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Controlled Atmosphere Storage of Fresh Black 'Gemlik' Olives

Mecit Hakan Ozer[1]*, Bulent Akbudak[1] and Bahattin Cetin[2]

[1]*Department of Horticulture, Faculty of Agriculture, Uludag University, 16059 Gorukle, Bursa, Turkey*
[2]*Department of Agricultural Economics, Faculty of Agriculture, Uludag University, 16059 Gorukle, Bursa, Turkey*

This study was carried out with fresh olives 'Gemlik' that were to be processed as a table black cultivar. Following harvest, the olives were transferred to a cold storage facility within a few hours and placed in plastic trays. The olives were stored at 5 ± 0.5°C and 90–95% RH for 9 weeks under different controlled atmosphere (CA) combinations in plastic cells. During the period, physical and chemical analyses were conducted on samples at 3-week intervals. Our results indicate that olives can be stored, with acceptable quality losses for 6 weeks especially under 2% CO_2: 2% O_2: 96% N_2 CA. This situation may be beneficial for the prevention of chilling injury, the preservation of olives in the processing plant and the reduction of waste water.

Key Words: chilling injury, cold storage, controlled atmosphere, olive, wastewater.

Introduction

World olive production is nearly 15 million tons, although it fluctuates from year to year due to alternate bearing. Turkey generally ranks fourth or fifth in the world with an average production of 12% or 1.6 million tons (FAO Production Yearbook, 2001).

Forty percent of table olives in our country are supplied from the Marmara Region of which 95% is processed to brined black olives. In Bursa, the major olive-producing province in the Marmara Region, 7.88% of the cultivated land is reserved for olive cultivation, and 71896 tons of olives were produced in Bursa in 1999. 'Gemlik' is the most widely grown cultivar (Briefing Report of Agricultural Technical Office, 1999). Its popularity originates from its suitability to table-type consumption.

Olive fruits are generally harvested based on anthocyanin accumulation when they are still green, beginning to lose chlorophyll and firmness, and increasing in oil content. Mature black ones lack chlorophyll, but have more oil and are softer. Shulman and Lavee (1971, 1973, 1976) noted that as olives mature from the green to the black stage, anthocyanin and chlorophyll degrade while the fruit accumulates carbon dioxide and ethylene (C_2H_4).

The optimum means of maintaining eating quality is to process prime olives immediately after harvest. However, this is not always possible because olives arrive in such large quantities, exceeding the capacity of the processing plant within a short period and necessitating storage, albeit temporarily. In such a situation, the storage should be planned so as to maintain the fruit quality and minimize the spoilage. In the Basara area, fresh olives are generally stored in bins 20–30 cm deep in cold storage rooms with adequate air circulation (Olive Oil Quality Improvement International Olive Oil Council, 1996).

Kilic (1994) recommended that olives be processed within 14h after being harvested because any delay in the brining of olives leads to spoilage by bacterial colonies growing under the fruit skin (nail head). In the same year, Luh and Ferguson (1994), who observed surface pitting and spotting in olives stored at 10°C for 6 weeks or more, advised that olives be processed immediately after harvest or stored in brine. The pitting results from the death and collapse of epidermal cells, which create air pockets underneath the fruit skin (Kader et al., 1990).

Keeping olives that have been harvested in high quantities within a short time under inconvenient conditions and in batches leads to economic losses because of the reduction in weight and quality, as well as the decline in the flavour of the final product.

The increase in waste water that occurs at the brining stage can partially be overcome through cold storage of fresh olives without brine. The suitability of fresh olives to different storage temperatures and atmospheric combinations was investigated to evaluate this alternative potential (Kader et al., 1990) and carried out in California with the 'Manzanillo'. California is similar to our Bursa province in the amount of olives preserved as black or green-ripe types. In a study of the green olive 'Conservolea', it was found that olives could be stored for 37 days at 5°C in air or for 22 days at 7.5°C in 5% CO_2: 2% O_2: 93% N_2 (Nanos et al., 2002).

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* Corresponding author (E-mail: hozer@uludag.edu.tr).

When controlled atmosphere (CA) storage of fresh green olives is considered, it is noteworthy that the optimum conditions are 0–1% CO_2: 2–3% O_2, and the storage can be carried out for 12 and 9 weeks at 5 and 7.5°C, respectively (Kader, 2001; Kader et al., 1990; Sydney Postharvest Laboratory and Food Science, 2001). It was reported that fresh black types should be processed immediately after harvest, but that they could be stored for 4 weeks at 5°C and in 2% O_2 (Agar et al., 1998).

This study was carried out to establish an alternative solution to the loss of quality in the final product because of accumulation in processing plants caused by intensive production. We determined the storability of fresh olives in different controlled atmospheric combinations using the 'Gemlik' variety.

Materials and Methods

The reserarch was conducted in the Controlled Atmosphere Cold Storage Plant of the Department of Horticulture, Faculty of Agriculture, Uludag University, and used 'Gemlik' olives provided from a grower's orchard in Bursa province. There are 300–400 fruits in 1 kg of 'Gemlik' olives whose flesh/seed ratio is 80–86% (Barut, 1992). Olives were hand-harvested on December 24, towards the end of harvest at a firm black maturity according to Guillen (1988) and Institute of Turkish Standards (1997).

About 25 kg of harvested olives were stored. To eliminate those with defects, those of uniform colour and size were put into 500 g-plastic containers. The containers were randomly placed in cells of 120 L volume in which the atmospheric combination could be controlled. Each test, consisting of 8 kg of olive replicated 10 times, was exposed to 5 ± 0.5°C, 90–95% RH and a CO_2%:O_2% ratio of 0:21 (air, control), 2:2; 2:3; 2:4; 2:5 ± 0.1 to 0.2% recommended by Kader et al. (1990), Kader (1992). Agar et al. (1998), Kader (2001) and Nanos et al. (2002).

The controlled atmospheric combinations in plastic containers were kept constant by using a "BBG Goerz Metrowatt" (Servomex, UK) recorder, a "Servomex 1420" (Servomex, UK) O_2 analyzer and a "Servomex 1410" (Servomex, UK) CO_2 analyzer.

Physical and chemical analyses of samples were made at 3-week intervals to determine weight loss (%), dry matter (%), respiration rate ($\mu gCO_2 \cdot g^{-1}FW \cdot h^{-1}$), titratable acidity (%), pH, total soluble solids (%), total chlorophyll ($mg \cdot g^{-1}FW$), anthocyanin ($mg \cdot g^{-1}FW$), decay incidence, physiological disorders (%), overall appearance, and fruit skin colour (L, a, b).

Fresh weight losses were determined by subtracting the final from the initial weights. Dry matter content was calculated by drying the sample at 70°C to a constant weight. This was then subtracted from the initial FW according to Cemeroglu (1976).

Changes in the respiration rate of fruits within the period beginning with harvest until the end of storage were determined as $\mu gCO_2 \cdot g^{-1}FW \cdot h^{-1}$ by using the Claypool-Keefer method (Claypool and Keefer, 1942). Respiration rates were measured in an unheated laboratory, 10°C. The time between the transfer to normal air and the respiration was about 10 min.

For investigating titratable acidity, 20 g of fruit pulp was homogenized and the mixture brought to 100 mL by adding distilled water. A 20-g aliquot containing 3–4 drops of phenolphthalein was titrated with 0.1 N NaOH. The results were calculated as lactic acid, the most common acid in olives (Cemeroglu, 1992). pH measurements of fruits were made by using NEL 890 brand (Turkey) pH meter.

Fruit samples were blended in the laboratory and their total soluble solids contents were determined with N.O.W. (0–32) refractometers.

The samples taken from the skin and pulp were extracted with acetone (90%) and filtered. The absorbance values of the filtrate were monitored in a spectrophotometer (UV-120-01; Shimadzu, Japan) at 652 nm wavelength. The total chlorophyll was calculated according to Holden (1976).

To measure anthocyanin contents, the samples taken from the fruit pericarp were extracted in methanol:1% HCl and the absorbance values were recorded with a spectrophotometer at 530 nm. The results were stated as the optical absorbance value of 1 g of sample in 100 mL methanol-HCl (Shulman and Lavee, 1971).

The olives were evaluated for incidence and severity of physiological disorders, such as chilling injury (surface and internal browning) and surface pitting and spotting (nail head) (Kader et al., 1990). In addition, the fruits were graded on the extent of pathological spoilage (with visible mycelia growth). Those that underwent excessive water loss were evaluated as culls; their percentages of weight loss were determined as above.

For evaluation of overall appearance, stored samples were evaluated by 5 panelists and the quality scored as follows: 9-8, perfect; 7-6, good; 5-4, bad; 3-1, unusable. Skin colours of olives were determined by two readings on two different symmetrical faces of the fruit in each replicate by using Minolta CR-300 (Konica-Minolta, Japan).

Data were subjected to analysis of variance (ANOVA, Minitab); means were separated by the use of LSD test ($P < 0.05$).

Results

The effects of different atmosphere combinations on physical and chemical changes in fresh black olives during storage periods are shown in Table 1.

Weight loss

The data indicate that the largest weight loss at the end of 9-week storage occurred in air, whereas the lowest in 2% CO_2: 4% O_2 CA. All treatments resulted in significant reductions in dry matter ratios.

Table 1. Physical and chemical changes of 'Gemlik' olives during CA storage.

Stor. per. (week)	Treatment[z]	Weight loss (%)	Dry matter (%)	Respiration rate ($\mu gCO_2 \cdot g^{-1} FW \cdot h^{-1}$)	Titratable acidity (%)	pH	Total soluble solids (%)	Total chlorophyll ($mg \cdot g^{-1}$ FW)	Antho-cyanin ($mg \cdot g^{-1}$ FW)	DI and PD[y] (%)
0	0:21	0.00 e[x]	32.50 a	34.97 f	0.11 c	4.60 e	9.70 a	4.7 a	39.50 fgh	0.00 e
	2:2	0.00 e	32.50 a	34.97 f	0.11 c	4.60 e	9.70 a	4.7 a	39.50 fgh	0.00 e
	2:3	0.00 e	32.50 a	34.97 f	0.11 c	4.60 e	9.70 a	4.7 a	39.50 fgh	0.00 e
	2:4	0.00 e	32.50 a	34.97 f	0.11 c	4.60 e	9.70 a	4.7 a	39.50 fgh	0.00 e
	2:5	0.00 e	32.50 a	34.97 f	0.11 c	4.60 e	9.70 a	4.7 a	39.50 fgh	0.00 e
3	0:21	0.98 cde	33.30 a	124.70 def	0.08 c	5.46 d	9.90 a	3.4 a	51.80 e	0.00 e
	2:2	0.49 de	34.40 a	218.25 bcde	0.09 c	5.48 d	10.00 a	3.9 a	29.10 h	0.00 e
	2:3	0.48 de	32.50 a	196.05 cde	0.10 c	5.44 d	10.40 a	4.1 a	47.30 efg	0.00 e
	2:4	0.24 de	29.20 a	78.35 ef	0.10 c	5.62 bcd	9.70 a	3.9 a	36.40 gh	0.00 e
	2:5	1.09 cde	31.00 a	209.05 cde	0.09 c	5.59 cd	9.90 a	3.6 a	32.70 h	0.00 e
6	0:21	5.52 b	29.90 a	133.26 def	0.72 ab	5.84 abcd	10.30 a	3.1 a	56.80 cde	43.33 b
	2:2	1.10 cde	28.20 a	157.35 cdef	0.54 b	6.03 abcd	9.40 a	3.9 a	35.30 h	17.17 d
	2:3	0.72 de	30.40 a	67.75 ef	0.81 a	6.29 ab	10.20 a	3.7 a	49.80 ef	21.10 d
	2:4	0.48 de	28.20 a	116.95 def	0.49 b	6.25 abc	8.60 a	3.6 a	49.50 ef	19.27 d
	2:5	1.58cde	33.90 a	105.15 def	0.50 b	6.12 abcd	9.50 a	3.4 a	54.60 de	21.73 d
9	0:21	9.01 a	31.90 a	368.55 a	0.10 c	6.45 a	9.90 a	2.5 a	138.50 a	81.69 a
	2:2	2.69 c	31.20 a	286.50 abc	0.05 c	6.40 a	8.30 a	3.4 a	64.30 cd	30.53 c
	2:3	2.67 c	32.10 a	357.85 ab	0.06 c	6.08 abcd	9.30 a	3.1 a	66.70 c	34.53 c
	2:4	1.82 cde	30.20 a	217.98 bcde	0.06 c	6.29 ab	8.20 a	3.0 a	78.30 b	33.51 c
	2:5	1.95 cd	28.40 a	234.40 abcd	0.05 c	6.43 a	8.60 a	2.7 a	86.20 b	42.76 b
	LSD	1.64	5.99	131.60	0.24	0.61	2.15	4.24	10.94	4.68

[z] CO_2:O_2.
[y] DI: Disease infection, PD: Physiological disorders.
[x] Means within a column followed by different letters differ significantly at $P < 0.05$.

Respiration rate

Our data reveal that the respiration rates of olives in all treatments increased by the end of 9 weeks, but the difference among the CA treatments at the end of 3 or 6-week storage period were insignificant, but statistically significant at the end of 9 weeks. The highest respiration rate at the end of storage occurred in air, whereas the lowest was in 2% CO_2: 4% O_2 treatment.

Titratable acidity

Titratable acidity fluctuated during the three-week intervals, but the differences among treatments were found to be insignificant at the end of storage.

pH

pH changes fluctuated between sampling periods, but not among treatments.

Total soluble solids

Total soluble solids content among treatments also fluctuated during storage, but changes were not statistically significant among sampling times or treatments, except for the control.

Total chlorophyll

Total chlorophyll contents of treatments declined with prolonged storage. The highest total chlorophyll content at the end of storage was found in the 2% CO_2: 2% O_2 treatment, the lowest was in the control.

Anthocyanin

Total chlorophyll content increased with time, the highest anthocyanin at the end of 9 weeks was in the control; the lowest was in the 2% CO_2: 2% O_2 treatment. These differences between control and the other CA treatments were significant statistically.

Decay incidence and physiological disorders

No decayed fruits were noted at the end of the first 3 weeks, but they appeared during the following 6 weeks. Statistically significant differences were found among treatments. About 20% of the fruits in all CA treatments had spoiled, whereas 43% of the control fruits decayed. Most decays were surface pitting (nail head) rather than chilling injury. Internal browning was not observed, but symptoms of chilling and CO_2 injury (pitting) appeared. The ratio of decays and disorders that exceeded commercially unacceptable limits amounted to 25% and 82% in the control plots after 6 and 9 weeks of storage, respectively; the lowest percentage of spoiled fruits occurred in the 2% CO_2: 2% O_2 treatment. The distribution of decays in the control group in the form

of mycelium growth at the end of storage was determined as 70% *Penicillium* spp., 10% *Botrytis* spp., 10% *Alternaria* spp. and 10% *Fusarium* spp.

Overall appearance

No significant differences in the quality scores in the appearance of olives was recorded at the end of the first 3 weeks; this positive effect persisted to week 6 in CA treatments. The control group ranked the lowest quality (2.5 or unusable) at the end of storage, whereas the fruits in the 2% CO_2: 2% O_2 CA treatment ranked the highest (5.7 or good) (Fig. 1).

Fruit skin colour

Changes as increases and reductions in L values were observed during CA storage of olives; the lightest colour was obtained with 2% CO_2: 2% O_2 treatment after the 9 week-storage (Fig. 1). Similarly, increases and reductions, determined by a and b values of olives during the storage period, indicated that ripening and colour change in olives proceeded more slowly in the 2% CO_2: 2% O_2 treatment than in the other treatments.

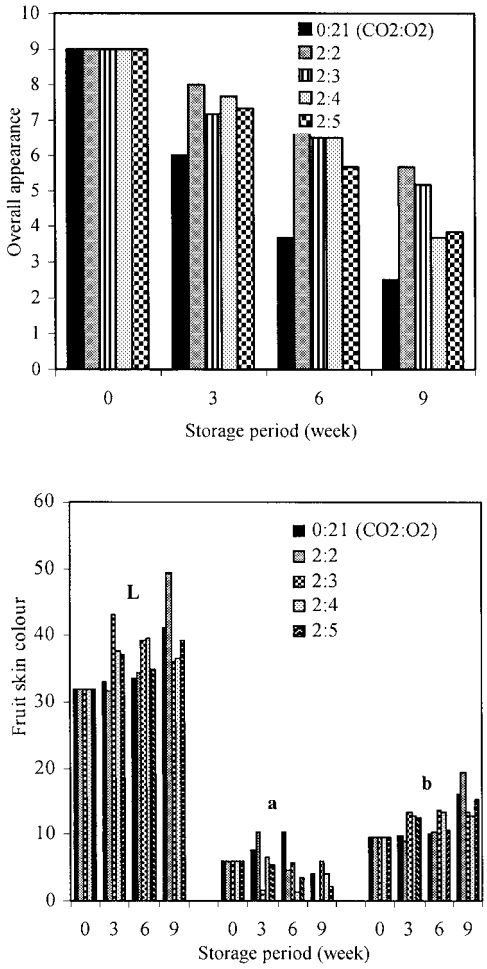

Fig. 1. Changes in overall appearance and fruit skin colour during CA storage of 'Gemlik' olives.

Discussion

The highest weight loss of 9.01% was observed in olives stored in air. This situation cannot be attributed to chilling injury or explained by their anatomical characteristics. Chilling was reported to hasten water loss in most products (Kays, 1997), but the typical symptoms of chilling injury were not encountered in any one of the treatments in our study. Hence, we propose that the difference in weight loss between the control and CA groups is on account of reduced respiration rates in the CA storage that retarded the ripening and senescence processes by slowing cell metabolism. Ke and Kader (1989) found that the respiration and metabolic rates decreased and slowed down under high CO_2 and low O_2 levels. Our respiration rate seems to confirm their findings as olives stored in air had the highest respiration rates. However, the high respiration rates at the end of the week 9 may have partly originated from the microorganisms. The developing mycelia are known to respire rapidly, and thus, raise the fruit temperature slightly higher than the ambient atmosphere. The weight losses determined at low levels under CA conditions in our study are in accordance with similar results of Nanos et al. (2002). Likewise, Jankovic and Drobnjak (1995) showed that CA storage could reduce the weight loss by 3–4 fold compared with normal atmosphere storage in apples.

In a study carried out at 0°C and 2.2°C with green 'Manzanillo' olives, chilling injury was determined after week 2 and week 5, respectively (Kader et al., 1990). Transferring the samples to higher temperatures hastened the spoilage. The main symptoms of chilling injury are internal and superficial browning; internal browning started around the pit and extended towards the skin, followed by superficial browning. No symptom of chilling injury was observed in the olives stored at 5, 7.5 or 10°C for 12 weeks in their study, although some fruits stored at 5°C and 2% O_2 for 12 weeks did not appear normal. However, this condition did not lower the external quality of olives. Thus, we agree with Kader et al. (1990) that to avoid chilling injury, fruit should not be stored below 5°C. Following their recommendations, we noted no symptoms of chilling injury in the CA treatments (2% CO_2: 2% O_2 and 2% CO_2: 3% O_2), but only in the air-stored ones. This situation is in accordance with Agar et al. (1999) who did not encounter the visual symptoms of chilling injury in black-ripe 'Manzanillo' olives stored under similar conditions.

Decay incidence and physiological disorders markedly increased with the prolonged storage period, but they were especially lower in fresh-black olives stored in 2% CO_2: 2% O_2, than in those stored in air or other CA treatments. This lowering influence of the O_2:CO_2 combination on decay incidence and physiological disorders in fresh-black 'Gemlik' olives is consistent with the results of Agar et al. (1999) who reported that

2 kPa O_2 in the storage atmosphere lowered the incidence of decay in black-ripe 'Manzanillo' olives more than in fruits stored in air. But our results are contrary to those of Garcia et al. (1994) who reported that 5 kPa O_2 in the storage atmosphere caused a higher incidence of decay in mature-green 'Picual' olives than that air. Garcia and Streif (1991) reported a noticeable decay in mature-green 'Gordal' olives stored in 1 kPa O_2 at 5°C and commented that possibly the 1 kPa O_2 atmosphere condition can increase the sensitivity of olives to cold temperature and induce anaerobiosis, which may be followed by fungal infection. No increase in sensitivity and related decay at significant levels was encountered in this study. This difference could originate from the higher sensitivity of green olives to chilling. Moreover, it is known that low O_2 and/or high CO_2 conditions, accompanied with critically low temperatures, increases the sensitivity to chilling in numerous products (Kader, 1986; Kader et al., 1990; Wang, 1982). Similarly, Nanos et al. (2002) reported the development of skin injury resulted from chilling injury after long term storage in only high CO_2 or in high CO_2-low O_2 combination in their study of the green olive variety 'Chandrolia' at 5°C. This situation explains the increase in decay ratios associated with high CO_2 ratio (2%) in our study (Table 1). The high rate (82%) of decays and spoilages and correlated high respiration rates (Table 1) observed at the end of storage (week 9) in the control olives may be attributed to fungal infections as well as senescence. The increase in decays-spoilages in CO_2 rates higher than 2% and correlated declines in quality scores of overall appearance especially after week 6 suggest that increased CO_2 level, especially over 5%, increased the physiological disorders markedly after week 6. Nevertheless, relatively low respiration rates in 4% or 5% CO_2 treatments could be explained on the basis of reduction in fungal and bacterial growths. Similar effects were observed in cherry, grape and strawberry by Kader (1992), Wills et al. (1981). These results approximate the results of Agar et al. (1999) who reported losses exceeding 25%. Sanchez-Raya (1983) reported that the respiration rate reached a peak at fruit maturity stage. It can be said that the high respiration rates in the 3rd and 6th weeks of our study originated from ripening, but those reached in the 9th week originated from senescence and spoilage.

One of the quality parameters to be taken into consideration in the storage of olives is the maintenance of the initial colour. Kader et al. (1990), found that fresh-green 'Manzanillo' olives stored in 2% CO_2 maintained their initial colour after 12 weeks. Likewise, 'Gemlik' olives stored at 2% CO_2: 2% O_2 maintained the initial colour best. Nanos et al. (2002) came to the same conclusion with 'Conservolea' olives, but observed that the olives became russeted more rapidly in air or high CO_2 concentrations. That anthocyanin accumulation and chlorophyll breakdown proceeded more rapidly in the control olives indicates that CA treatments (low O_2 and high CO_2) are preferable for prolonged storage. Romero et al. (1997) reported similar results with olives stored in sterile water to respiration, changes in titratable acidity, pH, and total soluble solids content.

It is possible to store fresh black 'Gemlik' olives up to 6 weeks at 5 ± 0.5°C and in 2% CO_2: 2% O_2 CA conditions within acceptable quality losses, and minimize chilling injury and physiological disorders. Our findings should be beneficial to prevent quality losses when a backlog of fresh olives arrives at the processing plant, and help to reduce waste water.

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ブラックオリーブ‘Gemlik’生果のCA貯蔵

Mecit Hakan Ozer[1], Bulent Akbudak[1] and Bahattin Cetin[2]

[1]*Department of Horticulture, Faculty of Agriculture, Uludag University, 16059 Gorukle, Bursa, Turkey*

[2]*Department of Agricultural Economics, Faculty of Agriculture, Uldag University, 16059 Gorukle, Bursa, Turkey*

食卓用ブラックオリーブとして加工される‘Gemlik’の生果を用いて本研究を行った．オリーブ果実を収穫後数時間以内に低温貯蔵施設のプラスチックトレイの上に移した．プラスチック容器に入れたオリーブについて，温度 5±0.5 ℃，相対湿度 90–95% で，異なるガス組成（CA 条件）下で 9 週間の貯蔵をおこなった．貯蔵期間中 3 週間毎に，生理学的及び化学的分析を行った．その結果，CA 条件が特に 2% CO_2: 2% O_2: 96% N_2 の場合に，6 週間貯蔵中の品質低下を，受け入れられるレベルにまで抑えることができた．本研究成果は低温障害の回避や加工プラントにおけるオリーブの貯蔵および水分溶出の低減に有効である．

注）本摘要は，Abstract を編集委員会が和訳したものである．