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Effect of Olive Ripening Degree on the Oxidative Stability and Organoleptic Properties of Cv. Nostrana di Brisighella Extra Virgin Olive Oil

ANNALISA ROTONDI,*,† ALESSANDRA BENDINI,§ LORENZO CERRETANI,§ MATTEO MARI,† GIOVANNI LERCKER,§ AND TULLIA GALLINA TOSCHI§

Institute of Biometeorology, National Research Council, Via Gobetti 101, 40129 Bologna, Italy, and Department of Food Science, University of Bologna, P.zza Goidanich 60, 47023 Cesena (FC), Italy

The evaluation of the influence of olive ripening degree on the stability of extra virgin olive oils by the determination of the oxidative stability index, the DPPH• radical test, and the quali-quantitative analysis of phenolic compounds, as well as the study of the variation of their sensory profiles, plays a key role in the assessment of the overall olive oil quality. Olives of the cv. Nostrana di Brisighella grown in the north-central Italian region of Emilia-Romagna were picked at four different stages of ripeness and immediately processed in an experimental mill. The polar extracts of oil samples were submitted to spectrophotometric analysis of total phenols and *o*-diphenols and to liquid chromatographic determination of their quali-quantitative profile (HPLC-DAD/MSD). To attain a complete description of oil samples, fatty acid composition, ultraviolet indices (K_{232}, K_{270}, and ΔK), free acidity degree, and peroxide value were also determined according to the European Union methods stated in Regulation 2568/91 (*1, Off. J. Eur. Communities* **1991**, *L248*, 1−82). Sensory quantitative descriptive analysis (QDA) and triangular tests were performed to establish the influence of olive ripening degree on the resulting oil's organoleptic properties. The evolution of the analytical parameters studied shows that the ripeness stage of Nostrana di Brisighella olives that yields the best oil corresponds to a Jaén index value between 2.5 and 3.5. Oils produced from olives harvested within this time frame present a superior sensory profile accompanied by the highest possible chemical and nutritional properties.

KEYWORDS: Extra virgin olive oil; oxidative stability; sensory analysis; phenols; DPPH•; olive ripening degree

INTRODUCTION

The quality and uniqueness of specific extra virgin olive oils are primarily determined by genetic, climatic, and pedologic factors. The cv. Nostrana di Brisighella is exclusively found in Brisighella, a circumscribed area in the north-central Italian region of Emilia-Romagna. The monovarietal oil obtained from these olives has been distinguished with a European Protected Designation of Origin (POD) trademark since 1996. This oil's peculiar sensory properties, together with its high oxidative stability during storage, make it particularly appreciated by consumers, notwithstanding its very high price.

The stability of extra virgin olive oils is mainly due to their relatively low fatty acids' unsaturation level and the antioxidant activity of some of their unsaponificable components. There is increasing interest in the phenols found in olive oil because of their intrinsic biological properties as well as their contribution to the color, flavor, and shelf life of the finished product. Some of the most representative phenolic compounds in olive oils can be grouped in three classes: the oleuropein and ligstroside aglycons and their derivatives (SIDs), the simple phenols (SPs) derivatives from phenylethyl alcohol, cinnamic, and benzoic acids, and other phenolic compounds recently identified as lignans (Ls) (*2*). The changes in fruit chemical composition that take place during ripening as well as their influence on the properties of extracted oils have been studied by several authors (*3*, *4*).

Studies conducted on the change of the phenolic substances have indicated that during olive ripening, the concentration of phenols progressively increases to a maximum level at the "half pigmentation" stage, decreasing sharply as ripening progresses (*3*). An appropriate index of fruit ripening must be established specifically for each individual olive cultivar; in fact, during ripening and processing, several chemical and enzymatic reactions may take place, yielding phenols of lower molecular weight. Although several studies on the relationships between the content of these compounds and the oxidative stability of the oils as well as the influence of the ripening degree and olive cultivar used on the phenolic content of the finished oil have been reported in the literature (*5*−*8*), there is much still to be learned.

Recently, phenols in food have gained much attention owing to their antioxidant properties and their possible beneficial

* Corresponding author (telephone +039-51-6399006; fax +039-51-6399024; e-mail a.rotondi@ibimet.cnr.it).
† National Research Council.
§ University of Bologna.

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implications for human health, a consequence of their demonstrated biological activity in the prevention of cancer and cardiovascular disease (*9*, *10*).

This study aims to evaluate the effect of olive ripening stages on the overall flavors as well as on the oxidative stability of Nostrana di Brisighella monovarietal extra virgin olive oil.

MATERIALS AND METHODS

Samples, Standards, and Solvents. The present study was carried out in a commercial olive (*Olea europaea* L. cv. Nostrana di Brisighella) orchard located in the Emilia-Romagna region. Ten adult 50-year-old olive trees were identified and carefully marked. Olive samples were hand-picked at four different stages of ripeness index (RI_I, RI_{II}, RI_{III}, and RI_{IV}) based on the degree of skin and pulp pigmentation. RI was determined according to the method developed by the Agronomic Station of Jaén defining the RI as function of fruit color in both skin and pulp (*11*). Immediately, olives were processed using an experimental oil mill (Olio Mio Baby 50; Toscana Enologica Mori, Firenze, Italy) equipped with a hammer crusher, a vertical mixer, and a two-phase decanter. Both time and temperature during oil extraction were standardized. Only healthy fruits without any kind of infection or physical damage were used in these experiments.

The standards used for spectrophotometric phenol quantification (gallic acid) and the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) were produced by Sigma-Aldrich Inc., St. Louis, MO. All of the solvents used were of analytical or HPLC grade (Merck & Co. Inc., Darmstadt, Germany).

Analytical Indices. Free acidity, peroxide value, and UV spectrophotometric indices (K_{232}, K_{270}, ΔK) were evaluated according to the official methods described in Regulation EC 2568/91 (*1*) of the Commission of the European Union. All parameters were determined in triplicate for each sample.

Evaluation of Oxidative Stability by Oxidative Stability Instrument Index (OSI). An eight-channel OSI (Omnion, Decatur, IL) was used. The instrument was set at 110 °C and a 120 mL min^{-1} air flow rate, following the analytical protocol described in Bendini et al. (*12*). The OSI was determined four times for each sample and the mean value expressed as OSI time in hours.

Extraction of the Phenolic Fraction. According to Pirisi et al. (*13*), 2 g of the oil sample was added to 1 mL of *n*-hexane and 2 mL of a methanol/water (60:40, v/v) solution in a 10 mL centrifuge tube. After vigorous mixing, they were centrifuged for 3 min at 3000 rpm. The hydroalcoholic phase was collected, and the hexane phase was re-extracted twice with 2 mL of methanol/water (60:40, v/v) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 2 mL of *n*-hexane to remove the residual oil, then concentrated and evaporated in vacuo at 35 °C. The dry extracts were resuspended in 0.5 mL of a methanol/water (50:50, v/v) solution and filtered through a 0.2 μm nylon filter (Whatman Inc., Clifton, NJ) before being analyzed by HPLC-DAD/MSD, by UV−vis spectrometry, and by $DPPH^{\bullet}$ radical test.

Chromatographic Analysis by HPLC-DAD/MSD. HPLC analyses were carried out using an HP 1100 series instrument (Agilent Technologies, Palo Alto, CA), equipped with a binary pump delivery system, a degasser, an autosampler, an HP diode array UV−vis detector (DAD), and an HP mass spectrometer detector (MSD) equipped with a Luna C18 (Phenomenex, Torrance, CA) column of 5 μm particle size and 25 cm × 3.00 mm i.d. All solvents were filtered through a 0.45 μm nylon filter disk (Lida Manufacturing Corp., Kenosha, WI) prior to use. The mobile phase flow rate was 0.5 mL min^{-1}. The wavelengths were set at 280 nm for phenolic acids, alcohols, and secoiridoids and at 350 nm for flavonoids. The injection volume was 10 μL. All of the analyses were carried out at room temperature. The gradient elution (**Table 1**) was carried out using water/formic acid (99.5:0.5, v/v) as mobile phase A and acetonitrile as mobile phase B of the solvent system. The MS analyses were carried out using an electrospray (API-ES) interface operating in negative mode using the following conditions: drying gas flow, 9 L min^{-1}; nebulizer pressure, 50 psi; gas drying temperature, 350 °C. The average was calculated by four replications for each sample and expressed as area in mAU.

Table 1. HPLC Gradient Composition

time (min)	A (%)	B (%)	time (min)	A (%)	B (%)
0	95	5	42	69	31
5	93	7	45	68	32
10	91	9	48	66	34
15	88	12	50	65	35
18	85	15	55	60	40
20	84	16	60	50	50
25	82	18	70	5	95
30	80	20	75	95	5
32	78	22	80	postrun	
35	75	25			
38	72	28			
40	70	30			

Spectrophotometric Determination of Total Phenols. The total phenols (TP) content of the extracts was determined according to the Folin−Ciocalteu spectrophotometric (Shimadzu spectrophometer UV−vis 1204, Kyoto, Japan) method at 750 nm (*14*), using a gallic acid calibration curve ($r^2 = 0.996$). The results were expressed as milligrams of gallic acid per kilogram of oil. The spectrophotometric analysis was repeated three times for each type of extract.

Spectrophotometric Determination of *o*-Diphenols. A 0.5 mL sample of each phenolic extract was dissolved in 20 mL of methanol/water (1:1, v/v), and 4 mL of the resulting solution was added to 1 mL of a 5% solution of sodium molybdate dihydrate in ethanol/water (1:1, v/v) and shaken vigorously. After 15 min, the absorbance at 370 nm was measured using gallic acid for the calibration curve ($r^2 = 0.998$). The results were expressed in milligrams of gallic acid per kilogram of oil. The spectrophotometric analysis was repeated three times for each type of extract.

Antiradical Efficiency of Phenolic Extracts. An ethanolic solution of $DPPH^{\bullet}$ radical (2.9 mL; 15 μM) was added to 0.1 mL of the sample extract to be tested. After 30 min of incubation at 25 °C, absorbance was read at 515 nm and compared to a control sample prepared with 0.1 mL of methanol/water (50:50, v/v) solution. For each phenolic extract, nine dilutions with methanol (1:2, 1:5, 1:10, 1:15, 1:20, 1:40, 1:50, 1:80, and 1:100, v/v) were prepared to determine the antiradical power (ARP) value. The ARP value is equivalent to the reciprocal of EC_{50} ($1/EC_{50}$), which corresponds to the phenolic extract concentration able to reduce 50% of the $DPPH^{\bullet}$ radical content.

Fatty Acid Methyl Ester (FAME) Analysis by Capillary Gas Chromatography (CGC). FAMEs, from the oil samples, were obtained by alkaline treatment with 1 M KOH in methanol (*15*). CGC analyses were carried out using a Carlo Erba HRGC (Carlo Erba, Milan, Italy) with a flame ionization detector (FID) equipped with a 0.25 μm (f.t.), 50 m × 0.25 mm i.d., CP Sil-88 capillary column (Superchrom S.r.l., Milan, Italy) 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 temperature, 220 °C; detection temperature, 230 °C; initial oven temperature, 170 °C maintained for 2 min, followed by a ramp to 230 °C at 2.5 °C min^{-1}, then maintained for 8 min. The analysis was repeated four times for each sample. The data obtained were analyzed with Turbochrom Navigator (r.6.1.2.0.1) software (Perkin-Elmer Instruments LLC, Norwalk, CT).

Sensory Analysis. Sensory analysis was performed by a fully trained analytical taste panel recognized by the International Olive Oil Council (IOOC). The panel evaluated all oil samples following an incomplete randomized block design. Because the main objective of the sensory IOOC method T20/Doc. n.15/Rev 1 (*16*) is to give a commercial classification of the oils, a panel test was established for the present study using a standard profile sheet IOOC method T20 modified by IBIMET-CNR in order to obtain a complete description of the organoleptic properties of the oils sampled. By comparison with the IOOC method's sheet, this modified sheet also provides for the collection of different pleasant flavor intensities perceived by orthonasal, retronasal, and gustative routes as well as the overall pleasantness of the oils. Median of sensory data and robust standard deviation were also calculated (*17*).

Table 2. Chemical Parameters Determined in Oils of Cv. Nostrana di Brisighella Extracted from Olives at Different Ripeness Indices[a]

	I	II	III	IV
harvest date	Oct 22, 2002	Nov 7, 2002	Nov 19, 2002	Dec 3, 2002
ripeness index	2.38	4.21	4.86	5.11
free acidity (%)	0.19 b	0.26 a	0.26 a	0.27 a
POV (mequiv of O_2/kg)	6.03 b	7.39 a,b	7.94 a	6.22 b
K_{232}	1.33 a	1.60 a	1.39 a	1.54 a
K_{270}	0.08 b	0.11 a	0.09 b	0.09 b
ΔK	–0.004 a	–0.003 b	–0.003 b	–0.003 b
TP (mg of gallic acid/kg)	441.43 a	379.51 b	277.43 c	209.57 d
o-diphenols (mg of gallic acid/kg)	212.19 a	228.06 a	153.50 b	127.47 c
SPs (area in mAU)	735.96 c	1682.33 a	1212.65 b	1260.19 b
SIDs (area in mAU)	7796.28 a	5435.00 b	4697.80 b	2778.97 c
Ls (area in mAU)	2512.63 a	2501.90 a	2369.92 a	2579.93 a
ARP	4.02	3.99	2.44	2.05
OSI time (h)	47.09 a	43.10 b	38.43 c	35.30 d
C18:1/C18:2	19.66 a	14.54 b	13.45 c	13.33 c

[a] Ripeness index, ripeness index values of olive fruits; POV, peroxide values; K_{232}, K_{270}, and ΔK, UV spectrophotometric indices; TP, total phenols; *o*-diphenols, *o*-diphenols determined by spectrophotometry; SPs, simple phenols analyzed by HPLC; SIDs, secoiridoid derivates (HPLC); Ls, lignan derivates (HPLC); ARP, antiradical power; OSI, oxidative stability index; C18:1/C18:2, ratio of oleic acid to linoleic acid; I–IV, different harvest dates. Different letters in the same row indicate significantly different values ($P < 0.05$).

A discrimination method (triangular test) (*18*) was applied to identify different sensory profiles among oils obtained from olives at different stages of ripeness. Three samples for each thesis were presented simultaneously, of which two of them were identical. Each taster was asked to identify the different sample. Each oil sample was analyzed by 10 tasters during three different sessions. The sample sets were randomly distributed among the assessors. The test supervisor chose a significance level of 5%.

Statistical Analysis. Chemical data were analyzed using the SPSS r.11.0.0 statistical software (SPSS Inc., Chicago, IL). The significance of differences at a 5% level among means was determined by one-way ANOVA, using Tukey's test. Data were also analyzed by principal components and classification analysis (Statistica 6.0, Statsoft Inc., Tulsa, OK) to evidence the correlation between the analyses and the differences on the samples. Values of sensory attributes were computed using analysis of variance (ANOVA) and tests of least significant differences ($P = 0.05$; SAS version 6.12).

RESULTS AND DISCUSSION

Analytical parameters considered in this work (**Table 2**) of all samples of cv. Nostrana di Brisighella olive oils were widely within estimated limits of Reg. 2568/91 (*1*), so oils could be labeled as "extra virgin" according to European Union rules. The main increase in both free acidity and RI occurred between the first and second ripeness stages, where the RI increased considerably from 2.38 to 4.21, and did not increase significantly afterward; the free acidity value rose from 0.19 to 0.26 and remained at that level throughout to the last stage of ripening (RI_{IV}) (**Table 2**). As shown in **Table 3**, this trend was also confirmed by the high positive correlation ($r^2 = 0.95$) found between RI and free acidity. A slight increase in free acidity as ripening progressed was also observed in cv. Correggiolo (*19*) and Cornicabra monovarietal oils (*20*). The peroxide value did not show significant differences as ripening progressed, reaching values never above 8, which represent a good level of olive oil freshness index. As suggested by official methods the aforementioned parameters (free acidity and peroxide number) together with spectrophotometric indices are valuable olive oil freshness indices. The low values of K_{232}, K_{270}, and ΔK also confirmed the good overall quality of these oils at each olive ripening stage.

Previous studies on the shelf life of Nostrana di Brisighella olive oil emphasized its high content of antioxidant compounds such as tocopherols (*21*) and some phenols, which make this oil particularly resistant to oxidation during storage (*22*).

Table 3. Correlations between RI and Some Analytical Parameters (Second Column) and between OSI Time and the Same Analytical Parameters (Third Column) of Cv. Nostrana di Brisighella Extra Virgin Olive Oils[a]

	ripeness index	OSI time (h)
ripeness index	1.00	–0.94*
free acidity (%)	0.95*	–0.82*
POV (mequiv of O_2/kg)	0.45	–0.19
K_{232}	0.38	–0.26
K_{270}	0.12	0.14
ΔK	0.92*	–0.88*
TP (mg of gallic acid/kg)	–0.88*	0.98*
o-diphenols (mg of gallic acid/kg)	–0.67*	0.85*
SPs (area on mAU)	0.63*	–0.38
SIDs (area on mAU)	–0.92*	0.95*
Ls (area on mAU)	–0.08	0.01
ARP	–0.80*	0.95*
OSI time (h)	–0.94*	1.00
C18:1/C18:2	–0.99*	0.89*

[a] Values marked with an asterisk are correlated by Pearson test ($P < 0.05$).

Moreover, the higher levels of phenolic substances present in this olive oil may enhance its nutritional properties and further contribute to the well-known positive health effects of the Mediterranean lifestyle.

The total oil phenols (TP) content significantly decreased during olive ripening, reaching 47.4% at RI_{IV} stage. The reduction in total phenols content during ripening was also demonstrated by a negative correlation ($r^2 = -0.88$) between TP and RI (**Table 3**).

The *o*-diphenol family can be identified as the main source of the overall antioxidant activity of extra virgin olive oils (*23*). Nostrana di Brisighella olive oils showed high initial levels of *o*-diphenols, which underwent a significant decrease at the III stage of olive ripening to a value of 127.47 mg of gallic acid/kg of oil, which corresponds to a decrease of 60% from the initial content observed at the first stage of ripening.

The SID levels present in the oils sampled decreased as ripening progressed, as shown by the spectrophotometric values found through the TP and *o*-diphenols evaluations (**Table 2**). Moreover, the SIDs' trend showed a positive high correlation with the OSI time values ($r^2 = 0.95$), whereas it showed an opposite correlation with RI ($r^2 = -0.92$) (see **Table 3**). The Ls levels did not show significant differences during the ripening

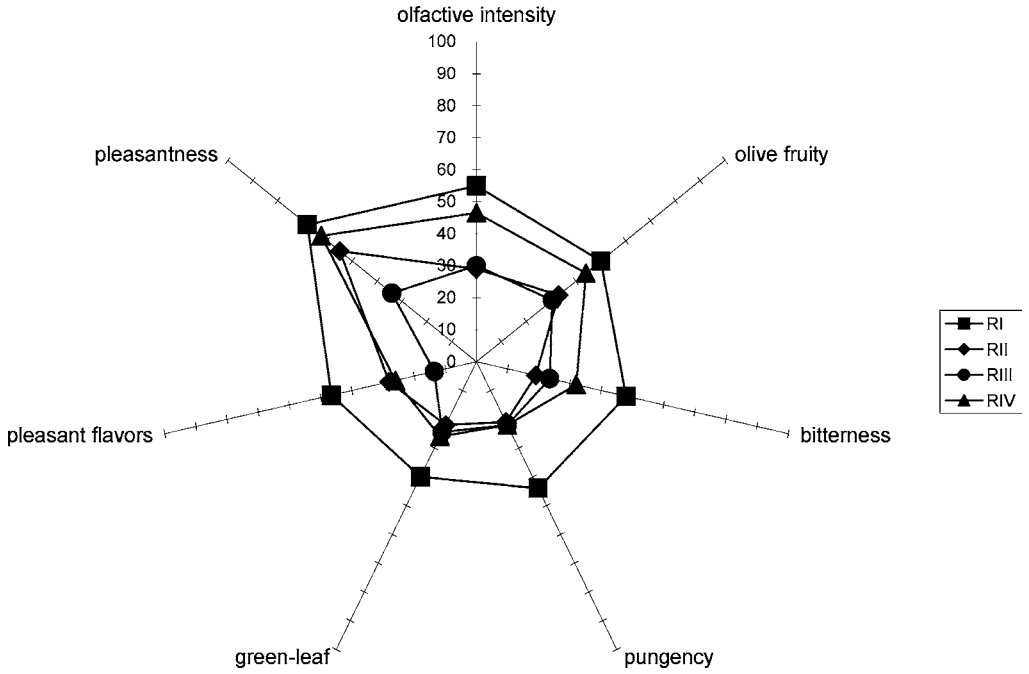

Figure 1. Sensory profiles of cv. Nostrana di Brisighella extra virgin olive oils obtained from olives at different olive ripening indices.

stage. Regarding the amount of simple phenols (SPs), the highest value was found for samples taken at the second harvest time.

Two different analytical methods, ARP and OSI, were respectively applied on the phenolic extract and oils samples, to evaluate the sensitivity to oxidative phenomena. As shown in **Table 2**, the antioxidant activity shown by the ARP index appears to follow a decreasing trend from its high value of 4.02 at the first stage of ripeness to 2.05 at the last harvest date. This trend was corroborated by the negative correlation ($r^2 = -0.80$) observed between ARP value and ripeness index (**Table 3**). The antioxidant activity as measured by DPPH$^\bullet$ reduction has been proposed as an index to differentiate extra virgin olive oils (*23*).

Several authors found a clear correlation between phenols content and OSI (*24*−*26*). Nostrana di Brisighella olives are characterized by their high level of phenols that protect the extracted oils from oxidation during storage.

Nostrana di Brisighella oils obtained from olives harvested at the first ripeness index, corresponding to an $RI_I = 2.3$, exhibited the highest stability of any of the oils tested in this study, with an OSI time = 47.09 h. This value underwent a gradual decrease in subsequent ripeness stages, where we determined a 75% decrease at the RI_{IV}, the highest level of olive pigmentation (RI = 5.11). Even at this high RI, the corresponding OSI = 35.30 h was still very high when compared to the average OSI values of commercial extra virgin olive oil. Although the ripening process of Nostrana di Brisighella olives is slow and gradual, it was important to describe the marked decrease of total phenols content, and consequently the decrease of OSI, as ripening progressed.

The fatty acid composition of olive oil is an important parameter in the length of shelf life that is quantitatively affected by two main factors: the olive variety used in the production of the oil and the ripening stage at which the olives are harvested (*27*). Changes observed from first harvest to last harvest in the oleic/linoleic acid ratio show a decreasing trend during ripening, which was also confirmed by a good negative correlation ($r^2 = -0.99$) between this ratio and the RI (**Table 3**).

It is clear that the oil produced from olives at the initial ripeness stage (RI_I), thanks to the higher absolute level of phenolic compounds and the oleic acid/linoleic acid ratio, ensures the best quality from the oxidative stability point of view. This was also demonstrated by the high correlations ($r^2 = 0.98$ and 0.89) observed between the OSI and the TP levels and C18:1/C18:2 ratios, respectively (**Table 3**). Among freshness parameters, only free acidity and ΔK values showed a clear correlation with the OSI.

Phenolic compounds are closely associated with the nutritional and sensory qualities of food, contributing directly or indirectly to desirable or undesirable aromas and flavors (*28*).

Quantitative descriptive sensory analysis evidenced a clear decreasing trend of the positive olive oil descriptors as olives ripened. None of the samples of Nostrana di Brisighella oils included in this study presented any sensorial defects. Olive fruity, expressed as olfactory intensity value, bitter, pungent, green-leaf, and pleasant gustative attributes showed a statistically significant highest intensity at the first stage of olive ripening when compared to the other oils' sensory profiles. Moreover, the olive fruity attribute, expressed as olfactory-gustative overall satisfaction, exhibited the highest value in oils produced from olives at the first stage of olive ripeness, making this oil statistically different only from the oils obtained from olives harvested at RI_{II} and RI_{III} (**Figure 1**). Also, pleasant flavors, mainly ascribable to grassy, artichoke, and green tomato attributes, were significantly higher in oil obtained from olives harvested at R_I. The organoleptic properties of oils extracted from olives at any of the subsequent harvests exhibited statistically significant losses of some pleasant attributes such as bitterness, pungency, green-leaf aroma, and pleasant flavors. In the case of the olive fruity attribute we observed a significant decrease in oils produced from olives harvested at RI_{II} and RI_{III}, whereas at the last stage of ripeness the intensity of the olive fruity attribute increased again, allowing the oil to reach a panel score similar to the one recorded for the oils obtained from olives at the first stage of pigmentation. Nevertheless, it is important to emphasize that the results of the triangular test confirmed the statistical results of sensory quantitative descriptive analysis (QDA): in fact, comparison with triangular tests oils obtained from the first and fourth olive ripening stages, 100% of the

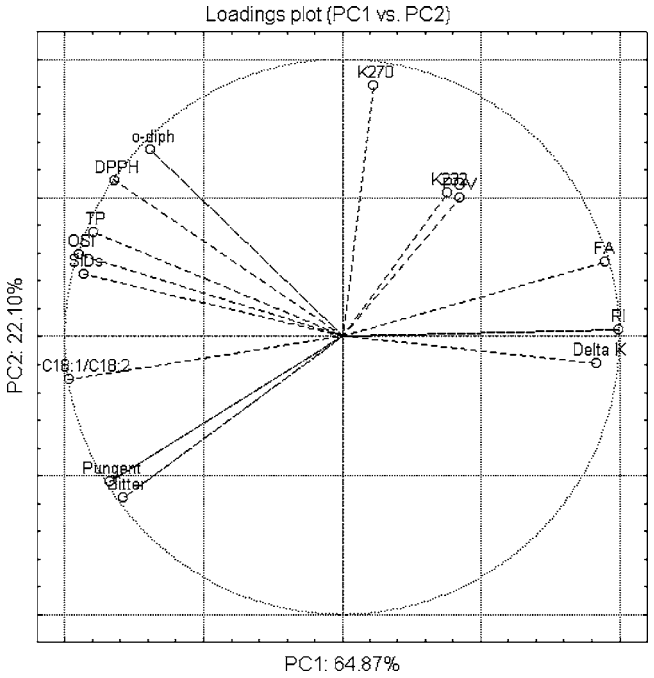

Figure 2. Score plot of following parameters: RI, ripeness index; FA, free acidity; POV, peroxide values; K_{232}, K_{270}, and ΔK, UV spectrophotometric indices; TP, total phenols; *o*-diph, *o*-diphenols; SPs, simple phenols; SIDs, secoiridoid derivates; Ls, lignan derivates; OSI, oxidative stability index; C18:1/C18:2, ratio of oleic acid to linoleic acid ratio; POV (mequiv of O_2/kg).

assessors correctly identified all of the samples provided during the panel tests.

The decrease of bitterness and pungency is also related with the reduction in TP and *o*-diphenols levels (*29*). In the present study, we observed a weak positive correlation between the SIDs content and the bitter ($r^2 = 0.57$) and pungent ($r^2 = 0.65$) sensory attributes.

Principal component discriminant analyses showed that RI, free acidity, POV, K_{232}, K_{270}, ΔK, TP, *o*-diphenols, SPs, SIDs, Ls, OSI, and C18:1/C18:2 ratio were able to distinguish oils obtained at the olive RI_I from all the other oils sampled. The oil produced from olives at RI_{II} was discriminated from that of the other samples. Oils from olives at RI_{III} and RI_{IV} did not exhibit significant statistical differences. The first two discriminant functions of the statistical analysis explained 86.71% of the total variance, as the plot of discriminant functions showed the oil obtained from RI_I well separated from the other oils (**Figures 2** and **3**).

The effect of fruit ripeness on the chemical and sensorial characteristics of the oil has been extensively investigated for several cultivars such as Casaliva and Leccino (*30*, *31*).

In general, the chemical relationship to the sensory characteristics of the oil indicates that the optimal ripeness corresponds to incomplete pigmentation of the fruit surface, even if it strongly depended on the individual olive cultivar. A previous study on cv. Nostrana di Brisighella showed that in order to obtain the best sensorial oil quality, the olive RI cannot exceed a value of 3.5 (*22*).

The data from the present study clearly emphasize the importance of identifying the optimal RI for Nostrana di Brisighella olives. In fact, the earliest harvest date, corresponding to an RI_I of 2.38, gave the best results in terms of all the parameters considered. In oil extracted from olives picked 15 days later at an RI_{II} of 4.21, a noticeable increase in linoleic acid levels, a decrease in total polyphenol content, and a lower

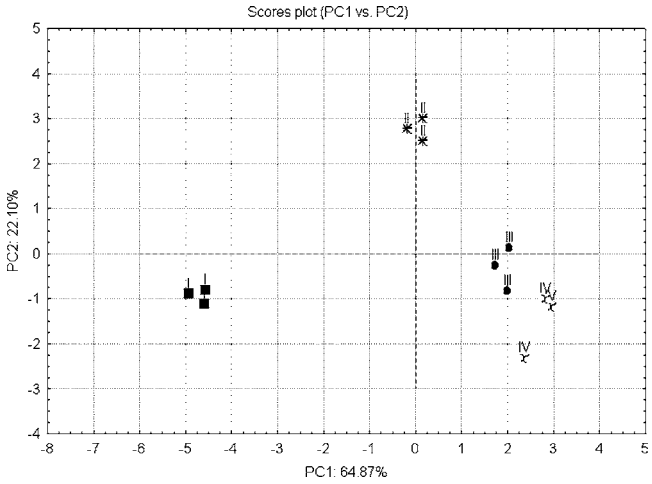

Figure 3. Principal component analysis of chemical and sensory profile of Nostrana di Brisighella extra virgin olive oil produced from olives of different RIs. I–IV represent different harvest dates.

resistance to forced oxidation (expressed as OSI) were observed. Also, the marked decrease in some positive attributes such as bitterness, pungency, grassiness, and green-leaf observed in oils obtained from olives with RIs above 3.5 confirmed the overall worsening of the oils' sensory profile. The increase of the sensory intensity of olive fruitiness at the last stage of olive pigmentation (RI_{IV}) can be explained by a different perception of its intensity by the assessors, probably due to the concurrent marked decrease in other strong attributes such as bitterness and pungency. This will be explained in a further study carried out by the authors on the analytical determination and quantification of aromatic volatile substances responsible for the sensory attributes of olive oils.

In conclusion, we suggest that for the production of optimal extra virgin olive oil, Nostrana di Brisighella olives growing in the Emilia-Romagna region should be harvested at an RI between 2.5 and 3.5. Further studies on this interesting olive cultivar will include the determination of changes of phenolic substances during oil storage as well as their role in the autoxidation reactions.

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