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Variations of phenolic compounds and sensory properties of virgin olive oils from the variety "Istrska belica"

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ABSTRACT
The olive variety "Istrska belica" is well known for its numerous positive properties, such as resistance to low temperature and high oil content. The aim was to determine the variations in the levels of phenolic compounds and sensory properties during storage of "Istrska belica" virgin olive oil. The profile of the phenolic compounds and sensory properties of "Istrska belica" olive oil were further compared with those for other varieties, including "Leccino" and "Maurino". The content of phenolic compounds of the olive oils decreased after 1 year and 2 years of storage. After 2 years of storage, the levels of oleuropein and the ligstroside derivatives significantly decreased, while the end-stage compounds tyrosol and hydroxytyrosol increased. These data show that after 1 year of storage, the "Istrska belica" olive oil preserves similar intensities for bitterness and pungency, and similar oleuropein and ligstroside derivatives levels. In contrast to the other oils, the intensities of bitterness and pungency of "Istrska belica" olive oil decreased greatly only after 2 years of storage. Moreover, the phenolic compounds content, and oleuropein and ligstroside derivatives levels, and the intensities of bitterness and pungency were highest in fresh "Istrska belica" olive oil, compared to other olive oils analysed. Overall, "Istrska belica" olive oil has important advantages over olive oil from other varieties that are grown in the Istria region.

Section: RESEARCH PAPER

Keywords: olive oil; phenolic compounds; sensory properties; "Istrska belica"

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1. INTRODUCTION

The olive (Olea europaea L.) variety "Istrska belica" is the most widely spread olive variety in Slovenian olive groves. The quality of the olive oil from "Istrska belica" is distinguished by its rich aroma, which reminds the user of the fresh, optimal ripe olive fruit, combined with freshly mown grass. The high content of phenolic compounds gives the "Istrska belica" oil its characteristic bitter taste and pungent tactile sensation.

Diets containing the phenolic compounds found in olive oil can have health benefits, which include the reduction of risk factors for coronary heart disease, protection against several types of cancers, and modification of immune and inflammatory responses [1]-[19]. According to European Union Regulation (UE) 432/2012, phenolic compounds can be cited according to the indication: "Olive oil phenolic compounds contribute to the protection of blood lipids from oxidative stress". This claim demonstrates the importance of these phenolic compounds.

The phenolic compounds in olive oil are secondary metabolites that arise through the conversion of complex substances produced by olive trees, and they can be classified as lignans, flavonoids and secoiridoids. Virgin olive oil contains at least 30 different phenolic compounds [1]. The most common lignans in olive oil are pinoresinol, acetoxypinoresinol and hydroxypinoresinol [11], and the most common flavonoids are luteolin and apigenin [20]. While lignans and flavonoids are also in other foods, such as wine, secoiridoids are specific for olive oil [21], [22]. The two main secoiridoids in olive oil are ligstroside and oleuropein, and their conversion products give olive oil its unique aroma and taste. During the olive-pressing process or if the drupes are injured, ligstroside and oleuropein in the fresh drupes can enter different transformation-reaction pathways, such as their enzymatic and chemical transformation to aldehyde or hydroxy forms [23]. One possible transformation pathway is autooxidative decay as a consequence of the protection of phenolic compounds against harmful oxidative changes [24]. This conversion is gradual and continues in the olive oil throughout its use. During olive oil storage, hydrolytic mechanisms that lead to the release of simple phenols, such as hydroxytyrosol and tyrosol, from the complex phenols, such as the secoiridoids, can occur [25]-[27]. The newly formed substances from these secoiridoids have amphiphilic characteristics, and are thus partitioned between the oily layer and the vegetation water, and are concentrated in the water fraction through their polar functional group [1]. As long as these secoiridoids do not get transformed into their final forms (i.e., the aromatic alcohols tyrosol and hydroxytyrosol), the olive oil preserves its freshness, fruitiness and harmony. It should be noted that when the transformation pathway is reaching its end and the olive oil has already lost its freshness and antioxidative properties, the content of total phenolic compounds can be relatively high. Therefore, it is crucial to determine the relative amounts of each of these compounds, and not just the total sum of all of the phenolic compounds.

Oxidation of olive oil begins as soon as it has been extracted. Endogenous enzymes in the olive fruit are involved in the phenolic profile and in its qualitative and quantitative modification during the processing of virgin olive oils [28]. However, olive oil is more severely affected during its storage. The decomposition of the phenolic compounds depends on oxygen, light, temperature, metals, pigments, unsaturated fatty acid content and composition, and quality and kind of natural antioxidants present [29], [30]. Part of the low quantity of water in virgin olive oil is free and available for chemical and enzymatic reactions. This low quantity of water keeps hydrophilic phenols in solution, which is where the decomposition process can occur for the phenols and triacylglycerols during olive oil storage [31], [1]. Furthermore, according to Frankel [30], the hydrophilic antioxidants, such as the polar phenols that are oriented at the air-oil interface, can better protect against oxidation compared to the lipophilic antioxidants, like the tocopherols, which remain in solution in the olive oil. Moreover, Aparicio et al. [32] measured the correlation between the oxidative stability of virgin olive oil and several compositional variables. They showed that phenols, o-diphenols, and the oleic/ linoleic ratio have the highest stability values, followed by chlorophylls, total tocopherols and caratenoids. According to Tsimidou et al. [33], hydroxytyrosol is the most active antioxidant compound in virgin olive oil. Carrasco-Pancorbo et al. [34] showed that the hydroxytyrosol oleuropein-aglycone di-aldehyde (3,4-DHPEA-EDA; i.e., elenoic acid linked to hydroxytyrosol) and oleuropein aglycon have the strongest antioxidant power. Elenoic acid, which does not have a phenolic ring, was one of the compounds investigated in virgin olive oil that has the weakest antioxidant activity.

Extra virgin olive oil can be characterized by a unique combination of aroma and taste that is highly appreciated [35], [36]. The method of sensory evaluation of virgin olive oils, introduced in 1991 by Commission Regulation (EEC) No. 1348/2013 annex XII (revision of Reg. EEC 2568/91), lays down the procedure for evaluating the sensory attributes of virgin olive oils and quality classification (categorization). It specifies the criteria for sensory evaluation of virgin olive oil, as well as providing a special vocabulary and standardized conditions for evaluation. However, the panel test, performed according to EU procedures, it is an expensive process that requires accredited assayers and is not easily accessible to the companies that have limited production, and therefore alternative tools have been developed [37]. Many studies have tried to clarify the relationships between the sensory attributes in virgin olive oil and the phenolic compounds that are responsible for its aroma and taste [1], [36], [38]-[41]. Some studies have suggested that secoiridoid derivatives of hydroxytyrosol are the main contributors to olive oil bitterness [1]. Caponio et al. [38] showed that the bitter to pungent taste can be ascribable to oleuropein aglycon. Furthermore, oleuropein and its aglycon decrease as the ripening of olives progresses [38]. Rotondi et al. [39] confirmed the relationship between the decrease in bitterness and pungency and the reduction in total phenols and diphenol levels. In particular a positive correlation between the content of oleuropein and ligstroside derivatives and the bitterness and pungency was shown. Frank and co-authors [40] reported that when an isomer (or isomers) of oleuropein aglycon was prepared by β-glucosidase hydrolysis of oleuropein isolated from olives and evaluated by assessors, it was defined as bitter. Using the same evaluation technique, no bitterness was observed for hydroxytyrosol or elenolic acid. According to Andrewes et al. [41], the dialdehyde form of decarboxymethyl ligstroside aglycone (p-HPEA-EDA) is the key source of the pungent sensation found in olive oil, while 3,4-DHPEA-EDA produces very little burning sensation. Moreover, Beauchamp et al. [5] assessed the pungent intensity of p-HPEA-EDA isolated from different virgin olive oils, and confirmed that p-HPEA-EDA is the principal agent responsible for throat irritation. Gutierrez-Rosales et al. [42] concluded that the chromatographic peaks corresponding to 3,4-DHPEA-EDA, oleuropein-aglycone mono-aldehyde (3,4-DHPEA-EA) and p-HPEA-EDA are mainly responsible for the bitter taste of virgin olive oil. Overall, some phenols mainly define the bitterness of olive oil, while others define the perception of pungency, and these might be related to the olive variety.

In the present study, the levels of 14 phenolic compounds and the total sum of all of the phenolic compounds included were determined in 167 samples. In all of the samples, an International Olive Council (IOC) recognized panel tested the sensory properties, as the bitterness, pungency, olive fruity, defects and sensations that resemble olive fruit, green-leaf, tomato, almond, artichoke and vanilla. The aim of the present study was to determine the changes in the phenolic compounds and sensory properties during the storage of the oil of the "Istrska belica" variety, which is specific and very important for the region of Istria. The phenolic profile and sensory properties of "Istrska belica" oil were further compared to the phenolic compounds and sensory properties determined for the oil of other varieties grown in the study area. In addition, the aim was to define the correlations between the content of phenolic compounds and the sensory properties of the fresh oil, and after 1 year and 2 years of storage. To the best of our knowledge, this is the first investigation into the effects of storage on the content of the phenolic compounds and the sensory properties as tested for the “Istrska belica” variety, the oil of which has a characteristic high content of phenolic compounds and a bitter taste.

2. METHODS

2.1. Collection and storage of samples

More than 80 samples of fresh olive oil were randomly collected from Slovenian producers. During this 3-year project, the determination of the phenolic compounds and the sensory analyses were carried out on both fresh oil and stored oil produced in Slovenia. Therefore, the first year samples (2011) were analysed after 1 year and 2 years of storage, and the second year samples (2012) were analysed after 1 year of storage.

Olive fruit samples were hand-picked from olive trees at the same optimal maturity level for each cultivar taking into consideration the index of maturity and the hardness of the fruit [43]. Healthy fruits, without any kind of infection or physical damage, were used for the oil production. The collected samples of extra virgin olive oils were produced using modern production technology with a two-phase decanter. During the production process the temperature was monitored and maintained at 27 °C. The oils were produced according to good manufacturing practice guide [44]. The chemical analyses of fresh olive oil samples were performed after the extraction process in each particular year. All the olive oil samples were stored at 20°C in the closed dark bottles in the same place.

2.2. Sensory analysis

The sensory characteristics were determined by a panel composed of eight trained assessors. The sensory properties of fruitiness, bitterness, pungency and other specific characteristics of selected oils were assessed based on the method defined in Annex XII of EEC Regulation No 2568/91, 640/2008, which includes the use of a 10-cm linear scale for intensity determination.

2.3. Determination of phenolic compounds

The phenolic compounds in the olive oil, such as the natural and oxidised derivatives of oleuropein and ligstroside, lignans, flavonoids and phenolic acids, were extracted using 60 % (w/w) aqueous methanol solution, and analysed by reverse phase high-performance liquid chromatography (HPLC), according to COI/T.20/Doc No 29 [42]. The HPLC system (Agilent 1100) was equipped with a thermostated autosampler, a binary pump system (BinPump G1312A), and a diode array detector (G1315B). A Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250 × 4.6 mm i.d.; Torrance, CA, USA) was used. The analyses were performed according to a modified method published by the IOC [42]. Detection was at 280 nm, with the exception of the flavonoids luteolin and apigenin, which were detected at 340 nm. Calibration curves for tyrosol (mass fraction from 30 to 800 mg/kg; $y = 0.0811a$) were constructed using standard compounds. All of the phenolic compounds were determined according to the IOC publication and quantified using the response factor for tyrosol [42]. In this study, the following groups of phenolic compounds were determined: 1) The sum of oleuropein and the ligstroside derivatives; 2) the sum of tyrosol and hydroxytyrosol; 3) the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids; and 4) the total phenolic compounds (mg/kg). The term “total phenolic compounds” refers to the “biophenolic minor polar compounds” determined according to the IOC method [45].

2.4. Statistical analysis

All of the data were analysed using the STATA13/SE software. The distribution of the total phenolic compounds, oleuropein, the ligstroside derivatives, tyrosol and hydroxytyrosol determined in the fresh olive oils and after 1 year and 2 years of storage are given as box plots. The normality of the variable distributions was determined using Shapiro–Wilk tests. The correlations between the levels of the determined phenolic compounds and the sensory parameters were evaluated. Spearman rank correlations were used for bivariate comparisons. Due to the significant correlations between the variables of the different phenolic compounds, factor analysis was applied. After running the factor analysis, rotation of the factor loads was performed to provide a clearer pattern. From the different concentraions of the specific phenolic compounds, new variables were created ($n=5$) for “eigenvalues” >1. Wilcoxon–Mann–Whitney tests were applied for comparisons of two different groups, and Kruskal Wallis tests were applied for comparisons of three different groups. The level of statistical significance was set to $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Phenolic compounds

The phenolic compounds were quantified using the response factor for tyrosol [41]. The sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids (1), the sum of oleuropein and the ligstroside derivatives (2), and the sum of tyrosol and hydroxytyrosol (3) determined in all the fresh olive oils from three years of sampling varied from 145 mg/kg to 966 mg/kg (median, 417 mg/kg), 83 mg/kg to 584 mg/kg (median, 251 mg/kg), and 2 mg/kg to 97 mg/kg (median, 9 mg/kg), respectively. It is important to note that the composition of the phenolic compounds can vary widely according to each olive variety. The contents of the flavonoids luteolin and apigenin in the fresh olive oil from “Istrska belica” were in the ranges of 2.6 mg/kg to 5.8 mg/kg ($n = 20$) and 0.9 mg/kg to 1.9 mg/kg ($n = 20$), respectively. The same flavonoids contents for “Leccino” oil ranged from 1.5 mg/kg to 4.3 mg/kg ($n = 11$) and 0.3 mg/kg to 0.8 mg/kg ($n = 11$), and for “Maurino” oil they ranged from 0.8 mg/kg to 2.0 mg/kg ($n = 4$) and 0.2 mg/kg to 0.6 mg/kg ($n = 4$). The “Istrska belica” oil lignans ranged from 22 mg/kg to 70 mg/kg ($n = 20$), with “Leccino” oil as 11 mg/kg to 33 mg/kg ($n = 15$), and “Maurino” oil as 46 mg/kg to 51 mg/kg ($n = 6$). The dialdehyde forms of both decarboxymethyl oleuropein aglycone (DMO-Agl-dA) and decarboxymethyl ligstroside aglycone (DML-Agl-dA) for “Istrska belica” oil varied from 23 mg/kg to 124 mg/kg ($n = 20$), with “Leccino” from 17 mg/kg to 274 mg/kg ($n = 15$), and “Maurino” from limit of detection (LOD) to 119 mg/kg ($n = 6$). The oxidised aldehyde and hydroxyl forms of oleuropein aglycone (O-Agl-dA) and ligstroside aglycone (L-Agl-dA) were lower compared to DMO-Agl-dA and DML-Agl-dA, and for “Istrska belica” oil they varied from 5.6 mg/kg to 87 mg/kg ($n = 20$), with “Leccino” from 1.5 mg/kg to 21 mg/kg ($n = 15$), and “Maurino” from <LOD to 30 mg/kg ($n = 6$). As expected, the fresh olive oil from “Istrska belica” showed the highest median for the total phenolic compounds (median, 616 mg/kg; minimum, 324 mg/kg; maximum, 787 mg/kg; $n = 20$) and for the sum of oleuropein and the ligstroside derivatives (median, 366 mg/kg; minimum, 165 mg/kg; maximum, 515 mg/kg; $n = 20$). Statistical analysis showed that the differences in the

content of the total phenolic compounds and oleuropein and the ligstroside derivatives in the fresh olive oil from 'Istrska belica" compared to those determined in the fresh olive oil from the other varieties ($median_{total}$, 360 mg/kg; $median_{sec}$, 212 mg/kg; n = 61) were significant (z_{total} = -5.049; p_{total} <0.005; z_{sec} = -4.063; p_{sec} <0.005). This confirms that the high content of phenolic compounds is a specific characteristic of the "Istrska belica" variety, compared to the levels determined in the other varieties grown in these Slovenian olive groves.

The phenolic compounds contents of these olive oils varied greatly according to crop year (Table 1, Figure 1). The variations in the sum of oleuropein and the ligstroside derivatives, the sum of tyrosol and hydroxytyrosol, and the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids in the fresh olive oils from the crop years 2011, 2012 and 2013 are shown in Figure 1. These levels (medians, 346 mg/kg, 13 mg/kg, 410 mg/kg, respectively) were highest in the crop year 2012, compared to the years 2011 and 2013. Statistical analysis (Wilcoxon–Mann–Whitney tests) showed that these differences were significant for the sum of oleuropein and the ligstroside derivatives (z = -4.655; p <0.005) and for the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids (z = -4.572; p <0.005), with marginal significant for the sum of tyrosol and hydroxytyrosol (z = -2.04; p = 0.04). The highest levels of the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids in crop year 2012 might have been due to the extreme weather conditions, with a drought for crop year 2012 (Figure 1). Less than 50% of normal rainfall fell in the crop year 2012 in the study area [46]. The variations in the levels of tyrosol and hydroxytyrosol are not so obvious, because most of these were probably the end-products of the decomposition pathways of oleuropein and the ligstroside derivatives, and their concentrations might not be directly influenced by the different conditions across the crop years. These variations might be greater after a time of storage longer than 3 years.

The sum of oleuropein, the ligstroside derivatives, tyrosol and hydroxytyrosol in the fresh olive oils following 1 year and 2 years of storage are shown in Figure 2. While the levels of oleuropein and the ligstroside derivatives decreased significantly across the years, the end-stage compounds tyrosol and hydroxytyrosol only increased after 2 years of storage (Figure 2). The sum of oleuropein and the ligstroside derivatives (median, 251 mg/kg), and the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids (median, 308 mg/kg) were higher in the fresh oils compared to the levels of oleuropein and the ligstroside derivatives (median, 162 mg/kg) and the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids (median, 226 mg/kg) in the oils stored for 1 year and for 2 years. The sum of tyrosol and hydroxytyrosol was higher in the oils stored for 2 years (median, 32 mg/kg), compared to fresh and 1-year-stored oils (median, 14 mg/kg). Statistical analysis (Wilcoxon–Mann–Whitney tests) showed that these differences were significant in the case of the sum of oleuropein and the ligstroside derivatives (z = 5.226; p <0.005), the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids (z = 4.322; p <0.005), and the sum of tyrosol and hydroxytyrosol (z = -4.970; p <0.005). The relatively high content of the sum of oleuropein, the ligstroside derivatives, tyrosol, hydroxytyrosol, lignans and phenolic acids in the 2-year-stored oils (median, 296 mg/kg; minimum, 138 mg/kg; maximum, 594 mg/kg) is in agreement with the concept that when the transformation pathway of the phenolic compounds is reaching its end, oleuropein and the ligstroside derivatives become substituted with the end-stage compounds tyrosol and hydroxytyrosol.

3.2. Sensory properties

A high content of phenolic compounds gives an oil its characteristic bitter taste and pungent tactile sensation. According to the present study, the intensity of bitterness (median, 3.9) and pungency (median, 4.6) in the olive oils was highest in the "Istrska belica" oil compared to the oils of the other varieties, such as "Leccino" ($median_{bitterness}$, 3.3; $median_{pungency}$, 3.9) and "Maurino" ($median_{bitterness}$, 3.5; $median_{pungency}$, 4.0). Statistical analysis (Wilcoxon–Mann–Whitney tests) showed that the differences between the intensity of bitterness and pungency in the olive oils from "Istrska belica" and the other analysed oils were significant ($z_{bitterness}$ = -3.111. $p_{bitterness}$ = 0.002; $z_{pungency}$ = -3.688, $p_{pungency}$ <0.005). Moreover, the olive oil from "Istrska belica" had a wide range for its sensory profile. In the olive oil from "Istrska belica" there were tastes reminiscent of artichoke, almond, tomato, green-leaf and vanilla. In comparison, in olive oil from "Maurino" there were only tastes reminiscent of green-leaf and almonds.

As for the phenolic compounds in olive oil, the sensory properties of olive oils can vary greatly on a yearly basis. The highest medians of bitterness (median, 3.7; minimum, 1.6; maximum 4.4) and pungency (median, 4.6; minimum, 1.9; maximum, 5.4) in these olive oils were found for the fresh olive oil from crop year 2012. The bitterness and pungency of olive oils are influenced by the phenolic compounds in the olive oil. Therefore, the highest median score of bitterness and pungency might be due to the extreme weather conditions, which included a drought in crop year 2012 [46]. However, the highest bitterness and pungency in 2012 were only evident for oils from varieties such as "Istrska belica", "Črnica" and "Leccio del corno", while for many varieties included in this study, this was not noted (Figure 3). This might be because the specific composition of phenolic compounds of the olive oils depends on the variety. However, due to the relatively low numbers of samples for each variety, with exception of "Istrska belica", "Maurino" and "Leccino", further investigations are necessary to confirm these observations.

Bitterness and pungency are highly dependent on the phenolic compounds in olive oil, and these sensory properties also decreased after 2 years of storage (Figure 4). Like the variation in bitterness and pungency according to the crop year that depended on the specific varieties (Figure 3), the variation in the bitterness and pungency according to the time of storage was also highly dependent on the variety. "Istrska belica" preserved a similar intensity of bitterness and pungency after 1 year of storage, compared to the fresh oil. This was not seen for varieties such as "Leccino" and "Maurino", where bitterness and pungency decreased greatly after only 1 year of storage (Figure 4). The intensities of bitterness and pungency in the olive oil from "Istrska belica" decreased greatly only after 2 years of storage. These findings are in agreement with the content of oleuropein and the ligstroside derivatives in "Istrska belica" in oil from the crop year 2011 (Table 1). Therefore, it is important to emphasise the advantages that this domesticated variety provides compared to the other varieties that are grown in this study region.

Table 1. Mean data (±standard deviation/ range) for the sums of the compounds determined (as indicated) in the fresh olive oils and after 1 and 2 years of storage, for the oils of the different olive varieties analysed.

Storage (years)	Variety	Crop year	n	Sum of oleuropein and the ligstroside derivatives (mg/kg)	Sum of TyrOH and Tyr (mg/kg)	Sum of oleuropein and ligstroside derivatives, Tyr, TyrOH, lignans and phenolic acids (mg/kg)	Total phenolic compounds (mg/kg)
Fresh	"Istrska belica"	2011	6	251 ±58	11 ±2	321 ±68	491 ±105
		2012	8	429 ±74	21 ±19	514 ±93	664 ±118
		2013	6	373 ±70	12 ±7	449 ±79	604 ±105
	"Leccino"	2011	6	197 ±78	6 ±2	225 ±82	293 ±105
		2012	5	340 ±178	21 ±21	389 ±170	465 ±188
		2013	4	211 ±127	6 ±3	239 ±124	302 ±137
	"Maurino"	2011	2	141 ; 225	8 ; 10	200 ; 289	200 ; 289
		2012	2	251 ; 326	7 ; 8	309 ; 383	308 ; 383
		2013	2	121 ; 160	2 ; 8	180 ; 210	180 ; 210
	"Buga"	2011	2	207 ; 240	7 ; 12	253 ; 276	327 ; 349
		2012	1	326	9.0	375	461
		2013	1	189	6.0	229	292
	"Črnica"	2011	2	157; 189	20 ; 20	222 ; 271	306 ; 377
		2012	1	364	40	469	573
		2013	2	142 ; 261	29 ; 97	283 ; 318	358 ; 424
	"Leccio del corno"	2011	1	159	6.0	219	318
		2012	1	367	5	424	516
		2013	1	128	2	157	253
	"Leccione"	2011	1	184	12	227	360
		2012	1	285	21	335	450
		2013	1	394	19	446	556
	"Arbequina"	2011	1	187	2	226	271
		2012	0	/	/	/	/
		2013	0	/	/	/	/
	"Komuna"	2011	1	266	15	343	492
		2012	0	/	/	/	/
		2013	0	/	/	/	/
	"Mata"	2011	1	213	11	249	362
		2012	0	/	/	/	/
		2013	1	166	21	232	295
	Mixed	2011	6	171 ±46	8 ±2	226 ±50	320 ±72
		2012	5	328 ±46	9 ±3	385 ±51	492 ±62
		2013	5	288 ±205	9 ±3	351 ±241	482 ±310
	All	2011	31	198 ±59	9 ±4	250 ±67	351 ±108
		2012	25	356 ±104	17 ±16	422 ±115	533 ±144
		2013	24	259 ±138	14 ±19	318 ±155	426 ±201
1	"Istrska belica"	2011	6	247 ±56	37 ±10	345 ±69	508 ±86
		2012	8	318 ±83	42 ±25	396 ±103	566 ±124
	"Leccino"	2011	6	159 ±54	13 ±6	194 ±62	270 ±80
		2012	5	232 ±121	23 ±10	273 ±119	375 ±167
	"Maurino"	2011	2	104 ; 148	24 ; 36	177 ; 234	288 ; 321
		2012	2	176 ; 227	20 ; 25	244 ; 285	368 ; 400
	"Buga"	2011	2	189 ; 214	20 ; 26	242 ; 265	335 ; 376
		2012	1	212	17	254	343
	"Črnica"	2011	2	142 ; 148	27 ; 32	215 ; 233	309 ; 342
		2012	1	317	54	412	587
	"Leccio del corno"	2011	1	107	20	177	282
		2012	1	257	19	304	452
	"Leccione"	2011	1	140	43	217	364
		2012	1	180	30	223	339
	"Arbequina"	2011	1	173	6	220	270
		2012	0	/	/	/	/
	"Komuna"	2011	1	259	46	369	512
		2012	0	/	/	/	/
	"Mata"	2011	1	155	24	198	316
		2012	0	/	/	/	/
	Mixed	2011	6	126 ±45	22 ±7	192 ±48	290 ±70
		2012	5	215 ±47	26 ±9	276 ±53	402 ±63
	All	2011	31	166 ±64	25 ±12	233 ±80	340 ±113
		2012	25	252 ±88	31 ±18	314 ±102	447 ±140
2	"Istrska belica"	2011	6	142 ±33	65 ±19	256 ± 56	458 ±108
	"Leccino"	2011	6	106 ±63	18 ±8	145 ±65	237 ±88
	"Maurino"	2011	2	48 ; 143	32 ; 48	129 ; 241	212 ; 345
	"Buga"	2011	2	135 ; 188	27 ; 40	190 ; 237	296 ; 368
	"Črnica"	2011	2	101 ; 144	32 ; 41	168 ; 239	287 ; 353
	"Leccio del corno"	2011	1	96	32	178	335
	"Leccione"	2011	1	78	64	/	/
	"Arbequina"	2011	1	171	10	225	267
	"Komuna"	2011	1	173	68	288	473
	"Mata"	2011	1	102	33	153	257
	Mixed	2011	6	89 ±30	22 ±7	166 ±35	272 ±49
	All	2011	31	114 ±46	25 ±12	189 ±63	312 ±109

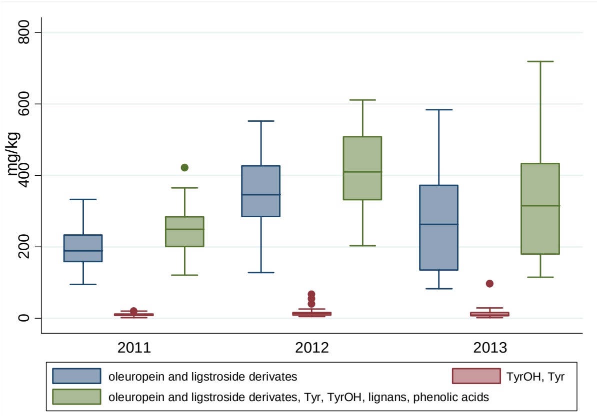

Figure 1. Box plot of the sum of oleuropein and the ligstroside derivatives, the sum of oleuropein, the listroside derivatives, tyrosol (Tyr), hydroxytyrosol (TyrOH), lignans and phenolic acids, and the sum of Tyr and TyrOH in the fresh olive oil from crop years 2011, 2012 and 2013.

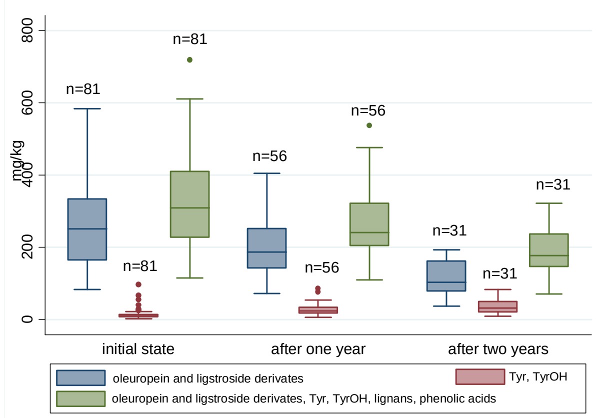

Figure 2. Box plot of the sum of oleuropein and the ligstroside derivatives, the sum of oleuropein, the ligstroside derivatives, tyrosol (Tyr), hydroxytyrosol (Tyr-OH), lignans and phenolic acids, and the sum of Tyr and Tyr-OH in the fresh olive oil and after 1 year and 2 years of storage.

3.3. Correlations between sensory profiles and phenolic compounds in the olive oils Managing frames

As indicated in the literature, the relationship between the sensory parameters in virgin olive oils and the levels of total phenolic compounds is well known [36], [1]. The significant positive correlation between the levels of oleuropein and the ligstroside derivatives and bitterness and pungency is in agreement with the literature [1], [39], [40], based on the present data, it is very difficult to make assumptions about which phenolic compound is attributed to the chemo-aesthetic perception of pungency and bitterness. This is mainly because of the significant high correlations between the levels of specific assigned phenolic compounds in the collected samples. Due to these significant correlations, factor analysis was applied. The Spearman correlations between the pungency and bitternes was increasingly significant for the factors that included oleuropein aglycone, ligstroside aglycone, lignans (factor 1) and the dialdehyde forms of decarboxymethyl oleuropein aglycone and decarboxymethyl ligstroside aglycone (factor 2).

4. CONCLUSIONS

The content of the phenolic compounds and the sensory properties of these virgin olive oils varied greatly according to variety and crop year. According to our data, the content of total phenolic compounds, the levels of oleuropein and the ligstroside derivatives, the intensity of bitterness, and the pungency were greatest in "Istrska belica" fresh olive oil, compared to the other oil varieties analysed here. This study confirms that a high content of phenolic compounds and an astringent and bitter taste is a specific characteristic of "Istrska belica" olive oil. Moreover, "Istrska belica" olive oil has a wide range in its sensory profile, where the tastes are reminiscent of artichoke, almond, tomato, green-leaf, and vanilla.

The levels of the phenolic compounds and the sensory properties of these high quality olive oils are closely related. A significant positive correlation was found between the levels of oleuropein and the ligstroside derivatives and the bitterness and pungency. The total phenolic compounds of these olive oils decreased after 1 year and 2 years of storage. While the levels of oleuropein and the ligstroside derivatives significantly decreased with this storage, the end-stage compounds tyrosol and hydroxytyrosol increased after 2 years of storage. "Istrska belica" oil maintains similar levels of oleuropein and the ligstroside derivatives and of the intensity of bitterness and pungency after 1 year of storage, compared to the fresh oil. This was not evident for the other varieties involved in the present study. The intensities of bitterness and pungency of the "Istrska belica" olive oil decreased greatly only after 2 years of storage. Overall, it is important to note the advantages that "Istrska belica" olives have compared to the other varieties that are grown in Slovenian olive groves.

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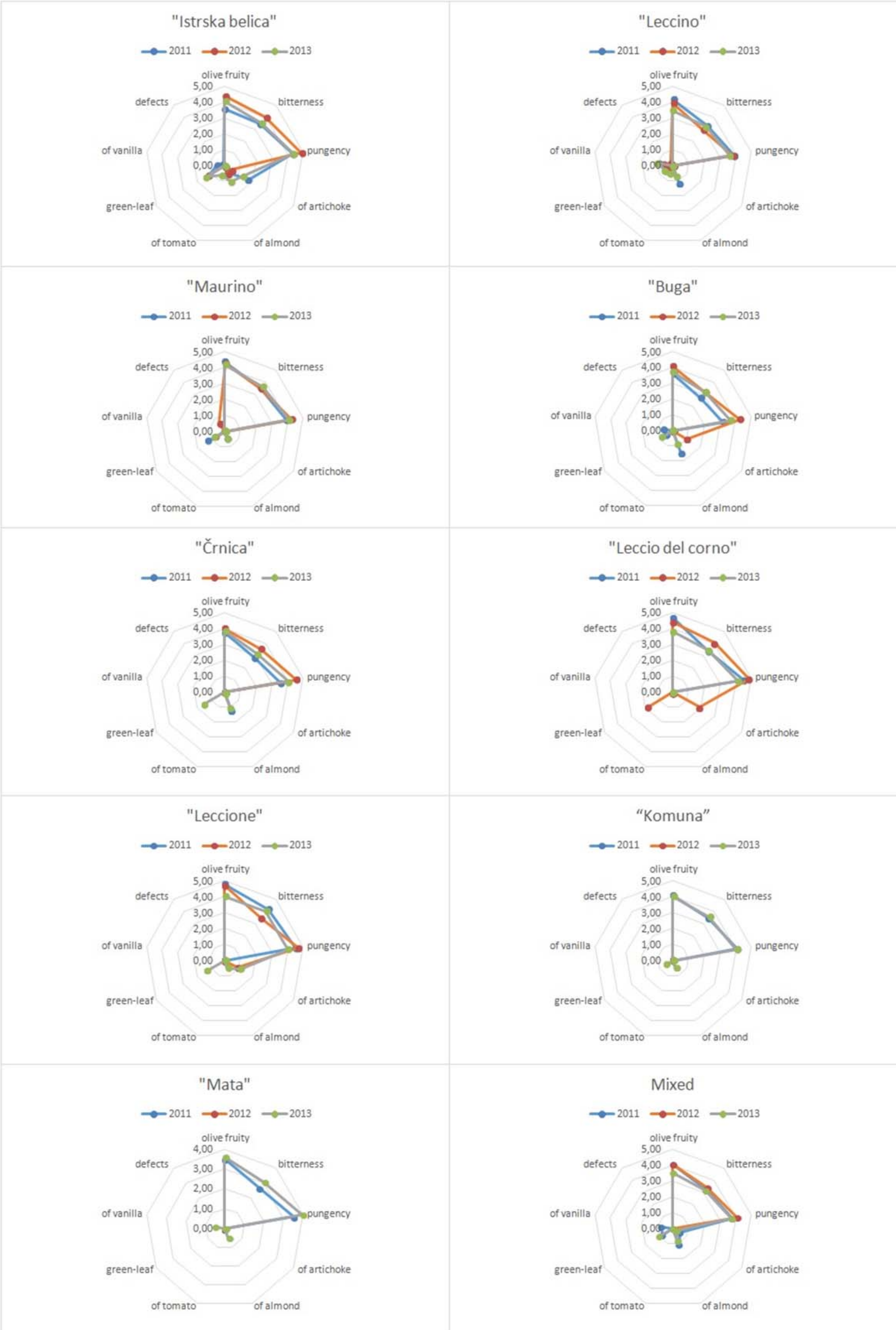

Figure 3. Sensory profiles of the various varieties and the mixed sample, as indicated, of the fresh olive oils from the crop years 2011, 2012 and 2013.

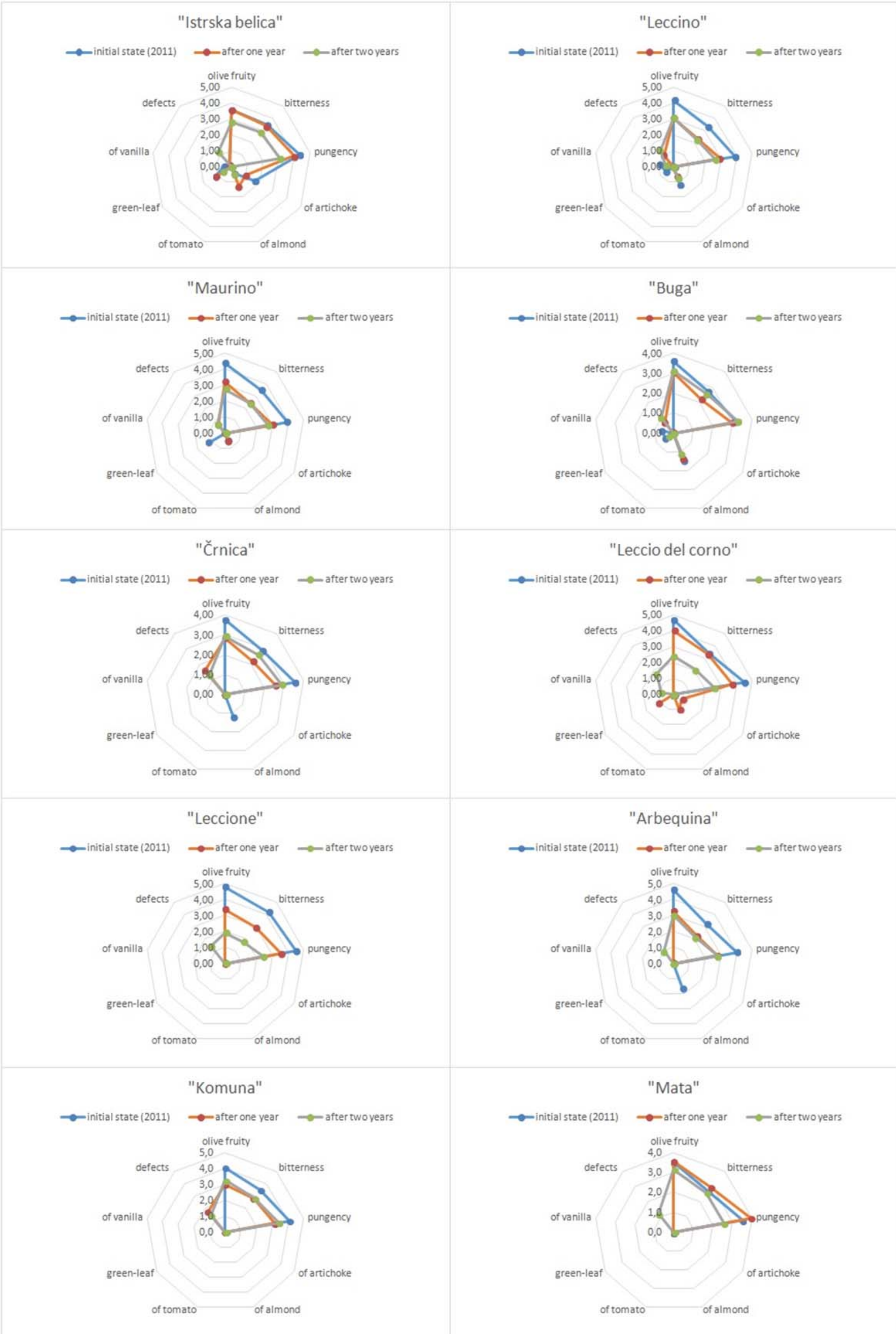

Figure 4. Sensory profiles of various varieties (as indicated) for the fresh olive oil from crop year 2011, after 1 year and 2 years of storage.

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