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Prediction of the antioxidant activity of extra virgin olive oils produced in the Mediterranean area

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ABSTRACT

A chemical characterisation was conducted on 75 commercial extra virgin olive oils (EVOO) produced in the years 2011–2012 in Southern Italy from five different olive monovarieties (*Coratina*, *Leccino*, *Maiatica*, *Ogliarola del Vulture* and *Ogliarola del Bradano*). The possibility of estimating the antioxidant activity of EVOO by using a chemical index as predictor of this property was considered. In order to build up and validate an antioxidant activity predictive model, the relationship between the antioxidant activity and the chosen chemical parameters was systematically investigated. The results indicated that oil antioxidant activity, measured as IC_{50}, could be satisfactorily predicted, for olive oils from the considered region, by using a simple index, such as the K_{225} value of oil samples, which represents a spectrophotometric index of the compounds responsible for oil bitterness measured at 225 nm.

1. Introduction

The consumption of extra virgin olive oil (EVOO) is becoming more important in daily diets due to its beneficial effects on human health. In fact, the health-promoting properties of EVOO concern the ability to prevent diseases that may be related to oxidative damage, such as coronary heart disease, stroke, and several types of cancers (Visioli & Bernardini, 2011). The protective role of EVOO is the result of its specific composition including high proportion of monounsaturated fatty acids (oleic acid), a balanced presence of polyunsaturated fatty acids and minor components, such as -tocopherol and phenolic compounds (Owen et al., 2000a, 2000b). Phenolic compounds act as antioxidants against reactive species through various mechanisms, preventing first chain initiation by scavenging initiating radicals, metal chelating, decreasing localised oxygen concentration, and decomposing peroxides (Owen et al., 2000a). Moreover, these substances are important not only for the nutritional quality, but also for the sensory quality and shelf-life of the oil.

The concentrations reported in literature for the antioxidant compounds are rather variable and for good quality oils are usually in the range 100–300 mg/kg for α-tocopherol and 200–1500 mg/kg for polar phenolic compounds (Hrncirik & Fritsche, 2005). The content of these moieties in virgin olive oil is influenced by variety, climatic conditions, fruit ripeness and oil extraction process. During storage, the presence of natural antioxidants depends on both the hydrolytic processes and the oxidation of the ortho-diphenolic fraction that occur in oils (Hrncirik & Fritsche, 2005).

Generally different approaches have been used to investigate and to predict antioxidant activity of food matrices and different tests have been used to measure radical-scavenging ability and the ability to inhibit the oxidation of a lipidic substrate (Milella et al., 2011; Padula et al., 2013; Russo, Bonomo, Salzano, Martelli, & Milella, 2012). The phenolic composition also represents an important characteristic in the evaluation of olive oil quality and it is related to the typical bitter taste of the olives. Bitterness is generally considered as a positive sensorial attribute of the oil and enhances the overall flavour with notes related to unripe olive fruit. Depending on the type of phenol content, rather than on the total phenol content, the intensity of bitterness of olive oils can be extremely variable. Therefore, it is important to establish the optimal level of bitterness in EVOO, depending on several factors, such as harvesting time, oil extraction system and olive variety (Favati, Condelli, Galgano, & Caruso, 2013).

Several studies have been carried out to investigate the antioxidant potential of commercial olive oils (Arslan & Schreiner, 2012; Baiano, Terracone, Viggiani, & Del Nobile, 2013; Bayram et al.,

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2012; Del Carlo et al., 2004; Gambacorta, Faccia, Trani, Lamacchia, & Gomes, 2012; Gargouri, Ammar, Zribi, Mansour, & Bouaziz, 2013; Loizzo, Di Lecce, Boselli, Menichini, & Frega, 2012; Taamalli, Gómez-Caravaca, Zarrouk, Seguira-Carretero, & Fernández-Gutiérez, 2010; Tura et al., 2007), but to our knowledge the literature focused on the prediction of antioxidant activity of EVOO is scarce (Del Carlo et al., 2004).

The aims of this work were to characterise the EVOO produced in the Mediterranean area, particularly in the Basilicata region (Southern Italy) and to evaluate the possibility of estimating its antioxidant activity by using chemical indices as predictors of this property. Furthermore, in order to build up and validate an antioxidant activity predictive model, the relationship between the antioxidant activity and the chosen chemical parameters has been systematically investigated.

2. Materials and methods

2.1. Sampling

A total of 75 commercial EVOO samples produced in the years 2011–2012 in the Basilicata region (South of Italy) were analysed. The oil samples were obtained from olive fruits (*Olea europea* L.) of different varieties (15 for each cultivar): *Coratina*, *Leccino*, *Maiatica*, *Ogliarola del Vulture* and *Ogliarola del Bradano*. The olives were processed by a two-phase centrifugal extraction. In order to characterise the oils, also to build up the predictive model based on the relationship between antioxidant activity and chemical parameters (building a predictive model) chemical analyses were performed. Furthermore, a total of 20 randomly chosen commercial EVOO oil samples, produced in the same area and years as described above, was used to validate the predictive model. Therefore, a total of 95 oil samples was evaluated.

All the samples were purchased directly from producers and stored at 15 °C in darkness using amber bottles sealed under N_2 prior to analysis.

2.2. Chemical analyses

2.2.1. Chemicals

All solvents and reagents were of analytical grade and were purchased from Carlo Erba (Milano, Italy). The -tocopherol standard was produced by Fluka (Buchs, Switzerland), while 1,1-diphenyl-*s*-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), β-carotene, chloroform, linoleic acid, Tween 20, methanol and ethanol were purchased from Sigma–Aldrich (Milano, Italy). Solid-phase extraction (SPE) C18 cartridges (6 mL) were obtained from Supelco (Milano, Italy). All analyses were run in triplicate.

2.2.2. Chemical parameters

Free acidity, peroxide values and spectrophotometric indices (K_{232}, K_{270} and ΔK) were determined according to the methods reported in the appropriate EU Regulation (2013). Free acidity was expressed as g of oleic acid per 100 g of oil, peroxide value as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 nm and 270 nm respectively, while ΔK was measured as: $\Delta K = K_m - [(K_{m-4} + K_{m+4})/2]$ where K_m is the extinction coefficient calculated at 268 nm.

The total polyphenol content in EVOO was determined following the method proposed by Favati, Caporale, and Bertuccioli (1994), and precisely by adding 2 mL of the phenolic oil purified extract to 0.5 mL of Folin–Ciocalteu reagent and 4 mL of a sodium carbonate aqueous solution (10% w/w), the volume was brought up to 20 mL with distilled water. The mixture was then stirred and allowed to stand in the dark for 90 min. Absorption at 765 nm was measured using a Cary 1E UV–Visible spectrophotometer (Varian, Leini, Italy). The phenolic content was expressed as mg gallic acid equivalents (GAE)/kg of oil.

The compounds responsible for oil bitterness were evaluated spectrophotometrically at 225 nm as absorbance (K_{225} values) with a Cary 1E UV–visible spectrophotometer (Varian, Leini, Italy), according to the method proposed by Gutiérrez Rosales, Perdiguero, Gutiérrez, and Olias (1992). -Tocopherol was determined by HPLC according to the method of Pocklington and Dieffenbacher (1988). Chlorophyll analysis was performed using the method proposed by Pokorny, Kalinova, and Dysseleri (1995) and the value was expressed as mg pheophytin/kg oil, while the carotenoids were analysed according to the method proposed by Minguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sanchez-Gomez, and Garrido-Fernàndez (1991), and values were expressed as mg lutein/kg oil.

2.2.3. In-vitro antioxidant activity

DPPH assay: Different volumes of oil (5, 10, 20, 40, 80, 100 μL) and BHT (50 ppm as external reference) were used. The volume was adjusted to 1 mL by adding ethanol to 0.5 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, 250 μL) and Tween 20 (5 μL). Tubes were stored at room temperature in the dark for 60 min (Gorinstein et al., 2003). The control was prepared as above without oil sample. The absorbance was monitored at 515 nm and radical scavenging was expressed as the half maximal inhibitory concentration (IC_{50}). The IC_{50} value denotes the volume (μL) of oil required to scavenge 50% of DPPH free radical. The antioxidant potential is inversely proportional to IC_{50} value.

Beta-carotene bleaching assay: The antioxidant activity was evaluated by the β-carotene-linoleic acid bleaching method (BCB) (Jayaprakasha & Jaganmohan Rao, 2000). β-Carotene solution (0.2 mg of β-carotene dissolved in 0.2 mL of chloroform), linoleic acid (20 mg) and Tween 20 (200 mg) were mixed. Chloroform was removed by using a rotary evaporator at 50 °C. Distilled water (50 mL) was added. Four millilitres of the emulsion were transferred into several tubes containing 0.2 mL of extra-virgin olive oil (5000 ppm in ethanol) or ethanol as control. BHT was used as positive control. The tubes were placed at 50 °C for 3 h. The absorbance was measured at 470 nm. Results were expressed as percent of β-carotene bleaching inhibition (AA%) and calculated as follows:

$$(A_{\beta-\text{carotene after 180 min}}/A_{\text{initial }\beta-\text{carotene}}) \times 100.$$

2.3. Statistical analysis

In order to study the effect of cultivar on EVOO quality characteristics, data were processed by analysis of variance (ANOVA), and the least significant difference (LSD) test was performed for comparison of means ($p \leqslant 0.05$). Correlation analysis ($p \leqslant 0.05$) regression analysis and cluster analysis were also applied to the data. Moreover, a Student *t*-test was performed to compare predicted and measured IC_{50} values. All statistical procedures were computed using the statistical package SYSTAT for Windows (ver. 10, 2003) (Systat Software, Chicago, IL).

3. Results and discussion

3.1. Chemical characterisation of EVOO samples

The quality characterisation of monovarietal olive oils obtained from 5 different cultivars has been investigated, taking into account not only the simple chemical parameters, such as total acidity and peroxide value, but also the bioactive compounds

(vitamin E, carotenoids and total polyphenols), as well as the antioxidant activity calculated with two different methods. The obtained data (Table 1) showed differences in content of the measured parameters among tested oils and were probably due to genetic factors, all oils being obtained from olive fruits cultivated in the same area, climate conditions, and processing techniques. In fact, the chemical and physical characterisation of the oils highlighted as the effect of the cultivar is significant on all parameters evaluated, apart from the content in carotenoids and the concentration of phenols.

All the olive oil samples had free acidity, peroxide values and spectrophotometric indices below the maximum limits established by EU Regulation (2013) for EVOO. Free acidity of the oils covered a range from 0.32% (*Coratina* oil) to 0.52% (*Maiatica* oil). The peroxide values of the samples ranged from 10.9 (*O. Vulture*) to 15.9 meq O_2/kg oil (*Maiatica*), while the specific ultraviolet absorbance K_{232} varied from 0.12 to 0.17, having the lowest values in *Maiatica* variety, while K_{270} ranged from 1.84 in *Coratina* to 2.03 in *Maiatica*.

The olive oil colour is directly linked to the chlorophyll and carotenoid contents, and it has been proposed as a characterising factor and as a quality index related to the oil extraction method and to olive variety (Taamalli et al., 2010). In the studied oils, only chlorophyll was significantly dependent on the cultivar, ranging from 7.90 to 20.0 mg/kg oil for *Leccino* and *O. Vulture* varieties, respectively.

α-Tocopherol is a compound affecting olive oil antioxidant properties. The daily consumption of about 50 mL of EVOO with the highest α-tocopherol concentration may be sufficient to fulfil the dietary recommendation for vitamin E (Bayram et al., 2012). Therefore, EVOO may be an important source of dietary vitamin E, especially in the Mediterranean diet. In the studied oils, the tocopherol content was highly variety dependent as previously reported (Arslan & Schreiner, 2012; Salvador, Aranda, Gomez-Alonso, & Fregapane, 2001); in particular, the highest concentration of vitamin E was detected in olive oil from *Coratina* (209 mg/L), *Leccino* (187 mg/kg) and *O. Vulture* (213 mg/L) and the lowest in *Maiatica* oil (151 mg/L).

The oil samples were also chemically characterised in terms of total phenol content and compounds responsible for of oil bitterness evaluated spectrophotometrically at 225 nm (K_{225} value). The oils obtained from *Coratina* variety were characterised by a high content of total polyphenols, more than 400 ppm for most of the samples analysed, according to the literature for this cultivar (Caponio, Gomes., & Pasqualone, 2001; Clodoveo, Delcuratolo, Gomes, & Colelli, 2007; Favati et al., 2013; Rotondi, Alfei, Magli, & Pannelli, 2010), while those obtained for all the other cultivars had an average total phenol content lower than 400 ppm, as also reported in the literature (García-González, Romero, & Aparicio, 2010; Rotondi et al., 2010). Regarding the K_{225} measures, the highest value was found also in *Coratina* oil samples (0.32), while the lowest was in *Maiatica* oils (0.12).

One-way ANOVA showed a significant effect of cultivar on K_{225} values (Table 1). A significant effect of olive variety on bitterness intensity evaluated spectrophotometrically has been also reported by other authors (Favati et al., 2013; Ilyasoglu, Ozcelik, Van Hoed, & Verhe, 2010; Škevin et al., 2003).

Conversely, the cultivar did not significantly affect the total phenol content of oils; this finding is in agreement with that reported by Favati et al. (2013). Taking into account the significant effect of cultivar on K_{225} value, it is reasonable to assume that cultivar influences the composition of the phenolic fraction, which is strongly related to the bitterness intensity.

Phenolic compounds in food have gained much attention owing to their antioxidant properties and their possible beneficial effects for human health, a consequence of their demonstrated biological activity in the prevention of cancer and cardiovascular disease (Visioli & Bernardini, 2011). In particular, there is an increasing interest in olive oil phenols due to their intrinsic biological properties. Olive oil phenols also contribute to the colour, flavour, and shelf life of the finished product. The stability of extra virgin olive oils is mainly due to their relatively low fatty acids unsaturation level, while the antioxidant activity is due to unsaponifiable components (Rotondi et al., 2004). Phenolic compounds can inhibit oxidation by a variety of mechanisms based on radical scavenging, hydrogen atom transfer and metal-chelating attributes.

There are many methods for total antioxidant determination (Gorinstein et al., 2003). In this study, the antioxidant activity by DPPH and BCB assays was determined. In particular, radical-scavenging activity in five Italian cultivars was carried out by DPPH assay, a simple test based on the colour change of the DPPH solution from purple to yellow due to reduction by the antioxidant. Results were expressed as IC_{50} values and the tested samples exhibited IC_{50} values from 31.9 to 53.4 μL, changing significantly among cultivars, as previously reported (Baiano et al., 2013). In particular, *Coratina* cultivar had the highest radical-scavenging activity (31.9 μL), while *Maiatica* cultivar showed the lowest (53.4 μL) (Table 1), corresponding respectively with highest (0.32) and lowest (0.12) K_{225} values. These differences in antioxidant capacity may depend on the composition and profile of phenolic compounds rather than total phenol level.

The lipid peroxidation inhibitory activity of the EVOOs was assessed by the BCB test. The β-carotene reacts with peroxyl radicals formed during lipid oxidation to form a stable β-carotene radical. This β-carotene/peroxyl adduct lacks the characteristic orange colour of native β-carotene so, as β-carotene scavenges peroxyl radicals, there is a colour decrease that is detectable at 470 nm.

Table 1
Effect of cultivar on extra virgin olive oil quality characteristics (n = 15 for each cultivar).

	Maiatica	*Coratina*	*O. Vulture*	*Leccino*	*O. Bradano*	*F*-value	*p*
Vitamin E (mg/L)	151 ± 6.3[a**]	209 ± 12.9[bc]	213 ± 14.9[bc]	187 ± 13.0[b]	223 ± 9.8[c]	5.90	0.00
Carotenoids (mg lutein/kg)	11.4 ± 1.40[a]	13.6 ± 1.07[a]	14.4 ± 1.28[a]	12.9 ± 1.39[a]	10.3 ± 1.15[a]	1.74	0.15
Chlorophyll (mg pheophytin/kg)	8.68 ± 1.40[a]	16.1 ± 1.90[b]	20.0 ± 4.57[b]	7.90 ± 1.05[a]	13.2 ± 1.51[ab]	4.30	0.00
Total polyphenols (mg/L)	350 ± 10.1[a]	408 ± 23.0[a]	393 ± 15.4[a]	384 ± 15.4[a]	391 ± 21.5[a]	1.94	0.15
K_{225} value	0.12 ± 0.01[a]	0.32 ± 0.03[c]	0.24 ± 0.01[b]	0.21 ± 0.01[b]	0.22 ± 0.01[b]	16.84	0.00
Peroxides (meq O_2/kg)	15.9 ± 1.48[a]	11.7 ± 0.96[b]	10.9 ± 0.70[bc]	15.4 ± 1.67[a]	12.8 ± 0.77[abc]	3.50	0.01
Free acidity (% oleic acid)	0.52 ± 0.05[a]	0.32 ± 0.04[b]	0.42 ± 0.04[ab]	0.39 ± 0.04[b]	0.35 ± 0.04[b]	3.02	0.02
K_{232}	0.12 ± 0.01[a]	0.16 ± 0.01[b]	0.17 ± 0.01[b]	0.16 ± 0.01[b]	0.15 ± 0.01[b]	7.11	0.00
K_{270}	2.03 ± 0.12[a]	1.84 ± 0.07[c]	1.90 ± 0.06[b]	2.01 ± 0.08[a]	1.93 ± 0.04[b]	7.71	0.00
IC_{50} (μL)	53.4 ± 2.75[a]	31.9 ± 4.32[b]	33.3 ± 1.43[b]	40.6 ± 1.82[b]	39.9 ± 4.30[b]	7.27	0.00
% AA*	12.3 ± 2.44[a]	30.5 ± 4.09[b]	10.6 ± 1.61[a]	22.8 ± 2.12[c]	24.1 ± 2.26[bc]	10.32	0.00

Data followed by different letters in the same column are significantly different (LSD test at $p \leqslant 0.05$).

* $(A_{\beta\text{-carotene after 180 min}}/A_{\text{initial }\beta\text{-carotene}}) \times 100$.

** Standard error.

Any antioxidant present that scavenges peroxyl radicals will compete with β-carotene and decrease the rate of its bleaching (Pietro & Bamforth, 2011). Analysing data, it was possible to note that *Coratina* cultivar has the highest antioxidant activity (30.5%), three times higher than *O. del Vulture* cultivar (10.6%) (Table 1).

It should be emphasised that the cultivar also has a significant effect on the antioxidant power. The differences found showed that the characterisation of the oils from a physico-chemical point of view allows differentiation of nutraceutical properties. There is increasing interest in the antioxidant properties of natural compounds and food components. It is obvious that it is advisable to control these characteristics during food processing in order to improve the nutritional quality of products. Therefore, the possibility to identify indices for the prediction of oil antioxidant properties is useful.

3.2. Cluster analysis

In order to verify if the beneficial properties on human health of EVOOs can be strictly related to the cultivar, a cluster analysis was performed. Fig. 1 shows the dendrogram obtained using Ward's method of agglomeration and Euclidean distances to measure the similarity between samples, considering as variables all the analysed chemical parameters. Two main clusters can be discerned, at a linkage distance of about 7.5. The cluster on the left of the dendrogram comprises 42 oil samples, whereas the cluster on the right comprises the remaining 32 samples. This last cluster includes a high number of *Maiatica* oils (87%), whereas 80% of *Coratina* and 80% of *O. Bradano* oils are enclosed in the first cluster. The oils from *Leccino* and *O. Vulture* cultivars are distributed in both clusters. These results demonstrate that chemical characteristics of EVOOs can be different, depending on the cultivar. However, other variables, such as the time of harvest of olives, the method of storage and the specific process conditions can have an important impact on these properties.

3.3. Correlation analysis

In order to identify a simple index that may be able to predict the oil antioxidant power, a correlation analysis among the different chemical parameters by calculating the Pearson coefficients has been conducted (Table 2, $p \leqslant 0.05$).

In literature a linear correlation between IC_{50} values and total polyphenols has widely been reported (Gargouri et al., 2013; Malencic, Maksimovic, Popovic, & Miladinovic, 2008; Mariod, Ibrahim, Ismail, & Ismail, 2009); also in this study, considering that the antioxidant potential is inversely proportional to IC_{50} value, a significant negative correlation either between IC_{50} and total polyphenols was recorded (−0.51), or with IC_{50} and vitamin E (−0.52); the best correlation was obtained between the K_{225} and the IC_{50} values, with a Pearson coefficient equal to −0.80.

On the other hand, there was no correlation between the total polyphenols and the antioxidant activity, expressed as BCB values. Moreover, a low correlation with K_{225} values (0.25) was observed; this is due to the different types of antioxidants that are assayed by the two methods. DPPH assay gives an indication of antioxidant activity of both lipophilic and hydrophilic compounds, while BCB method only gives an indication of the levels of lipophilic compounds. The affinity of the antioxidant for the lipid and thus the lipophilic nature of the molecules seems to be the determining factor (Milella, Bader, De Tommasi, Russo, & Braca, 2014). Other studies demonstrated that total phenol content is not correlated with BCB test (Mariod et al., 2009; Milella et al., 2014). Therefore, the different antioxidant methods can give back sensible differences due to the different reaction mechanism and different solvents involved. Moreover, differences can be more evident when the

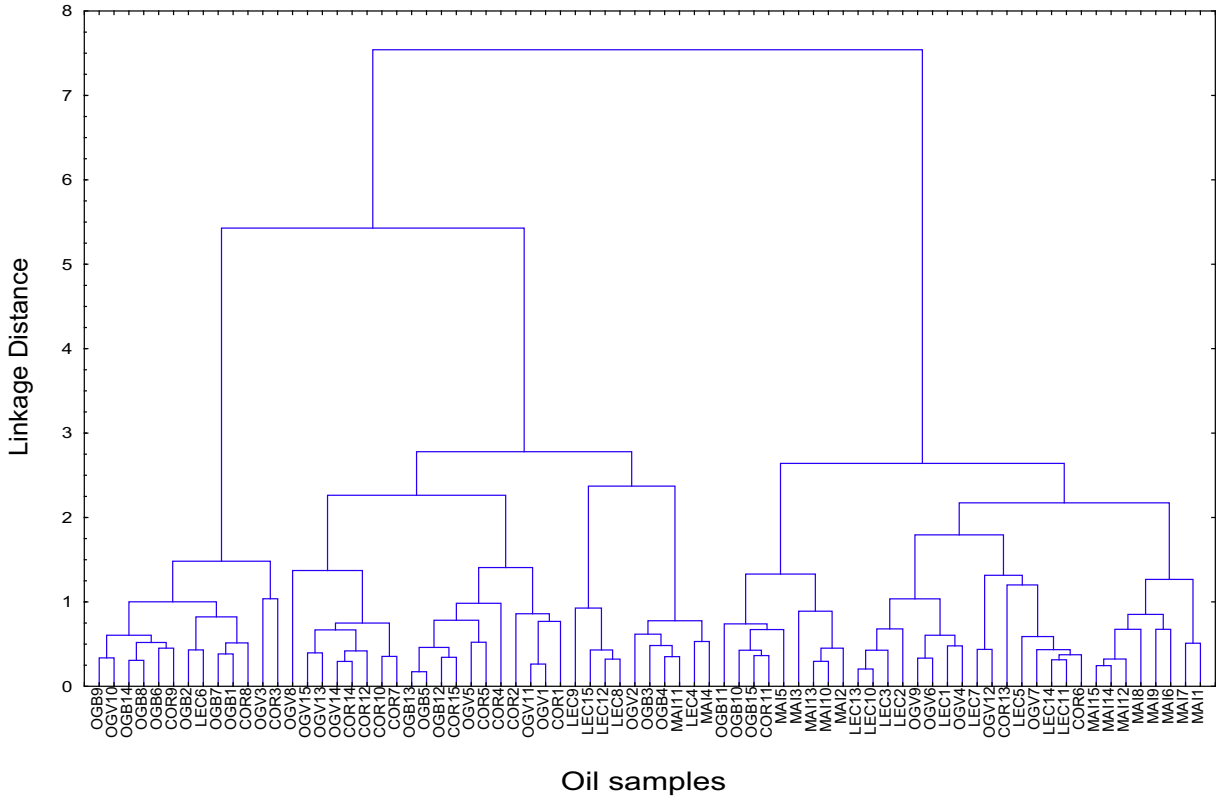

Fig. 1. Cluster analysis of oil samples (Euclidean distances, Ward's method). COR = Coratina, LEC = *Leccino*, MAI = *Maiatica*, OGB = *Ogliarola del Bradano*, OGV = *Ogliarola del Vulture*.

Table 2
Pearson correlation matrix of the studied parameters.

		1	2	3	4	5	6	7	8	9	10	11
1	Vitamin E (ppm)	1.000										
2	Carotenoids (mg lutein/kg)	−0.03	1.000									
3	Chlorophyll (mg pheophytin/kg)	0.35*	0.21	1.000								
4	Total polyphenols (ppm)	0.47*	0.04	0.27*	1.000							
5	K_{225} value	0.46*	0.09	0.26*	0.62*	1.000						
6	Peroxides (meq O_2/kg)	−0.14	0.04	−0.22	−0.15	−0.48*	1.000					
7	Free acidity (% oleic acid)	−0.37*	−0.08	−0.100	−0.34*	−0.58*	0.33*	1.000				
8	K_{232}	0.22	−0.05	0.21	0.23*	0.39*	−0.10	−0.07	1.000			
9	K_{270}	−0.000	0.05	−0.07	−0.05	−0.31*	0.80*	0.23*	0.19	1.000		
10	IC_{50}	−0.52*	−0.12	−0.33*	−0.51*	−0.80*	0.35*	0.54*	−0.40*	0.17	1.000	
11	% AA	0.54*	−0.20	−0.08	0.17	0.25*	−0.03	−0.33*	0.09	−0.02	−0.15	1.000

* Significant for $p < 0.05$.

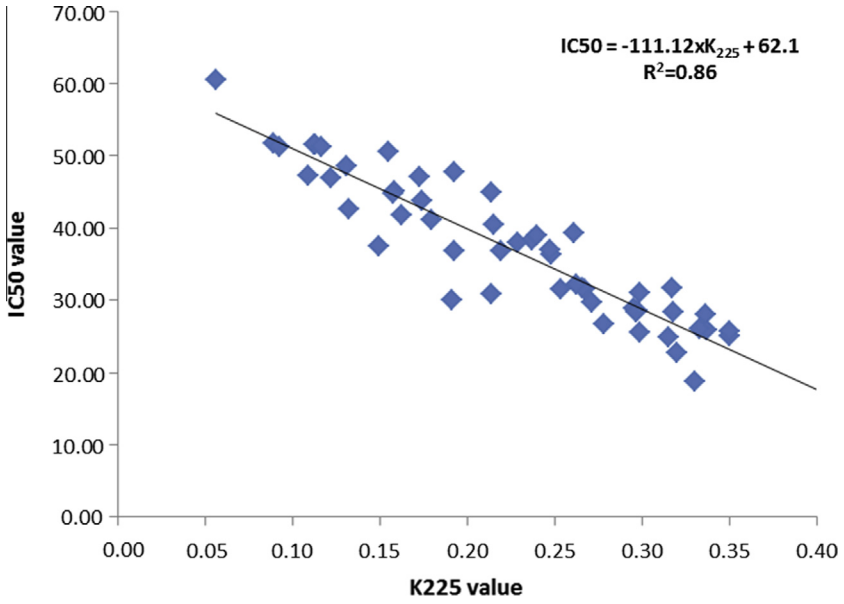

Fig. 2. Relationship between K_{225} value and IC_{50}.

antioxidant activity of a complex matrix, such as olive oil, is measured.

Several publications have previously demonstrated that phenolic compounds, such as the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4 dihydroxyphenylethanol-elenoic acid, 3,4-DHPEA-EDA), and an isomer of oleuropein aglycone (3,4-dihydroxyphenylethanol: 3,4-DHPEA-EA) are mainly responsible for oil antioxidant activity and these compounds are also related to the bitter taste of oil (Del Carlo et al., 2004; Favati et al., 2013; Gambacorta et al., 2012; Loizzo et al., 2012), confirming the role of each individual phenolic compound and not of total polyphenols in perceived bitterness of oil (Favati et al., 2013). Moreover, Gambacorta et al. (2012) showed that antioxidant activity is positively correlated with 3,4-DHPEA-EA phenolic compounds. 3,4-DHPEA-EA showed a positive contribution to antioxidant activity, in particular with IC_{50} values (Loizzo et al., 2012), as reported in our study. This could be due to the antioxidant activity of this compound being higher than that of hydroxytyrosol and shows a protective effect on oil oxidation that is similar to that of hydroxytyrosol. Other than being an effective antioxidant, 3,4-DHPEA-EA is present in high amounts in the phenolic fraction of olive oils, is the main polyphenol in some oil varieties and also is one of the major causes of the bitterness of the oil (Del Carlo et al., 2004). Therefore it is reasonable to assume that the bitterness index, expressed as K_{225} value, is correlated with the antioxidant activity of the oils. With the aim of extending the predictive capabilities of this simple parameter based on the probable correlation between bitterness and antioxidant activity, it is useful to verify the potential of K_{225} in predicting the antioxidant activity of the oils. The results of the Pearson correlation obtained between IC_{50} and K_{225} values corroborate this hypothesis.

3.4. Building predictive model

The results of the analysis correlation (Table 2) suggested the possibility of using the K_{225} values as a predictor of the antioxidant activity expressed as IC_{50}. Therefore, building of the predictive model was performed by relating K_{225} values to IC_{50} values. The obtained linear regression was significant with high r^2 value (0.86). Moreover, the standard error associated with the predictive model was 3.8. The goodness of fit was estimated too, with residual values never higher than 7.0 (absolute value).

On the basis of the results obtained, the oil radical scavenging activity, expressed as IC_{50} values, could be predicted by using the K_{225} values of oil samples in the following equation: $IC_{50} = -111.2K_{225} + 62.1$ (Fig. 2). The results of the regression encourage the use of K_{225} value for predicting oil antioxidant activity. Furthermore, in a recent paper, K_{225} values have been used as oil

Table 3
Predictive model validation. Comparison between predicted and measured IC_{50} (student *t*-test). Mean value ± regression standard error.

Samples	Predicted	Measured	Significance
1	29.3 ± 3.8	25.3 ± 1.2	**
2	43.9 ± 3.8	44.3 ± 2.8	ns
3	36.8 ± 3.8	34.7 ± 2.5	ns
4	43.7 ± 3.8	43.5 ± 3.4	ns
5	48.1 ± 3.8	48. 6 ± 5.3	ns
6	31.3 ± 3.8	31.4 ± 4.2	ns
7	37.7 ± 3.8	33.2 ± 3.5	**
8	46.0 ± 3.8	48.7 ± 5.4	ns
9	38.0 ± 3.8	39.5 ± 3.1	ns
10	35.4 ± 3.8	34.0 ± 3.8	ns
11	36.7 ± 3.8	37.9 ± 4.3	ns
12	40.5 ± 3.8	37.5 ± 3.9	*
13	36.2 ± 3.8	33.8 ± 2.8	ns
14	41.6 ± 3.8	44.6 ± 3.8	*
15	46.8 ± 3.8	48.2 ± 4.7	ns
16	49.5 ± 3.8	50.0 ± 5.6	ns
17	40.4 ± 3.8	40.8 ± 3.4	ns
18	42.0 ± 3.8	44.9 ± 4.6	*
20	30.8 ± 3.8	28.1 ± 2.0	ns

ns: not significant.
* ($p < 0.05$).
** ($p < 0.01$).

bitterness intensity predictors, with good results (Favati et al., 2013).

3.5. Predictive model validation

In order to allow a practical application and to verify the reliability of the results obtained, the proposed model was validated using unknown EVOO samples. Test set sample series were composed of 20 EVOO samples, four for each cultivar utilised in the study. The number of test set samples was assumed to be sufficient in order to validate the proposed predictive model, taking into account the number of samples utilised in other similar studies dealing with predictive models (Barbin, ElMasry, Sun, & Allen, 2012; Bassbasi, De Luca, Ioele, Oussama, & Ragno, 2014; Favati et al., 2013). The predictive capacity of the model was tested by comparing measured and predicted antioxidant activity, expressed as IC_{50}, in the test set samples. The data showed that the predicted values were not significantly different (*t*-test at $p < 0.05$) from the measured mean scores, except for five samples (1, 7, 12, 14 and 18) (Table 3). For three oil samples (1, 7 and 12), the predictive model overestimated the antioxidant activity, while in other two samples (14 and 18) the antioxidant activity was underestimated; however, it should be pointed out that in all cases the difference between the predicted and the measured mean value was less than 4.5. Thus, the risk of a limited underestimation or overestimation of the predicted antioxidant activity was reasonably assumed not to affect the reliability of the predictive model.

The results of this study confirm the suitability of the proposed method in predicting the antioxidant activity, measured as IC_{50}, of EVOO by using K_{225} values. The simplicity of the analytical method used and the good results of the validation tests of the predictive model, may allow the specific use of the proposed method in oil quality monitoring in terms of nutritional properties.

4. Conclusions

In this study, a chemical characterisation was performed on 75 commercial extra virgin olive oils from five different olive monocultivars produced in Southern Italy. The possibility of estimating the radical-scavenging activity of EVOO, measured as IC_{50}, by using a chemical index, the K_{225} value, as a predictor, has been evaluated. This parameter represents a spectrophotometric index of the compounds responsible for oil bitterness measured at 225 nm.

An antioxidant activity predictive model has been built; furthermore, the predictive capacity of the model has been validated by comparing predicted and measured antioxidant activity in a test set of unknown oil samples. The results indicated that the oil antioxidant activity could be satisfactorily predicted by using the K_{225} values of oil samples in the following equation:

$$\text{antioxidant activity } (IC_{50}) = -111.12K_{225} + 62.1.$$

The proposed predictive model can be used as a tool in the characterisation of EVOO samples from Basilicata region on the basis of nutritional properties.

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