# **Effect of Fruit Storage Conditions on Olive Oil Quality**

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**ABSTRACT:** "Koroneiki" olive fruit from trees grown in Crete were stored under five different conditions (0°C, air; 5°C, air; 5°C, 2% O<sub>2</sub> + 5% CO<sub>2</sub>; 7.5°C, air; 7.5°C, 2% O<sub>2</sub> + 5% CO<sub>2</sub>). Oil was obtained from fruit immediately after harvest and after fruit storage for 30 and 60 d. Olive oil quality was evaluated by determining acidity, peroxide value, absorption coefficients  $(K_{232}, K_{270})$ , phenol and chlorophyll content, fatty acid composition, and the resistance to oxidation by oven test. Olives stored at 7.5°C, even for 30 d, deteriorated from fungus development, and the obtained oil was of inferior quality with high acidity, peroxide value, and absorption coefficients. The same oil had high chlorophyll and phenol content, resulting in good oil resistance to oxidation. Olive oil from fruit stored at 0 or 5°C for 30 d had acceptable acidity, peroxide value, and absorption coefficients, but showed low resistance to oxidation, which was attributed to low chlorophyll and phenol content. This condition is further attributed to chilling injury caused by low storage temperatures. During storage, all treatments resulted in an increase of oleic acid, partly as a result of linoleic acid oxidation. *JAOCS 75,* 721–724 (1998).

**KEY WORDS:** Acidity, chilling injury, fatty acids, hydroperoxides, oil stability, *Olea europaea*.

The major Greek olive cultivar is "Koroneiki." Very often, "Koroneiki" fruit grown in southern Greece is harvested and stored in nets or sacks at ambient temperatures, which may exceed 10°C. These olives are mechanically damaged and deteriorate easily due to fungus growth (1,2). Rotten olives produce high acidity, low stability during heating, and generally low-quality olive oil (3–5).

To minimize olive deterioration before processing, either as oil olives or as table olives, storage of olive fruit at low temperatures has been studied in California (6,7) and in Spain (4,8). Other investigations (9,10) concluded that olives are sensitive to chilling at temperatures below 5°C. Variation in chilling sensitivity between cultivars also was found during storage, even at 10°C. The main chilling injury symptoms are internal browning around the pit, which progressively leads to external pitting and browning. It is known that these changes negatively affect table olive quality. However, the effect of olive fruit injury by low temperatures on the quality characteristics of the oil has not been studied. In other fruits,

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chilling causes an increase in unsaturated fatty acid content of the membranes (11,12). Olives grown at higher elevations (cooler temperatures) also contain a higher percentage of unsaturated fatty acids (13–15).

Reduced ambient  $O_2$  and/or increased  $CO_2$  concentration plus lower temperatures during storage have been shown to reduce the deterioration rate of various fruits and vegetables (16). These conditions especially are important for chilling sensitive crops. Ripening of "Manzanillo" olives grown in California is delayed by  $2\%$  O<sub>2</sub> alone or in combination with 5% CO<sub>2</sub> at 5°C (7). Previous reports showed that  $CO<sub>2</sub>$  above 5% or  $O_2$  below 2% were detrimental to the quality of olive fruit or oil (3,7–10). The objective of this study was to evaluate the effect of various storage temperatures and atmospheres on "Koroneiki" olives and on the quality characteristics of their oil.

### **EXPERIMENTAL PROCEDURES**

Olives (*Olea europaea* cv. Koroneiki) were harvested from mature trees in the area of Chania, Crete, Greece. During the first season, mature olives were harvested on January 22, 1995, transported to the Department of Pomology, Aristotelean University of Thessaloniki, where on January 24, they were sorted, packed in 2-kg replicates, and stored at 5 and 7.5°C. At each temperature, fruit were kept in air,  $2\%$  O<sub>2</sub>, 5% CO<sub>2</sub>, or 2% O<sub>2</sub> + 5% CO<sub>2</sub>. Each replicated treatment was conducted in a 400-L sealed metal chamber with 90 to 95% relative humidity. All chambers were connected with a  $CO<sub>2</sub>$  infrared gas analyzer and an  $O_2$  paramagnetic gas analyzer. Atmospheric conditions were regulated by computer with the addition of  $N_2$ , air, or  $CO_2$ . A 0.5%  $CO_2$  level was maintained by flushing the chamber air through a bath that contained 2 N KOH. Ethylene produced by the olives was removed by  $KMnO<sub>4</sub>$  pellets present in each chamber. Three replicates for each treatment were removed after 20 and 40 d of storage. During the second season, olives were harvested on December 12, 1995, transported, sorted, packed in replicates as described above, and then stored at 0, 5, or 7.5°C. Fruit stored at 0°C were kept in air, while at the other two temperatures, fruit were kept in air or 2%  $O_2$  + 5%  $CO_2$ , where controlled atmospheres were reached and regulated as described above. Three replicates for each treatment were removed after 30 and 60 d of storage.

Fruit weight losses were followed by weighing certain replicates initially and at every fruit exit. Fungus growth on olives at each exit was graded on scale of: 0 (no fungus development) to 5 (samples were 100% rotting and sporulating).

The oil of each 2-kg replicate was obtained as follows: fruit were first crushed in a laboratory blender, and the resulting paste was mixed for 30 min in the presence of warm (30 $^{\circ}$ C) distilled water (about 30% of the paste w/w) to facilitate oil separation. Olive oil was obtained after centrifugation of the paste at 3000 rpm for 10 min and stored in bottles at 2°C until it was analyzed.

Total titratable acidity, peroxide value, and chlorophyll content were determined by AOCS (17) methods. Phenols were determined according to the colorimetric method described by Gutfinger (18) and expressed as ppm of caffeic acid. The specific absorbance values  $K_{232}$  and  $K_{270}$  were determined according to the method proposed by the International Olive Oil Council and described elsewhere (19). Oil (1 g) plus cyclohexane was mixed vigorously in a 100-mL volumetric flask. The absorbance at 232 and 270 nm was recorded against a blank (pure cyclohexane). Oil resistance to oxidation was determined by heating the oil samples at 63°C (oven test) and periodically measuring the peroxide value of each sample.

The fatty acids of each oil sample were methylated according to the method described by the *Official Journal of European Communities* (20) by mixing 0.5 g of oil sample with 0.3 g (approximately 0.4 mL) of sodium methylate (95%) in a glass vial. This vial was closed and immersed in a water bath at 85 to 90°C for 2 h with periodic shaking to facilitate the reaction. The esterification process was completed when the contents of the vial were clear after sedimentation of the glycerine and reagent residues. Fatty acid analysis followed with the injection of  $1 \mu L$  of cooled fatty acid methyl ester in a Varian 3700 gas chromatograph (Varian, Sunnyvale, CA), equipped with chromosorb (80/100 mesh, DGS 10%, 1%  $H_3PO_4$ ) and flame ionization detector. The chromatograph operated with  $N_2$  as carrier gas with a flow rate of 20 mL/min and column temperature 190°C, injection temperature 220°C, and detector temperature 250°C. Each lipid present in the sample was quantitated with the DAPA software package (DAPA Scientific Pty, Ltd., Kalamunda, West Australia). The ratio  $(C_{18:1} + C_{18:2} + C_{18:3})/(C_{16:0} + C_{18:0})$  was used to further compare treatments.

Analysis of variance was carried out for all data. A 5% level of least significant difference (LSD) and Duncan's multiple range test were used to establish differences between the means. All means were calculated from three replicates, and each quality parameter was measured twice for each replicate.

## **RESULTS AND DISCUSSION**

"Koroneiki" olives did not show chilling injury symptoms at 5°C in air, 2% O<sub>2</sub>, or 2% O<sub>2</sub> + 5% CO<sub>2</sub> and at 7.5°C in 2% O<sub>2</sub> or 2%  $O_2$  + 5%  $CO_2$ , even after 40 d of storage (first season's results, not shown). Storing "Koroneiki" olives in 5%  $CO<sub>2</sub>$ 

# **TABLE 1**





*a* Temperature values are in units of °C.

*<sup>b</sup>*Index: 0, no rot; 1, few rotting olives without sporulation; 2, 30% rotting but few sporulating; 3, 50% rotting and sporulating; 4, 75% rotting and sporulating; 5,100% rotting and sporulating.  $c_2$ % O<sub>2</sub> plus 5% CO<sub>2</sub>.

atmospheres resulted in poor-quality oil. It also became obvious that respiration of ripe "Koroneiki" olives increased the  $CO<sub>2</sub>$  content of the storage room.

Fruit weight losses increased with time in storage. Highest losses occurred at 7.5°C in air (Table 1). Weight losses decreased with lower storage temperatures and controlled atmospheres. Fungus development (rot index) also was highest at 7.5°C in air or controlled atmospheres. Olives stored at 5°C in air after 60 d of storage had some fungus development, but olives stored at 0°C in air or 5°C in 2%  $O_2$  + 5% CO<sub>2</sub> were almost unaffected by fungi, even after 60 d of storage.

Oil acidity from freshly harvested olives was below 1%, a value that classifies olive oil as extra virgin. Oil acidity increased during storage of olives (Fig. 1). It was highest when olives were stored at 7.5°C and decreased with lower storage temperature. For each temperature, oil obtained from olives stored in controlled atmospheres had lower acidity than oil from air-stored olives. The increase in acidity was probably the result of fungal lipase activity (1).

Peroxide value of oils obtained from olives stored at various temperatures and controlled atmospheres increased during storage, compared to oil from freshly harvested olives



**FIG. 1.** Changes in acidity of oil during storage of olives at various conditions. Temperature values are in units of °C. FFA, free fatty acid; LSD, least significant difference.



**FIG. 2.** Changes in peroxide value (PV) of oil during storage of olives at various conditions. For other abbreviation see Figure 1.

(Fig. 2). The increase was significant only for olives stored at 7.5°C.

The ultraviolet absorption coefficient  $K_{232}$  did not change in oil from olives stored at 0 or 5°C but increased at 7.5°C (Fig. 3), which indicates the formation of conjugated hydroperoxides (4). The same pattern was found for the  $K_{270}$ absorption coefficient, probably due to conjugated diene secondary products.

Chlorophyll content was low in oil obtained from 0°Cstored olives, even after 30 d of storage, probably due to chilling. Oil from 5°C-stored olives had slightly less chlorophyll content than oil from freshly harvested olives, while oil from 7.5°C-stored olives had similar chlorophyll content as oil from freshly harvested olives (data not shown).

Phenol content was low in the oil obtained from  $0^{\circ}$ Cstored olives, probably due to chilling, after 30 d of storage, and in the oil from olives stored for 60 d at  $5^{\circ}$ C in  $2\%$  O<sub>2</sub> +  $5\%$  CO<sub>2</sub>. All other treatments did not affect oil phenol content (Fig. 4).



**FIG. 3.** Changes in the ultraviolet absorption coefficient  $K_{232}$  of oil obtained from olives stored at various conditions for 30 and 60 d. For abbreviation see Figure 1.



**FIG. 4.** Changes in phenol content of oil during storage of olives at various conditions. For abbreviation see Figure 1.

Resistance to oxidation of oil from olives stored 30 d at 0°C was lost after 14 d of oven heating (Fig. 5) and from olives stored for 60 d at 0°C from the first day of heating (data not shown). This was attributed to the decrease of natural antioxidants (phenols) and chlorophyll. The latter acts as an antioxidant in the dark (21). Oil oxidation of olives stored for 30 d at 5°C in 2%  $O_2$  + 5%  $CO_2$  was increased after 35 d of heating. Oil from olives stored for 60 d at the same storage conditions was oxidized after 28 d of heating (data not shown). The resistance of oil to oxidation did not change, even after 57 d of heating, when it was obtained from olives stored at 5°C in air or 7.5°C in air or 2%  $O_2$  + 5%  $CO_2$ (Fig. 5).

The main fatty acids determined in olive oil from freshly harvested olives were oleic acid (83%), palmitic acid (11.5%), and linoleic acid (3.2%). When olives were stored at low temperatures and controlled atmospheres for 30 and 60 d, palmitic acid content decreased slightly to around and below 10%, and linoleic acid content decreased to below 3%. Oleic



**FIG. 5.** Resistance to oxidation of oil (change in PV) obtained from olives stored for 30 d at various conditions. For abbreviation see Figure 2.

acid content increased 1 to 2% with storage regardless of storage temperature, atmospheric composition, and duration in storage, except when olives were completely deteriorated after 60 d of storage at 7.5°C. Also, the unsaturated/saturated fatty acid ratio increased in oil from stored olives from 6.5 to 7.7, regardless of treatment.

Oil obtained from olives stored at 7.5°C for 30 d was of inferior quality due to increased oil oxidation. Therefore, this temperature is not recommended for storage of this oil-producing cultivar. However, oil obtained from olives stored at these storage conditions had high phenol and chlorophyll content, which resulted in good stability during heating. Shorter storage time of fruit at 7.5°C or control of fungus development during fruit storage could result in a better overall oil quality. Storage of olives at 7.5°C in controlled atmospheres had similar fungus development, but the oil quality, although still unacceptable, was better than that of oil from olives stored at 7.5°C in air.

Olives stored at 0°C had no fungus development, and the oil had low acidity, low peroxide value, and low absorbance coefficients  $(K_{232}, K_{270})$ . Based on these results, the oil was rated of good quality (virgin oil) when fruit was stored for 30 d. However, olives stored at this temperature were severely injured due to chilling and showed almost complete external discoloration after 30 d of storage. Due to chilling, chlorophyll and phenol content were substantially reduced, resulting in low oil resistance to oxidation. These changes due to chilling of oil-producing olive cultivars are detrimental to oil quality. Therefore, oil cultivars that are chilling-sensitive should not be stored at chilling temperatures for long periods of time.

Olives stored at 5°C in air had low fungus development and produced relatively good oil quality after 30 d of storage, although oil acidity slightly increased. Prolonged storage at these conditions caused further deterioration in oil quality due to high oil acidity. Storage of olives at 5°C in controlled atmospheres accelerated chilling and caused deterioration of oil quality. Results were similar but to a lesser degree than those obtained from olives stored at 0°C. These negative changes also can be attributed to high percentage of  $CO<sub>2</sub>$  in storage (3,8).

In conclusion, storage of olives at 7.5°C resulted in high fungus development and low oil quality. This temperature could be used for olive fruit storage if fungus development could be suppressed. Storage of olives at  $0^{\circ}$ C was detrimental to oil quality due to chilling injury, because the natural antioxidants present in the olive fruit were destroyed and oil resistance to oxidation was diminished. The best storage condition for "Koroneiki" olives was at 5°C in air because, even after 30 d of storage, oil obtained from these olives was of good quality.

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