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# Effects of Fly Attack (*Bactrocera oleae*) on the Phenolic Profile and Selected Chemical Parameters of Olive Oil

Ana Maria Gómez-Caravaca,[†] Lorenzo Cerretani,*[,‡] Alessandra Bendini,[‡] Antonio Segura-Carretero,*[,†] Alberto Fernández-Gutiérrez,[†] Michele Del Carlo,[§] Dario Compagnone,[§] and Angelo Cichelli[||]

Department of Analytical Chemistry, Faculty of Sciences, University of Granada, C/Fuentenueva s/n, E-18071 Granada, Spain, Department of Food Science, University of Bologna, Piazza Goidanich 60, I-47023 Cesena (FC), Italy, Department of food Science, University of Teramo, Via C. Lerici 1, I-64023 Mosciano Stazione (TE), Italy, and Department of Science, University of Pescara and Chieti, Viale Pindaro 42, I-65127 Pescara, Italy

The phenolic fraction of virgin olive oil influences both its quality and oxidative stability. One of the principal threats of the quality of olive fruit is the olive fly (*Bactrocera oleae*) as it alters the chemical composition. The attack of this olive pest has been studied in order to evaluate its influence on the quality of virgin olive oil (free acidity, peroxide value, fatty acid composition, water content, oxidative stability, phenols, and antioxidant power of phenolic fraction). The study was performed using several virgin olive oils obtained from olives with different degrees of fly infestation. They were acquired in different Italian industrial mills from the Abruzzo region. Qualitative and quantitative analyses of phenolic profiles were performed by capillary electrophoresis−diode array detection, and electrochemical evaluation of the antioxidant power of the phenolic fraction was also carried out. These analyses demonstrated that the degree of fly attack was positively correlated with free acidity ($r = 0.77$, $p < 0.05$) and oxidized products ($r = 0.58$, $p < 0.05$), and negatively related to the oxidative stability index ($r = -0.54$, $p < 0.05$) and phenolic content ($r = -0.50$, $p < 0.05$), mainly with secoiridoid compounds. However, it has been confirmed that the phenolic fraction of olive oil depends on several parameters and that a clear correlation does not exist between the percentages of fly attack and phenolic content.

**KEYWORDS: Virgin olive oil; phenols; qualitative parameters; oxidative stability; capillary electrophoresis; olive soundness**

## INTRODUCTION

Virgin olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions that do not alter its properties and must not undergo any treatments other than washing, decantation, centrifugation, or filtration (*1*). These processes maintain volatile and other minor compounds such as phenols that enhance the characteristic flavor of virgin olive oil (*2*).

Stability is not a standard parameter used to measure quality. However, it provides information about the hypothetical shelf life of the oil. In particular, lower stability indicates a poorer quality (e.g., greater acidity, higher peroxide values and extinction coefficients, and lower sensorial score). It has been shown that 78% of the stability, evaluated by Rancimat, is due to the combined effect of two variables, namely, phenolic compounds and the oleic/linoleic (O/L) ratio.

Phenolic compounds can be active as antioxidants and also can inhibit the free radical chain reaction (*3*). Their antioxidant properties and in particular their hydrogen-donating capacities are modulated by the presence of different chemical groups in the phenol backbones. Mainly, phenolic compounds having an *o*-catechol group in their structure such as those found in virgin olive oil (**Figure 1**), such as hydroxytyrosol and its oleosidic forms, are powerful antioxidants (*4, 5*). Using different assays, Carrasco-Pancorbo et al. (*3*) evaluated the antioxidant capacity of different phenolic compounds and concluded that among them, hydroxytyrosol, oleuropein, and decarboxy-methyl oleuropein aglycons with an o-catecholic structure exhibited the

* To whom correspondence should be addressed. Tel: +390547338121. Fax: +390547382348. E-mail: lorenzo.cerretani@unibo.it.
[†] University of Granada.
[‡] University of Bologna.
[§] University of Teramo.
[||] University of Pescara and Chieti.

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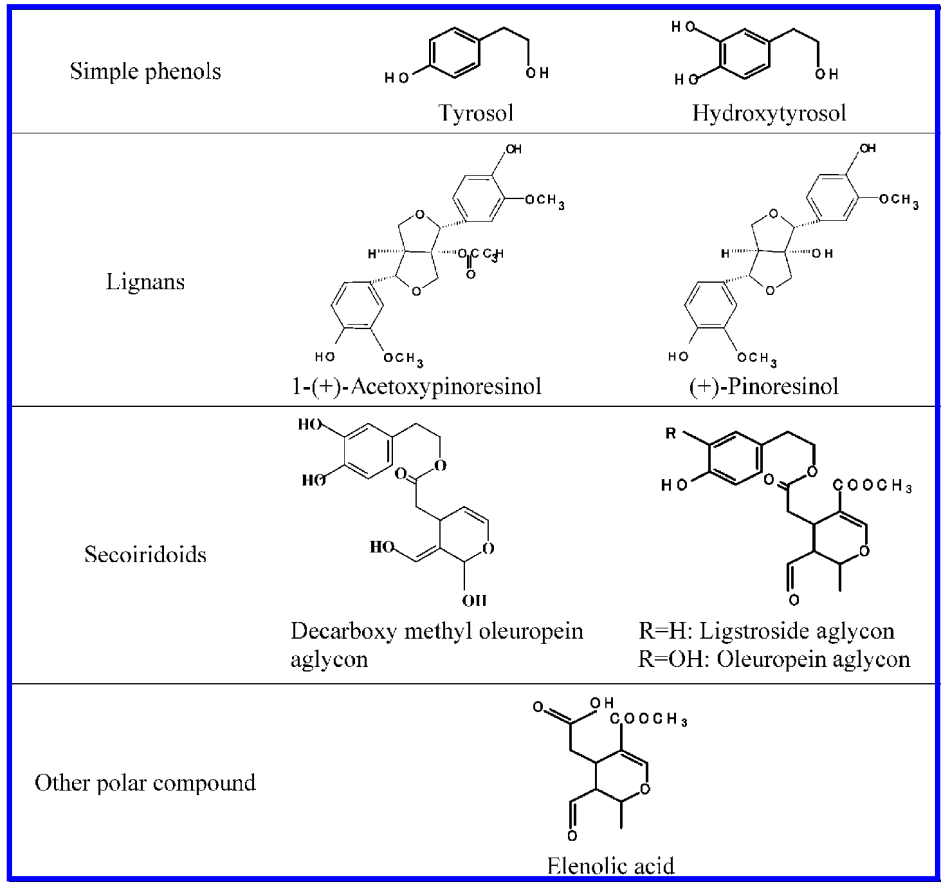


**Figure 1.** Structures of the phenolic compounds under study.

strongest antioxidant activity. In contrast, monohydroxylated phenols as tyrosol and ligstroside aglycon had very poor radical-scavenging activity.

Phenolic compounds have a positive effect on the health, sensory properties, and oxidative stability of olive oil (*2, 6–9*). But despite that it is well known that the phenolic fraction is influenced not only by the olive cv. but also by climatic and environmental conditions (*10, 11*), agronomic practices, and technological process (*10–14*).

Plants are subject to attacks from different organisms and as a result have evolved a complex, integrated defense system against potential pathogenic organisms to ensure survival, growth, and development. It has been shown that plants respond to pathogenic attack by synthesizing compounds that activate the defense system in fruits (*15*).

The quality of virgin olive oil is strongly related to the health status of the fruit from which it is extracted. One of the most detrimental enemies of the quality of olive oil is the olive fruit fly (*Bactrocera oleae*). This insect can reduce oil yield, affect quality parameters (acidity, peroxide value, ultraviolet (UV) absorbance, and organoleptic quality), and negatively alter the chemical composition (sterols, phenols, fatty acid, and volatile fraction) (*12, 16–24*). The severity of the negative effects depends on the stage of the development of the olive fly, the intensity of the attack, and olive variety. It has been shown that olive oils produced from fruits that have been attacked by the olive fly present an increase in acidity, peroxide values, and UV absorbance. Moreover, it has been demonstrated that the phenolic content and total amount of volatile compounds decrease, and no significant variations can be observed in fatty acid composition (*24, 25*). While there are several publications about the influence of *Bactrocera oleae* on the qualitative parameters of olive oil, potential variations in the phenolic profile have not been considered in depth.

Traditionally, free acidity and peroxide values have been considered the qualitative chemical parameters of virgin olive oil (*1*). Nevertheless, in recent years the evaluation of phenolic compounds as a qualitative parameter has been proposed (*26*).

The first aim of this investigation was to assess different qualitative parameters of olive oil in various commercial olive oil samples depending on the percentage of fly attack. The second aim was related to the study of changes in the quality and oxidative stability of these olive oils, with particular emphasis to correlations with the phenolic profile and antioxidant power of the phenolic fraction. This statistical treatment of data allowed us to determine which qualitative parameter of olive oil was more robust and less influenced by all the variables.

## MATERIALS AND METHODS

**Reagents, Stock Solutions, and Reference Compounds.** 3,4-Dihydroxyphenylacetic acid (dopac) was obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA), and oleuropein (oleuropein glucoside) was obtained from Extrasynthèse (Genay, France). The stock solutions of these two analytes were prepared in methanol/water (50/50, v/v) at a concentration of 500 $\mu$g/mL in the case of dopac and 6000 $\mu$g/mL for oleuropein glucoside. Dopac was used for the quantification of simple phenols present in the extracts of olive oil, and oleuropein glucoside was used to make the calibration curves for the quantification of lignans and complex phenols.

Sodium hydroxide was purchased from Merck (Darmstadt, Germany); sodium tetraborate (borax) was obtained from Sigma and was used as running buffer at different concentrations and pH values.

Double-deionized water with a conductivity less than 18.2 MΩ was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Hydranal-Titran 2 and Hydranal-solvent oil (solvents used to measure

**Table 1.** Information Relative to Olives (Cultivars, Area of Production, Healthy State) and Corresponding Oil Samples (Code and Technological System of Their Production)

| code | olive varieties | town of production | tech. system[a] | % fly attack |
|---|---|---|---|---|
| S1 | Dritta, Leccino | Loreto Aprutino (PE) | C | 2% |
| S2 | Dritta, Intosso | Città S. Angelo (PE) | C | 2.5% |
| S3 | Intosso | Città S. Angelo (PE) | C | 2.5% |
| S4 | Leccino | Loreto Aprutino (PE) | C | 2.5% |
| S5 | Dritta, Leccino | Loreto Aprutino (PE) | C | 4% |
| S6 | Dritta | Loreto Aprutino (PE) | P | 5% |
| S7 | Dritta | Loreto Aprutino (PE) | P | 5% |
| S8 | Dritta | Loreto Aprutino (PE) | P | 5% |
| S9 | Dritta | Loreto Aprutino (PE) | P | 5% |
| S10 | Carpinetina | Farindola (PE) | P | 5% |
| S11 | Leccino | Rocca S. Giovanni (CH) | C | 5% |
| S12 | Gentile | Rocca S. Giovanni (CH) | C | 5% |
| S13 | Gentile | Rocca S. Giovanni (CH) | C | 5% |
| S14 | Dritta, Leccino | Morro d'oro (TE) | D+C | 5% |
| S15 | Tortiglione | Cologna (TE) | D+C | 7.5% |
| S16 | Mix | Ortona (CH) | C | 7.5% |
| S17 | Leccino | Casoli (CH) | C | 7.5% |
| S18 | Gentile | Casoli (CH) | C | 10% |
| S19 | Mix | Orsogna (CH) | C | 10% |
| S20 | Mix | Morro d'oro (TE) | C | 10% |
| S21 | Gentile | Crecchio (CH) | C | 15% |
| S22 | Intosso | Casoli (CH) | C | 15% |
| S23 | Mix | Guardiagrele (CH) | P | 25% |
| S24 | Dritta | Loreto Aprutino (PE) | C | 25% |
| S25 | Leccino | Crecchio (CH) | C | 30% |
| S26 | Mix | Crecchio (CH) | P | 35% |
| S27 | Dritta | Cappelle (PE) | P | 35% |
| S28 | Mix | Orsogna (CH) | C | 35% |
| S29 | Mix | Cepagatti (PE) | C | 45% |
| S30 | Gentile, Leccino | Rocca S. Giovanni (CH) | C | 60% |
| S31 | Mix | Orsogna (CH) | C | 60% |
| S32 | Mix | Cologna (TE) | C | 85% |

[a] P, pressure system; C, continuous system; D+C, destoner plus continuous system.

the water content with the volumetric titration of Karl Fisher) were from Riedel-deHaën (Seelze, Germany).

All solvents used were analytical or HPLC grade (Merck & Co. Inc., Darmstadt, Germany).

**Samples.** Thirty-two virgin olive oils produced from different industrial mills located in the Abruzzo region (Italy, December 2006) were analyzed. Samples differed in the percentage of fly attack, variety of olive cultivars, and technological system used (pressure or centrifugation, with or without a destoning phase) as reported in **Table 1**. The degree of infestation was calculated as the number of damaged olives per 100 fruits, considering both the presence of exit holes and grubs.

**Free Acidity and Peroxide Value (PV).** These parameters were determined according to the official methods described in European Regulation EEC 2568/91 and amendments (*27*). PV was expressed as mequiv $O_2$ $kg^{-1}$ of oil. The samples were stored in the absence of light and at room temperature in order to measure PV after three months of storage. Relative standard deviation (RSD) of the free acidity method was 1.7, and RSD of the PV method was 1.4.

**Determination of Water Content in Virgin Olive Oil.** The water content was analyzed with a TitroMatic 1S instrument (Crison Instruments, S.A.; Alella, Barcelona, Spain). This measurement uses a Karl Fischer titration based on a bivoltametric indication (2-electrode potentiometry). A solution of chloroform/Hydranal-solvent oil (a methanolic solvent) 2:1 (v/v) was used to dissolve the sample, and Hydranal-Titran 2 was used as a titrating reagent. Each sample was introduced three times, and the quantity of the sample was measured with the back weighting technique. The sample was dissolved in a solution of chloroform/Hydranal-solvent oil, and the titrating reagent was added until the equivalence point was reached. The quantity of water was expressed as mg of water/kg of oil ($n = 3$). RSD of the water method was 3.5.

**Fatty Acid Composition.** The fatty acid composition of oil samples was determined as methyl esters by capillary gas chromatography (GC) (Clarus 500 GC Perkin-Elmer Inc., Shelton, CT) analysis after alkaline treatment, according to Bendini et al. (*28*). Alkaline treatment was carried out by mixing 0.05 g of oil dissolved in 2 mL of *n*-hexane with 1 mL of 2 N potassium hydroxide in methanol according to Christie (*29*).

**Oxidation Stability Index (OSI) Time.** These analyses were carried out in an eight-channel OSI instrument (Omnion, Decatur, IL, USA). Virgin olive oil samples (5.0 ± 0.1 g) were heated at 110 °C under atmospheric pressure, and air (150 mL $min^{-1}$ of flow rate) was allowed to bubble through the oil. Under these conditions, the oxidative process reaches its final steps, and the short-chain volatile acids produced are recovered and measured conductimetrically in distilled water. The time required to produce a sudden increase in conductivity (due to volatile acid formation) determines an induction period (OSI time), expressed in hours and hundredths of hours, which can be used to measure the stability of oil.

**Extraction of Polar Phenolic Fraction.** Phenolic compounds were extracted from virgin olive oil by a liquid−liquid extraction method according to Pirisi et al. (*30*). The dry extracts were dissolved in 0.5 mL of a methanol/water (50/50, v/v) solution and filtered through a 0.2 $\mu$m syringe filter (Whatman Inc., Clinton, NJ, USA). Extracts were frozen and stored at −43 °C.

**Electrophoretic Procedure.** Capillary electrophoretic separation was performed by the capillary zone electrophoresis method proposed by Carrasco-Pancorbo et al. (*31*). A Beckman 5500 capillary electrophoresis instrument connected to a diode array detector was used. This method uses a capillary with 50 $\mu$m i.d. and a total length of 47 cm (40 cm to the detector) with a detection window of 100 × 200 $\mu$m, and a buffer solution containing 45 mM sodium tetraborate pH 9.3

**Antioxidant Power (AOP) Determination.** Phenolic extracts were measured in a FIA apparatus at a potential set at 0 mV vs Ag/AgCl. The apparatus consists of a Minipuls II peristaltic pump (Gilson, France), a high pressure injection valve model 7125 (Rheodine, USA) equipped with a 20 $\mu$L loop, an electrochemical cell model UniJet (BAS, West Lafayette, USA) mounted with a glassy carbon working electrode (3 mm diameter), and an amperometric detector AMEL 559 HPLC detector (AMEL, Milan, Italy) linked to a chart recorder (RC 102; Pharmacia, Sweden). The flow rate of phosphate buffer (pH 7.4) was 150 $\mu$L $min^{-1}$. All extracts were injected in triplicate. The current produced during the electrochemical oxidation of the phenolic compounds was recorded. Quercetin was used as the reference compound, and the concentration of phenolic compounds was expressed as $\mu$g/mL quercetin equivalent (QE); AOP was expressed as $QE_0$, corresponding to QE.

**Statistical Analysis.** Data were analyzed using Statistica 6.0 (Statsoft, Tulsa OK, USA) statistical software. The values reported are the averages of at least three repetitions ($n = 3$), unless otherwise stated. Tukey's honest significant difference (HSD) multiple comparison (one-way ANOVA) and Pearson's linear correlations are both at $p < 0.05$.

## RESULTS AND DISCUSSION

**Free Acidity and PV.** The free acidity values of the oils studied ranged from 0.14% and 3.81%. Taking into account the acidity of the oil samples (*32*), there were 25 oils with very low acidity values (≤0.8%) that could be classified as extra virgin olive oils; five samples (S22, S25, S26, S27, and S32) with an acidity between 0.8−2% that were defined as virgin olive oils, and finally two oils (S30 and S31) with an acidity higher than 2% that were considered as lampante olive oils. We demonstrated that the majority of oils with a fly attack more than 30% had an acidity higher than 0.8%, which means that these oils belonged to the category of virgin or lampante instead of extra virgin. Furthermore, the two samples that had suffered a fly attack higher than 50% had a very high acidity value and because of this belonged to the category of lampante olive oils.

Regarding PV, the freshly pressed samples showed values from 5.3 to 19 mequiv $O_2$ $kg^{-1}$ oil and an average value of 9.90. These values are slightly higher than those usually obtained

**Table 2.** Chemical Characteristics of Olive Oil Samples[a]

| sample | FA | PV | PV 3 | $H_2O$ | OSI | O/L | AOP |
|---|---|---|---|---|---|---|---|
| S1 | 0.5 | 8.4 | 10.0 | 1557 | 28.5 | 10.1 | 96.6 |
| S2 | 0.3 | 6.6 | 9.1 | 1121 | 40.6 | 10.8 | 104.0 |
| S3 | 0.2 | 5.9 | 9.2 | 904 | 35.4 | 9.7 | 71.4 |
| S4 | 0.3 | 9.9 | 8.2 | 1342 | 42.1 | 13.7 | 54.8 |
| S5 | 0.4 | 9.3 | 9.9 | 1585 | 28.8 | 10.7 | 45.7 |
| S6 | 0.5 | 10.2 | 11.0 | 1946 | 22.0 | 10.2 | 82.5 |
| S7 | 0.6 | 8.8 | 10.0 | 2053 | 23.6 | 10.7 | 103.3 |
| S8 | 0.6 | 8.4 | 13.2 | 1938 | 17.5 | 11.4 | 31.9 |
| S9 | 0.7 | 10.9 | 10.2 | 2095 | 17.5 | 10.8 | 52.4 |
| S10 | 0.4 | 7.9 | 11.1 | 2389 | 29.8 | 10.3 | 34.8 |
| S11 | 0.3 | 11.3 | 9.0 | 1407 | 23.7 | 11.7 | 20.9 |
| S12 | 0.4 | 7.9 | 12.0 | 1421 | 14.0 | 4.5 | 37.0 |
| S13 | 0.6 | 12.2 | 13.0 | 1479 | 12.2 | 4.8 | 19.1 |
| S14 | 0.7 | 7.1 | 10.0 | 927 | 18.2 | 10.6 | 12.7 |
| S15 | 0.2 | 8.8 | 7.2 | 951 | 20.6 | 4.2 | 167.0 |
| S16 | 0.4 | 9.7 | 10.7 | 974 | 20.8 | 7.7 | 42.2 |
| S17 | 0.3 | 6.1 | 8.2 | 1463 | 38.5 | 14.3 | 5.2 |
| S18 | 0.1 | 5.3 | 6.4 | 1002 | 27.1 | 12.2 | 30.3 |
| S19 | 0.6 | 13.8 | 11.0 | 1079 | 20.5 | 12.3 | 11.4 |
| S20 | 0.3 | 7.8 | 10.7 | 820 | 23.9 | 8.1 | 52.7 |
| S21 | 0.6 | 9.8 | 16.8 | 1365 | 8.3 | 4.4 | 10.5 |
| S22 | 0.9 | 9.1 | 15.0 | 1517 | 20.6 | 11.3 | 42.9 |
| S23 | 0.3 | 7.5 | 8.2 | 3332 | 28.5 | 12.8 | 51.7 |
| S24 | 0.8 | 11.2 | 12.0 | 1863 | 31.7 | 11.4 | 42.8 |
| S25 | 1.0 | 12.1 | 15.5 | 1366 | 17.2 | 11.5 | 18.5 |
| S26 | 0.9 | 11.3 | 12.2 | 2068 | 16.9 | 12.6 | 20.8 |
| S27 | 1.2 | 19.0 | 20.2 | 1573 | 12.5 | 11.0 | 13.2 |
| S28 | 0.7 | 8.3 | 10.0 | 1084 | 24.8 | 12.3 | 26.3 |
| S29 | 0.5 | 10.4 | 14.8 | 1257 | 18.7 | 9.5 | 41.9 |
| S30 | 3.8 | 14.8 | 17.4 | 1146 | 7.5 | 4.9 | 9.3 |
| S31 | 2.3 | 11.9 | 17.4 | 1176 | 12.5 | 11.3 | 13.9 |
| S32 | 1.9 | 14.9 | 15.2 | 1192 | 9.1 | 8.2 | 10.6 |

[a] FA, free acidity percentage (g oleic acid on 100 g of oil); PV and PV3, peroxide values (mequiv $O_2$ $kg^{-1}$ oil) measured on fresh oils and after three months of oil storage; O/L, ratio between oleic and linoleic acids; $H_2O$, water content (mg $H_2O$ $kg^{-1}$ oil); OSI, oxidative stability index (hours); AOP, antioxidant power expressed as QE0 quercetin equivalent with potential set to 0 mV ($\mu$g quercetin $mL^{-1}$ phenolic extract).

**Table 3.** Quantification Express as mg Analyte $kg^{-1}$ Olive Oil of the Different Phenols by CE (mean ± SD, $n = 7$) [a]

| sample | simple phenols[b] | lignans[c] | secoiridoids[c] | total |
|---|---|---|---|---|
| S1 | 4.4 ± 0.6 | 12.6 ± 3.2 | 150.0 ± 7.2 | 167.0 ± 4.9 |
| S2 | 1.9 ± 0.2 | 11.9 ± 2.7 | 264.5 ± 9.3 | 278.4 ± 9.2 |
| S3 | 4.6 ± 0.2 | 3.7 ± 0.5 | 233.9 ± 11.4 | 242.2 ± 11.9 |
| S4 | 4.7 ± 0.2 | 8.9 ± 0.6 | 193.8 ± 8.6 | 207.5 ± 9.3 |
| S5 | 4.2 ± 0.4 | 13.4 ± 3.8 | 139.4 ± 9.9 | 156.9 ± 9.8 |
| S6 | 5.1 ± 0.2 | 11.7 ± 2.3 | 98.3 ± 11.6 | 115.1 ± 11.3 |
| S7 | 4.0 ± 0.3 | 8.8 ± 2.0 | 80.0 ± 10.5 | 92.8 ± 11.9 |
| S8 | 2.4 ± 0.9 | 10.8 ± 2.6 | 74.8 ± 5.2 | 88.0 ± 6.5 |
| S9 | 3.5 ± 0.3 | 10.6 ± 2.00 | 53.7 ± 9.2 | 66.1 ± 9.8 |
| S10 | 4.6 ± 0.4 | 6.2 ± 1.4 | 141.0 ± 17.7 | 151.8 ± 19.2 |
| S11 | 2.7 ± 0.1 | 5.8 ± 0.4 | 39.3 ± 2.3 | 48.4 ± 3.1 |
| S12 | 3.4 ± 0.2 | 27.5 ± 1.5 | 63.7 ± 2.8 | 98.1 ± 4.5 |
| S13 | 2.9 ± 0.2 | 19.7 ± 1.2 | 37.5 ± 0.2 | 62.1 ± 1.4 |
| S14 | 2.3 ± 0.0 | 7.8 ± 0.2 | 24.4 ± 0.9 | 34.6 ± 1.1 |
| S15 | 5.2 ± 0.2 | 29.5 ± 1.5 | 125.1 ± 2.8 | 165.5 ± 3.7 |
| S16 | 6.9 ± 0.3 | 19.5 ± 1.3 | 116.8 ± 8.0 | 144.8 ± 9.8 |
| S17 | 1.7 ± 0.1 | 3.1 ± 0.4 | 144.8 ± 9.7 | 149.6 ± 10.1 |
| S18 | 3.7 ± 0.1 | 2.6 ± 0.4 | 77.6 ± 2.8 | 83.9 ± 3.2 |
| S19 | 2.1 ± 0.1 | 2.3 ± 0.3 | 20.0 ± 0.5 | 24.7 ± 0.9 |
| S20 | 3.4 ± 0.2 | 6.5 ± 0.4 | 102.6 ± 4.6 | 112.5 ± 4.8 |
| S21 | 1.8 ± 0.6 | 7.6 ± 1.6 | 24.2 ± 1.1 | 33.6 ± 1.4 |
| S22 | 1.8 ± 0.1 | 10.3 ± 0.7 | 115.1 ± 7.3 | 127.2 ± 7.4 |
| S23 | 4.0 ± 0.3 | 6.7 ± 0.8 | 87.6 ± 8.9 | 98.3 ± 10.0 |
| S24 | 5.4 ± 0.2 | 9.6 ± 1.1 | 158.4 ± 5.7 | 173.4 ± 5.9 |
| S25 | 1.4 ± 0.5 | 3.5 ± 0.3 | 15.1 ± 1.0 | 20.0 ± 1.7 |
| S26 | 1.1 ± 0.0 | 2.6 ± 0.2 | 18.0 ± 1.3 | 21.7 ± 1.5 |
| S27 | 2.4 ± 0.8 | 16.7 ± 3.3 | 39.7 ± 3.4 | 58.8 ± 3.2 |
| S28 | 2.6 ± 0.1 | 3.3 ± 0.3 | 40.7 ± 0.8 | 47.2 ± 0.7 |
| S29 | 2.9 ± 0.1 | 8.6 ± 1.3 | 66.6 ± 4.4 | 78.1 ± 4.1 |
| S30 | 1.5 ± 0.1 | 3.8 ± 0.4 | 20.0 ± 0.4 | 25.3 ± 0.5 |
| S31 | 1.9 ± 0.1 | 1.2 ± 0.3 | 11.6 ± 0.7 | 14.7 ± 0.9 |
| S32 | 1.0 ± 0.0 | 3.7 ± 0.1 | 14.2 ± 0.3 | 18.9 ± 0.3 |

[a] Simple phenols, sum of tyrosol and hydroxytyrosol; lignans, sum of pinoresinol and acetoxypinoresinol; secoiridoids, sum of seven peaks: OA (a) + DOA, DOA (b), Lig Agl (b), OA (b), EA (a), OA (c) + Lig Agl (c) + DOA (c) + EA (b,c), DOA (d) + EA (d). [b] Quantified with a calibration curve of 3.4-dihydroxyphenilacetic acid at $\lambda = 200$ nm. [c] Quantified with a calibration curve of oleuropein glucoside at $\lambda = 200$ nm.

from fresh olive oils (*11, 13*). Another evaluation of PV was carried out after three months of storage because this period is long enough to observe the beginning of the oxidative reactions and to see differences in the PV. As shown in **Table 2**, after three months of storage the average of PV reached a mean of 11.70. The samples attacked to a higher degree were for the most part those that presented a stronger increase in the PV.

As reported by other authors (*12, 16–25*) *Bactrocera oleae* attack has been positively correlated with both free acidity ($r = 0.77$, $p < 0.05$) and PV ($r = 0.58$, $p < 0.05$). These correlations were higher when the analyses were carried out after three months of oil storage: $r = 0.78$ for acidity (data not shown) and $r = 0.63$ for PV (in both cases for $p < 0.05$). In particular, when the percentage of infested olives was modest (<10%), the oxidative status of corresponding virgin olive oil was not affected. These results are in accordance with previous studies (*24, 25*), which emphasized that free acidity and peroxides together with sensory characteristics are the principal quality parameters and are related to fruit integrity. Fly attack can be considered an influential factor in the premature aging process of virgin olive oil. In fact, this kind of olive infestation may cause an acceleration of oxidative and hydrolytic degradation favored by the presence of exit holes that expose the olive pulp to the action of microorganisms and oxygen.

**Water Content.** The water content of samples varied from 800 to 3330 ppm, with an average value of 1481 ppm. It is important to highlight that no influence of fly attack was found on the water content of olive oils.

Nevertheless, water is affected by the processing system of olive fruits (continuous, pressure, or traditional). Generally, samples with a higher water content were those produced with a traditional processing system (average value 2170 ppm), whereas samples obtained by continuous processing systems had a lower content of water (average value 1250 ppm).

**OSI.** This parameter varied greatly from 7.5 h (S30) to 42.1 h (S4). As has been previously noted, OSI depends on several factors (*8, 14*). Aparicio et al. attributed the oxidative stability to several variables; however, fatty acid composition and the content of phenolic compounds are those with the greatest influence. The oxidation rates of linoleic and oleic acids explain why stability is higher when the content of the monounsaturated acids is high and the content of polyunsaturated acids is low. Thus, the O/L ratio has the most marked relationship with stability (*8*). An olive oil has a good stability index if this value is higher than 7 (*33*), although samples S12, S13, S15, S21, and S30 had a ratio lower than 5. The infestation did not cause significant changes in the fatty acid composition, which was affected mostly by olive ripening and olive cultivar (S12, S13, S21, and S30 were produced by *Gentile* olive cv).

In general, our results agree with those previously reported. In fact, positive correlations with OSI have been found for both the phenolic content and O/L ratio ($r = 0.81$, $p < 0.05$; $r = 0.57$, $p < 0.05$), whereas the oxidative stability was negatively correlated with the degree of olive infestation ($r = -0.54$, $p < 0.05$). The lower stability with increased infestation may be

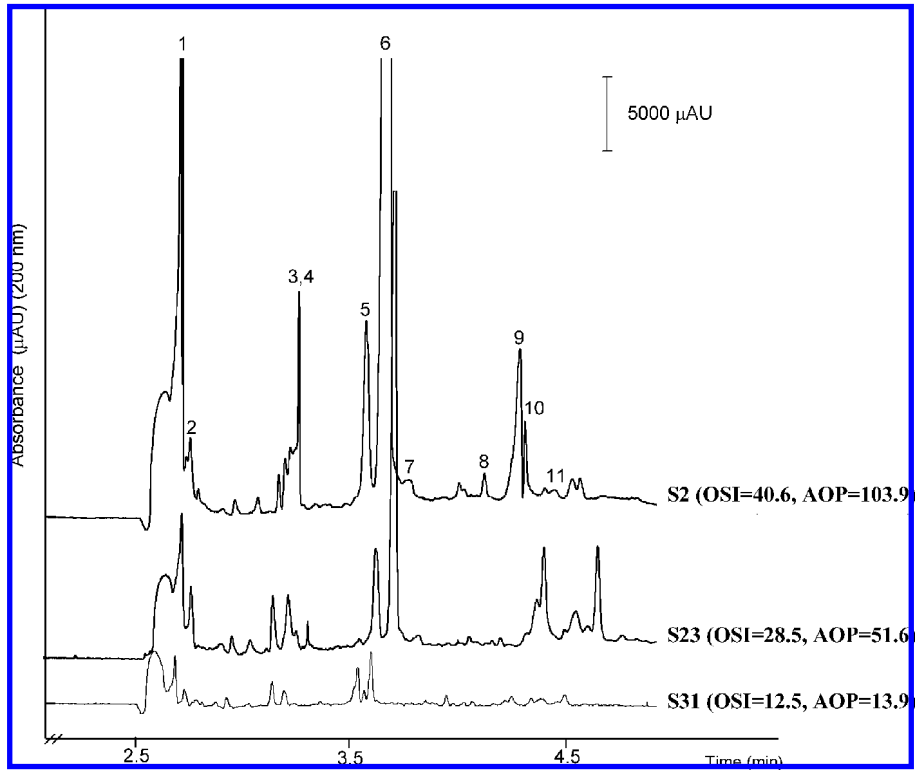


**Figure 2.** Overlay of electropherograms relative to phenolic extracts of three oil samples (S2, S23, and S31) differing in phenol content, values of oxidative stability (OSI, in hours) and antioxidant power (AOP, in quercetin equivalent), and the degree of fly attack (2.5%, 25%, and 60%).

explained by the decrease in phenols and *o*-diphenols in damaged olives. Additionally, we noted that a negative correlation exists between the PV and OSI ($r = -0.61$, $p < 0.05$), which was more pronounced after three months of storage of olive oil ($r = -0.71$, $p < 0.05$).

**Phenolic Compounds.** The content of phenolic compounds presented a high variability depending on the variety of olives, different typology of transformation and production system (**Table 3**). In general, samples strongly attacked by the fly showed a particularly low phenolic content; for example, oil obtained from olives with a percentage of attack higher than 30% always showed phenolic content values lower than 80 mg $kg^{-1}$ of olive oil. It can be observed that the *o*-diphenol content showed a high correlation with OSI values, whereas it was negatively influenced by the degree of olive infestation ($r = 0.86$, $p < 0.05$; $r = -0.50$, $p < 0.05$). Among the *o*-diphenols, the oleuropein (OA) and decarboxy-methyl oleuropein aglycons (DOA), belonging to secoiridoids, were highly involved in both of these effects (in particular, OA(a) + DOA(a) $r = 0.74$, $p < 0.05$; $r = -0.53$ $p < 0.05$).

Therefore, fly attack resulted in the loss of phenols, *o*-diphenols, and in particular of some secoiridoid derivatives. Consequently, when phenols decrease as the percentage of infested olives increases, the stability of the resulting oils is compromised. It is likely that this effect is due to an increase of polyphenoloxidase activity caused by larval damage to tissues and by the presence of exit holes that expose the olive pulp to oxygen (*24*).

Different samples with low, medium, and high percentages of fly attack were chosen (**Figure 2**), and an evident decrease of phenolic content was observed when the percentage of fly attack increases. However, it is not possible to state that a direct correlation exists between phenolic content and the percentage of fly attack because as we said before, there are many variables that influence the phenolic profile (cultivar, growing area, climatic conditions, peaking system, technological plant, oil stored, etc.) (*31*).

If we consider different real samples, the influence of all other variables determines a large qualitative and quantitative variation on the phenolic profile. Therefore, we conclude that phenolic content itself cannot be considered as a process parameter or as an indicative marker of the percentage of fly attack. For example, samples from the same cultivar and with the same percentage of fly attack, 5, 6, 7, 8, and 9, present different behaviours regarding their phenolic content.

**Antioxidant Power.** AOP varied from 5.19 (S17) to 167.01 (S15) $\mu$g $mL^{-1}$ quercetin equivalents. As previously observed (*32*), the AOP values of the phenolic fraction of virgin olive oils are closely related to their radical scavenging activity as they represent the most readily oxidizable compounds. The AOP shows a weak positive correlation with OSI ($r = 0.38$, $p < 0.05$), confirming the influence of the phenolic fraction, particularly the readily oxidizable, on the oxidative stability of olive oil. Our results showed a positive correlation between AOP and the phenol and *o*-diphenol contents ($r = 0.69$, $p < 0.05$; $r = 0.59$, $p < 0.05$) as well as simple phenols ($r = 0.55$; $p < 0.05$). Significant positive correlation also exists between AOP and individual phenols, or groups of phenols. An especially high influence was exerted by OA(a) + DOA(a), which also showed a negative correlation with percentage of fly attack ($r = -0.53$, $p < 0.05$). In addition, we observed that a negative correlation exists between the PV and AOP ($r = -0.38$; $p < 0.05$) and that this correlation is still more prominent between AOP and PV after three months of oil storage ($r = -0.49$; $p < 0.05$). The contribution of the quantity of phenolic compounds to oxidative stability and their different levels of AOP are evident in **Figure 2** in which several electropherograms (S2, S23, and S31) have been overlaid. These findings suggest that the AOP value may be used as a predictive index of antioxidant capacity that could be exerted by phenols during virgin olive oil storage.

Nowadays, the content of phenolic compounds is increasingly used in industrial mills as a useful parameter to evaluate the quality of olive oil. This correlation is right because it is obvious that phenolic content influences the stability of olive oil and the sensory characteristics. However, this parameter is not that important for evaluating the health status of the olives (attacked by *Bactrocera oleae*).

The aim of this work was to find the most appropriate method to know if an olive oil comes from olives attacked by the olive fly. There has been evidence that a simple parameter such as free acidity is stronger and more useful for judging the quality of an olive oil right after production because it is independent of all of the other technological parameters.

It is important to highlight that this is the first time that the effect of fly attack on the phenolic fraction, considering several percentages of attack, has been studied. Although it is not possible to say that a direct correlation exists between these two parameters, it has been proved that olive oils from olives with a high percentage of fly attack (S30, S31, and S32, more than 50% of fly attack) present a very low amount of phenols.

This argument is even more important if we observe that the quality of the row material is highly correlated with oil stability after a few months of storage. Peak identification numbers: **1**, Lig Agl (a); **2**, TY, **3**, Pin; **4**, Ac Pin; **5**, OA (a) + DOA (a); **6**, DOA (b); **7**, Lig Agl (b); **8**, OA (b); **9**, EA (a); **10**, OA (c) + Lig Agl (c) + DOA (c) + EA (b,c); **11**, HYTY. Detection wavelength: 200 nm.

## ABBREVIATIONS USED

O/L, oleic/linoleic acid ratio; UV, ultraviolet; PV, peroxide value; RSD, relative standard deviation; GC, gas chromatography; OSI, oxidative stability index; AOP, antioxidant power; QE, quercetin equivalent; HSD, honest significant difference; TY, tyrosol; HYTY, hydroxytyrosol, Pin, pinoresinol; Ac Pin, acetoxypinoresinol; OA, oleuropein aglycon, DOA, decarboxymethyl oleuropein aglycon; Lig Agl, ligstroside aglycon; EA, elenolic acid.

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