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Research Article

Phenolic Compounds, Antioxidant Activity, and Other Characteristics of Extra Virgin Olive Oils from Italian Autochthonous Varieties *Tonda di Villacidro*, *Tonda di Cagliari*, *Semidana*, and *Bosana*

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Extra virgin olive oils (EVOOs) from the fruits of Italian autochthonous varieties *Tonda di Villacidro*, *Tonda di Cagliari*, *Semidana*, and *Bosana* were investigated to promote their quality aspects. All the analyzed EVOOs showed low values of acidity (≤0.45%) and of peroxide value (≤6.22 mEq O_2/kg). There were no relevant differences in fatty acids and triacylglycerols composition among the four EVOOs. Tocopherols determined by HPLC-FL revealed that *Bosana* oil was characterized by the highest α-tocopherol level (213.3 ± 55.4 mg/kg). Chlorophylls, carotenoids, and total phenol (TP) contents as well as antioxidant activity (FRAP, DPPH$^{\bullet}$, and ABTS$^{\bullet+}$ assays) of the oils hydrophilic fractions (HFs) were assessed by spectrophotometric methods. Some differences concerning the antioxidant activity and the TP content were observed: *Bosana* oil HF activity was the most pronounced (1.17 ± 0.37 mmol TEAC/kg) and it contained the highest TPs amount (335.20 ± 121.34 mg/kg). HFs phenolic composition was determined by HPLC-DAD. The main identified phenols were secoiridoids, dominating in *Bosana* oil, such as decarboxymethyl ligstroside aglycone (*p*-HPEA-EDA, 35.8 ± 19.9 mg/kg) and oleuropein aglycone (3,4-HPEA-EA up to 84.7 mg/kg). In summary, all the four varieties showed good characteristics for the use as quality EVOO.

1. Introduction

Extra virgin olive oil (EVOO) is a worldwide recognised high valuable food product and there are constant efforts to enhance its quality and to preserve it from adulteration. In the last decades, several analyses to identify different olive oil cultivars and to verify the presence of any adulteration have been developed [1, 2]. The quality of olive oil is directly connected with the variety of the olives and there is a strong link between the cultivar and the territory of cultivation [3, 4]. Extra virgin olive oil from Sardinia (Italy) is protected by the European Union "Protected Designation of Origin" (PDO "*Sardegna*") and it can be obtained from several autochthonous olives as *Tonda di Villacidro*, *Tonda di Cagliari* (also known as *Nera di Gonnos*), *Semidana*, and *Bosana* [5]. More than 20 cultivars were defined so far in Sardinia and wild olive forms are still widely represented [6]. Archaeological evidence proves that olive oil extraction was performed in ancient times, long before different olive cultivars were introduced by Phoenicians, Romans, and Spanish peoples [6]. The PDO "*Sardegna*" was created mainly to promote the sensory properties, but also to preserve the health benefits of the most typical olive oils of the region. The EVOOs obtained from *Tonda di Villacidro*, *Tonda di Cagliari*, *Semidana*, and *Bosana* have specific sensory characteristics and a long tradition in Sardinia [6–9]. Good yield and quality were observed in *Semidana* and

Bosana for oil production, while *Tonda di Cagliari* resulted as interesting dual-purpose cultivars [7]. Some data on the physical-chemical parameters of these monovarietal oils can be found in scientific databases. The *Bosana* cultivar has been shown to have a high content of phenolic compounds [10]. Nevertheless, data in the main Sardinian EVOO are fragmentary and, to the best of our knowledge, no data on *Tonda di Villacidro* has been published so far.

The aim of this paper is to perform a chemical characterization of *Tonda di Villacidro*, *Tonda di Cagliari*, *Semidana*, and *Bosana* EVOOs by (1) determination of basic technological characteristics (acidity, peroxide value, K_{232}, and K_{270}) according to EU regulations; (2) the fatty acids (GC-FID/MS) and triacylglycerols profiles (HPLC-DAD); (3) total phenols, chlorophylls, and carotenoids evaluation by UV/vis including targeted tocopherols analysis by HPLC-FL; (4) HPLC-DAD targeted phenolics analysis of the hydrophilic oil fractions (HFs) and assessing of the HFs antioxidant activity (with $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and FRAP assays).

2. Material and Methods

2.1. Chemicals and Reagents. All chemicals and solvents used in this study were of analytical grade. Methanol, *n*-hexane, ethylacetate, acetonitrile, chloroform, gallic acid, sodium carbonate, sodium chloride and potassium hydroxide α-tocopherol, γ-tocopherol, squalene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu's reagent, β-carotene, and chlorophyll *a* were purchased from Sigma-Aldrich (Milan, Italy). Primary reference standard (purity > 99.9%) of phenolic compounds, triacylglycerols, and fatty acids methyl esters were obtained from Sigma-Aldrich, Merck, and Carlo Erba (Milan, Italy). Ultrapure water (18 MΩ·cm) was obtained with a Milli-Q Advantage A10 System apparatus (Millipore, Milan, Italy).

2.2. Olive Oil Samples and Hydrophilic Fraction Extraction. Extra virgin olive oils ($n = 61$) from *Tonda di Villacidro* ($n = 15$), *Tonda di Cagliari* ($n = 14$), *Semidana* ($n = 14$), and *Bosana* ($n = 18$) cultivar were obtained from olive fruits by local groves in Sardinia (Italy) in the years 2013-2014. Four batches of healthy olive fruits (each ca. 200 kg) were harvested in the same ripening index (RI = 4.3 ± 0.2) [11], processed within 24 h to olive oils, and stored in dark glass bottles at 12 ± 1°C until analysis. Sensory analysis of the samples was performed under the conditions described within the EC Regulation 640/2008 [12] and the samples were analyzed within 3 months from their production. Hydrophilic fractions (HFs) of the oils were prepared as reported by Tuberoso et al. [13].

2.3. Determination of Oil Technological Parameters. Free acidity (% of oleic acid (%18 : 1)), peroxide value (mEq O_2/kg of the oil), and UV absorption characteristics (K_{232} and K_{270}) were determined according to the European Union Commission Regulations EC 1989/2003 [14]. K_{232} and K_{270} were determined using 1% solution of the oil in cyclohexane with an UV-visible spectrophotometer Varian Cary 50 (Varian, Leini, TO, Italy) at 232 and 270 nm in a 10 mm quartz cuvette. All parameters were determined in triplicate for each sample.

2.4. Fatty Acids and Squalene. A transmethylation technique followed by GC-FID/MS determination was used [13, 15]. The percentage composition of the oils was calculated from GC peak areas without using correction factors. The quantitative analysis of squalene was performed using the internal standard method (with squalane) and results were expressed as mg squalene/kg of sample.

2.5. Triacylglycerols. The analysis of triacylglycerols (TAG) was performed with an HPLC-UV method [13]. Calibration graphs were constructed with the external standard method by measuring peak area versus concentration ($r = 0.9982$–0.9997). The concentrations of the compounds were calculated in mg/kg and data were expressed in weight percentages. Standard solutions of LLL, LLO, LLP, OOL, POL, OOO, OOP, PPO, and OOS were prepared in acetone. The use of equivalent carbon number (ECN = CN − 2*n*, where CN is the number of carbon atoms and *n* is the number of double bonds) allowed the attribution of compounds of which no analytical standards were found. In this way LnLL, LnLno, LnLnLn, LLnLn, OLLn, PLLn, OOLn, POLn, LPP, PSL, and SSLn were identified and the quantification was performed using the calibration curves of the TAG standard with the closest chemical structure and ECN number.

2.6. Targeted Phenolic Compounds Analysis. Detection and quantitative analyses of HF phenolic compounds were carried out using a LC-DAD method as described by Šarolić et al. [15]. Calibration curves were constructed with the external standard method, correlating the area of the peaks with the concentration. All compounds were dosed using the calibration curve built with the respective standard, except oleuropein and ligstroside derivatives that were dosed using oleuropein calibration curve. The correlation values were comprised between 0.9993 and 0.9998. Oleuropein and ligstroside derivatives and acetoxypinoresinol were tentatively identified by comparison with literature data [16–18].

2.7. Determination of Tocopherols, Total Chlorophylls, and Carotenoids. An HPLC system connected to a spectrofluorometer detector was used to dose α- and γ-tocopherols [15]. Standard solutions were prepared in acetone, while working solutions were prepared to appropriate dilution with the eluent mobile phase. Linearity in the range 0.1–6 mg/kg was 0.9998. Total chlorophylls and carotenoids were estimated spectrophotometrically reading the absorbances at two different wavelengths (464 nm for carotenoids and 669 nm for chlorophylls) [14]. Chlorophyll a and β-carotene stock standard solutions were prepared in acetone, as well as working solutions, which were prepared with proper dilutions (0.1–2.0 mg/kg, $r = 0.9996$, and 0.02–0.50 mg/kg, $r = 0.9995$, for chlorophyll a and β-carotene, resp.).

2.8. Determination of Total Phenolic Content (Folin-Ciocalteu's Assay). Total phenolic content of the HF was estimated spectrophotometrically with modified Folin-Ciocalteu's

Table 1: Technological characteristics of *Tonda di Villacidro*, *Tonda di Cagliari, Semidana*, and *Bosana* EVOO.

Fatty acid	*Tonda di Villacidro* Area [%]				*Tonda di Cagliari* Area [%]				*Semidana* Area [%]				*Bosana* Area [%]			
	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD
Acidity[a] (%)	0.14	0.61	0.43	0.19	0.12	0.58	0.45	0.19	0.12	0.53	0.37	0.14	0.15	0.59	0.41	0.18
Peroxide value[b] (mEq O_2/kg)	3.37	6.49	4.73	1.97	2.11	5.62	3.47	2.02	3.97	7.11	5.45	2.16	4.52	7.65	6.22	1.36
K_{232}[c]	1.98	2.24	2.13	0.11	1.80	1.83	1.81	0.02	1.95	2.11	2.03	0.11	1.85	2.25	2.06	0.13
K_{270}[d]	0.09	0.14	0.12	0.02	0.08	0.14	0.11	0.03	0.13	0.16	0.15	0.02	0.13	0.18	0.15	0.01

[a]Threshold value for EVOO is ≤0.8; [b]threshold value for EVOO is ≤20; [c]threshold value for EVOO is ≤2.5; [d]threshold value for EVOO is ≤0.22 [EEC Regulation 2568/91].

Table 2: Fatty acid composition of *Tonda di Villacidro*, *Tonda di Cagliari, Semidana*, and *Bosana* EVOO (%, w/w).

	Fatty acid	*Tonda di Villacidro* Area [%]				*Tonda di Cagliari* Area [%]				*Semidana* Area [%]				*Bosana* Area [%]			
		Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD
M	Myristic (C14:0)	0.1	0.1	0.1	0.0	0.2	0.2	0.2	0.0	0.1	0.1	0.1	0.0	0.1	0.4	0.1	0.1
P	Palmitic (C16:0)	14.9	16.8	15.8	0.7	13.3	17.5	15.7	1.1	14.1	16.1	15.2	0.6	11.1	16.0	14.1	1.1
Po	Palmitoleic (C16:1 *n*-7)	1.0	1.4	1.2	0.2	0.8	1.7	1.2	0.3	1.0	1.3	1.1	0.2	0.1	5.1	3.2	1.8
Ea	Heptadecanoic (C17:0)	0.2	0.3	0.2	0.0	0.1	0.2	0.1	0.0	0.1	0.2	0.1	0.0	tr	0.2	0.1	0.0
S	Stearic (C18:0)	1.9	2.9	2.3	0.3	1.6	2.4	2.0	0.2	1.8	2.5	2.2	0.3	1.1	3.0	2.2	0.4
O	Oleic (C18:1 *n*-9)	55.2	65.5	60.7	3.3	53.8	69.5	61.0	3.4	58.3	66.0	62.0	2.5	59.8	73.4	66.4	3.0
V	Vaccenic (C18:1 *n*-7)	2.3	3.3	3.0	0.3	1.9	3.5	2.7	0.4	2.5	3.2	2.9	0.3	1.5	3.4	2.5	0.4
L	Linoleic (C18:2 *n*-6)	10.8	19.8	15.5	3.0	9.9	22.1	15.5	2.4	12.4	17.4	15.1	2.0	9.1	17.0	12.5	1.8
Ln	Linolenic (C18:3 *n*-3)	0.6	9.0	3.0	3.5	0.4	0.8	0.6	0.1	0.7	1.0	0.9	0.1	0.4	1.0	0.7	0.1
A	Arachidic (C20:0)	0.3	0.4	0.4	0.1	0.2	0.4	0.3	0.1	0.2	0.4	0.3	0.1	0.2	0.7	0.4	0.1
Ec	Eicosenoic (C20:1 *n*-11)	0.2	0.2	0.2	0.0	0.1	0.3	0.2	0.0	0.2	0.2	0.2	0.0	0.2	0.4	0.2	0.1
Be	Behenic (C22:0)	0.1	0.2	0.1	0.0	0.1	0.7	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.5	0.1	0.1
Lg	Lignoceric (C24:0)	tr	0.2	0.1	0.1	tr	0.8	0.2	0.2	0.1	0.3	0.1	0.1	tr	0.8	0.2	0.2

Min = minimal percentage. Max = maximal percentage. Mean = average percentage. SD = standard deviation. tr = trace (<0.05%).

method [19]. The total polyphenol content results, expressed as mg/kg of gallic acid equivalent (GAE), were obtained using a calibration curve of a freshly prepared gallic acid standard solution (5–100 mg/kg, $r = 0.9998$).

2.9. Free Radical Scavenging Activity (DPPH$^\bullet$ and ABTS$^{\bullet+}$ Assays) and Total Antioxidant Activity (FRAP Assay). The in vitro HF antiradical activity was assessed with the DPPH$^\bullet$ spectrophotometric method and the obtained data were expressed as Trolox equivalent antioxidant capacity (TEAC) [15]. A Trolox calibration curve in the range 0.02–1.00 mM was prepared ($r = 0.9998$), and data were expressed in Trolox equivalent antioxidant capacity (TEAC, mmol/kg). The ABTS assay was performed according to Re et al. [20] with slight modification [19], and data were expressed as TEAC values. The FRAP assay was performed preparing a ferric complex TPTZ and Fe^{3+} [19–21]. Quantitative analysis was performed according to the external standard method ($FeSO_4$, 0.1–2.0 mmol/kg, $r = 0.9999$), correlating the absorbance with the concentration, and results were expressed as mmol/kg of Fe^{2+}.

3. Results and Discussion

EVOOs quality parameters (free acidity, peroxide value, and UV absorption characteristics K_{232} and K_{270}) were determined according to the analytical methods described in the European Union Commission Regulations EEC/2568/91 and EEC/1429/92 [22, 23]. Table 1 shows that all the analyzed EVOOs exhibit low average values of acidity (≤0.45%), peroxide value (≤6.22 mEq O_2/kg), and K_{232}/K_{270} absorption (≤2.13 and ≤0.15, resp.), prerequisite for the commercial category "extra virgin" olive oil, according to European Union regulations (EEC Regulation 2568/91) [22]. These values meet also the requirement of the PDO "*Sardegna*" production specification [5].

The analysis of fatty acids and triacylglycerols allowed finding in EVOO 13 fatty acids and 16 different triacylglycerols. Table 2 shows the fatty acid composition of *Tonda di Villacidro*, *Tonda di Cagliari*, *Semidana*, and *Bosana* EVOO. No significant differences were observed between these oils concerning the amount of each quantified fatty acid, and among these oleic, palmitic, and linoleic acid were the most concentrated, consistent with other previously published data

Table 3: Triacylglycerols composition of *Tonda di Villacidro*, *Tonda di Cagliari, Semidana*, and *Bosana* EVOO (%, w/w).

Triacylglycerols		*Tonda di Villacidro*				*Tonda di Cagliari*				*Semidana*				*Bosana*			
ECN		Area [%]				Area [%]				Area [%]				Area [%]			
		Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD
LLL[a]	42	0.1	0.5	0.3	0.2	0.2	0.5	0.4	0.1	tr	0.8	0.4	0.2	tr	0.6	0.3	0.1
LnLO	42	0.2	0.5	0.4	0.1	0.2	0.5	0.4	0.1	0.2	0.6	0.3	0.1	0.1	0.5	0.3	0.1
PLLn	42	tr	0.3	0.2	0.1	0.1	0.3	0.2	0.1	tr	0.4	0.2	0.1	tr	0.3	0.2	0.1
LLO[a]	44	2.1	5.9	3.7	1.0	3.3	5.4	4.1	1.0	2.1	7.2	4.8	1.0	1.9	7.0	3.6	1.1
LnOO	44	0.7	1.2	0.9	0.2	0.7	1.2	1.0	0.2	0.5	1.3	0.9	0.2	0.6	1.6	1.0	0.2
LLP[a]	44	1.1	8.8	3.8	2.4	1.5	8.0	3.6	2.6	1.1	8.2	2.8	1.9	0.4	5.6	1.8	1.1
LnOP	44	0.9	2.3	1.4	0.5	0.5	2.4	1.4	0.7	0.5	2.2	1.0	0.5	0.5	2.7	1.0	0.5
LOO[a]	46	11.2	19.8	15.6	3.1	12.5	17.4	14.9	1.6	12.3	19.6	14.3	1.6	10.9	19.8	14.0	2.3
LOP[a]	46	2.0	4.2	3.0	0.7	2.3	9.7	3.6	2.5	2.2	11.2	6.1	3.4	1.5	9.9	4.1	2.7
PLP	46	0.2	0.6	0.4	0.1	0.2	1.1	0.4	0.3	0.2	1.4	0.7	0.4	0.1	1.2	0.4	0.3
OOO[a]	48	15.8	34.5	25.4	4.8	20.1	31.7	26.1	4.2	16.5	34.6	23.2	3.6	23.7	39.0	31.3	3.8
POO[a]	48	19.1	23.7	21.6	1.4	15.2	21.2	19.9	2.0	14.5	22.8	18.4	2.8	14.2	23.9	19.5	2.7
POP[a]	48	0.3	1.6	0.9	0.4	0.5	1.4	0.8	0.3	tr	1.4	0.7	0.3	tr	3.1	0.6	0.4
PSL	48	1.0	4.2	2.7	1.4	0.7	3.5	2.0	1.0	0.5	4.0	1.7	1.2	0.3	3.8	1.6	1.0
SSLn	48	0.5	2.8	1.7	1.0	0.4	3.2	1.3	1.1	0.4	3.1	1.0	1.0	0.2	3.4	1.1	0.9
SOO[a]	50	2.1	6.2	4.0	1.6	1.0	5.1	2.8	1.5	0.8	5.8	2.0	1.6	0.5	7.1	2.8	1.6

[a]Compounds identified by comparison with pure standard. tr = trace < 0.05%.
L: linoleic, Ln: linolenic, P: palmitic, O: oleic, and S: stearic acids.

Table 4: Lipophilic antioxidant compounds of *Tonda di Villacidro*, *Tonda di Cagliari, Semidana*, and *Bosana* EVOO (mg/kg).

	Tonda di Villacidro				*Tonda di Cagliari*				*Semidana*				*Bosana*			
	(mg/kg)				(mg/kg)				(mg/kg)				(mg/kg)			
	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD
Squalene	4676.7	8672.0	6232.8	1330.8	3273.7	9964.0	5106.2	1865.7	4640.3	7705.0	6023.2	1296.4	1837.4	9691.0	4559.9	1809.2
α-Tocopherol	112.9	226.1	172.2	46.1	144.2	170.0	159.3	12.7	136.6	260.5	181.5	35.0	158.2	268.4	213.3	55.4
γ-Tocopherol	9.7	22.1	15.3	4.7	5.1	12.0	8.8	3.0	3.1	18.4	9.1	4.7	2.5	22.3	8.7	4.7
Chlorophylls	4.2	25.1	8.8	6.4	7.4	12.6	10.5	2.0	3.2	17.5	6.5	3.8	2.8	23.9	10.8	4.9
Carotenoids	5.3	54.6	34.3	20.6	7.2	88.6	47.6	40.1	1.2	67.6	20.9	26.3	1.0	106.0	26.0	28.5

[24, 25]. *Bosana* oil showed a slightly higher amount of oleic acid (66.4 ± 3.0%): this is reflected in the analysis of triacylglycerols content (Table 3), which consists mostly in OOO, POO, and LOO, in all the EVOO samples, but in particular in that of *Bosana* variety.

Antioxidant compounds are fundamental for both oil stability and nutritional aspects. Lipophilic antioxidant compounds (α- and γ-tocopherols, squalene, chlorophylls, and carotenoids) were quantified by different analytical methods and the results are reported in Table 4. Squalene, which is an important molecule that gives oils some pharmacological properties [26], dominates in all samples (about 5000 mg/kg), followed by tocopherols, carotenoids, and chlorophylls. *Tonda di Villacidro* and *Semidana* EVOO exhibited the highest amount of squalene (6232.8 ± 1330.8 and 6023.2 ± 1296.4 mg/kg, resp.), whereas *Bosana* EVOO showed the highest amount of tocopherols (213.3 ± 55.4 and 8.7 ± 4.7 mg/kg for α- and γ-tocopherol, resp.). As regards these compounds, the varieties included in the EVOO PDO "*Sardegna*" production specification must have an amount in tocopherols ($\alpha + \gamma$) at least of 100 mg/kg [5] and this value is exceeded in all the analyzed samples (Table 4).

The average level of total phenols (TPs) found in oils (ca. 240 mg GAE/kg) resulted to be on the average compared to other olive oils analyzed in other studies [27–29]. The PDO "*Sardegna*" production specification requires an amount of TP higher than 100 mg GAE/kg [5] and all the four analyzed EVOOs meet this requirement (Table 5). The antioxidant activity of the polar compounds extracted in the HF was evaluated by FRAP, DPPH$^{\bullet}$, and ABTS$^{\bullet+}$ assays (Table 5). *Bosana* EVOO showed the highest TP concentration (335.20 ± 121.34 mg GAE/kg), and this data is correlated with the highest antiradical (1.17 ± 0.37 mmol TEAC/kg, DPPH$^{\bullet}$ assay, and 1.19 ± 0.43 mmol TEAC/kg, ABTS$^{\bullet+}$ assay) and total antioxidant (2.38 ± 0.52 mmol Fe^{2+}/kg, FRAP assay) activities (Table 5).

The hydrophilic extracts obtained from four monovarietal EVOOs were also analyzed by LC-DAD to quantify the main phenolic compounds and the results are shown in Table 6. Significant differences among most of the phenolic

Table 5: Total phenolic content and antioxidant activities of hydrophilic fraction of *Tonda di Villacidro*, *Tonda di Cagliari, Semidana*, and *Bosana* EVOO.

Assay	*Tonda di Villacidro*				*Tonda di Cagliari*				*Semidana*				*Bosana*			
	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD
Total phenols (mg GAE[a]/kg)	158.40	219.50	182.35	22.54	142.80	388.00	261.18	83.83	133.10	249.20	179.73	42.64	249.40	421.00	335.20	121.34
DPPH$^{\bullet}$[b] (mmol TEAC/kg)	0.08	0.52	0.25	0.16	0.13	0.52	0.29	0.14	0.10	0.64	0.29	0.18	0.51	1.68	1.17	0.37
ABTS$^{\bullet+}$[b] (mmol TEAC/kg)	0.09	0.33	0.22	0.09	0.19	0.52	0.33	0.12	0.11	0.77	0.34	0.22	0.55	1.88	1.19	0.43
FRAP[c] (mmol Fe^{2+}/kg)	0.39	1.03	0.88	0.23	0.52	1.57	1.02	0.36	0.36	1.66	0.99	0.39	1.42	2.95	2.38	0.52

[a]GAE: gallic acid equivalent. [b]DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ values are expressed as TEAC millimolar concentration, obtained from a Trolox solution having an antiradical capacity equivalent to that of the dilution of the EVOO. [c]FRAP value is expressed as Fe^{2+} millimolar concentration, obtained from a $FeSO_4$ solution having an antioxidant capacity equivalent to that of the dilution of the EVOO.

Table 6: Phenolic compounds isolated from *Tonda di Villacidro*, *Tonda di Cagliari, Semidana*, and *Bosana* EVOO hydrophilic fraction.

Compounds	*Tonda di Villacidro* (mg/kg)				*Tonda di Cagliari* (mg/kg)				*Semidana* (mg/kg)				*Bosana* (mg/kg)			
	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD	Min	Max	Mean	±SD
Hydroxytyrosol[a]	tr	2.7	0.4	0.9	0.6	12.8	2.4	3.6	tr	7.5	2.1	2.6	3.4	26.9	9.0	8.6
Tyrosol[a]	nd	2.5	1.2	1.0	0.3	12.2	5.3	3.2	tr	8.8	4.5	2.9	11.8	36.7	22.5	11.6
Vanillic acid[a]	nd	nd	nd		tr	0.8	0.3	0.2	0.3	3.9	1.3	1.4	tr	1.6	0.6	0.3
p-Coumaric acid[a]	nd	0.1	0.0	0.1	0.1	0.7	0.3	0.2	0.0	4.6	1.5	1.8	tr	3.2	0.4	0.4
3,4-DHPEA-EDA[b]	0.4	19.7	6.8	7.4	0.4	1.9	1.0	0.7	3.3	22.0	11.6	7.9	2.0	67.6	9.8	13.3
Pinoresinol[a]	nd	nd	nd		nd	nd	nd		tr	32.3	12.4	14.0	4.1	22.9	9.8	7.6
p-HPEA-EDA[b]	3.4	23.3	10.3	7.9	0.5	31.0	10.6	10.5	2.5	29.2	13.6	9.8	11.2	66.4	35.8	19.9
Luteolin[a]	1.0	4.4	2.5	1.8	2.1	16.1	5.5	3.3	2.3	4.5	3.4	1.0	0.2	7.1	2.8	1.7
Acetoxypinoresinol[b]	2.3	8.4	5.4	2.5	nd	nd	nd		0.5	17.4	8.4	6.4	tr	10.7	5.3	3.3
3,4-HPEA-EA[b]	0.3	5.1	2.2	1.7	1.6	40.7	14.8	14.6	1.0	73.2	20.8	25.7	22.3	145.0	84.7	55.4
Apigenin[a]	0.9	6.1	2.0	2.0	1.1	6.5	1.9	1.4	0.6	5.9	1.0	2.0	0.2	2.1	0.8	0.4

Min = minimal value; Max = maximal value; mean = average value; SD = standard deviation; nd = not detected (<LOD); [a]identification based on RT and UV-Vis spectra of pure compounds; [b]tentative identification by UV-Vis spectra and comparison of retention times with the literature data; 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (decarboxymethyl oleuropein aglycone); *p*-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol (decarboxymethyl ligstroside aglycone); 3,4-DHPEA-EA, oleuropein aglycone.

constituent levels were observed from the extracts. Taking into account that the mechanical extraction process (grinding, malaxing, and separation) was very similar for both oils, differences in phenolic compounds amount can be primarily due to the variety but can be affected also by year, peculiar atmospheric conditions, agronomic practices, storage, and maturation grade [30–32]. Targeted analysis showed that the most abundant secoiridoids of the samples were dialdehydic form of elenolic acid linked to hydroxytyrosol or tyrosol (p-HPEA), respectively, assigned as 3,4-DHPEA-EDA, 3,4-HPEA-EA, and *p*-HPEA-EDA. Other phenolics such as vanillic acid, *p*-coumaric acid, flavones (luteolin and apigenin), and lignans (pinoresinol and 1-acetoxypinoresinol) were found in almost all analyzed samples. *Bosana* HF showed the highest amount of the targeted phenolic compounds dosed (142.2 ± 4.7 mg/kg), confirming that the oil obtained from this cultivar is one of the richest in polyphenols [10]. This extracts were characterized statistically by the highest amount of 3,4-HPEA-EA and 3,4-DHPEA-EDA (84.7 ± 55.4 and 35.8 ± 19.9 mg/kg, resp.). This HF was also the richest in hydroxytyrosol and tyrosol, phenolic compounds highlighted for their health benefits [33, 34].

4. Conclusions

In this work were highlighted the chemical characteristics of the four EVOOs that are included in PDO "*Sardegna*." They exhibited all parameters within the limits for the EVOO category; moreover, all the EVOOs showed levels of tocopherols and phenolic compounds higher than the minimum reported in the PDO "*Sardegna*" production specification, therefore satisfying the parameters of the Protected Designation of Origin. Inside each variety, some peculiarities in the analytical data can be noticed, but the strong variability makes them not significant. The analysis of fatty acids and triacylglycerols composition does not help to clearly differentiate the four Sardinian varieties. The phenolic profile of the EVOO's HF may be more useful. *Bosana* HFs confirm data that were

previously reported in the literature, proving to be the oil rich in antioxidants (lipophilic and hydrophilic) and then with a huge antioxidant activity. Anyway, all the HFs from four varieties showed a good antioxidant capacity and it may be interesting to appreciate the product not only from the sensorial point of view, but also from that of nutraceutical use.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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