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Study of the combined effects of ripeness and production area on Bosana oil's quality

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ABSTRACT

The effects of olive ripeness, areas of production and their interaction on the chemical and sensory characteristics of cv. Bosana oil were assessed. The study was carried out in three areas of the Sassari province, Sardinia (Italy), at three stages of maturation. The results indicated the independence of the two factors: ripeness influenced saturated fatty acids, pigment content and deacetoxy oleuropein aglycone (DAOA) content and didn't affect the sensory characteristics, while production area influenced unsaturated fatty acids, content of vanillic acid and some sensory characters. In order to verify the interdependency of the two factors, statistical analyses (two-way ANOVA) were performed. Our study showed that a thoughtful planning of harvest times and production area could allow to obtain Bosana virgin olive oil of the highest quality. Furthermore, utilizing cultivars that maintain the properties of their oils even at late dates of harvest, it would be possible to optimize harvest times.

1. Introduction

Bosana cultivar, widely grown in different environments of the vast Sardinian territory, produces oils of high quality, rich in phenols such as secoiridoids (Tuberoso, Jerković, Maldini, & Serreli, 2016) and characterized by medium olive fruity and grassy sensory characters, with prevalent scents of thistle and artichoke and hints of almond and tomato, with a medium intensity of bitter and pungent notes (Rotondi, Alfei, Magli, & Pannelli, 2010).

The quality of extra virgin olive oil (EVOO) is directly related to the cultivar of the olives, and there is a strong link between cultivar and growth environment (Tuberoso et al., 2016), but it is however influenced by all the factors intervening during the entire production process. In fact, affecting the oil quality are primary factors (D'Imperio et al., 2010), that are thus not manageable, such as olive cultivar and pedoclimatic conditions, and manageable secondary factors such as ripening, agronomic practices, technological features of the milling process and oil storage conditions (Fregapane & Salvador, 2013; Inglese et al., 2011). Furthermore all these factors interact with each other, resulting in a complex multivariate matrix affecting VOO quality (Inglese et al., 2011).

The primary factor growth environment, namely pedoclimatic characteristics, is crucial in expressing the characteristics and quality typical of a given olive cultivar, as it influences the parameters drupe development, colouring trend and content of fatty acids and phenols (Di Vaio, Nocerino, Paduano, & Sacchi, 2013), as well as of wax esters, tocopherols and sterols (Piscopo, De Bruno, Zappia, Ventre, & Poiana, 2016). The same cultivar grown in different areas produces oils with different characteristics (Di Vaio et al., 2013); this peculiarity strictly connects the oil to its production territory, and increases the number of oil typologies available to consumers.

Among the secondary factors olive ripeness degree is one of the most studied due to its interdependence with other factors; ripeness is in fact influenced both by genetic matrix and environmental conditions. During ripening the chemical composition of olive fruit changes due to different metabolic activities (Yorulmaz, Poyrazoğlu, Özcan, & Tekin, 2012); hence, oils produced using olives at different ripening degrees may present different chemical and sensory characteristics. Thus choosing the right ripening degree is pivotal in determining the balance between oil quality and quantity (Dağdelen, Tümen, Özcan, & Dündar, 2013). Ripening affects pigments composition; in fact as ripening progresses the photosynthetic activity decreases, and thus the content of both chlorophylls and carotenoids decreases as well (Baccouri et al., 2008; Criado, Motilva, Goñi, & Romero, 2007). Ripening affects the

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fatty acid composition, essential parameter for the evaluation of oil quality due to its influence on the oxidative processes (Rotondi et al., 2004a). As the ripeness proceeds, a decreasing trend for palmitic and linoleic acids and an increasing trend for oleic acid were found by Fuentes de Mendoza et al. (2013), moreover Beltrán, del Rio, Sánchez, and Martínez (2004) described a rise in oleic acid content.

Ripening influences the concentration of the phenolic fraction, directly related to the oxidative stability of olive oils (Machado, Felizardo, Fernandes-Silva, Nunes, & Barros, 2013) and to the oil biological properties (antioxidant, anti-inflammatory, chemopreventive and anti-cancer; Servili et al., 2009). In fact, during ripening demethyloleuropein's content increases while oleuropein's content decreases (Gómez-Rico, Fregapane, & Salvador, 2008); the total phenolic fraction, in particular the secoiridoid compounds, decreases as well in EVOO obtained from olives collected at progressing stages of maturation (Morelló, Romero, & Motilva, 2004). Finally ripening influences the sensory properties of EVOO: the characters olive fruitiness, bitterness and pungency have a lower intensity in oils produced from olives at an advanced stage of ripening, independently from the cultivar (Brkić Bubola, Koprivnjak, Sladonja, Škevin, & Belobrajić, 2012). In fact the bitter and pungent tastes in oils are due to the presence of secoiridoid compounds, thus the decline of the secoiridoid content during ripeness is reflected in the decreasing trend of those organoleptic properties, and oils from earlier stages of maturation are also high-value nutritional products (Servili et al., 2009).

The factors affecting EVOO quality need to be considered in conjunction to avoid an underestimation of their possible interdependencies, as reported by Lukić et al. (2017) in a work on the interaction of fruit ripening and malaxation parameters.

In this work we studied the chemical and sensory characteristics of Bosana olive oils produced in 3 areas belonging to the production area of the Protected Designation of Origin (PDO) "Sardegna", and from olives at three stages of ripeness, to evaluate the single effects of the factors date of harvest and production area, and to verify if there was a combined effect of these factors on the final oil quality.

2. Materials and methods

2.1. Sampling and fruit analysis

The study was carried out during two consecutive crop seasons on cv. Bosana olive plants cultivated in 3 areas Alghero (AHO), Ittiri (ITR) and Sassari (SS) of the Sassari province, in the north-west of Sardinia island (Italy). The three areas chosen are characterized by a vast diffusion of olive culture and differ for their climatic and orographic characteristics (Canu et al., 2015). Three harvests for each year were carried out at different dates chosen after monitoring the drupe ripening stage, in order to standardize RI at collection date in each area under study (November the 15th, December the 14th, January the 11th). Olive fruits were harvested manually from five trees, at least 50 years old, from each macro area, collecting a total of 30 kg that constituted the sample from which the oil was extracted. The Ripening Index (RI) was calculated on 100 drupes for each sample by dividing the drupes in 7 classes according to the different skin and pulp pigmentation and following the formula:

$$RI = (A*0 + B*1 + C*2 + D*3 + E*4 + F*5 + G*6 + H*7)/100$$

where A, B…, H is the number of fruits in each category.

The method was developed by the Agronomic Station of Jaén defining the RI as function of fruit colour in both skin and pulp (Uceda & Hermoso, 1998).

The crude fat was determined in triplicate by extracting 20 g of grinded olive sample with diethyl ether, using a Soxhlet apparatus (Soxtec 2050, Foss-Tecator, Foss North America, MN, USA).

2.2. Olive processing and oils storage

A low scale continuous mill (Oliomio®; Toscana Enologica Mori, Firenze, Italy) equipped with an horizontal malaxator and a two phase decanter was used. Olive samples were processed within 24 h from harvest. For each sample the temperature (below 27 °C) and the time of malaxation (20 min), the speed of the decanter (4200 rpm) and the flux of water in the separator (0.8 L h^{-1}) were standardized in order to minimize the variability due to the extraction procedures. Oils samples were filtered through cotton filters, poured in dark glass bottles, keeping the headspace to a minimum, and stored in a temperature controlled cupboard set at 15 ± 1 °C until chemical and sensory analyses that took place two months after olive processing

2.3. Chemical analyses on the olive oil

2.3.1. Fatty acids profiles

Fatty acids profiles were evaluated according to Commission Regulation EC No 1513/2001 and following amendments of the Council of the European Union using a Chrompack CP 9000 gas chromatograph with a flame ionization detector (FID) equipped with a capillary column (Stabilwax, Restek Corporation, USA) with helium as the carrier gas (flow rate = 1 mL min^{-1}; split ratio of 1:20, v:v). Chromatographic parameters were as follows: injection and detection temperature 250 °C; 230 °C; column oven temperature, 240 °C. All parameters were determined in triplicate for each sample.

2.3.2. HPLC analysis of the phenolic fraction

The phenolic fraction was extracted by LLE in triplicate according to Pirisi, Cabras, Falqui Cao, Migliorini, and Mugelli (2000) but using 8 g of oil and maintaining the ratio oil/solvent. The chromatographic conditions are reported by Morrone et al. (2016). HPLC analysis was carried out using a Schimadzu LC-10ADvp equipped with a low pressure gradient unit, FCV-10Alvp (Schimadzu), degasser Flow154, (Gastorr), and a column oven CTO-10A (Schimadzu). The wavelengths were set at 280 nm for phenolic alcohols and secoiridoids, and at 330 nm for flavonoids and phenolic acids. Identification of phenolic compounds was carried out by the comparison with the retention time and spectra of the standard compounds and with data literature. Hydroxytyrosol was quantified using the tyrosol calibration curve; derivatives of oleuropein and ligstroside were quantified using an oleuropein calibration curve; tyrosol, vanillin, vanillic acid, o-cumaric acid, luteolin and apigenin were quantified using the calibration curve of the relative standard.

2.3.3. Pigments analysis

For the quantitative analysis of tocopherols, lutein and β catotene, 2 ml of oil sample were filtered through a PTFE syringe filter of 0.2 µm pore size (gyroDisc 25 mm Orange Scientific) and directly injected in HPLC (Rotondi, Bertazza, & Magli, 2004b); for instrument details see Section 2.3.2. Analytes were separated on a C18 column 150 × 4.6 (inertsil ODS-2, Alltech), the flow rate was 1 ml min^{-1}, the injection volume was 20 µl and the column temperature was 25 °C. The eluent used were: A methanol:water 80:20 (v/v) and B methanol:tetrahydrofuran 20:80 (v/v).

2.4. Sensory analysis

Sensory analysis was performed by 8 people belonging to a fully-trained analytical taste panel of the Agency for Agrofood Section Services of Marche region (ASSAM), recognized by the International Olive Oil Council (IOC) of Madrid, Spain, and by the Italian Ministry for Agriculture, Food, and Forestry Policy. The panel evaluated all oil samples following an incomplete randomized block design. Since the main objective of the sensory UE method reported in Commission Regulation EC 2568/91 and subsequent amendments (Commission

Table 1
Ripening index (RI) and oil content of olive samples collected at three stages of ripeness (I, II and III) and in three areas of Sardinia Alghero (AHO), Ittiri (ITR) and Sassari (SS). The data are presented as mean ± standard deviation. The variability, expressed as percent of the total sum of the squares (TSS), is reported in brackets. Oil is reported as percentage on dry matter.

	Ripeness				Production area				Ripeness x production area
	I	II	III	Stat. sign and variab. as %TSS	AHO	ITR	SS	Stat. sign and variab. as %TSS	Stat. sign and variab. as %TSS
RI	1.37 ± 0.16a	2.42 ± 0.27b	4.07 ± 0.22c	*** **(95.92%)**	2.88 ± 1.05	2.39 ± 1.12	2.60 ± 1.14	ns (1.82%)	ns (2.26%)
% oil	36.16 ± 0.88	38.93 ± 2.10	42.59 ± 2.43	ns (39.45%)	40.65 ± 4.55	37.44 ± 4.82	39.58 ± 6.50	ns (20.98%)	ns (39.57%)

Different letters (a, b, c) within a row indicate significant difference at 5% level for the ripeness factor while Greek letters (α, β, γ) within a row indicate significant difference at 5% level for the production area factor; values highlighted in bold are statistically significant. *, **, *** and ns significant F-values; the *P < .05, **P < .01 or ***P < .001 levels, ns not significant.

Regulation EC 796/02) is to give a commercial classification of the oils, a panel test was established for the present study using a standard profile sheet (International Olive oil Council, 1996) modified by IBIMET-CNR and ASSAM (Rotondi et al., 2010).

2.5. Statistical analysis

The data collected were elaborated using Microsoft® Excel 2007/ XLSTAT© (Version 2009.3.02, Addinsoft, Inc., Brooklyn, NY, USA). The significance of differences among means at a 5% level was determined by two-way ANOVA, in order to examine treatment (date of harvest and growing area) interdependences, followed by a Tukey's Honestly Significant Difference (HSD) test.

3. Results and discussion

3.1. Ripening index and oil content

The RI determined on the basis of skin and pulp pigmentation are shown in Table 1, where the clear influence of maturation on this index is shown (95.92% of variability expressed as percent of the total sum of the squares). Conversely, not statistically significant differences were found for oil accumulation, even if a decreasing trend related to ripening was detectable. This disagreement between veraison and oil accumulation is probably attributable to genetic factors related to the studied cultivar Both oil quantity and RI did not show statistically significant differences in the three production areas under study, therefore the differences in the chemical and sensory characteristics emerging from the comparison of the produced oils will be more easily attributable to the different production area.

3.2. Fatty acids

Palmitic acid showed a statistically significant decreasing trend during maturation (Table 2), in agreement with other authors (Fuentes de Mendoza et al., 2013). However Brkić Bubola et al. (2012) reported, in a study on two cultivars of Istria region, a decreasing trend in palmitic acid for one of the cv (Črna), while no differences during maturation were recorded for the other cv (Buža), suggesting an influence of both the genetic and the environmental factor. The variability of palmitic acid, expressed as percentage of the total sum of the squares, was mostly related to the date of harvest (79.73%).

Oleic acid's content did not vary significantly according to ripeness (Table 2), in agreement with Bengana et al. (2013), who reported no accumulation during ripeness; however both a decreasing (Desouky, Haggag, Abd El-Migeed, & El-Hady, 2009) and an increasing trend during ripening (Fuentes de Mendoza et al., 2013) were reported by other authors. Nevertheless, oleic acid together with linolenic acid varied significantly according to the production area, in accordance with Piscopo et al. (2016); in fact both their variability is due for more of the 80% by the area of production. Oils produced in the Alghero area showed a lower content of oleic acid and a higher content of linoleic acid respect to oils produced both in Ittiri and Sassari; this difference is probably due to the warmer temperature of the area (Morrone, 2015) (Fig. 1), as reported by Lombardo et al. (2008).

The parameters related with the fatty acid composition, namely saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), poly unsaturated fatty acids (PUFA), the ratio MUFAs/PUFAs is of a great importance due to its nutritional value and its influence on the oxidative stability of olive oil. The SFA were mainly affected by ripeness stage (88.64%), since this class includes palmitic and stearic acid. Both MUFA and PUFA, as well as their ratio were affected by the production area, with oils from olives produced in Alghero statistically different from oils deriving from drupes produced in Sassari and Ittiri; in fact oils produced in Alghero presented lower MUFA and higher PUFA contents. Hence, the production area influenced the unsaturated part of fatty acid profile while the saturated part was affected by ripeness; moreover, the interaction between the factors was never significant, thus the two factors studied were independent.

3.3. Pigment profiles

Both the quality and the quantity of pigments present in Bosana olive oil were not influenced by the production area (Table 3), in agreement with a work carried out in Sicily by Cerretani, Motilva, Romero, Bendini, and Lercker (2008); the same authors didn't find a clear influence of the ripening stage on the concentration of chlorophylls and carotenoids on oils from different Italian regions, and attributed this result possibly to the limits of the Jaèn method. However our results showed a clear influence of ripening on the chlorophylls and carotenoids content (Table 3), in agreement with what reported by Brkić Bubola et al. (2012). Carotenoids including β-carotene decreased significantly with the progress of maturation, as reported by Roca and Minguez-Mosquera (2001) in a study on drupes. No significant interaction between ripeness and production area was established for the oil pigment's content.

3.4. Phenolic content

The main phenolic compound found in our study on monovarietal EVOO obtained from cv. Bosana olives was DAOA (Table 4), in agreement with reports for the same cultivar (Cerretani et al., 2006). The DAOA presented a decreasing trend during ripening and only at the first date of harvest the content was statistically higher compared to the other dates. The same trend was observed for the total of secoiridoids compounds (ΣSID_S), since the DAOA is the most represented secoiridoid. DAOA's variability depended for the 73.42% on fruit ripeness and only for the 16.24% on the production area.

The content of the simple phenols hydroxytyrosol and tyrosol was on average 5.47 and 3.69 mg kg^{-1} respectively, in agreement with

Table 2
Fatty acid profiles of the oils obtained at three stages of ripeness (I, II and III) in the three production areas of Alghero (AHO), Ittiri (ITR) and Sassari (SS). The data are presented as means ± standard deviation. The variability, expressed as percent of the total sum of the squares (TSS), is reported in brackets.

	Ripeness				Production area				Ripeness x Production area
	I	II	III	Stat. sign and variab. as %TSS	AHO	ITR	SS	Stat. sign and variab. as %TSS	Stat. sign and variab. as %TSS
C 16	13.45 ± 0.61a	13.07 ± 0.7ab	12.08 ± 0.66b	* **(79.73%)**	13.26 ± 0.85	12.63 ± 0.86	12.71 ± 0.86	ns (18.51%)	ns (1.76%)
C16:1	0.83 ± 0.08	0.75 ± 0.11	0.73 ± 0.05	ns (50.71%)	0.77 ± 0.11	0.72 ± 0.08	0.81 ± 0.07	ns (35.42%)	ns (13.87%)
C18	2.62 ± 0.17	2.62 ± 0.37	2.15 ± 0.25b	ns (62.57%)	2.4 ± 0.36	2.67 ± 0.35	2.3 ± 0.26	ns (29.04%)	ns (8.39%)
C18:1	70.76 ± 2.11	70.39 ± 2.43	72.00 ± 2.37	ns (12.34%)	68.62 ± 1.27β	71.60 ± 1.81α	72.94 ± 1.04α	** **(84.69%)**	ns (2.97%)
C18:2	10.36 ± 1.80	11.09 ± 1.99	11.12 ± 1.91	ns (4.9%)	12.87 ± 1.01α	10.41 ± 1.2.β	9.29 ± 0.89β	*** **(89.12%)**	ns (5.98%)
C18:3	0.72 ± 0.04	0.72 ± 0.07	0.66 ± 0.06	ns (35.52%)	0.74 ± 0.05	0.68 ± 0.08	0.68 ± 0.02	ns (37.54%)	ns (26.94%)
C20	0.52 ± 0.13	0.55 ± 0.13	0.47 ± 0.11	ns (58.71%)	0.53 ± 0.14	0.52 ± 0.12	0.49 ± 0.1	ns (23.1%)	ns (18.19%)
C20:1	0.35 ± 0.06	0.39 ± 0.08	0.38 ± 0.09	ns (46.07%)	0.40 ± 0.07	0.36 ± 0.08	0.36 ± 0.09	ns (41.93%)	ns (12%)
ΣSFA	16.63 ± 0.53a	16.28 ± 0.69a	14.75 ± 0.72b	** **(88.62%)**	16.23 ± 1.04	15.87 ± 1.09	15.56 ± 1.07β	ns (9.86%)	ns (1.52%)
ΣMUFA	72.01 ± 2.11	71.60 ± 2.39	73.20 ± 2.43	ns (12.11%)	69.87 ± 1.23β	72.76 ± 1.86α	74.19 ± 1.06α	** **(85.05%)**	ns (2.84%)
ΣPUFA	11.08 ± 1.82	11.81 ± 2.02	11.78 ± 1.92	ns (4.37%)	13.61 ± 1.04α	11.09 ± 1.16β	9.97 ± 0.88β	*** **(89.18%)**	ns (6.45%)

Different letters (a, b, c) within a row indicate significant difference at 5% level for the ripeness factor while Greek letters (α, β, γ) within a row indicate significant difference at 5% level for the production area factor; values highlighted in bold are statistically significant. *, **, *** and ns significant F-values; the *P < .05, **P < .01 or ***P < .001 levels, ns not significant.

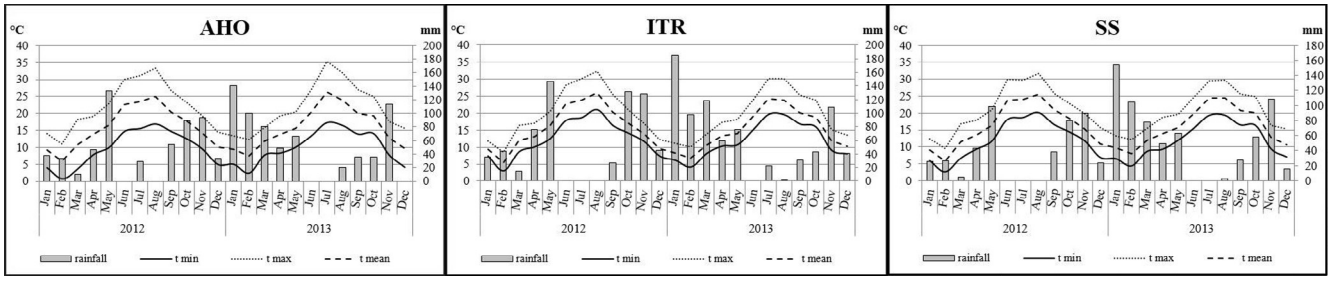

Fig. 1. Rainfall (mm) and temperatures (minimum, maximum and mean; °C) of the two years under study (2012–2013) in Alghero (AHO), Ittiri (ITR) and Sassari (SS) areas.

values reported for other cultivars (Bengana et al., 2013; Jiménez, Sánchez-Ortiz, Lorenzo, & Rivas, 2013). No statistically significant differences in the content of phenolic alcohols during maturation were detected, however, the found trend, that increased up to the second ripening stage and then decreased, is in accordance with the results of Dağdelen et al. (2013) in a study on Turkish varieties. The area of production seemed to affect only the vanillic acid content, being the cause of 85.97% of the variability. Several authors (Gómez-Rico, Salvador, La Greca, & Fregapane, 2006; Marsilio et al., 2006) reported an increase in vanillic acid and vanillin in virgin olive oils from irrigated olive trees; thus, considering that in our study none of the fields was subjected to irrigation, the higher amount of rainfall could be the cause of this difference in the oils from Alghero (Morrone, 2015) (Fig. 1).

The flavones concentration was not influenced significantly by the two factors under study, even if both luteolin's and apigenin's contents increased during the maturation process, in accordance with other studies (Jiménez et al., 2013).

Thus for all the above considered compounds, we found no interaction between the factors production area and ripening stage.

3.5. Sensory analysis

The results of the sensory analysis of cv. Bosana oils are shown in Table 5. The sensory profile of monovarietal Bosana oil is described by Rotondi et al. (2010) as medium olive fruity, grassy with prevalent scents of thistle and artichoke and hints of almond and tomato, with a medium intensity of bitter and pungent notes. The results here

Table 3
Pigment content of the oils at three stages of ripeness (I, II and III) and for the three production areas (AHO, Alghero, ITR, Ittiri, SS, Sassari). The data are presented as means ± standard deviation. The variability, expressed as percent of the total sum of the squares (TSS), is reported in brackets.

	Ripeness				Production Area				Ripeness × Production area
	I	II	III	Stat. sign and variab. as %TSS	AHO	ITR	SS	Stat. sign and variab. as %TSS	Stat. sign and variab. as %TSS
β Carotene	4.01 ± 0.85a	2.18 ± 0.95b	0.72 ± 0.36c	*** **(68.35%)**	1.86 ± 1.30	2.41 ± 1.60	2.63 ± 1.89	ns (8.53%)	ns (23.12%)
Σ Chlorophylls	7.63 ± 3.08a	3.14 ± 0.70b	1.02 ± 0.50b	*** **(86.86%)**	3.22 ± 2.10	3.80 ± 3.10	4.76 ± 4.70	ns (4.62%)	ns (8.52%)
Σ Carotenoids	9.31 ± 2.44a	6.75 ± 2.60ab	3.37 ± 1.05b	** **(84.94%)**	5.15 ± 2.67	7.02 ± 3.14	7.26 ± 3.86	ns (12.82%)	ns (2.25%)
ΣChloro/ΣCarot	0.81 ± 0.15a	0.50 ± 0.13b	0.29 ± 0.07c	*** **(89.54%)**	0.58 ± 0.23	0.46 ± 0.22	0.56 ± 0.31	ns (5.19%)	ns (5.27%)

Pigment content is expressed as mg of relative standard compound per kg of oil. Different letters (a, b, c) within a row indicate significant difference at 5% level for the ripeness factor while Greek letters (α, β, γ) within a row indicate significant difference at 5% level for the production area factor; values highlighted in bold are statistically significant. *, **, *** and ns significant F-values; the *P < .05, **P < .01 or ***P < .001 levels, ns not significant.

Table 4
Phenolic content of the oils at three stages of ripeness (I, II and III) and for the three production areas of Alghero (AHO), Ittiri (ITR) and Sassari (SS). The data are expressed as means ± standard deviation. The variability, expressed as percent of the total sum of the squares (TSS), is reported in brackets.

	Ripeness				Production area				Ripeness × production area
	I	II	III	Stat. sign and variab. as %TSS	AHO	ITR	SS	Stat. sign and variab. as %TSS	Stat. sign and variab. as %TSS
OhTy	5.09 ± 2.25	9.32 ± 14.83	3.35 ± 0.98	ns (19.61%)	9.56 ± 14.64	4.27 ± 2.43	3.93 ± 2.04	ns (20.82%)	ns (59.57%)
TY	3.08 ± 0.78	4.51 ± 4.19	3.6 ± 1.89	ns (11.99%)	5.39 ± 3.75	2.70 ± 0.88	3.09 ± 1.76	ns (48.17%)	ns (39.84%)
Vanillic acid	0.55 ± 0.44	0.76 ± 0.35	0.71 ± 0.38	ns (10.77%)	1.03 ± 0.24α	0.45 ± 0.34β	0.53 ± 0.28αβ	* **(85.97%)**	ns (3.26%)
Vanillin	0.30 ± 0.11	0.22 ± 0.07	0.2 ± 0.05	ns (48.95%)	0.28 ± 0.12	0.22 ± 0.06	0.21 ± 0.06	ns (25.05%)	ns (26.00%)
DAOA	393.23 ± 104.03a	227.8 ± 40.09b	200.29 ± 62.63b	*** **(73.42%)**	226.61 ± 94.41	270.13 ± 82.69	324.57 ± 144.77	ns (16.24%)	ns (10.34%)
(+)-pinoresinol	9.94 ± 4.12	9.39 ± 3.07	9.82 ± 5.54	ns (1.51%)	9.21 ± 3.35	8.72 ± 4.16	11.22 ± 4.96	ns (31.17%)	ns (67.32%)
Luteolin	2.94 ± 1.2	4.43 ± 1.99	6.55 ± 2.73	ns (76.40%)	5.28 ± 3.35	3.66 ± 2.00	4.97 ± 1.94	ns (17.21%)	ns (6.39%)
Apigenin	2.17 ± 1.97	3.32 ± 3.02	3.76 ± 3.09	ns (38.28%)	2.39 ± 1.17	2.76 ± 2.77	4.10 ± 3.63	ns (45.81%)	ns (15.91%)
ΣSIDs	512.35 ± 121.87a	347.53 ± 61.78b	317.53 ± 71.89b	** **(69.48%)**	332.91 ± 96.83	395.74 ± 87.48	448.76 ± 158.57	ns (21.24%)	ns (9.28%)

Hydroxytyrosol (OhTy) is expressed as mg/kg tyrosol; tyrosol (TY) is expressed as mg/kg of tyrosol; deacetoxy oleuropein aglycon (DAOA) and the sum of secoiridoids (ΣSIDs) are expressed as mg/kg of oleuropein, while the other compounds are expressed as mg/kg of relative standard. Different letters (a, b, c) within a row indicate significant difference at 5% level for the ripeness factor while Greek letters (α, β, γ) within a row indicate significant difference at 5% level for the production area factor; values highlighted in bold are statistically significant. *, **, *** and ns significant F-values; the *P < .05, ** P < .01 or ***P < .001 levels, ns not significant.

presented matched the sensory description given above. A number of authors reported that olive ripeness has a strong impact on the sensory characteristics of EVOO (Brkić Bubola et al., 2012; Jiménez et al., 2013; Rotondi et al., 2004a); however, in our study none of the sensory descriptors was affected by the ripening degree. Our findings suggest that harvesting the crops in November or in January didn't affect the oil sensory profile. Conversely, the area of production influenced significantly the sensory profile, with respect to the artichoke and the pungent scents. In detail, oil produced in the Alghero area had less pronounced pungent and artichoke hints, probably due to more frequent rainfalls (Fig. 1) as also reported by Pannelli, Servili, Selvaggini, Baldioli, and Montedoro (1994).

4. Conclusion

In this study a chemical and sensory characterization of Bosana EVOOs was carried out in a wide time window (from November to January). Ripeness significantly influenced some of the chemical characteristics (saturated fatty acids, carotenoids including β-carotene, the ratio of chlorophylls/carotenoids, the content of DAOA and the sum of secoiridoid compounds), with the main differences being observed between the first and the second date of harvest. However oil produced at all the dates of harvest met the EFSA requirement for the health claim (EFSA Journal, 2011).

Olives didn't reach the overmaturation phase even at the January harvest, when the drupes were however characterized by a high RI, as demonstrated by the oil accumulation that kept increasing. The Jaén index is thus strictly cultivar dependent, because for several cultivars veraison is not directly proportional to the internal drupe metabolic processes. Unlike other cultivars studied, for which a strong depletion of the chemical characters and sensory profile occurred as the ripeness proceeded, the chemical qualities and the intensity of the sensory attributes in Bosana's oils were maintained. The gradual trend of cv. Bosana's ripening could allow olive growers a wider time range for olive harvesting without compromising the oil sensory properties, and could allow to improve the management of harvesting and processing operations even in unfavourable climates, such as the heavy rainfalls during the period of harvest common in the Sardinia region.

The production area significantly affected the content of oleic and linoleic acid as well as their ratio, the MUFA, PUFA and their ratio, the content of vanillic acid and the sensory attributes pungent and artichoke note. Thus in different areas it is possible to produce different typologies of oil, in detail the area of Alghero produced oils with notes of bitter, spicy and artichoke less intense than oils produced in Ittiri and Sassari. Such diversification of oil typologies could satisfy the different tastes of consumers. It is important to underline that the interaction between the factors ripeness stage and production area was never significant: the two factors under study can be considered as totally independent from each other.

The effective interdependence of the factors production area and ripening phase, influencing oil chemical and sensory properties, should be tested on different cultivars in areas where olive groves are widespread, with the aim to obtain monovarietal EVOOs with different chemical and sensory characters. Furthermore, using cultivars that maintain the properties of their oils even at late dates of harvest, it would be possible to optimize harvest times in large orchards, and thus to optimize food chain best practice as well.

Further studies on cv. Bosana with later dates of harvest will let us establish the overmaturation phase and hence help determine the optimum ripening stage for this cv., in order to obtain an EVOO which still retains all its chemical and sensory properties.

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Table 5
Intensities of sensory characteristics of the oils at different stages of ripeness (I, II and III) and deriving from the production areas Alghero (AHO), Ittiri (ITR) and Sassari (SS). The variability, expressed as percent of the total sum of the squares (TSS), is reported in brackets.

	Ripeness				Production area				Ripeness × production area
	I	II	III	Stat. sign and variab. as %TSS	AHO	ITR	SS	Stat. sign and variab. as %TSS	Stat. sign and variab. as %TSS
Olive fruity	4.98	4.95	4.40	ns (26.87%)	4.36	4.88	5.10	ns (14.30%)	ns (58.83%)
Grass	2.60	2.55	2.09	ns (26.84%)	2.25	2.37	2.61	ns (48.24%)	ns (25.42%)
Almond	2.35	2.38	2.20	ns (12.48%)	2.16	2.37	2.39	ns (12.09%)	ns (75.43%)
Artichoke	2.62	2.45	2.09	ns (17.06%)	1.77 α	2.74 β	2.66 αβ	* **(51.82%)**	ns (31.12%)
Bitter	5.16	4.62	4.17	ns (39.29%)	3.98	4.83	5.14	ns (34.46%)	ns (26.25%)
Pungent	5.08	4.85	4.36	ns (23.60%)	4.07 α	5.06 αβ	5.15 αβ	* **(68.86%)**	ns (7.54%)

Different letters (a, b, c) within a row indicate significant difference at 5% level for the ripeness factor while Greek letters (α, β, γ) within a row indicate significant difference at 5% level for the production area factor; values highlighted in bold are statistically significant. *, **, *** and ns significant F-values; the *P < .05, **P < .01 or ***P < .001 levels, ns not significant.

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