LA), 89% (DR) and 90% (OB) of oleuropein in the total phenols. Oleuropein was also the main OL secoiridoid in OL from Tunisian cultivars (Ben Mohamed et al. 2018) in which represented up to 85% of the total identified phenols. IC was marked with the lowest oleuropein (3 133 mg 100 g^{-1} DM) while OB with the highest oleuropein content (9 544 mg 100 g^{-1} DM). Oleuropein content in OL can range from 2 470–14 320 mg 100 g^{-1} DM (Talhaoui et al. 2015b). A high level of oleuropein in OB, as well as in DR, IB and LE may be a result of specific varietal interaction with a highly sunny orchard position and cold season sampling. Namely, exposure to UV-B radiation can induce the synthesis of phenols in OL (Talhaoui et al. 2015b), while a higher oleuropein in OL was reported during the cold season (Talhaoui et al. 2015a; Romero et al. 2017).

The flavonoids amount was also significant and ranged from 8% (IB and OB) to 15% (IC) of total phenols. Glycosylated flavonoids were more abundant than aglycones, which constituted up to 13% and 1.7% of total phenols, respectively. The sugar moiety on flavonoid molecules provides structural stability during storage in plant tissues and may influence their absorption from plant foods (Marín et al. 2015). Luteolin-7-O-glucoside was the main flavonoid in all cultivars and ranged from 398.7 mg 100 g^{-1} to 710.7 mg 100 g^{-1} DM. This is consistent with previous reports (Ben Mohamed et al. 2018; Orak et al. 2019). DR and IB were characterised by a higher amount of rutin (123.7 mg 100 g^{-1} and 157.3 mg 100 g^{-1} DM, respectively). Ben Mohamed et al. (2018) also detected luteolin-7-O-glucoside and rutin as main flavonoids in OL from Tunisian cultivars.

Phenolic alcohols and acids were present in lower amounts. The tyrosol content was lower than hydroxytyrosol in all cultivars (from 3.5- to 9-fold in IB and DR, respectively). Verbascoside in OL samples varied markedly, from 49.4 mg 100 g^{-1} to 196.6 mg 100 g^{-1} DM. Medina et al. (2019) have already reported a similarly wide range of verbascoside in OL (36–231 mg 100 g^{-1} DM).

Talhaoui et al. (2015b) proposed OL phenols as chemotaxonomic markers for the classification and discrimination among cultivars of the same geographical origin. In this study, the CDA was applied to reduce the multidimensionality of the sets of results by creating the new variables named canonical discriminant functions (CDFs), which best separate the predefined groups of samples (cultivars). The first two CDFs (CDF1 and CDF2) explained 98.3% of the total variance (Figure 1), and the difference among cultivars was significant (Wilks' lambda = 0.00 and $P < 0.01$ for CDF1 and CDF2). CDFs are linear functions of original variables, with original variables having different contributions to the differentiation of the groups. The CDF1 was influenced most heavily by hydroxytyrosol, tyrosol, verbascoside and oleuropein, while luteolin-7-O--glucoside, apigenin-7-O-glucoside, oleuropein and

Czech Journal of Food Sciences, 39, 2021 (5): 393–401

https://doi.org/10.17221/185/2020-CJFS

Table 1. Olive leaves (OL) of six Croatian cultivars – Phenolic composition (mg 100 g^{-1} DM) [n = 3 (3 extractions × 1 analytical determination); mean ± SD]

Phenols	Cultivar					
	DR	IB	IC	LA	LE	OB
Oleuropein	7 057.6 ± 136.4^{a}	7 073.0 ± 87.9^{a}	3 133.1 ± 95.7^{b}	5 957.6 ± 323.8^{c}	7 423.7 ± 208.8^{a}	9 544.4 ± 587.0^{d}
Luteolin-7-O-glucoside	465.9 ± 13.4^{a}	398.7 ± 8.1^{b}	436.4 ± 12.2^{ab}	583.7 ± 20.6^{c}	633.9 ± 19.4^{d}	710.7 ± 7.9^{e}
Luteolin	25.4 ± 1.4^{a}	16.5 ± 0.5^{b}	35.6 ± 0.6^{c}	21.8 ± 1.2^{d}	29.4 ± 2.4^{e}	14.6 ± 0.7^{b}
Apigenin-7-O-glucoside	29.1 ± 1.2^{ac}	21.7 ± 0.2^{a}	21.6 ± 0.1^{a}	29.9 ± 1.0^{ac}	68.8 ± 3.0^{b}	31.9 ± 7.9^{c}
Apigenin	1.4 ± 0.0^{a}	1.4 ± 0.1^{a}	1.3 ± 0.0^{a}	0.5 ± 0.0^{b}	1.4 ± 0.1^{a}	0.2 ± 0.0^{c}
Catehin	37.7 ± 1.3^{a}	34.7 ± 2.0^{a}	26.7 ± 1.0^{b}	37.8 ± 1.3^{a}	37.7 ± 1.4^{a}	49.2 ± 0.3^{c}
Rutin	123.7 ± 1.1^{a}	157.3 ± 1.5^{b}	35.7 ± 4.9^{c}	57.8 ± 8.3^{cd}	74.7 ± 8.8^{d}	91.9 ± 15.3^{d}
Hydroxytyrosol	40.5 ± 2.3^{a}	36.0 ± 2.4^{ab}	37.0 ± 0.9^{ab}	34.7 ± 0.7^{b}	50.3 ± 2.0^{c}	33.1 ± 0.6^{b}
Tyrosol	4.5 ± 1.3^{a}	10.3 ± 2.0^{b}	7.1 ± 1.3^{ab}	7.9 ± 2.2^{ab}	7.8 ± 0.4^{ab}	5.6 ± 1.5^{a}
Verbascoside	99.5 ± 0.8^{a}	49.4 ± 0.9^{bc}	78.9 ± 4.2^{ad}	69.3 ± 5.4^{cd}	196.6 ± 22.7^{e}	84.5 ± 5.3^{ad}
Vanillin	1.4 ± 0.2^{ab}	1.0 ± 0.0^{ab}	0.8 ± 0.0^{b}	1.9 ± 0.8^{a}	1.1 ± 0.3^{ab}	3.6 ± 0.3^{c}
Phenolic acids*	2.0 ± 0.6^{a}	2.1 ± 0.5^{a}	3.9 ± 1.0^{b}	2.6 ± 0.6^{ab}	2.5 ± 0.7^{ab}	2.5 ± 0.7^{ab}
Total**	7 888.6 ± 146.2^{a}	7 802.2 ± 98.1^{a}	3 818.1 ± 116.2^{b}	6 805.8 ± 347.0^{c}	8 527.8 ± 263.2^{a}	10 572.3 ± 580.1^{d}

$^{a–e}$Mean values labelled by different lowercase letters in the same row are significantly different at $P < 0.05$ according to Tukey's test; *sum of 4-hydroxybenzoic acid, vanillic, ferrulic and caffeic acid; *sum of 4-hydroxybenzoic acid, vanillic, ferrulic and caffeic acid; **sum of all identified phenols; DM – dry mass; SD – standard deviation; DR – Drobnica; IB – Istarska bjelica; IC – Istarska crnica; LA – Lastovka; LE – Levantinka; OB – Oblica

https://doi.org/10.17221/185/2020-CJFS

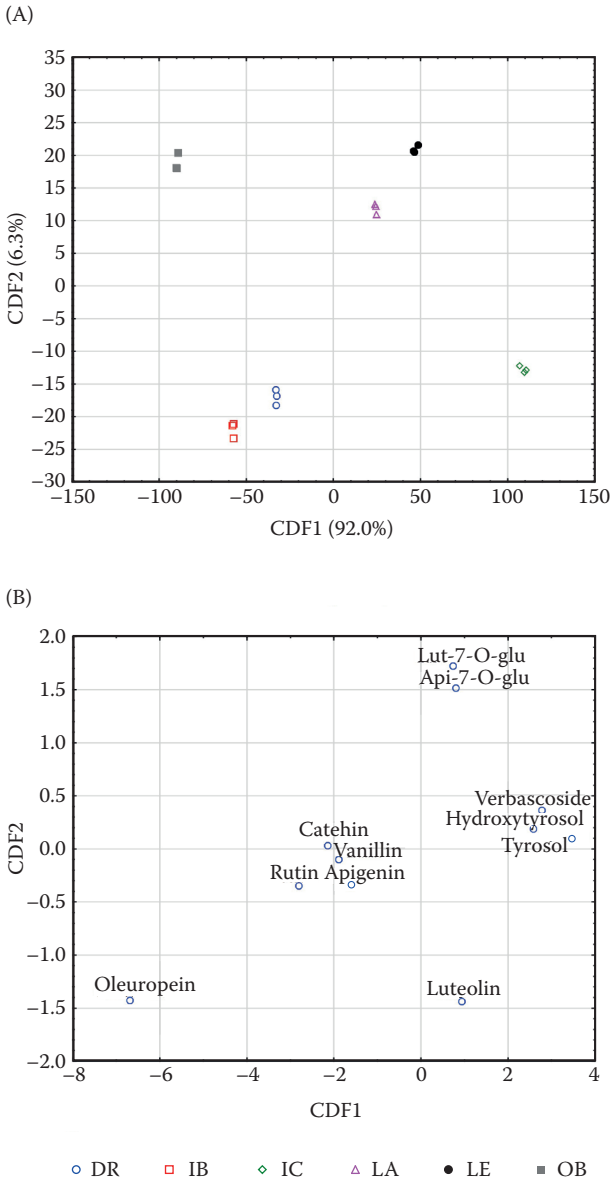

Figure 1. 2D plots obtained by canonical discriminant analysis (CDA): (A) classification of cultivars by the first two canonical discriminant functions (CDF1, CDF2), (B) individual phenols of olive leaves (OL) included in the model (lut-7-O-glu – luteolin-7-O-glucoside, api-7-O-glu – apigenin-7-O-glucoside)

DR – Drobnica; IB – Istarska bjelica; IC – Istarska crnica; LA – Lastovka; LE – Levantinka; OB – Oblica

luteolin had the highest impact on the CDF2. Distinct discrimination among cultivars was observed. IC extracts were grouped by the higher luteolin and tyrosol contents. LA and LE shared common characteristics of the higher luteolin-7-O-glucoside and apigenin-7-O--glucoside but low oleuropein content, which separated them from other cultivars. Conversely, IB and DR were clustered by the higher oleuropein and lower luteolin--7-O-glucoside and apigenin-7-O-glucoside. OB was separated from other cultivars by the lower luteolin and tyrosol (Figure 1).

Regarding the phenols transfer rate of green and white tea infusions, cold extraction has proved to be a good but more time-consuming alternative to hot brewing (Castiglioni et al. 2015; Pastoriza et al. 2017). In order to test a feasible practical application, a restricted time for OL cold extraction (30 min) was compared to the two most common hot extraction conditions (75 °C or 100 °C/3 min). Cold extraction of total phenols was significantly less effective (an average transfer rate of 40%) than hot extraction (63% and 76% at 75 °C and 100 °C, respectively) (Table 2). Regardless of the cultivar or extraction method, the main phenol in OLI was oleuropein (78.5–324.7 mg L^{-1}), followed by luteolin-7-O-glucoside (6.6–23.5 mg L^{-1}) and rutin (1.4–7.0 mg L^{-1}). OLI of LE (100 °C/3 min) were the richest source of oleuropein and luteolin-7-O-glucoside, while cold prepared OLI of IC had the lowest content of oleuropein and rutin. A very high transfer rate in all six cultivars to cold OLI has been detected for tyrosol (130–257%) and vanillin (111–241%), as well as to hot OLI (tyrosol 129–295% and vanillin 74–230%). The hydrosolubility of these two compounds (Noubigh et al. 2009) does not explain such marked changes of its transfer rates. An increase of tyrosol in OLI may be due to the hydrolysis of more complex phenols (tyrosol is an integral part of ligstroside). However, the same trend was not observed for the other phenols (e.g. hydroxytyrosol) potentially released from the complex phenols (oleuropein and verbascoside).

The health claim related to virgin olive oil [Commission Regualtion (EU) No. 432/2012] states that daily intake of at least 5 mg of hydroxytyrosol and its derivatives contribute to the protection of low-density lipoprotein (LDL) particles from oxidative damage. Amounts contained in one cup of hot OLI are multiple higher than the health claim minimum content (Table 3). Even simple short-term cold preparation contributes to obtaining the OLI containing 3- to 7-fold higher values. Since such high levels may result in unacceptable sensory properties of OLI, i.e. intense bitterness and astringency (Medina et al. 2019), these aqueous beverages could be used as an ingredient in fruit smoothies, juices and similar products. Moreover, cold preparation could be advantageous over hot preparation since there is no energy consumption for heating and subsequent cooling.

Czech Journal of Food Sciences, 39, 2021 (5): 393–401

https://doi.org/10.17221/185/2020-CJFS

Table 2. Olive leaves infusions (OLI) of six Croatian cultivars – Phenolic composition (mg L^{-1} of leaf infusion) and their transfer rates from olive leaves (OL) to the water (%) [n = 3 (3 preparations × 1 analytical determination); mean ± SD]

Cultivar	Temperature (°C)/time (min)*	Phenols (mg L^{-1})													
		oleuropein		luteolin-7-O--glucoside		luteolin		apigenin-7-O--glucoside		apigenin		catehin		rutin	
		(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)
DR	25/30	141.2 ± 6.5^{xAB}	40	7.1 ± 0.3^{xAB}	31	0.1 ± 0.0^{xA}	9	0.4 ± 0.0^{xA}	25	0.01 ± 0.0^{xA}	8	1.5 ± 0.2^{xAB}	81	3.8 ± 0.2^{xA}	61
	75/3	220.7 ± 25.6^{yA}	63	13.2 ± 1.2^{yA}	57	0.3 ± 0.0^{yA}	24	0.9 ± 0.2^{yA}	60	0.01 ± 0.0^{xA}	12	1.4 ± 0.2^{xAE}	77	4.9 ± 0.1^{yA}	80
	100/3	277.8 ± 17.9^{yA}	79	15.9 ± 1.3^{zAB}	68	0.7 ± 0.1^{zAC}	53	0.9 ± 0.1^{yA}	62	0.01 ± 0.0^{xA}	12	1.6 ± 0.1^{xA}	86	5.2 ± 0.2^{yA}	85
IB	25/30	125.5 ± 2.5^{xB}	35	6.6 ± 0.6^{xA}	33	nd	0	0.3 ± 0.0^{xA}	27	0.01 ± 0.0^{xAC}	15	1.5 ± 0.1^{xAB}	87	4.3 ± 0.4^{xA}	55
	75/3	228.7 ± 14.8^{yAE}	65	11.9 ± 0.3^{yA}	60	0.1 ± 0.0^{xB}	12	0.6 ± 0.0^{yAB}	53	0.10 ± 0.0^{yB}	76	1.6 ± 0.1^{xAC}	95	6.5 ± 0.1^{yB}	83
	100/3	280.7 ± 22.5^{zA}	79	13.9 ± 1.0^{zB}	70	0.2 ± 0.1^{yB}	27	0.7 ± 0.0^{yB}	61	0.10 ± 0.0^{yB}	89	1.6 ± 0.1^{xA}	95	7.0 ± 0.4^{yB}	89
IC	25/30	78.5 ± 2.2^{xC}	50	7.3 ± 0.4^{xAB}	34	0.2 ± 0.0^{xBD}	13	0.3 ± 0.0^{xA}	24	0.10 ± 0.0^{xB}	78	1.3 ± 0.1^{xA}	95	1.4 ± 0.1^{xB}	79
	75/3	104.7 ± 2.4^{yB}	67	12.1 ± 0.7^{yA}	55	0.4 ± 0.1^{yC}	25	0.5 ± 0.0^{yB}	44	0.10 ± 0.0^{xB}	94	1.2 ± 0.0^{xB}	87	1.7 ± 0.1^{yC}	98
	100/3	129.5 ± 6.4^{zB}	83	15.6 ± 0.8^{zAB}	71	0.7 ± 0.1^{zC}	39	0.7 ± 0.1^{zB}	63	0.10 ± 0.0^{xB}	111	1.2 ± 0.1^{xB}	93	1.9 ± 0.1^{yC}	106
LA	25/30	102.5 ± 6.2^{xD}	35	8.9 ± 0.8^{xB}	31	0.2 ± 0.0^{xB}	17	0.2 ± 0.0^{xA}	15	0.02 ± 0.01^{xC}	84	1.5 ± 0.2^{xyAB}	82	2.0 ± 0.1^{xC}	72
	75/3	163.0 ± 5.0^{yC}	55	14.1 ± 0.7^{yAC}	48	0.2 ± 0.0^{xA}	22	0.5 ± 0.1^{yB}	31	0.02 ± 0.00^{xA}	84	1.4 ± 0.0^{xBE}	71	2.8 ± 0.1^{yD}	99
	100/3	208.4 ± 18.1^{zC}	70	20.3 ± 1.4^{zCD}	69	0.4 ± 0.1^{yAB}	40	0.9 ± 0.1^{zA}	60	0.04 ± 0.01^{xC}	138	1.8 ± 0.1^{yAC}	95	3.7 ± 0.5^{zD}	132
LE	25/30	177.8 ± 10.8^{xE}	48	12.0 ± 1.1^{xC}	38	0.4 ± 0.0^{xC}	25	1.1 ± 0.1^{xB}	32	0.10 ± 0.0^{xD}	100	1.8 ± 0.1^{xB}	94	3.0 ± 0.0^{xD}	80
	75/3	288.6 ± 26.8^{yD}	78	19.7 ± 1.6^{yB}	62	0.5 ± 0.1^{xyC}	36	1.7 ± 0.2^{yC}	51	0.10 ± 0.0^{xB}	90	1.9 ± 0.1^{xyCD}	101	3.4 ± 0.3^{xyE}	93
	100/3	324.7 ± 9.2^{yA}	88	23.5 ± 0.6^{zC}	74	0.7 ± 0.1^{yAC}	47	2.1 ± 0.1^{zC}	61	0.10 ± 0.0^{xB}	100	2.0 ± 0.0^{yCD}	104	3.7 ± 0.3^{yD}	100
OB	25/30	154.7 ± 14.5^{xA}	32	11.1 ± 0.7^{xC}	31	0.2 ± 0.0^{xD}	32	0.3 ± 0.1^{xA}	22	nd	108	2.3 ± 0.1^{xC}	92	2.5 ± 0.2^{xCD}	54
	75/3	269.5 ± 11.4^{yDE}	57	16.9 ± 1.1^{yBC}	48	0.2 ± 0.0^{yAB}	24	0.6 ± 0.1^{yAB}	38	nd	87	2.0 ± 0.0^{yD}	82	3.3 ± 0.3^{yE}	75
	100/3	284.6 ± 29.0^{yA}	60	17.6 ± 2.1^{yAD}	50	0.2 ± 0.0^{xyB}	28	0.6 ± 0.0^{yB}	39	nd	194	2.0 ± 0.0^{yD}	81	3.1 ± 0.1^{yD}	68

To be continued

https://doi.org/10.17221/185/2020-CJFS

Table 2. To be continued

Cultivar	Temperature (°C)/time (min)*	Phenols (mg L^{-1})											
		hydroxytyrosol		tyrosol		verbascoside		vanillin		phenolic acids**		total***	
		(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)
DR	25/30	1.2 ± 0.0^{xAC}	61	0.5 ± 0.0^{xAB}	257	0.4 ± 0.3^{xA}	8	0.1 ± 0.0^{xA}	170	0.1 ± 0.0^{xA}	98	156.5 ± 7.0^{xAB}	40
	75/3	1.7 ± 0.1^{yAB}	85	0.6 ± 0.0^{xA}	267	2.4 ± 0.2^{yAB}	48	0.1 ± 0.0^{xAB}	147	0.1 ± 0.0^{xA}	109	246.4 ± 27.3^{yA}	62
	100/3	2.2 ± 0.0^{zAC}	108	0.6 ± 0.1^{xA}	295	4.5 ± 0.4^{zA}	90	0.1 ± 0.0^{xAB}	151	0.1 ± 0.0^{xA}	104	309.6 ± 20.1^{zA}	79
IB	25/30	1.2 ± 0.0^{xAC}	65	0.6 ± 0.1^{xA}	130	nd	0	0.1 ± 0.0^{xA}	241	0.1 ± 0.0^{xA}	81	140.3 ± 2.2^{xBD}	36
	75/3	1.6 ± 0.1^{yAC}	91	0.6 ± 0.0^{xAB}	129	0.9 ± 0.3^{xAD}	36	0.1 ± 0.0^{xAB}	205	0.1 ± 0.0^{xA}	97	252.9 ± 15.5^{yA}	65
	100/3	2.1 ± 0.0^{zAC}	115	0.7 ± 0.0^{xAB}	149	2.2 ± 0.2^{yB}	91	0.1 ± 0.0^{xAB}	214	0.1 ± 0.0^{xA}	97	309.4 ± 24.3^{zA}	79
IC	25/30	1.6 ± 0.1^{xB}	87	0.6 ± 0.1^{xA}	186	2.8 ± 0.7^{xB}	73	0.1 ± 0.0^{xA}	238	0.2 ± 0.0^{xB}	83	94.4 ± 2.8^{xC}	49
	75/3	1.9 ± 0.0^{yB}	103	0.8 ± 0.1^{xB}	225	3.1 ± 0.3^{xB}	78	0.1 ± 0.0^{xA}	230	0.1 ± 0.0^{xA}	59	126.6 ± 3.4^{yB}	66
	100/3	2.4 ± 0.1^{zA}	130	0.7 ± 0.1^{xAB}	200	3.3 ± 0.3^{xC}	85	0.1 ± 0.0^{xA}	200	0.2 ± 0.0^{xB}	81	156.3 ± 7.9^{zB}	82
LA	25/30	1.1 ± 0.0^{xC}	65	0.7 ± 0.1^{xA}	187	nd	0	0.1 ± 0.0^{xA}	141	0.1 ± 0.0^{xA}	42	117.4 ± 7.3^{xCD}	35
	75/3	1.5 ± 0.0^{yC}	87	0.6 ± 0.0^{yA}	163	0.7 ± 0.1^{xAD}	21	0.1 ± 0.0^{xAB}	103	nd	0	185.0 ± 5.7^{yC}	54
	100/3	2.2 ± 0.1^{zAC}	124	0.9 ± 0.1^{zB}	243	3.1 ± 0.2^{yBC}	89	0.1 ± 0.0^{xAB}	122	0.1 ± 0.0^{xA}	41	241.8 ± 19.2^{zC}	71
LE	25/30	1.8 ± 0.1^{xB}	70	0.6 ± 0.0^{xAB}	151	3.2 ± 0.3^{xB}	33	0.1 ± 0.0^{xB}	161	0.1 ± 0.0^{xA}	41	201.6 ± 12.5^{xE}	47
	75/3	2.6 ± 0.1^{yD}	104	0.6 ± 0.1^{xA}	147	7.3 ± 1.7^{yC}	76	0.1 ± 0.0^{xA}	138	0.1 ± 0.0^{xA}	51	326.6 ± 30.7^{yD}	77
	100/3	3.2 ± 0.3^{zB}	129	0.7 ± 0.2^{xAB}	192	9.9 ± 0.7^{yD}	102	0.1 ± 0.0^{xA}	131	0.1 ± 0.0^{xA}	58	370.8 ± 10.7^{yD}	87
OB	25/30	1.2 ± 0.1^{xC}	70	0.4 ± 0.1^{xB}	163	nd	0	0.2 ± 0.1^{xC}	111	nd	0	172.9 ± 14.9^{xA}	33
	75/3	1.6 ± 0.1^{yAC}	100	0.5 ± 0.1^{xyA}	209	0.3 ± 0.2^{xD}	6	0.2 ± 0.0^{xB}	94	nd	0	295.2 ± 12.8^{yAD}	56
	100/3	1.8 ± 0.2^{yC}	107	0.6 ± 0.1^{yAB}	240	2.1 ± 0.2^{yB}	51	0.1 ± 0.0$^{x B}$	74	nd	0	312.8 ± 31.6^{yA}	59

$^{x, y, z}$Different lowercase letters with mean values in the same column and within each cultivar indicate significant differences among preparation conditions; $^{A–E}$uppercase letters in the same column and within each preparation condition indicate significant differences among cultivars according to Tukey's test at $P < 0.05$; *infusions preparation: 1.0 g dry leaves/200 mL of distilled water; **sum of 4-hydroxybenzoic acid, vanillic, ferrulic and caffeic acid; ***sum of all identified phenols; SD – standard deviation; nd – not detected; DR – Drobnica; IB – Istarska bjelica; IC – Istarska crnica; LA – Lastovka; LE – Levantinka; OB – Oblica

https://doi.org/10.17221/185/2020-CJFS

Table 3. Olive leaves infusions (OLI) of six Croatian cultivars – Sum of hydroxytyrosol derivatives per 200 mL of infusion (mg 200 mL^{-1})

Cultivar	Hydroxytyrosol derivatives in infusion*		
	25 °C/30 min	75 °C/3 min	100 °C/3 min
DR	28.6	45.0	56.9
IB	25.3	56.2	57.0
IC	16.6	21.9	27.0
LA	20.7	33.1	42.7
LE	36.5	59.7	67.6
OB	31.2	54.3	57.7

*Infusions preparation: 1.0 g dry leaves/200 mL of distilled water; values are sum of means of hydroxytyrosol, oleuropein and verbascoside; DR – Drobnica; IB – Istarska bjelica; IC – Istarska crnica; LA – Lastovka; LE – Levantinka; OB – Oblica

CONCLUSION

OL of six Croatian cultivars grown in an organic orchard showed remarkable quantitative differences in the phenolic profiles. Distinct discrimination among cultivars was achieved by the CDA. Short time cold OLI preparation resulted in a considerable transfer of phenols, although lower compared to hot preparation. One cup of cold OLI is sufficient to achieve several times higher intake of hydroxytyrosol derivatives than minimally required for the beneficial health effect.

Acknowledgement. We especially extend our appreciation to Modro-zelena zadruga (Kula Norinska, Croatia) and MEng Jana Klanjac for their technical support and assistance in olive leaf sampling.

REFERENCES

Abaza L., Taamalli A., Arráez-Román D., Segura-Carretero A., Fernández-Gutierrérez A., Zarrouk M., Ben Youssef N. (2017): Changes in phenolic composition in olive tree parts according to development stage. Food Research International, 100: 454–461.

Amany M.B., Shaker M.A. (2018): Olive leaves healthy alternative for green tea. Current Trends in Biomedical Engineering & Biosciences, 15: 555919.

Araki R., Fujie K., Yuine N., Watabe Y., Nakata Y., Suzuki H., Isoda H., Hashimoto K. (2019): Olive leaf tea is beneficial for lipid metabolism in adults with pre-diabetes: An exploratory randomised controlled trial. Nutrition Research, 67: 60–66.

Ben Mohamed M., Guasmi F., Ben Ali S., Radhouani F., Faghim J., Triki T., Kammoun N.G., Baffi C., Lucini L., Benincasa C. (2018): The LC-MS/MS characterisation of phenolic compounds in leaves allows classifying olive cultivars grown in South Tunisia. Biochemical Systematics and Ecology, 78: 84–90.

Casazza A., Aliakbarian B., Comotto M., Monteiro Souza P., Perego P. (2017): Olive leaves infuse and decoct production: Influence of leaves drying conditions and particle size. Chemical Engineering Transactions, 57: 1807–1812.

Castiglioni S., Damiani E., Astolfi P., Carloni P. (2015): Influence of steeping conditions (time, temperature, and particle size) on antioxidant properties and sensory attributes of some white and green teas. International Journal of Food Sciences and Nutrition, 66: 491–497.

Ferdousi F., Araki R., Hashimoto K., Isoda H. (2018): Olive leaf tea may have hematological health benefit over green tea. Clinical Nutrition, 38: 2952–2955.

Güzelmeriç E., Çevik D., Yeşilada E. (2020): Quality assessment of marketed food supplements and herbal tea products of olive leaf in Turkey. Journal of Research in Pharmacy, 24: 159–169.

Marín L., Miguélez E.M., Villar C.J., Lombó F. (2015): Bioavailability of dietary polyphenols and gut microbiota metabolism: Antimicrobial properties. BioMed Research International, 2015: 1–18.

Marinova D., Ribarova F., Atanassova M. (2005): Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy, 40: 255–260.

Medina E., Romero C., García P., Brenes M. (2019): Characterisation of bioactive compounds in commercial olive leaf extracts, and olive leaves and their infusions. Food & Function, 10: 4716–4724.

Mujić I., Živković J., Nikolić G., Vidović S., Trutić N., Kosić U., Jokić S., Ruznić A. (2011): Phenolic compounds in olive leaf extract as a source of useful antioxidants. Croatian Journal of Food Technology, Biotechnology and Nutrition, 6: 129–133.

Nicolì F., Negro C., Vergine M., Aprile A., Nutricati E., Sabella E., Miceli A., Luvisi A., De Bellis L., Paiva-Martins F. (2019): Evaluation of phytochemical and antioxidant properties of 15 Italian *Olea europaea* L. cultivar leaves. Molecules, 24: 1998.

Noubigh A., Abderrabbaa M., Provost E. (2009): Salt addition effect on partition coefficient of some phenolic compounds constituents of olive mill wastewater in 1-octanol-water system at 298.15 K. Journal of the Iranian Chemical Society, 6: 168–176.

Orak H.H., Karamać M., Amarowicz R., Orak A., Penkacik K. (2019): Genotype-related differences in the phenolic com-

https://doi.org/10.17221/185/2020-CJFS

pound profile and antioxidant activity of extracts from olive (*Olea europaea* L.) leaves. Molecules, 24: 1130.

Pasković I., Lukić I., Žurga P., Majetić Germek V., Brkljača M., Koprivnjak O., Major N., Grozić K., Franić M., Ban D., Marcelić Š., Ban S.G. (2020): Temporal variation of phenolic and mineral composition in olive leaves is cultivar dependent. Plants, 9: 1099.

Pastoriza S., Pérez-Burillo S., Rufián-Henares J.Á. (2017): How brewing parameters affect the healthy profile of tea. Current Opinion in Food Science, 14: 7–12.

Peršurić Ž., Saftić L., Klisović D., Kraljević Pavelić S. (2019): Polyphenol-based design of functional olive leaf infusions. Food Technology and Biotechnology, 57: 171–182.

Romero C., Medina E., Mateo M.A., Brenes M. (2017): Quantification of bioactive compounds in Picual and Arbequina olive leaves and fruit. Journal of the Science of Food and Agriculture, 97: 1725–1732.

Souilem S., Fki I., Kobayashi I., Khalid N., Neves M.A., Isoda H., Sayadi S., Nakajima M. (2016): Emerging technologies for recovery of value-added components from olive leaves and their applications in food/feed industries. Food and Bioprocess Technology, 10: 229–248.

Talhaoui N., Gómez-Caravaca A.M., Roldán C., León L., De la Rosa R., Fernández-Gutiérrez A., Fernández-Gutiérrez A., Segura-Carretero A. (2015a): Chemometric analysis for the evaluation of phenolic patterns in olive leaves from six cultivars at different growth stages. Journal of Agricultural and Food Chemistry, 63: 1722–1729.

Talhaoui N., Taamalli A., Gómez-Caravaca A.M., Fernández-Gutiérrez A., Segura-Carretero A. (2015b): Phenolic compounds in olive leaves: Analytical determination, biotic and abiotic influence, and health benefits. Food Research International, 77: 92–108.

Received: July 23, 2020
Accepted: June 29, 2021