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Sterol, triterpen dialcohol and fatty acid profile of less- and well-known Istrian monovarietal olive oil

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ABSTRACT

The aim of this study was to characterize monovarietal olive oils obtained from two less known autochthonous cultivars (Bova and Buža puntoža) on the basis of sterols, triterpene dialcohols and fatty acids profile for the first time, and three monovarietal olive oils obtained from the most widespread autochthonous cultivars (Buža, Istarska bjelica and Rosinjola) grown in Istria, Croatia. β-sitosterol, Δ-5-avenasterol and campesterol were the most abundant sterols in all samples. Campesterol, β-sitosterol and Δ-5-avenasterol most significantly differentiated investigated oils. Bova oil had the highest amount of total sterols (2964 ± 458 mg/kg). Istarska bjelica oil had a peculiar sterol composition with exceptionally low β-sitosterol (67%), high Δ-5-avenasterol (27%) and the highest triterpene dialcohols percentages. Rosinjola and Istarska bjelica oils had the highest monounsaturated fatty acids level, Buža oil had the highest polyunsaturated fatty acids level, while Bova oil showed the highest level of saturated fatty acids. Tested oils obtained from the most abundant cultivars fulfilled all the demands of the current EU regulation required for virgin olive oil regarding sterols, triterpene dialcohols and fatty acids, but Bova and Buža puntoža oil slightly exceeded the upper limit for linolenic acid. Since cultivar is the source of natural variation of sterols, triterpene dialcohols and fatty acids in virgin olive oils, the knowledge about the content of these particular compounds in different monovarietal oils from autochthonous cultivars is important to determine possible disagreements with the demands of the current legislation required for virgin olive oils, in order to anticipate possible false results indicating adulteration..

Introduction

Virgin olive oil (*VOO*) is one of the main components of the Mediterranean diet highly appreciated for its delicious taste and aroma, as well as for its healthy and nutritional properties. These properties are mainly associated with its high monounsaturated oleic acid content as well as with a broad range of biological active minor components, such as phenols, tocopherols and sterols (Frankel et al., 2011). Furthermore, high concentration of monounsaturated fatty acids and antioxidative minor components as well as some sterols contribute to higher olive oil stability (Velasco and Dobarganes, 2002). The content and composition of those components depend on many environmental, agronomic and technological factors, such as geographic origin, cultivar, harvesting date and processing conditions (Koutsaftakis et al., 1999; Lukić et al., 2013). In the European Union as well as in other olive oil production countries, the content of sterols, triterpene dialcohols and fatty acids in different olive oil categories is limited by trade standards and regulations in order to prevent adulteration of *VOO* categories with some other oils (EEC, 1991; IOC, 2016). Moreover, there are investigations which

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confirm that there are cases in which oils obtained from specific cultivars or from certain geographical origin showed incompliance by the mentioned standards and regulations limits (Koutsaftakis et al., 1999; Rivera del Álamo et al., 2004; Mailer et al., 2010). For all these reasons, the knowledge about the content of these particular compounds in different monovarietal *VOO*s produced from autochthonous cultivars is important to determine eventual disagreements with the demands of the current legislation required for *VOO*s, as well as to estimate their health and nutritional properties (Manai-Djebali et al., 2012; Kosma et al., 2016).

In Croatia, olive plantations cover 19.100 ha (Statistical Yearbook, 2016) and olive trees are widespread in Istria and in Kvarner and Dalmatian coast and its islands. Istria region is a specific olive growing area since it is considered one of the marginal northern areas of olive cultivation and it is of great importance to establish a database with chemical and sensorial characteristics of monovarietal olive oils from this particular geographical region in order to anticipate possible false results indicating adulteration. Furthermore, characterization of Istrian monovarietal olive oil is important for selection of autochthonous cultivars adapted to the environmental conditions that could produce high quality extra virgin olive oils. A number of autochthonous olive cultivars have been found in Istria, but sterol and triterpene dialcohols composition of their monovarietal oils was not studied extensively and for some of them, as Bova and Buža Puntoža cultivars, not at all. Therefore, the aim of this study was to characterize monovarietal olive oils obtained from two less known autochthonous cultivars grown in Istria (Bova and Buža puntoža) on the basis of sterols, triterpene dialcohols and fatty acids profile for the first time, and monovarietal olive oils obtained from three most widespread autochthonous cultivars (Buža, Istarska bjelica and Rosinjola), grown in the same pedoclimatic conditions.

Materials and methods

Preparation of VOO samples

Investigated olive trees of five autochthonous cultivars Bova, Buža puntoža, Buža, Istarska bjelica and Rosinjola are grown in the same orchard located in the west part of Istrian region (Croatia). Approximately, 1.5 kg of olive fruits from each of the three trees per cultivar were handpicked at the same ripening index (RI = 2.5 - 3.0) at the end of October 2009, except Istarska bjelica cultivar which has slow skin pigmentation and changes colour later than other investigated cultivars so it was harvested in the middle of November at a lower ripening degree (RI = 1.1). RI of fruits was evaluated on the basis of the olive skin and pulp colour (Garcia & Yousfi 2005). In order to obtain monovarietal oils, fruits from each tree were processed separately under same conditions using an Abencor system (MC2 Ingenieria y Sistemas, Spain) within 24 hours after harvesting. Fruits were crushed with a hammer mill and olive paste was malaxed at 25 ± 1 °C for 45 min. After centrifugation at 3500 rpm for 60 seconds, extracted oil samples were decanted and stored at room temperature in taped dark bottles until analyses.

The analysis of sterols, triterpene dialcohols and fatty acid composition

Determination of the composition and content of sterols and triterpene dialcohols, as well as fatty acid composition, was conducted according to methods prescribed by EEC regulations (EEC, 1991) using an Agilent HP, 6890 gas chromatograph (Agilent technologies, CA, USA).

Statistical analysis

Mean values of concentrations and their standard deviations were calculated from three replicates (three samples for each investigated monovarietal olive oil). One-way analysis of variance (ANOVA) was carried out using the software package Statistica v. 9 (Stat-Soft, Tulsa, OK, USA) and Tukey's test was used to compare the means at the level of significance of $p < 0.05$.

Results and discussion

Sterols and triterpene dialcohols

Tested oils fulfilled all demands of the current international trade standard established by the IOC as well as EU legislation (EEC, 1991; IOC, 2016) required for *VOO*s regarding sterols and triterpene dialcohols (Table 1). Since this was the first attempt to characterize oils from Bova and Buža puntoža cultivars based on sterols and triterpene dialcohols, compliance with the regulation is also economically important. Among investigated monovarietal olive oils, Rosinjola oil had significantly the lowest relative amount of apparent β-sitosterol, even though not under the legal minimum for extra *VOO*s. Relative amount of campesterol was found to be significantly the highest in Rosinjola oil and close to the upper limit for *VOO*. In some samples of Spanish Cornicabra and Greek Koroneiki monovarietal *VOO*s a similar problem was encountered, but in those oils campesterol level exceeded the legal maximum of 4.0 % (Koutsaftakis et al., 1999, Rivera del Álamo et al., 2004).

Table 1. Sterols and triterpene dialcohols (erythrodiol and uvaol) composition (%) in different Istrian monovarietal virgin olive oils

	Bova	Buža puntoža	Istarska bjelica	Buža	Rosinjola	Limits for *EVOO***
Cholesterol	0.07±0.01[a]	0.13±0.09[a]	0.11±0.02[a]	0.10±0.02[a]	0.13±0.01[a]	≤ 0.5
Brassicasterol	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	≤ 0.1
24-Methylene-cholesterol	0.09±0.02[c]	0.14±0.01[bc]	0.30±0.03[a]	0.14±0.03[bc]	0.16±0.03[b]	
Campesterol	2.18±0.10[c]	1.97±0.02[d]	1.96±0.03[d]	2.87±0.04[b]	3.29±0.04[a]	≤ 4.0
Campestanol	0.03±0.01[b]	0.04±0.01[b]	0.07±0.00[a]	0.04±0.01[b]	0.05±0.01[b]	
Stigmasterol	0.70±0.10[ab]	0.53±0.04[b]	0.84±0.13[a]	0.54±0.04[b]	0.79±0.14[ab]	<Campesterol
Δ-7-Campesterol	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	
Δ-5,23-Stigmastadeniol	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	
Clerosterol	0.92±0.02[bc]	1.01±0.03[a]	0.90±0.03[b]	0.94±0.02[bc]	0.86±0.04[c]	
β-Sitosterol	87.89±1.10[a]	84.08±0.61[b]	67.07±0.22[c]	87.51±1.19[a]	82.05±0.91[b]	
Sitostanol	0.78±0.04[ab]	0.74±0.12[b]	0.96±0.10[a]	0.47±0.01[c]	0.65±0.08[bc]	
Δ-5-Avenasterol	5.97±1.02[c]	9.97±0.62[b]	26.58±0.14[a]	6.18±0.99[c]	10.61±0.84[b]	
Δ-5,24-Stigmastadienol	0.50±0.03[bc]	0.65±0.02[b]	0.77±0.01[a]	0.59±0.07[bc]	0.83±0.04[a]	
Δ-7-Stigmastenol	0.17±0.03[a]	0.15±0.04[a]	0.11±0.04[a]	0.12±0.03[a]	0.13±0.01[a]	≤ 0.5
Δ-7-Avenasterol	0.72±0.07[a]	0.60±0.03[ab]	0.31±0.03[d]	0.50±0.07[bc]	0.47±0.02[c]	
Apparent β-Sitosterol	96.05±0.04[b]	96.45±0.12[a]	96.29±0.18[ab]	95.70±0.17[bc]	95.00±0.18[d]	≥ 93.0
total sterols (mg/kg)	2963.67±457.77[a]	2157.67±180.36[b]	1450.67±42.45[c]	1567.33±68.66[bc]	1647.67±23.86[bc]	≥ 1000
Erythrodiol and uvaol	1.04±0.24[b]	0.53±0.05[c]	3.16±0.28[a]	0.55±0.18[bc]	0.64±0.02[bc]	≤ 4.5

Results are mean values of 3 independent repetitions ± SD, the means within each row for single cultivar, labelled by different letters, are significantly different (Tukey's test, $p < 0.05$). **Actual limits for extra virgin olive oil (*EVOO*) category according to the European Commission Regulation (EEC, 1991) and IOC trade standards (IOC, 2016).

Different monovarietal olive oils showed different sterol profile (Table 1), although β-sitosterol, Δ-5-avenasterol and campesterol were the most abundant sterols in all samples. Istarska bjelica oils were characterized by a peculiar sterol composition with exceptionally low β-sitosterol (67%) and high Δ-5-avenasterol percentages (27%). This is in line with previously published studies regarding oils from this cultivar (Koprivnjak, 1996). It may turn out to be a robust point for cultivar characterization and differentiation, exploitable for marketing purposes. Since Istarska bjelica olive cultivar is known as, a cultivar that ripens late, this sterol composition is even more surprising knowing that as maturation advances, percentages of β-sitosterol were mainly found to decrease while the percentage of Δ-5-avenasterol increased (Lukić et al., 2013). High Δ-5-avenasterol content could be looked upon as an additional value in Istarska bjelica oils. This sterol has an ethylidene functional group in the side chain of its molecular structure, which retards polymerisation and oxidation of triglycerides during frying at high temperatures. This is especially important knowing that other antioxidants, such as tocopherols and polyphenols, are heat sensitive, while other sterols without the mentioned structural feature exhibit adverse or no effects (Gordon & Magos, 1983; Gordon & Magos, 1984; Boskou, 1998). Furthermore, Istarska bjelica oils were differentiated with statistical significance from other varieties by the highest level of 24-methylene-cholesterol and campestanol as well as the lowest level of Δ-7-avenasterol. Considering Δ-5,24-stigmastadienol, statistically the highest levels in Rosinjola and Istarska bjelica oils were determined. Significantly, the highest relative amount of clerosterol was determined in Buža puntoža oil. Campesterol, β-sitosterol and Δ-5-avenasterol were three parameters that most significantly differentiated five investigated monovarietal olive oils.

Bova oil had significantly the highest amount of total sterols, which are beneficial for human health. Previous research about sensory characteristics of Bova oil demonstrated that this cultivar could be for Croatia a viable alternative to other traditionally cultivated olive cultivars expanding the offer on the market both at local and international level (Brkić Bubola et al., 2014). Since it has been observed that level of sterols rises during ripening to a certain point (Lukić et al., 2013), the lowest ripening degree of the fruits from which Istarska bjelica oil was obtained could be a reason for the lowest total sterol content determined in that oil.

Considering the level of erythrodiol and uvaol (Table 1), Istarska bjelica oil was the most distinctive among investigated oils, having the highest percentage for the sum of these two triterpene dialcohols. Considering that Istarska bjelica fruits were harvested at an earlier stage of maturity, and the relative amount of triterpene diols is supposed to rise with ripening and be more easily extracted due to the thinning of olive exocarp (Lukić et al, 2013), such result is somewhat unexpected.

Table 2. Fatty acid profile (%) in different Istrian monovarietal virgin olive oils

	Bova	**Buža puntoža**	**Istarska bjelica**	**Buža**	**Rosinjola**	**Limits for *EVOO*****
Myristic	0.02±0.01^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	≤ 0.03
Palmitic	17.02±0.11^{a}	15.69±0.78^{b}	12.76±0.21^{d}	13.24±0.38^{cd}	13.46±0.33^{c}	7.50-20.00
Palmitoleic	3.10±0.50^{a}	1.62±0.11^{b}	1.16±0.02^{c}	1.29±0.10^{b}	1.44±0.10^{b}	0.30-3.50
Heptadecanoic	0.04±0.01^{d}	0.13±0.01^{a}	0.06±0.01^{c}	0.09±0.00^{b}	0.04±0.00^{d}	≤ 0.40
Heptadecenoic	0.11±0.01^{c}	0.35±0.01^{a}	0.11±0.01^{c}	0.21±0.01^{b}	0.10±0.00^{c}	≤ 0.60
Stearic	2.10±0.16^{b}	1.82±0.02^{c}	3.46±0.11^{a}	1.85±0.03^{c}	2.06±0.03^{b}	0.50-5.00
Oleic	64.51±0.90^{c}	67.81±0.80^{b}	73.93±0.18^{a}	67.73±1.90^{b}	74.82±0.93^{a}	55.00-83.00
Linoleic	11.26±0.55^{b}	10.48±0.61^{b}	6.82±0.27^{c}	14.03±1.47^{a}	6.64±0.61^{c}	2.50-21.00
Linolenic	1.04±0.13^{a}	1.12±0.07^{a}	0.55±0.01^{c}	0.76±0.03^{b}	0.60±0.02^{b}	≤ 1.00
Arachidic	0.36±0.02^{c}	0.40±0.01^{b}	0.57±0.01^{a}	0.34±0.01^{c}	0.36±0.01^{bc}	≤ 0.60
Eicosenoic	0.27±0.01^{c}	0.35±0.01^{a}	0.32±0.00^{ab}	0.30±0.02^{bc}	0.29±0.01^{c}	≤ 0.50
Behenic	0.11±0.01^{c}	0.15±0.01^{b}	0.17±0.00^{a}	0.10±0.01^{c}	0.10±0.01^{c}	≤ 0.20
Lignoceric	0.06±0.01^{bc}	0.07±0.01^{b}	0.09±0.00^{a}	0.05±0.01^{c}	0.06±0.00^{bc}	≤ 0.20
∑SFA	19.71±0.28^{a}	18.27±0.06^{b}	17.11±0.11^{c}	15.68±0.39^{d}	16.10±0.29^{d}	
∑MUFA	67.99±0.94^{b}	70.13±0.72^{b}	75.52±0.16^{a}	69.52±1.83^{b}	76.65±0.85^{a}	
∑PUFA	12.30±0.67^{b}	11.61±0.68^{b}	7.36±0.27^{c}	14.79±1.44^{a}	7.25±0.60^{c}	
oleic/linoleic ratio	5.74±0.36^{b}	6.49±0.45^{b}	10.86±0.45^{a}	4.87±0.65^{b}	11.34±1.25^{a}	

Results are mean values of 3 independent repetitions ± SD, the means within each row for single cultivar, labelled by different letters, are significantly different (Tukey's test, $p < 0.05$). **Actual limits for extra virgin olive oil (*EVOO*) category according to the European Commission Regulation (EEC, 1991) and IOC trade standards (IOC, 2016).

Fatty acids profile

All investigated oils fulfilled the demands of the current EU legislation required for *VOO*s regarding fatty acids (EEC, 1991; IOC 2016), except Buža puntoža and Bova oil in the case of linolenic acid, which slightly exceeded the upper limit for this fatty acid (Table 2). Although high natural content of linolenic acid could be a specific characteristic of particular monovarietal oils, this could bring in question their authenticity confirmation according to this parameter. Rosinjola and Istarska bjelica oils showed the highest levels of monounsaturated fatty acids (MUFA), especially oleic acid (74%). Istrian region is considered one of the marginal northern areas of olive cultivation as regards mean temperatures (Moretini, 1972) and it is known that oils originated from colder growing area are generally richer in oleic acid. In a previous study on oils originated from Istrian region, level of oleic acid was usually higher than 70% (Koprivnjak et al., 2012), which was not confirmed in this study in the case of Bova, Buža puntoža and Buža oil. The reason for lower level of oleic acid in these oils could be in high temperatures measured in 2009 in the period of vegetation observed for Istrian region (according to data of Meteorological and Hydrological Service, Poreč station, Croatia; data not shown). In warmer cultivation conditions, the olive fruit reduces the synthesis of oleic acid and increases the synthesis of palmitic and linoleic acid (Panelli et al., 1993). Exactly these phenomena could be observed in the case of Bova, Buža puntoža and Buža oils. Therefore, Buža oil had the highest level of polyunsaturated fatty acids (PUFA) due to high level of linoleic acid and Bova oil showed the highest level of saturated fatty acids due to high level of palmitic acid compared to other investigated oils. Istarska bjelica and Rosinjola oils were characterized by the highest level of MUFA and by the lowest level of PUFA. These differences in fatty acid content according to cultivar could influence different oxidative stability of its oils. Oleic/linoleic ratio is usually considered as an oxidative stability parameter of *VOO* and high ratio (>7) favorably influences oil oxidative stability (Velasco and Dobarganes, 2002). High oleic/linoleic ratio detected in Rosinjola and Istarska bjelica oils indicated their higher oxidative stability when compared to other investigated oils.

Conclusion

Until now, sterol and triterpene dialcohols composition of monovarietal oils obtained from Istrian autochthonous olive cultivars were not studied extensively and this study represents the first attempt to characterize oils from Bova and Buža puntoža cultivars based on sterol and triterpene dialcohols composition. Investigated Istrian olive oils obtained from the most widespread autochthonous cultivars fulfill all the demands of the current EU legislation required for virgin olive oil regarding sterols, triterpene dialcohols and fatty acids, but for Buža puntoža and Bova oil obtained from less known autochthonous cultivars a deviation in the case of linolenic acid was found and this deviationcould lead to false conclusions about their authenticity. Since the regulatory limits are set mostly on the basis of predominant olive cultivars in the

European producing countries, it is clearly of great importance to notify the natural sterol and fatty acid profile of autochthonous olive cultivars present in specific geographical regions, such as Istria region which is a marginal northern area of olive cultivation, and to forestall the question of their authenticity. This study confirms that olive cultivar is a source of natural variation in the levels of sterol, triterpene dialcohols and fatty acids, but future extended researches which would investigate the influence of specific agroecological and pedoclimatic conditions on expression of sterol and fatty acids profile in virgin olive oils of Istrian autochthonous olive cultivars are needed.

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