

Alessandro Parenti^a
Paolo Spugnoli^a
Piernicola Masella^a
Luca Calamai^b
Ottorino Luca Pantani^b

^a Dipartimento di Ingegneria
Agraria e Forestale,
Facoltà di Agraria,
Università degli Studi di Firenze,
Firenze, Italia

^b Dipartimento di Scienza del
Suolo e Nutrizione della Pianta,
Facoltà di Agraria,
Università degli Studi di Firenze,
Firenze, Italia

Improving olive oil quality using CO₂ evolved from olive pastes during processing

The effect of blanketing with CO₂, naturally evolved during malaxation of olive pastes, on the quality of virgin olive oil was investigated at lab-scale. The O₂ depletion was monitored along with CO₂ emission to confirm the previously hypothesized accelerated respiration. Malaxation experiments were conducted for 180 min both in sealed (SC) and in the traditional open-to-air conditions to ascertain whether the oil quality was affected by O₂ concentration as afforded by CO₂ blanketing. The quality of olive oils obtained at different time intervals was monitored by total acidity, peroxide value (PV), specific extinction coefficients K₂₃₂ and K₂₇₀, total chlorophyll and total hydrophilic phenols, and HPLC hydrophilic phenols profile. A rapid decrease in oxygen concentration and a simultaneous increase in CO₂ concentration were recorded, confirming the accelerated respiration. The oil produced in SC showed a lower PV and K₂₃₂ coefficient and a higher chlorophyll (10–17 mg/kg) and hydrophilic phenols (110 mg/kg) concentration. No differences in total acidity and K₂₇₀ coefficient were observed. The hydrophilic phenols profile indicated that, at least for the Frantoio cultivar and an advanced ripeness state, the maximal extraction is generally achieved already after 20 min. Most of the individual hydrophilic phenols have higher concentrations (up to 50%) in SC.

Keywords: Olive oil, malaxation, carbon dioxide emission, quality, hydrophilic phenols.

1 Introduction

In a previous paper, it was shown that CO₂ emission spontaneously occurs during malaxation of olive pastes, probably as the result of accelerated respiration and, partly, of fermentation processes [1]. It was observed that, when malaxation was carried out in a sealed container, a huge CO₂ concentration builds up allowing the malaxation time to be prolonged without apparent paste browning. Moreover, the extraction of chlorophylls, *i.e.* highly lipophilic compounds, was largely increased, and the authors proposed the technological use of these natural CO₂ emissions to reduce oxidation processes in oil transformation plants, thus increasing oil quality through a proper engineering of the malaxation mixers. However, the large CO₂ emission was mainly attributed to respiration on the basis of the high emission rate and on the negligible presence of some fermentation by-products, and a direct monitoring of oxygen depletion during the process (which would have unambiguously demon-

strated the occurrence of respiration) was not reported. In addition, a wider evaluation of the effects on the olive oil quality, which included the evaluation of the oxidative state along with the concentration gain of minor components allowed by this technique, was not assessed. Particularly, these last compounds, even if their concentration is very low (about 2% of oil weight), are nevertheless crucial since they are responsible for the quality of virgin olive oils (VOO). This “minor” fraction includes more than 230 chemical compounds such as aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants. Among them, the hydrophilic phenols constitute a group of secondary plant metabolites that show peculiar sensory and healthy properties and include different classes of phenolic compounds such as phenolic acids, phenolic alcohols, hydroxy-isocromans, flavonoids, secoiridoids and lignans [2, 3]. They exhibit different octanol/water partition coefficients [4], and their occurrence is strictly related to the activities of various endogenous enzymes present in the olive drupes [5]. Therefore, the concentration of such molecules in VOO is strongly affected by the extraction conditions [2–6]. It is known that certain conditions, such as malaxation time and temperature, modify the phenolic fraction both in amount and composition [7]. In current oil processing plants, the olive pastes are malaxated in contact with air,

Correspondence: Alessandro Parenti, Dipartimento di Ingegneria Agraria e Forestale, Facoltà di Agraria, Università degli Studi di Firenze, Piazzale Cascine 15, 50144 Firenze, Italy. Phone: +39 055 3288319, Fax: +39 055 3288316, e-mail: alessandro.parenti@unifi.it

which is the main reason for the decay of the antioxidant activity of VOO [8]. It seems therefore reasonable that the hydrophilic phenols composition/amount could be improved by preserving the olive paste from the contact with O₂. Inert gases, as nitrogen or argon [6, 9–11], have been used to protect olive pastes from O₂ while the effects of CO₂ spontaneously produced by the pastes during the transformation process need deeper investigations.

The aim of this work was to assess, in a lab-scale experiment, the “quality” of VOO as influenced by two treatments: malaxation of olive pastes within an open-to-air chamber and within a sealed chamber, where blanketing with CO₂ spontaneously occurs. The VOO “quality” was evaluated through the concentration of hydrophilic phenols and through some analytical parameters related to oxidation state. In addition, the oxygen depletion was monitored during the process to confirm the occurrence of the respiration process previously hypothesized.

2 Materials and methods

2.1 Experimental procedure

The experimental design is summarized in Tab. 1. The olive drupes were harvested in early December 2004 from trees (cultivar Frantoio) cultivated near Florence, Italy: they were in good sanitary conditions and in an advanced state of ripeness (*i.e.* 100% of the drupes were fully black skin colored). Aiming to reduce the differences among different trials, all the experiments were carried out on a batch of 30 kg of olives, homogenized as they were harvested from different trees. To reduce alteration phenomena, the olive drupes were stored in aerated fruit baskets and malaxation experiments were run as soon as possible, *i.e.* within 3 days after the harvest. On each day, two sub-samples of 5 kg were treated as described hereafter.

The olive drupes were processed into paste with a lab-extrusion mill. Of the paste, 0.5 kg was discarded at the beginning and at the end of the crushing process, resulting in 4 kg of paste to be malaxated. This amount corresponds to a paste/gas ratio of about 4 : 1 in volume, *i.e.* the ratio commonly used in commercial malaxation mixers. The consistency and granulometry of the pastes obtained were comparable to those commonly produced in current olive mill plants. The olive pastes were then malaxated in a lab mixer purposely designed for this experiment and previously described [1]. The olive pastes were malaxated by the same device, with the contact with air being the only difference between the two sub-samples (sealed and open-to-air conditions). As shown in Tab. 1, malaxation temperature was maintained at 28 °C in all the trials, by means of a heat exchanger. The sub-samples processed in sealed conditions were run first and gas evolution (CO₂, O₂) was monitored by sampling air through a rubber sept in the upper part of the chamber. In all the trials, malaxation was prolonged for 180 min. This malaxation time is unrealistic for conventional industrial conditions (*i.e.* olive pastes in contact to air), which may vary between 30 and 60 min. However, the effect of extremely prolonged malaxation times in sealed conditions had not been investigated yet. So, aiming to evaluate the possible exploitation of the olive oil quality potential in this extremely long operative conditions, olive paste aliquots were withdrawn before malaxation (at $t = 0$) and after 20, 40, 60, 90, 120, 150 and 180 min in each trial, from a valve located in the lower part of the malaxation chamber. In this way, it was possible to compare the effects of the treatment either in correspondence of regular malaxation time (45 min) or along the entire malaxation experiments.

Eventually, the paste samples were centrifuged and the recovered oil was separated and stored at –20 °C in 15-mL plastic vials until analyzed.

The above-described procedure was repeated for 3 days and the effects of treatments (sealed apparatus vs. open-to-air) were evaluated through a paired *t*-test (3 + 3 replicates).

Tab. 1. Experimental design.

Day	Replicates	Cultivar	Apparatus	Paste [kg]	Head space [L]	Temperature [°C]	Malaxation time [min]
1 st	1	Frantoio	sealed	4.00	≈1	28	180
2 nd	2	Frantoio	sealed	4.00	≈1	28	180
3 rd	3	Frantoio	sealed	4.00	≈1	28	180
1 st	1	Frantoio	open	4.00	–	28	180
2 nd	2	Frantoio	open	4.00	–	28	180
3 rd	3	Frantoio	open	4.00	–	28	180

2.2 Chemical analysis

The quality of VOO resulting from each olive sub-sample was assessed by chemical analyses: free acidity, peroxide value (PV), UV absorption (*i.e.* specific extinction coefficients K_{232} and K_{270}), total chlorophyll, total phenols concentration and HPLC analysis of phenolic compounds.

The concentration measure of CO₂ and O₂ was done, in percentage, by a handheld gas analyzer type CheckPoint O₂/CO₂ (PBI-Dansensor A/S, Ringsted, Denmark). Before sampling, the air in the headspace of the chamber was homogenized by three quick aspiration and inflation cycles (50 mL each) with a 60-mL plastic syringe. Total acidity, PV and UV absorption were carried out according to the European Official Method of Analysis [12].

The total chlorophyll concentration in oil was obtained from the following formula [13]:

$$\text{total chlorophyll} = 345.3 [A_{670} - (A_{630} + A_{710})/2]/L$$

where total chlorophyll is expressed in mg/kg as pheophytin α , L is the optical path in mm, and A₆₇₀, A₆₃₀ and A₇₁₀ are absorbance units at 630, 670 and 710 nm, respectively.

Total hydrophilic phenols were extracted by liquid-liquid partition with an 80 : 20 methanol/water solution. The total phenol content of the extract was determined by the Folin–Ciocalteu spectrophotometric method at 765 nm, using gallic acid as calibration standard [14].

Phenolic extraction for HPLC analyses was performed by liquid-liquid extraction, following the procedure of Cortesi *et al.* [15] modified for small sample amounts. Briefly, the extraction was performed with an 80 : 20 methanol/water solution on a 200 mg aliquot of VOO, after the addition of 100 μ L of an 80 : 20 methanol/water solution of syringic acid at 20 mg/L as internal standard. The final extractant volume was 1 mL. The samples were agitated on a vortex mixer for 3 min, let sit for 15 min and again agitated on a vortex mixer for 3 min. Then the oil fraction (heaviest) was separated by centrifugation (10 min at 10,000 \times g) and the clear supernatant containing the hydrophilic phenols was injected into the HPLC system. The HPLC system consisted of a Perkin-Elmer 410 quaternary pump, a Series 200 autosampler and a 235C UV-DAD detector.

Analytical conditions were: HPLC column: Phenomenex Synergy C18 4.6 \times 15 cm; injection volume: 20 μ L; solvent: pH 2.5 H₂O/acetonitrile gradient as described by Cortesi *et al.* [15]; wavelength: 280 nm.

The identified phenolic compounds were: (*p*-hydroxyphenyl)ethanol (*p*-HPEA); (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA); dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA); dialdehydic form of decarboxymethyl elenolic acid linked to *p*-HPEA (*p*-HPEA-EDA); oleuropein aglycon (3,4-DHPEA-EA); ligstroside aglycon; and lignans [(+)-1-acetoxypinoresinol, (+)-pinoresinol]; as reported in Fig. 1.

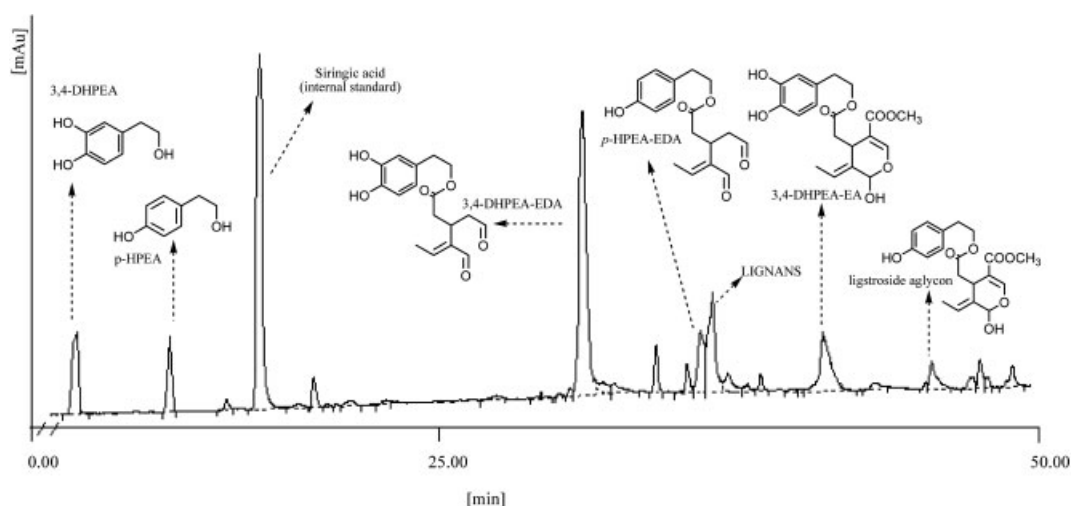


Fig. 1. HPLC chromatograms (at 280 nm) of phenolic extracts from virgin olive oil. 3,4-DHPEA, 3,4-dihydroxyphenyl)ethanol; *p*-HPEA, (*p*-hydroxyphenyl)ethanol; 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA; *p*-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to *p*-HPEA; lignans, (+)-1-acetoxypinoresinol, (+)-pinoresinol; 3,4-DHPEA-EA, oleuropein aglycon; ligstroside aglycon.

3 Results and discussion

3.1 Carbon dioxide emission and oxygen depletion

Carbon dioxide and O₂ concentration in the headspace of the sealed chamber during malaxation are reported in Fig. 2 as a function of time. All three malaxation trials showed essentially the same trend in CO₂ emission and O₂ depletion. The data of CO₂ emission fits a saturation equation $[y = (39.56x)/(9.13 + x)]$ with R^2 values of 0.99, and the general phenomenon is illustrated by the trend line shown in Fig. 2. In these trials, an initial rapid increase in CO₂ concentration was observed followed by a gradual decrease in emission rate. The final portion of the curve was characterized by CO₂ emission rates that were about 30–50 times lower than those present at the beginning. The small amounts of evolved CO₂, which were well above the detection limit clearly detectable, were probably the result of different biochemical processes. These results are in accordance with what was reported previously [1], where the initial portion of the curve was ascribed to the onset of accelerated cellular respiration after milling, as the result of the extensive contact with oxygen and the breakdown of cellular structures caused by crushing and successive malaxation. In this paper, differently from the previous one, the CO₂ emission was compared with a monitoring of O₂ concentration during malaxation (Fig. 2). In this case, the data fitted a power equation ($y = 21.32x^{-0.596}$) with R^2 values of 0.99 and showed a rapid decrease of O₂ concentration in the initial portion of the curves (the trend line in Fig. 2 is derived from the entire data set from the sealed trials) corresponding to the initial rapid CO₂ emission. The O₂ con-

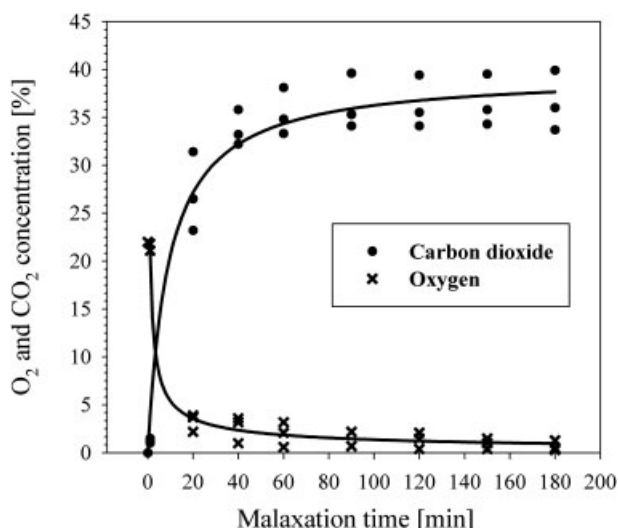


Fig. 2. Carbon dioxide emission and oxygen depletion during olive paste malaxation in sealed conditions.

centration was reduced to under 5% after about 20 min, then it gradually declined to 0. When the increase in CO₂ concentration (after logarithmic transformation) was plotted against the oxygen depletion, a straight line is obtained with a regression coefficient of 0.996 (Fig. 3). This indicates that over 99% of the variability in CO₂ emission is explained by the oxygen depletion. Therefore, these data strongly suggest the hypothesis that the general phenomenon of CO₂ emission is mainly due to the accelerated cellular respiration, as it involves a rapid depletion of O₂ in contact with the olive pastes during the malaxation process.

Malaxation performed under inert gases blanketing results in oils with higher phenolic content [9, 10], but some authors indicated that a partial oxidation of the fatty acid chains is necessary (especially in the initial part of malaxation) for the development of volatile compounds constituting the aroma. Therefore, it was proposed to use the time of exposure of olive pastes to air contact during malaxation to control the endogenous oxidative enzymatic activities and to manage O₂ control during malaxation by replacing the air in the malaxation chamber with inert gas (N₂) at suitable times. When malaxation is carried out in sealed conditions, a double protection effect may be expected: one due to progressive O₂ depletion and a second one due to CO₂ stratification on top of the paste surface (since CO₂ is the heaviest component of air, Mw: 44), improving the protection against oxidation. Furthermore, since the CO₂ emission is a spontaneous phenomenon related to both the biochemical activity of olive fruit

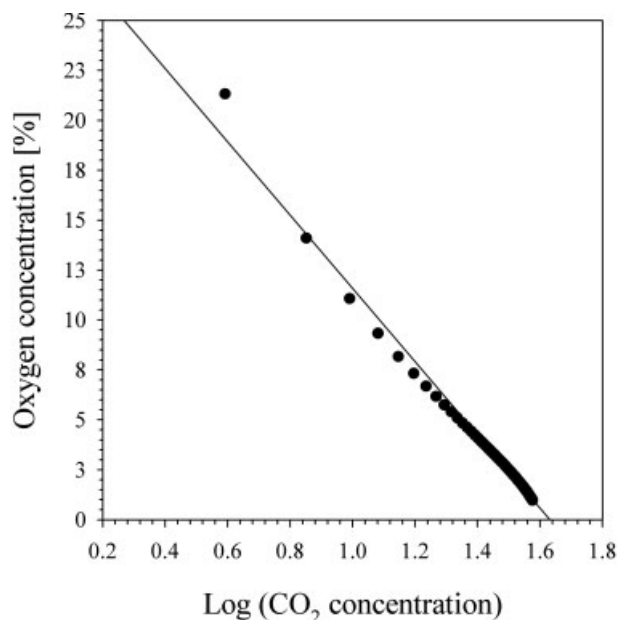


Fig. 3. Oxygen concentration plotted against CO₂ concentration (after logarithmic transformation). $R = 0.996$.

and the degree of contact between the enzymes and the substrates (presumably sugars and oxygen), it is probable that the operative conditions (*i.e.* the amount of olive pastes in the kneader resulting in different headspace volumes, temperature of malaxation and shaft mixer speed) could result in different dynamics of CO₂ emission and, ultimately, of O₂ depletion. Therefore it is possible to propose the CO₂ emission like a suitable new technological parameter to control the olive oil quality during processing as a function of a wide range of settings.

3.2 Effects on VOO quality

Free acidity, PV and UV indices are standard parameters currently considered by the EEC Regulation No. 2568/91 to evaluate the quality of VOO. Generally, technological treatments induce small variations in the free acidity which is inversely related to the quality of olive drupes. The oil sub-samples of each trial did not show differences either with the two methods of malaxation or at the different sampling times during paste processing (data not reported), confirming both the homogeneity of the initial batch of olive drupes and the goodness of the storage conditions.

The PV of the oils at the selected sampling times are reported in Tab. 2. Relatively small increments were recorded as a function of time for both malaxation conditions in comparison with the initial concentrations. Nevertheless, the oils from the sealed apparatus had PV values much lower (about 7 meqO₂/kg after 90 min) than those obtained open-to-air (about 12 meqO₂/kg at the same time). The differences were significant ($p < 0.05$)

already after 45 min of malaxation. Similar results were found for the K₂₃₂ values (Tab. 2) with significant differences ($p < 0.05$) of about 0.1, while for K₂₇₀, which is related to the secondary oxidation step of the oils [16], no significant difference ($p > 0.05$) between the two treatments were observed for all times (Tab. 2). Since PV and K₂₃₂ are related to the primary oxidation state of oil, the results indicate that the protection against oxidation due to the naturally evolved CO₂ was effective and resulted in a lower content of primary oxidation products. These characteristics are generally associated with VOO of improved quality [16].

In addition to hydrophilic antioxidant compounds, the concentration of lipophilic antioxidants (*e.g.* chlorophylls, tocopherols) is also an important aspect of VOO quality. Total chlorophyll concentration is an easy-to-determine parameter that is representative of VOO quality. Furthermore, it represents an antioxidant component that improves the stability of the oil in the dark [17, 18], is related to the commercial value of olive oil [19] and is a sensitive parameter towards the extraction techniques [20, 21]. Total chlorophyll concentrations measured on the oils are reported in Fig. 4. A general increase was observed in all trials as a function of malaxation time, thus confirming the results reported in previous experiments [1, 22]. However, extraction times being equal, there were large and significant ($p < 0.05$) differences of about 10–17 mg/kg between the trials conducted in the sealed apparatus and those open-to-air. The higher level of chlorophyll extraction due to the use of the sealed chamber probably resulted from an effective protection from oxidation by the rapid CO₂ emission of the olive paste.

Tab. 2. Oxidative state of the oil: PV, K₂₃₂ and K₂₇₀ extinction coefficients during malaxation in sealed conditions as compared to the control.

Time [min]	PV [meqO ₂ /kg] [#]			K ₂₃₂ [#]			K ₂₇₀ [#]		
	Sealed	Open	Mean difference	Sealed	Open	Mean difference	Sealed	Open	Mean difference
0	6.61(0.54)	6.30(0.25)	0.31(0.77)	1.90(0.02)	1.91(0.02)	0.02(0.03)	0.17(0.01)	0.17(0.01)	0.00(0.01)
20	6.68(0.07)	8.42(0.89)	1.73(0.82)	1.90(0.03)	1.97(0.04)	0.07(0.04)	0.16(0.01)	0.16(0.00)	0.00(0.01)
45	7.13(0.61)	10.88(1.55)	3.75(1.10)*	1.93(0.01)	1.98(0.01)	0.04(0.01)*	0.16(0.01)	0.17(0.01)	0.02(0.03)
60	7.48(0.56)	11.21(1.68)	3.72(1.14)*	1.92(0.01)	2.01(0.04)	0.09(0.03)*	0.16(0.01)	0.14(0.04)	0.02(0.04)
90	7.45(0.24)	12.25(0.57)	4.80(0.58)**	1.95(0.01)	2.02(0.03)	0.07(0.02)*	0.15(0.02)	0.18(0.01)	0.02(0.03)
120	8.09(1.53)	13.79(2.07)	5.70(2.12)*	1.94(0.02)	2.04(0.01)	0.10(0.01)**	0.17(0.01)	0.17(0.02)	0.00(0.02)
150	8.75(1.45)	13.31(1.30)	4.57(1.38)*	1.96(0.01)	2.02(0.02)	0.06(0.01)**	0.17(0.01)	0.17(0.01)	0.00(0.01)
180	9.50(1.29)	14.31(2.18)	4.81(1.75)*	1.93(0.02)	2.03(0.03)	0.10(0.01)**	0.16(0.01)	0.17(0.01)	0.00(0.02)

[#] Data are means of three independent replicates. Standard deviations are shown in parentheses. Within each row, mean difference values with one or two asterisks are significantly different from 0 (paired *t*-test; *, $p < 0.05$; **, $p < 0.01$).

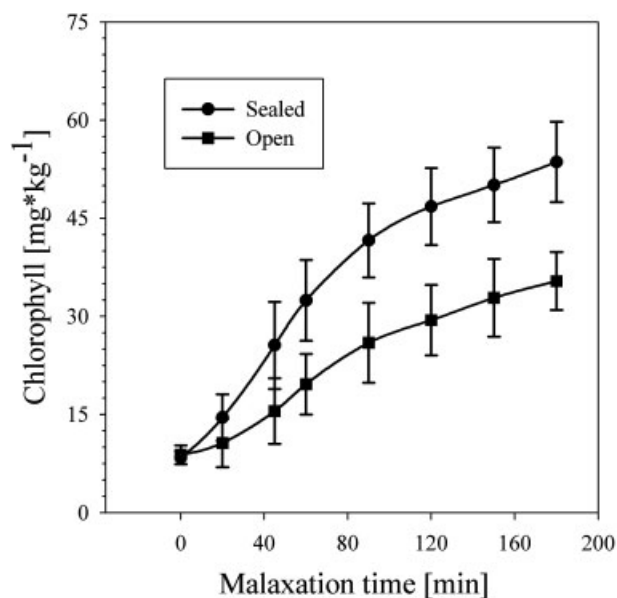


Fig. 4. Total chlorophyll concentration during malaxation. Data are means (\pm SD) of three independent replicates.

The protection against oxidation influenced also the total hydrophilic phenols concentration (Tab. 3). It is well known that these compounds are strictly related to extra VOO quality, in relation to sensory, healthy and stability properties [2, 23, 24]. In Tab. 3, the total hydrophilic phenols concentrations measured on the oil samples corresponding to the two kinds of malaxation are compared. In the “sealed” experiment, a sharp increase in concentration was observed in the first 20 min. There was a twofold significant increase (about 110 mg/kg at $p < 0.01$) of the hydrophilic phenols concentrations in the oils from the sealed trials in comparison with those coming from the open-to-air ones. These results may be explained in terms of O₂ concentration in the pastes during processing. Previous works have shown that the hydrophilic phenols concentration in olive oil is related to endogenous oxidoreductases (polyphenoloxidases and peroxidases) which can promote their oxidation [5]. A reduction of their enzymatic activity may be obtained by limiting the amount of oxygen in contact with the pastes during malaxation. Since the respiration processes result in a rapid depletion of O₂ concentration during malaxation and in an effective blanketing by the evolved CO₂, the oxidative reactions occurring in the pastes during processing are limited and the hydrophilic phenols amount in the oil increased. In a preliminary investigation [25], an increase of the hydrophilic phenols with time up to 60 min in the sealed apparatus was observed, whereas an initial increase for the first 20 min followed by a marked decrease was observed in the open-to-air conditions. The different patterns recorded in this set of trials are probably

Tab. 3. Total hydrophilic phenols concentration (colorimetric method) during malaxation in sealed conditions as compared to the control.

Time [min]	Total hydrophilic phenols [mg/kg] [#]		
	Sealed	Open	Mean difference
0	110.52(6.59)	111.14(13.95)	0.63(7.78)
20	223.57(22.30)	110.05(27.08)	113.52(4.84)**
45	222.32(15.00)	130.73(3.07)	91.59(12.94)**
60	225.79(17.02)	127.92(19.00)	97.87(2.01)**
90	219.04(9.30)	123.95(11.91)	95.08(8.55)**
120	215.96(22.63)	125.76(17.56)	90.21(16.56)**
150	213.60(8.63)	111.09(19.33)	102.51(11.23)**
180	207.16(11.20)	118.00(14.84)	96.06(10.21)**

[#] Data are means of three independent replicates. Standard deviations are shown in parentheses. Within each row, mean difference values with one or two asterisks are significantly different from 0 (paired *t*-test; *, $p < 0.05$; **, $p < 0.01$).

due to the different ripeness states of the olive drupes in the two experiments, as the olives were at full ripeness in the present experiment while they were at initial ripeness in the previous one [25]. The stiffness of the cell walls, *i.e.* weakening of the cell wall structures during ripening, may be responsible for such behavior [11, 26, 27].

This behavior is also revealed by the evolution of hydrophilic phenols (Fig. 5). A significantly higher concentration was recorded at all times for 3,4-DHPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA-EDA at $p < 0.01$ and lignans and ligstroside aglycon at $p < 0.05$, while *p*-HPEA and 3,4-DHPEA showed concentrations not significantly different from the VOO obtained open-to-air. As for the extraction profile with time, in sealed conditions all the identified phenolic compounds show a rapid increase after 20 min and either remain constant or slightly decrease thereafter. In the open-to-air conditions, a similar profile was found for 3,4-DHPEA-EA, 3,4-DHPEA and lignans, in contrast to 3,4-DHPEA-EDA and *p*-HPEA-EDA where a significant decrease is generally recorded with time from the beginning. The 3,4-DHPEA-EDA was the most abundant phenolic compound and accounted for over 40% of the total hydrophilic phenols. The malaxation in sealed condition afforded a doubled concentration of this compound in the VOO already after 20 min.

The hydrophilic phenols concentration profiles with time observed in this experiments differed from those reported in a preliminary experiment [25], where a continuous increase with time of all compounds was found. In par-

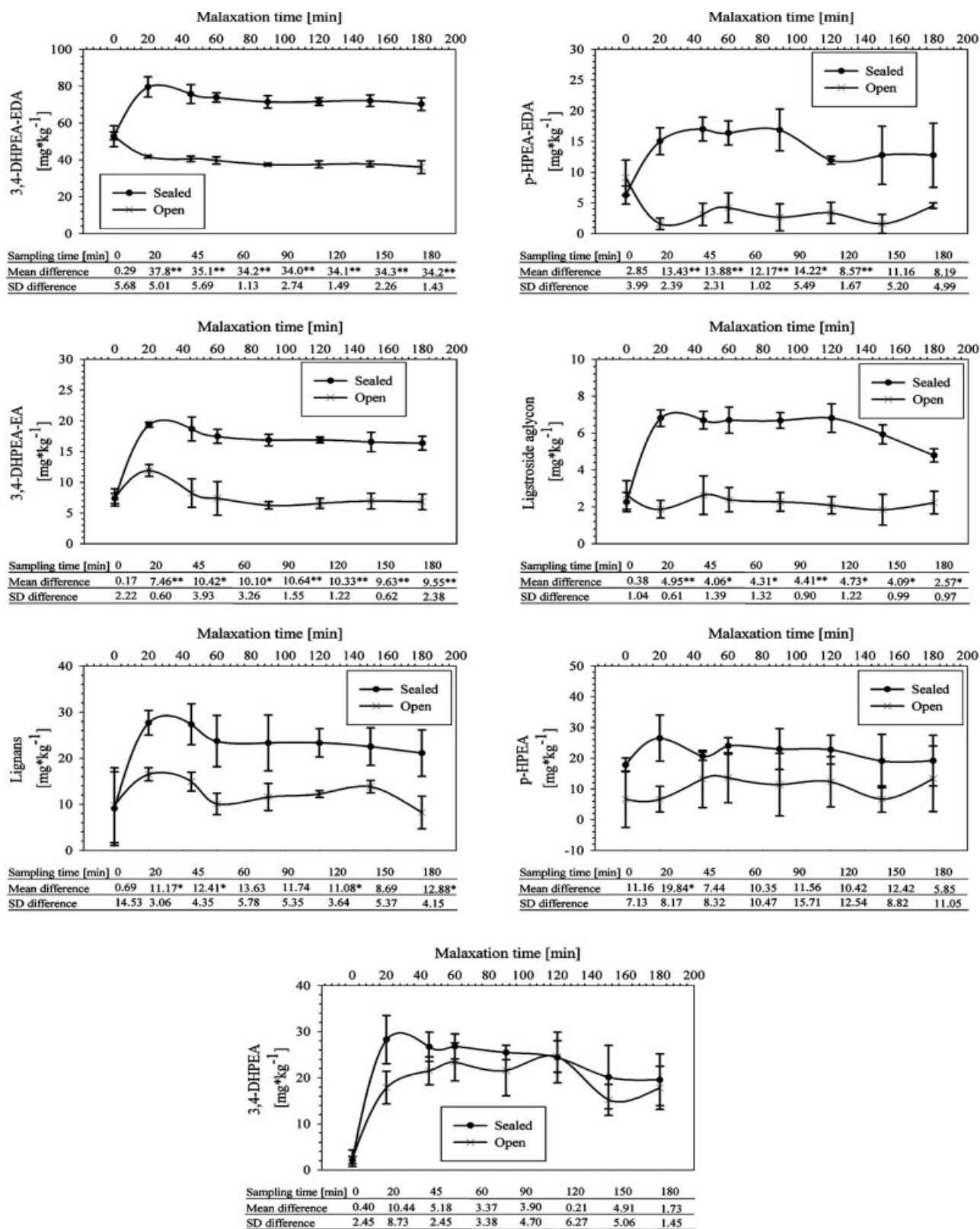


Fig. 5. HPLC phenolic profile during malaxation. Data are means (\pm SD) of three independent replicates. Where error bars are not visible, determinations were within the range of the symbols on the graph. Mean difference values with one or two asterisks are significantly different from 0 (paired *t*-test; *, $p < 0.05$; **, $p < 0.01$).

ticular, 3,4-DHPEA-EDA resulted in that case as the most abundant compound accounting almost entirely for the differences between VOO obtained in sealed vs. open-to-air conditions. As discussed for total hydrophilic phenols, this was mainly attributed to the ripeness state of the olive drupes. Further investigations are in progress to confirm this hypothesis.

4 Conclusions

The technological improvement of VOO transformation plants must engineer concept and principles aiming to maximize VOO quality. In this view, this research demonstrates that the natural CO₂ emission from olive pastes during the transformation process is due to an accelerated respiration process following the drupes crushing. In addition, this process can be successfully used to enhance the VOO quality with minimal changes in the malaxation mixer. The increment in VOO quality basically involves two aspects: reduction of the oxidation parameters (PV and K₂₃₂) and increase in extraction of antioxidant compounds (chlorophyll, phenolic compounds). However, the quality differences that can be achieved are related to the ripeness state of the olive drupes and probably to the cultivars. Further investigations are needed to manage the extraction system settings as related to the above-mentioned olive characteristics. Future developments of this research will consider the implementation of these concepts at plant scale, and the study of the effects of operative set-ups (malaxation time and temperature) to maximize VOO quality. In addition, the sensory evaluation and the analysis of the volatile fractions should be considered along with the chemical analyses to obtain a complete assessment of the potential of this innovation.

References

- [1] A. Parenti, P. Spugnoli, P. Masella, L. Calamai: Carbon dioxide emission from olive oil pastes during the transformation process: Technological spin offs. *Eur Food Res Technol.* 2006, **222**, 521–526.
- [2] M. Servili, R. Selvaggini, S. Esposito, A. Taticchi, G. F. Montedoro, G. Morozzi: Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. *J Chromatogr A.* 2004, **1054**, 113–127.
- [3] N. Uccella: Olive biophenols: Biomolecular characterization distribution and phytoalexin histochemical localization in the drupes. *Trends Food Sci Technol.* 2001, **11**, 315–327.
- [4] P. S. Rodis, V. T. Karathanos, A. Mantzavinou: Partitioning of olive oil antioxidants between oil and water phases. *J Agric Food Chem.* 2002, **50**, 596–601.
- [5] G. F. Montedoro, M. Baldioli, R. Selvaggini, A. L. Begliuomini, A. Taticchi, M. Servili: Relationships between phenolic composition of olive fruit and olive oil: The importance of the endogenous enzymes. *Acta Hort.* 2002, **586**, 551–556.
- [6] M. Servili, G. F. Montedoro: Contribution of phenolic compounds to virgin olive oil quality. *Eur J Lipid Sci Technol.* 2002, **104**, 606–613.
- [7] F. Angerosa, R. Mostallino, C. Basti, R. Vito: Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chem.* 2001, **72**, 19–28.
- [8] M. Servili, M. Baldioli, E. Federici, G. F. Montedoro: Effetto dei fenomeni ossidativi in fase di gramolatura sulle caratteristiche qualitative dell'olio vergine di oliva. *Proceedings of CISETA Congress.* In: *Ricerche e Innovazioni nell'Industria Alimentare*, vol. 4. Chiriotti Editori, Pinerolo (Italy) 2000, pp. 294–304.
- [9] M. Servili, R. Selvaggini, A. Taticchi, S. Esposito, G. F. Montedoro: Air exposure time of olive pastes during the extraction process and phenolic and volatile composition of virgin olive oil. *J Am Oil Chem Soc.* 2003, **80**, 685–695.
- [10] M. Servili, R. Selvaggini, A. Taticchi, S. Esposito, G. F. Montedoro: Volatile compounds and phenolic composition of virgin olive oil: Optimization of temperature and time exposure of olive pastes to air contact during the mechanical extraction process. *J Agric Food Chem.* 2003, **51**, 7980–7988.
- [11] E. Vierhuis, M. Servili, M. Baldioli, H. A. Schols, A. G. J. Voragen, G. F. Montedoro: Effect of enzyme treatment during mechanical extraction of olive oil phenolic compounds and polysaccharides. *J Agric Food Chem.* 2001, **49**, 1218–1223.
- [12] EC 1991 Commission Regulation EEC 2568/91 and later amendments. *Official J Eur Communities.* 1991, L248(9).
- [13] J. Pokorny, L. Kalinova, P. Dysseler: Determination of chlorophyll pigments in crude vegetable oils. *Pure Appl Chem.* 1995, **67**, 1781–1787.
- [14] C. Capannesi, I. Palchetti, M. Mascini, A. Parenti: Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chem.* 2000, **71**, 553–562.
- [15] N. Cortesi, P. Rovellini, P. Fusari: Determination of biophenols minor polar compounds in virgin olive oil. *Riv Ital Sost Grasse.* 2002, **79**, 145–150.
- [16] R. Aparicio, J. Harwood: *Handbook of Olive Oil – Analysis and Properties.* Aspen Publishers, Gaithersburg, MD (USA) 2000, pp. 129–151.
- [17] Y. Endo, R. Usuki, T. Kaneda: Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. *J Am Oil Chem Soc.* 1985, **62**, 1375–1378.
- [18] Y. Endo, R. Usuki, T. Kaneda: Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophylls. *J Am Oil Chem Soc.* 1985, **62**, 1387–1390.
- [19] M. I. Minguez-Mosquera, L. Rejano-Navarro, B. Gandul-Rojas, A. H. Sanchez-Gomez, J. Garrido-Fernandez: Color-pigment correlation in virgin olive oil. *J Am Oil Chem Soc.* 1991, **68**, 332–336.
- [20] M. Roca, M. I. Minguez-Mosquera: Change in the natural ratio between chlorophylls and carotenoids in olive fruit during processing for virgin olive oil. *J Am Oil Chem Soc.* 2001, **78**, 133–138.
- [21] P. Luaces, A. G. Pérez, J. M. Garcia, C. Sanz: Effects of heat-treatments of olive fruit on pigment composition of virgin olive oil. *Food Chem.* 2005, **90**, 169–174.

- [22] A. Ranalli, L. Pollastri, S. Contento, E. Iannucci, L. Lucera: Effect of olive paste kneading process time on the overall quality of virgin olive oil. *Eur J Lipid Sci Technol.* 2003, **105**, 57–67.
- [23] R. Mateos, A. Cert, M. C. Perez-Camino, J. M. Garcia: Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. *J Am Oil Chem Soc.* 2004, **81**, 71–75.
- [24] K. L. Tuck, P. J. Hayball: Major phenolic compounds in olive oil: Metabolism and health effects. *J Nutr Biochem.* 2002, **13**, 636–644.
- [25] A. Parenti, P. Spugnoli, P. Masella, L. Calamai: Utilizzo dell'anidride carbonica emessa dalle paste di oliva durante la trasformazione per migliorare la qualità dell'olio. Primo contributo – Prove di laboratorio. *Riv Ital Sost Grasse.* 2005, **82**, 283–289.
- [26] I. Mafra, B. Lanza, A. Reis, V. Marsilio, C. Campestre, M. De Angelis, M. A. Coimbra: Effect of ripening on texture microstructure and cell wall polysaccharide composition of olive fruit (*Olea europaea*). *Physiol Plant.* 2001, **111**, 439–447.
- [27] A. Ranalli, L. Pollastri, S. Contento, L. Lucera, P. Del Re: Enhancing the quality of virgin olive oil by use of a new vegetable enzyme extract during processing. *Eur Food Res Technol.* 2003, **216**, 109–115.

[Received: August 3, 2006; accepted: September 5, 2006]