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# Effect of different temperatures and storage atmospheres on *Coratina* olive oil quality

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## Abstract

Olives (*Olea europaea* cv. *Coratina*) used for oil production were stored for 30 days at three different temperatures and under different atmospheres (ambient temperature, 5 °C with a flux of humidified air, 5 °C with a flux of  $3\%O_2 + 5\%CO_2$ ). The olives were kept in jars used for fruit storage, each with a capacity for 1.5 kg of olives.

Conventional analyses (acidity, peroxide value, specific extinction coefficient at 232 nm and 270 nm) and non conventional (polar compounds) analyses were carried out in order to measure the hydrolytic and oxidative degradation of the oils obtained from the olives. Storage at 5 °C, both under a flow of humidified air and a flow of  $3\%O_2 + 5\%CO_2$ , produced oils that maintained their initial chemical qualities until the end of the experimentation; whereas storage of olives at room temperature led to their deterioration after 15 days of storage.

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#### 1. Introduction

Olive oil quality is directly related to the physiological conditions of the olives from which the oil is extracted. Olive processing in important producing countries (such as Spain, Italy, and Greece) is often not well synchronized with crop harvests (Garcia and Streif, 1991; Gutierrez, Perdiguero, Garcia, and Castellano, 1992). Olives are often piled into large heaps and stored at ambient temperature for up to several weeks prior to processing for oil extraction (Garcia, Gutierrez, Castellano, Perdiguero, and Albi, 1996), and this is when the greatest deterioration takes place (Olias and Garcia, 1997). Pressure within the olive pile during storage may cause secretion of fluid from the olives thereby providing an optimum medium for the growth of fungi and bacteria (Olias and Garcia, 1997). Under these conditions, anaerobiosis can occur in the inner part of the pile while aerobic losses occur in the outer part (Garcia and Streif, 1991). Furthermore, heat production from respiratory activity may accelerate the deterioration of the fruit (Garcia and Streif, 1991) and eventually cause the breakdown of cell structure (Gutierrez, Perdiguero, Garcia et al., 1992). Oil extracted from these damaged olives can be high in acidity and low in stability (Garcia, Gutierrez, Castellano et al., 1996) and can develop a great amount of volatile acids (acetic or butyric) that cause a characteristic musty smell (Olias and Garcia, 1997). In a few days, the physical and chemical structure of the olives is altered and the oil extracted from them has a very poor quality. This type of oil must be refined before consumption. (Gutierrez, Perdiguero, Garcia et al., 1992). A great number of different methods for olive storage have been experimented to find ways to counter this important

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problem (Castellano, Garcia, Morilla, Perdiguero, and Gutierrez, 1993; Garcia, 1993a, 1993b; Garcia, Gutierrez, Barrera, and Albi, 1996; Garcia et al., 1994; Kader, 1986; Kader, Nanos, and Kerbel, 1989, 1990; Koprivnjak, Conte, and Totis, 2002; Pereira, Casal, Bento, and Oliveira, 2002; Petruccioli and Parlati, 1987).

Very few articles concerning polar compound concentrations or HPSEC analyses of virgin oils from stored *Picual* olives are available (Perez-Camino, Garcia, & Castellano, 1992) and overall, little information is found on the storage of Italian cultivars. It is possible that mill olive varieties behave differently under refrigeration. Our study was conducted to identify the optimum pre-processing storage temperature and atmospheric composition to preserve the quality of *Coratina* olives and oil.

# 2. Materials and methods

#### 2.1. Experimental material

Olive fruits (*Olea europaea* L.) from the *Coratina* variety were harvested in olive groves in the same area near Foggia (Apulia – Italy) during the crop season 2002/2003. The olives were randomly picked at the industrial optimal ripening stage, according to their skin colour. Harvesting was done by hand, using rakes. Olives were sorted to obtain fruits of uniform size and colour and then distributed randomly in 1.5 kg lots that were placed into 14 glass jars.

# 2.2. Storage treatments

A group of six jars of olives were kept at 5 °C and connected to a continuous humidified air flow or a  $3\%O_2 + 5\%CO_2$  flow-through-system at a flow rate of 500 ml min<sup>-1</sup>. Another group of six jars of olives were kept at 20 °C in normal ambient air (control) to verify the deterioration rate at an ambient temperature. Two replicates were used per treatment. Each sample was subjected to three analytical determinations. All data points in the figures are the mean  $\pm$  SD. Quality evaluations were made initially on day 1 and after 15 and 30 days of olive storage.

# 2.3. Sample preparation

After washing and leaf-removal, each batch was hammer-crushed in a pilot plant. After malaxation for 30 min at 25 °C, the oil was extracted by means of a laboratory basket centrifuge. All the oil samples were filtered through cotton and stored at -20 °C in the dark in amber glass bottles without head space until analysis.

# 2.4. Percent free fatty acids, peroxide value and ultra-violet light absorption

Free fatty acids value, peroxide value and UV light absorption ( $K_{232}$ ,  $K_{270}$ ) were determined following the offi-

cial analytical methods described in EC Regulation 2568/ 91 and subsequent modifications ECC/796/02 and ECC/ 1989/03.

#### 2.5. Total phenol content

Phenolic compounds were isolated from a solution of oil in hexane by triple-extraction with water-methanol (60:40 v/v). Total phenols, expressed as gallic acid equivalents (ppm), were determined with a UV visible spectrophotometer (Beckman) at 765 nm using the Folin-Ciocalteu reagent (Swain and Hillis, 1969).

# 2.6. Oxidative stability

Oxidation induction time was evaluated by the Rancimat method. Stability was expressed as the oxidation induction time (*h*), measured with the Rancimat apparatus (Metrohm AG, Herison, Switzerland) (T = 120 °C; air flow rate =  $20 \ 1/h$ ).

#### 2.7. Polar compounds and triglyceride oligopolymers

Polar compounds (PC) were determined in each sample by means of silica gel column chromatography as described by the IUPAC method (IUPAC, 1987). PC were analyzed by high-performance size exclusion chromatography (HPSEC) to determine oxidized triglycerides (ox-TG), oligopolymers, and partial glycerides.

The chromatographic system consisted of a Perkin– Elmer pump, series 10, a 7125 S sample injector (Rheodyne), a 50 µl injector loop, and a series of three PL-gel columns (Perkin–Elmer Ltd., Beaconsfield, Great Britain) of 0.75 cm i.d.  $\times$  30 cm length. The columns were packed with highly cross-linked styrene divinylbenzene copolymers with a particle diameter of 5 µm and pore diameters of 500, 500, and 100 Å, respectively. A PL-gel guard column (Perkin–Elmer Ltd.) of 7.5 mm i.d.  $\times$  5 cm length was used. The detector was a differential refractometer (Shimadzu RID 6A, Shimadzu Corp., Japan) connected to an integrator. The elution solvent used was CH<sub>2</sub>Cl<sub>2</sub> for HPLC at a flow rate of 1.0 ml/ min.

The procedures for identifying the peaks on each chromatogram and for the quantitative assessment of the classes of compounds under investigation were carried out as described elsewhere (Gomes, 1992; Gomes and Caponio, 1999).

#### 2.8. Statistical analysis

Statistical analysis was performed using Microsoft Excel software. Significant differences between treatments were determined using one-way ANOVA followed by "Duncan's test" (p < 0.05) carried out on the Software Stat-graphics Plus (Manugistics, MD, USA).

# 3. Results and discussion

#### 3.1. Percent free fatty acids

Virgin olive oil contains about 98% neutral lipids, mainly triglycerides (96–97%) followed by small quantity of diglycerides (1–2%) and a variable quantity of free fatty acids which are used as a marker of oil quality (Olias and Garcia, 1997).

Fig. 1A shows the changes in the percent free fatty acids (FFA) (% oleic acid) of the oils obtained from olives stored at the different temperatures and under different atmospheres. The oils extracted immediately post-harvest

had an average FFA value of  $0.25\% \pm 0.04$  and were therefore categorized as extra virgin olive oil. During the initial 15 days of storage, the FFA values of the oils from all three treatments were lower than 0.8%, which is the limit set for 'extra' virgin olive oil quality in ECC/1989/03. The oils obtained from the olives stored at 5 °C for 30 days had FFA values within the limit of the extra virgin category irrespective of storage atmosphere. By contrast, the oil extracted from the olives kept at ambient temperature had an FFA value >2 and was classified as "lampante" olive oil". Garcia, Gutierrez, Castellano et al. (1996) reported that *Picual* olives stored at 5 °C for 45 days retained FFA value levels <1%, this



Fig. 1. Changes in the percent free fatty acids (% oleic acid) (A), in the peroxide value (meq/kg) (B), in the content of conjugated fatty acids measured by the specific extinction coefficient at 232 nm (*K*232) (C), in the content on carbonylic compounds measured by the specific extinction coefficient at 270 nm (*K*270) (D), in the total phenol content (ppm) (E) and in the stability to oxidation (hours) (F) of oils obtained from olives "*Coratina*" stored at different temperatures and atmospheres. Each data point represents the mean of two replicates ( $\bullet$ , 3%O<sub>2</sub> + 5%CO<sub>2</sub> at 5 °C;  $\blacksquare$ , humidified air 5 °C;  $\blacktriangle$ , ambient 20 °C).

result is similar to our observations with *Coratina* olives The increase in the FFA value of an oil during storage is positively related to increasing storage temperature (Garcia et al., 1994; Gutierrez, Perdiguero, Garcia et al., 1992). Gutierrez, Perdiguero, Garcia et al. (1992) reported that the increase in the acidity of oils extracted from fruit stored at 20 °C correlated well with decay incidence. In general, the first action of a parasitic microorganism in an oil-rich tissue is the to induce hydrolytic activity by lipases which leads to the release of fatty acids from the triacylglycerol molecules of the oil. The free fatty acids are easily metabolized by microorganisms. Fruit storage at 5 °C allowed the FFA of the oil to be maintained at 0.8% for up to 30 days.

#### 3.2. Peroxide value

The peroxide value (PV) is a measure of primary oxidation. Fig. 1B shows the changes in the PV (meq  $O_2/kg$ ) of oils obtained from olives stored at the different temperatures and under different atmospheres. The PV of the oils extracted immediately after the harvesting was 2.0 meq O<sub>2</sub>/kg. Cold storage at 5 °C significantly delayed the rise in the peroxide value of the oils. The PV of the oil obtained from olives stored at 20 °C for 15 days was double that of the baseline values. At the end of observation trial (30 days), none of the oils analyzed exceeded the maximum peroxide value for extra virgin olive oil  $(20 \text{ meq } O_2/\text{kg})$  since the highest PV obtained was on average 5.8 meg  $O_2/kg$  for oils extracted from olives stored at ambient temperature for 30 days. This result is due to the high phenol content of the Coratina cultivar which preserves the oil from oxidative phenomena.

#### 3.3. Specific extinction coefficient at 232 nm and 270 nm

The  $K_{232}$  parameter is mainly indicative of the conjugation of trienes and also of the presence of carbonylic compounds. The maximum permitted values of  $K_{232}$  and  $K_{270}$ for extra virgin olive oils are 2.50 and 0.20, respectively. Fig. 1C and D show the changes in  $K_{232}$  and  $K_{270}$  of oils obtained from olives stored at different temperatures and under different atmospheres. The  $K_{232}$  of the oils extracted immediately post-harvest was on average 1.63 and the  $K_{270}$ was 0.13. The  $K_{232}$  did not change markedly in the oil from olives stored at 5 °C. The oil from olives stored at 20 °C had a higher  $K_{232}$  value (2.27 after 30 days). A  $K_{232}$  value of 2.50, which is the limit for extra virgin olive oil, was not exceeded irrespective of storage atmosphere and temperature. Storage of olives at a low temperature had a positive effect on the  $K_{270}$  of the oils; 0.22 (the limit for extra virgin olive oil) was not surpassed in any of the treatments with the exception of the oil obtained from olives stored at 20 °C (on average 0.43  $\pm$  0.06). Our results for the  $K_{270}$  values are similar to those of previous reports (Garcia, Gutierrez, Castellano et al., 1996; Gutierrez, Perdiguero, Garcia et al., 1992).

#### 3.4. Total phenol content

Virgin olive oil contains phenolic compounds responsible for its fragrance and peculiar flavour, (Gutierrez, Perdiguero, Gutierrez, and Olias, 1992; Servili et al., 2004). These substances also contribute to the oxidative stability of the oil (Baldioli, Servili, Perretti, and Montedoro, 1996; Gutfinger, 1981; Papadopoulus and Boskow, 1991) and protect consumers against cancer and atherosclerosis (La Vecchia et al., 1995; Trichopoulou et al., 1995; Visioli, Bellosta, and Galli, 1997). Fig. 1E shows the changes in the total phenol content (mg of gallic acid/kg of oil) of the oils obtained from olives stored at different temperatures and under different atmospheres. The total phenols decreased in the oils obtained form olives kept at the higher temperature and in ambient air, where the concentration of the oxygen was higher than the controlled atmosphere.

During storage fluid exuding from the olives may provide an optimum medium for the growth of fungi and bacteria (Olias and Garcia, 1997). Pseudomonas and other soil bacteria are able to metabolize a wide variety of aromatic compounds, such as phenol and its derivatives (Watanabe, Hino, Onodera, Kajie, and Takahashi, 1996). Low temperatures may have a bacteriostatic action that controls microbial development. Moreover, breakdown of the cells may favour contact of the phenolic substances with the oxidative enzymes. Olives contain oxidoreductases, such as polyphenoloxidase and peroxidase, that may oxidize polyphenols and impair the health-related qualities and sensory characteristics of olive oil (Servili, Selvaggini, Taticchi, Esposto, and Montedoro, 2003), the activity of these enzymes is known to decrease at low temperatures. The results we obtained are consistent with these observations.

# 3.5. Oxidative stability

The antioxidant activity of hydrophilic phenols of virgin olive oil has been extensively studied (Servili et al., 2004). As reported by several investigators, the concentration of phenolic compounds, evaluated colorimetrically and expressed as total phenols, shows a correlation with the shelf -life of virgin olive oil as tested by accelerated methods such as Rancimat (Gutierrez Gonzales-Quijano, Janer del Valle, Janer del Valle, Gutierrez Rosales and Vazquez Roncero, 1977).

Fig. 1F shows the changes in oxidation stability the of the oils obtained from olives stored at different temperatures and under different atmospheres. Fig. 2 relates total phenols to the corresponding induction times for the oils obtained from the olives stored under the three conditions described. The overall values show that a direct correlation exists between the two parameters. The regression line computed yielded  $r^2 = 0.88$  (p < 0.05).

#### 3.6. Polar compounds and triglyceride oligopolymers

The HPSEC method measured the triglyceride oligopolymers (PTGs), oxidized triglycerides (ox-TGs)



Fig. 2. Correlation between total phenols and induction time.

and diglycerides (DGs). PTGs are absent or present only in trace amounts in good quality olive oils. Ox-TGs include all forms of oxidation of triglycerides and are an important class of degradation substances so their determination helps to better define the oxidative degradation of an oil. Fig. 3A shows the changes in the ox-TG (%) of oils obtained from olives stored at different temperatures and under different atmospheres. Determination of DGs in oils is useful to quantify the hydrolytic degradation of an oil. Fig. 3B shows the changes in the DGs (%) of oils obtained from olives stored at different temperatures and under different atmospheres.

Table 1 shows the ANOVA analysis of oxidized triglycerides of oils extracted from olives immediately post-harvest and of oils extracted from olives after 15 and 30 days of storage at different temperatures and under different atmospheres. The data represent the mean (M) (g/100 g)of oil)  $\pm$  Standard deviation (SD). Duration of olive storage significantly affected the amount of ox-TG (p < 0.01%) in all the experimental conditions considered. The ox-TG values differed in a statistically significant way (p < 0.01) for the three conditions applied also over the same duration of storage. At the end of the storage period, the lowest ox-TG was obtained when the drupes were stored at 5 °C in humidified air and at 5 °C under controlled atmosphere. When the drupes were stored in air at 20 °C the oils extracted contained much higher amounts of ox-TG. An average 30% more was found than in the oils obtained from olives stored at 5 °C in humidified air and an average 12% more was found than in the oils obtained from olives stored at 5 °C in controlled atmosphere. When the percent values of ox-TG were plotted against the corresponding induction times a linear trend of the experimental data was depicted for all the series of samples examined (Fig. 4). The figure shows that a inverse correlation was found ( $p \le 0.05\%$ ). The fact that the amount of ox-TG is inversely correlated to the induction time is further support of the value of this parameter in defining the oxidative degradation of oils and in predicting their shelf life.



Fig. 3. Changes in oxidized triglycerides (%) (A) and in diglycerides (%) (B) of oils obtained from olives "*Coratina*" stored at different temperatures and atmospheres. Each data point represents the mean of two replicates ( $\bullet$ , 3%O<sub>2</sub> + 5%CO<sub>2</sub> at 5 °C;  $\blacksquare$ , humidified air 5 °C;  $\blacktriangle$ , ambient 20 °C).

Table 1

ANOVA analysis of oxidized triglycerides of oils extracted from olives immediately post-harvest and of oils extracted from olives after 15 and 30 days of storage at different temperatures and under different atmospheres

Storage treatment Days	3%O <sub>2</sub> + 5% CO <sub>2</sub> at 5 °C M ± SD	Humidified air at 5 °C $M \pm$ SD	Ambient 20 °C $M \pm SD$
0	$0.43 \pm 0.01 \mathrm{aA}$	$0.43\pm0.01\mathrm{aA}$	$0.43\pm0.01 aA$
15	$0.64 \pm 0.01 \mathrm{aB}$	$0.58\pm0.01\mathrm{bB}$	$0.79\pm0.01\mathrm{cB}$
30	$0.74 \pm 0.02 \mathrm{aC}$	$0.64\pm0.01 \mathrm{bC}$	$0.83\pm0.02\text{cC}$

Data represents mean (M) (g/100 g of oil) and standard deviation (SD).

Significant differences in the same row are showed by different small letters (P < 0.01).

Significant differences in the same column are showed by different capital letters ( $P \le 0.01$ ).



Fig. 4. Correlation between oxidized triglycerides and Induction time.

# 4. Conclusions

This experimentation provided evidence that, after thirty days, oils obtained from olives stored at 5 °C preserved the best characteristics compared to those obtained from olives kept at ambient temperature. Ensuring a controlled atmosphere during storage did not produce any remarkable advantage over storage in humidified air at 5 °C. Therefore, considering the cost of controlled atmosphere technology, the best option for storing *Coratina* olives is to simply keep them at cold temperatures.

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