Influence of Storage Temperature on Fruit Ripening and Olive Oil Quality

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Olives (*Olea europaea* cv. Picual) used for oil production were stored for 60 days at three different temperatures (ambient, 5 °C, and 8 °C) in containers used for fruit storage, each with a capacity for 64 kg of olives. The quality of both the fruits and the oils extracted from these fruits was analyzed. Fruit storage at 5 °C maintained the initial sensorial and chemical qualities of the oil for 45 days, but at 8 °C, these qualities were maintained for only 15 days. At room temperature, these qualities deteriorated just after 7 days of storage.

Keywords: *Olea europaea; postharvest; refrigeration; cold storage*

INTRODUCTION

Spanish olive production ($\sim 3 \times 10^6$ Tm) exceeds the processing capacity of the mills, making it necessary to store the fruit for periods that may range from weeks to months (Kiritsakis and Markakis, 1991). The fruit is normally piled in the open air in great heaps and deteriorates rapidly as a result of the joint action of pathogenic microorganisms and the internal processes of senescence. Both of these processes are accelerated by the raised temperature that is caused by fermentation of the fruit, and by the considerable mechanical damage that the fruit suffers as a consequence of compression. The degradation seen in the fruit begins as a loss of flesh texture and browning of the skin and ends with the complete decomposition of the olive. The oils extracted from these fruits usually have high acidity, low stability, and a characteristic musty smell. For these reasons, these oils need to be refined before consumption, with the consequent increase in cost and loss of commercial value.

Studies carried out with table olives demonstrated that olive varieties vary in their sensitivity to chilling injury and responses to storage atmosphere (Maxie, 1964; Woskow and Maxie, 1965; Kader, 1985; Kader et al., 1990). However, all the varieties exhibited severe incidence of chilling injury when were stored at temperatures ≤2.2 °C. On a laboratory scale, using boxes capable of holding only 2 or 4 kg of olives and refrigerating at 5 °C in an air atmosphere is effective for maintaining fruit and oil quality (García and Streif, 1991; Gutiérrez et al., 1992; Castellano et al., 1993). Nevertheless, from an industrial point of view, these small containers cannot be employed in practice because the cost of fruit storage increases as the capacity of the storage containers decreases. However, the negative effects of softening or fermentation induced by fruit compression are direct consequences of the increase of fruit accumulation in large containers and are responsible for the bad quality of the oils obtained. To know if storage of olives at temperatures higher than 5 °C would also be effective for maintaining fruit and oil quality would be of economic interest because the energy required for reducing the storage temperature by only 1 °C is considerable on the industrial scale. The present study was undertaken to determine the effects of three different temperatures on the storage of Picual variety olives, the variety most widely cultivated in Spain, in plastic containers commonly used in fruit handling.

MATERIALS AND METHODS

Olive fruits (*Olea europaea* cv. Picual) were handpicked, a method of harvesting traditionally used in olive-producing countries to minimize fruit deterioration, and distributed randomly in 60 × 40 × 40-cm plastic containers, each with a capacity for 64 kg of olives. Three different storage conditions were tested: two in refrigerated rooms [5 and 8 °C with a relative humidity (RH) of 95%] and a third at ambient conditions (12 ± 4 °C; RH, 70%). Three containers were used for each treatment at each sampling. Samples were taken at 0, 7, 14, 30, 45, and 60 days of storage. The percentage of rotten fruits was quantified (fruits with visible mycelial growth). Triplicate samples of 100 olives were examined per treatment. Each replicate was taken randomly from a different container. Resistance of the flesh to penetration was determined in the same olives with Zwick 3300 densimeter (Zwick Gmbh & Company, Ulm, Germany) with a 5-mm diameter disk (force required to depress the disk 2.4 mm into the fruits), and the results are expressed in N/cm^2.

The ripening index of fruits was calculated with a subjective evaluation of the color of the olive skin and flesh (Uceda and Frías, 1975). This system is routinely used by the oil industry to characterize the degree of ripeness of olives arriving at the mill. The procedure consists of distributing 100 fruits in eight groups, according to the following characteristics: group 0, bright green skin; group 1, green-yellowish skin; group 2, green skin with reddish spots; group 3, reddish-brown skin; group 4, black skin with white flesh; group 5, black skin with <50% purple flesh; group 6, black skin with ≥50% purple flesh; and group 7, black skin with 100% purple flesh. The ripeness index is determined by the following equation: ripeness index = $\sum(in_i)/100$, where i is the number of the group and n_i, the number of olives in it. The evaluation was performed in triplicate.

The oil from the three containers of each treatment was extracted separately at each sampling date with an Abencor analyzer (Comercial Abengoa S.A., Sevilla, Spain). This unit consists of three basic elements: they are a mill, a thermobeater, and a pulp centrifuge (Martínez et al., 1975).

The titratable acidity was calculated according to the following procedure. An oil sample (20 g) from each of the three replicates of each treatment was placed in an Erlenmeyer flask. Then, 125 mL of a previously neutralized solvent mixture was added. The mixture consisted of equal parts by volume of ethanol and diethyl ether and a phenolphthalein indicator (1% in ethanol) in the ratio 2 to 125 mL of the solvent mixture. When the sample was completely dissolved, it was

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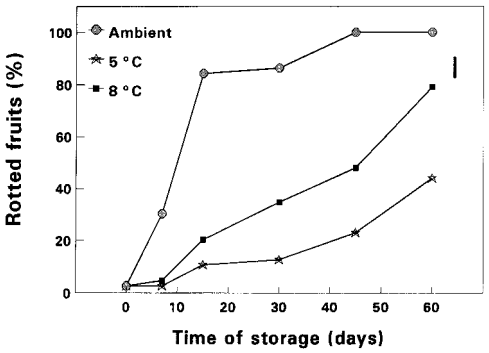

Figure 1. Changes in the incidence of decay (% rotted fruits) of olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

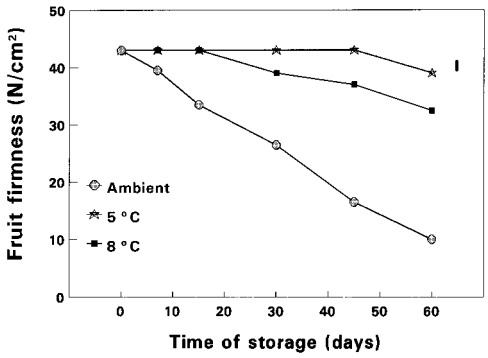

Figure 2. Changes in the firmness (N/cm^2) of olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

titrated with 0.1 N KOH to the first permanent pink color of the same intensity as that of the neutralized solvent before it was added to the sample. The results are expressed as percent of free oleic acid present in the oil.

The peroxide value was calculated according to the following procedure. An oil sample (5 g) from each of the three replicates of each treatment was placed in a 250-mL Erlenmeyer flask, and the sample was dissolved in 30 mL of a 3:2 acetic acid: chloroform solution. Subsequently, 0.5 mL of saturated KI solution was added, and the mixture was allowed to stand for 1 min in darkness. Then, 30 mL of distilled water and 0.5 mL of fresh prepared 0.5% starch indicator solution were added. Finally, the mixture was titrated with 0.1 N sodium thiosulfate until the blue color just disappears. The results are expressed as milliequivalents of oxygen per kilogram of oil.

The coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) were measured according to the following procedure. An oil sample (0.25 g) from each of the three replicates of each treatment was placed in a 25-mL graduated flask and diluted to 25 mL with spectrophotometrically pure cyclohexane. The sample was then homogenized, and the resulting solution was used to fill a cuvette. Absorbance in a spectrophotometer at 232 and 270 nm was determined, using the same solvent as a reference.

Stability was measured in triplicate by the Rancimat method (Läubli and Bruttel, 1986; Gutiérrez, 1989). Evaluation of bitterness was carried out in triplicate, using the coefficient of specific extinction of the oils at 225 nm, according to the method of Gutiérrez et al. (1992). Sensorial quality was evaluated in triplicate by a 12-member analytical panel of the Instituto de la Grasa, according to the method described in Annex XII of the European Economic Community Rules (2568/91). Each oil was graded according to a scale of nine points, one being the value for very poor quality and nine for optimum quality.

Analysis of variance was carried out on all data. A 5% level of least significant difference (lsd), calculated by Duncan's multiple range test, was employed to establish differences between the means obtained for the treatments.

RESULTS AND DISCUSSION

The percentage of decayed fruit differed significantly ($p \leq 0.05$) according to storage temperature (Figure 1). The higher the storage temperature, the greater the amount of rotten fruits. More than 25% of the olives stored at ambient temperature showed rotting after 7 days of storage and 100% after 30 days. The percentages of decay in fruit stored at 8 and 5 °C were significantly different ($p \leq 0.05$) starting at 15 days of storage, when 20% of those stored at 8 °C and 10% of those stored at 5 °C showed decay. Infection developed preferentially in those fruits with physiological disorders or mechanical damage. Therefore, the harvesting method largely determines the proliferation of microbial infection during fruit storage.

Storage temperature also determined the speed of fruit softening (Figure 2). Fruits stored at 5 °C maintained the initial value of firmness for at least 45 days. In contrast, the fruit texture fell rapidly at ambient temperature, losing almost 50% of firmness at 30 days. At 8 °C, a moderate, but statistically significant ($p \leq 0.05$) decrease was recorded. Low temperatures delay both softening caused by the endogenous activity of the enzymes involved in fruit ripening and that caused by the exogenous action of the pathogens.

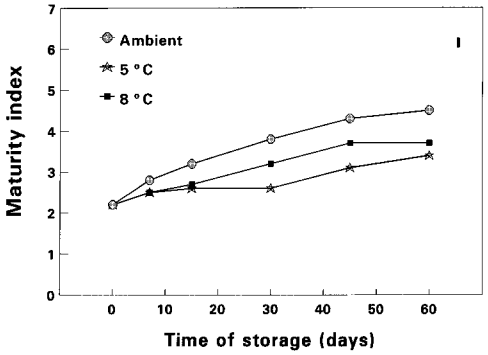

Figure 3. Changes in the maturity index on a subjective scale according to the skin color of the fruit (0, bright green skin; 1, green-yellowish skin; 2, green skin with reddish spots; 3, reddish-brown skin; 4, black skin with white flesh; 5, black skin with < 50% purple flesh; 6, black skin with ≥ 50% purple flesh; and 7, black skin with 100% purple flesh) of olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

Maturation development of fruits was significantly ($p \leq 0.05$) delayed by refrigeration at 5 or 8 °C from the very beginning of storage (Figure 3). To reach the level of maturity corresponding to a reddish-brown skin color (number 3 in the maturity index), the fruits stored at ambient temperature took 15 days, the ones stored at 8 °C took 30 days, and the fruits stored at 5 °C reached this level only after 45 days. Low temperatures delay the destruction of the chlorophyll pigments and their substitution by anthocyanins in the cells of the olive skin during fruit maturation.

The increase in titratable acidity of the oils is the main effect of fruit decay. In general, the first action of a parasitic microorganism in an oil-rich tissue is the development of hydrolytic activity of lipases. These lipases release fatty acids from the triacylglycerol molecules of the oil. Free fatty acids are easy metabolized. As a consequence, after their extraction, the oils showed a titratable acidity value in direct proportion with the percentage of decay of the fruits from which they were extracted. For this reason, the changes in titratable acidity (Figure 4) and the changes in decay during fruit storage showed a similar profile (Figure 1). Fruit storage at 5 °C allowed the titratable acidity of the oil to be maintained at ≤1% for up to 45 days. This

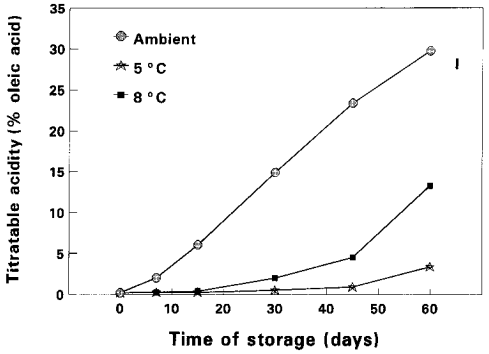

Figure 4. Changes in the titratable acidity (% oleic acid) of oils obtained from olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

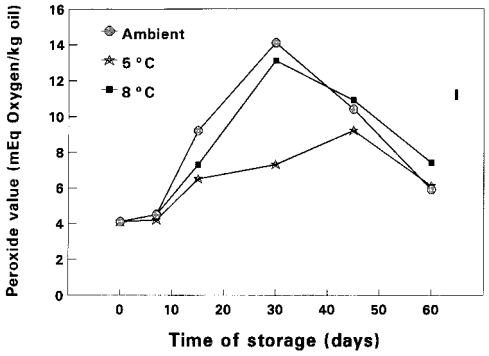

Figure 5. Changes in the peroxide value (mequiv of oxygen/kg of oil) of oils obtained from olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

value is the limit accepted for the titratable acidity of the best commercial quality olive oil ("extra" in the European market). The temperature of 8 °C was not effective enough to stop or slow down the hydrolytic changes for longer than 3 weeks, and the quality "extra" was maintained during that period. The fruits maintained at ambient conditions produced "extra" oils for only a few days of storage.

The peroxide values of the oils obtained from fruits stored at different treatments followed similar trends (Figure 5). After remaining practically constant for 1 week, there was a sharp rise in the peroxide value up to 30 days in the samples stored at ambient temperature and at 8 °C. This trend was followed by a decrease until 60 days. The oils obtained from fruits stored at 5 °C had the lowest levels of peroxides during the period studied. Initially, these levels showed a gradual rise until 45 days and finally a decrease until 60 days. The decrease observed in the samples was probably due to the breakdown of the peroxides formed, which would follow the pathway of fatty acid oxidation. The limiting value accepted for "extra" quality olive oil (20 mequiv of oxygen/kg of oil) was not exceeded in any of the samples analyzed.

The oils obtained from olives stored at different temperatures showed similar K_{232} values for 30 days of fruit storage. Subsequently, the samples differed significantly ($p \leq 0.05$) according to the temperature at which fruit was stored (Figure 6). The lowest values were found in the oils obtained from olives stored at 5 °C, which maintained their initial levels of conjugated fatty acids. The limiting value of 2.40 for "extra" olive oils was not exceeded by the oils, irrespective of the fruits from which they were extracted. The maintaining of the initial content of conjugated diunsaturated fatty acids in the oils is related with the low level observed

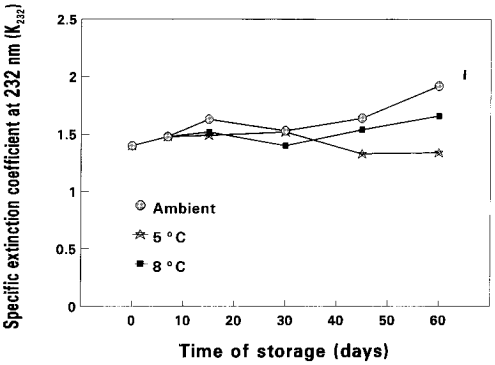

Figure 6. Changes in the content of conjugated fatty acids measured by the specific extinction coefficient at 232 nm (K_{232}) of oils obtained from olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

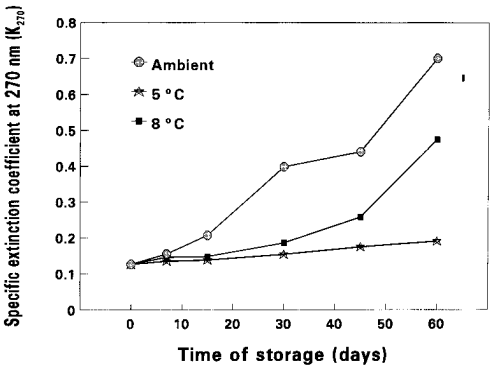

Figure 7. Changes in the content on carbonylic compounds measured by the specific extinction coefficient at 270 nm (K_{270}) of oils obtained from olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

for the peroxide value because the conjugation of double bonds in the fatty acids occurs prior to their peroxidation.

Temperature of fruit storage significantly affects ($p \leq 0.05$) the content of carbonylic compounds (aldehydes and ketones), measured by their K_{270} values, of the oils obtained from stored olives (Figure 7). The oils extracted from fruits stored at ambient conditions always showed the highest values of K_{270}, which were significantly higher ($p \leq 0.05$) after 15 days of storage. On the other hand, the oils extracted from fruits stored at 5 °C had the lowest values for this parameter during storage, becoming significantly ($p \leq 0.05$) lower than the samples from fruit stored at 8 °C after 30 days. From the commercial point of view, the limiting level accepted for "extra" quality oil (0.20) was only maintained during storage in the samples extracted from fruits refrigerated at 5 °C. This level deteriorated after 15 days and after 30 days in oils obtained from olives stored at ambient temperature and at 8 °C, respectively. The increase of K_{270} observed after 30 days of storage explains the decrease observed in peroxides at the same time. These molecules are quickly hydrolyzed and oxidized and are transformed into fatty acid derivatives with shorter carbon chains.

The oils obtained from olives stored at 5 °C had lost 35% of their initial stability after 60 days of fruit storage, but the losses for those stored at 8 °C and ambient temperature were 70 and 93%, respectively (Figure 8). This temperature-dependent loss of stability is due to thermocatalyzed hydrolytic and oxidative processes, which act on the oil contained in the olives during their ripening, either as a consequence of their own metabolism or as a result of pathogenic activity.

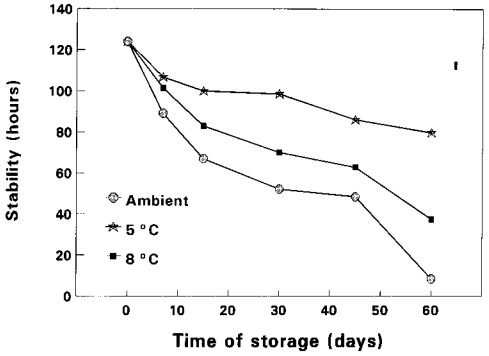

Figure 8. Changes in the stability to oxidation (hours) of oils obtained from olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

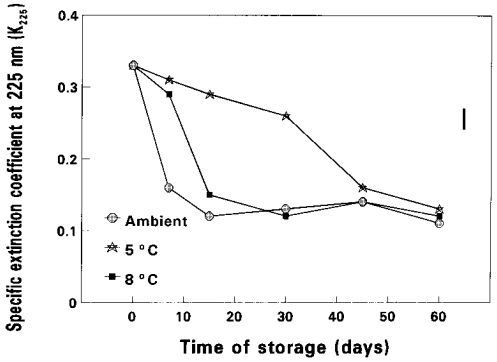

Figure 9. Changes in the content on bitterness compounds objectively measured by the specific extinction coefficient at 225 nm (K_{225}) of oils obtained from olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

Bitterness of the extracted oils (measured by K_{225}) decreased sharply in those oils obtained from fruit stored at ambient conditions or at 8 °C and slowly in the oils extracted from fruit stored at 5 °C (Figure 9). The K_{225} values of the samples seemed to heat towards an asymptotic value of 0.12, in which all the treatments coincided after 45 days of storage. Excess bitterness is a traditional problem for the marketing of oils obtained from the olives, especially those of the Picual variety. Fruit storage at 8 °C for 15 days allowed oils to be obtained with a significant ($p \leq 0.05$) reduction of their initial content of compounds responsible for the bitter taste.

Although, in general, the sensorial quality decreases with storage time, the extent and rate of this decrease depends on the conditions under which the olives have been kept (Figure 10). In the oil from olives stored at ambient temperature, the loss of quality is sharp after the first week, losing the quality of "extra" ($\geq$6.5) after 10 days and reaching a very poor score of 1.5 after 30 days [a score of 1.5 corresponds to oils that cannot be used for human consumption without previous refining ("lampant" quality)]. In the oils from olives stored at 8 °C, the decrease is less sharp and begins at 15 days. These oils were no longer "extra" at 30 days, and became "lampant" ($\leq$3) at 60 days. In contrast, the oils from olives stored at 5 °C, although their scores decrease slightly throughout storage, remain "extra" until 45 days.

According to the results obtained, refrigeration at 5 °C was the most suitable treatment assayed for obtaining the best oil quality after prolonged fruit storage. However, if the delay expected before the oil extraction were only ~15 days, 8 °C storage temperatures may also be used. Temperatures >8 °C must be avoided for fruit storage.

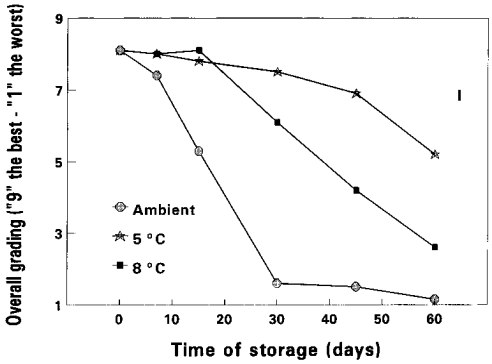

Figure 10. Changes in the sensorial evaluation (overall grading on a subjective scale where 9 means the best and 1 the worst) of oils obtained from olives stored at different temperatures. Vertical bar represents least significant difference at $p \leq 0.05$.

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