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Article in Journal of Horticultural Science and Biotechnology · July 2003

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Impact of controlled atmosphere storage conditions on storability and consumer acceptability of sweet cherries ‘Regina’

By J. HARB¹, J. STREIF^{2*} and A. SAQUET²

¹Department of Biology and Biochemistry, Birzeit University, P. O. Box 14, Birzeit-West Bank, Palestine

²Institut für Obstbau-Bavendorf, Universität Hohenheim, D-88213 Ravensburg, Germany

(e-mail: streif@uni-hohenheim.de)

(Accepted 26 March 2003)

SUMMARY

Sweet cherries obtained from a rain-protected orchard were subjected to various controlled atmosphere storage conditions for up to seven weeks. Quality parameters responded differentially to various CA-conditions: changes in fruit firmness, sugar content and colour were mostly not significant. However, the storage conditions exerted significant impact on other parameters such as respiration rate, RQ, ATP- and ADP-concentrations. Air-stored fruits respired at significant higher rates than CA or ultra low oxygen-stored fruits. Moreover, the impact of CA-storage conditions persisted even after a conditioning period of 36 h at room temperature. The ATP and ADP concentrations of sweet cherries behaved similarly, where a combination of reduced O₂ and very high CO₂ concentration (12% or above) resulted in highly reduced ATP-concentration in the fruit tissue. A taste panel considered fruits stored under 6% CO₂ + 2% O₂ as good, although there was a strong indication that it is better to store sweet cherries for a limited time (up to five weeks) under a low O₂ concentration combined with a high CO₂ concentration (12%) or under a high O₂ concentration combined with a very high CO₂ (18%).

The short shelf-life of sweet cherries restricts their marketability span, primarily due to fungal decay, blackening of fruit stalks, weight loss and rapid acid decrease (Lurie and Aharoni, 1997; Wermund and Lazar, 2003). Therefore, various postharvest techniques are recommended to achieve extended shelf-life. Rapid cooling is considered to be the most important mean. Resnizky and Sive (1989) reported that cold storage of cultivars Rainier and Bing could be prolonged for four weeks when fruit were cooled rapidly with a hydro-cooler. The short shelf-life of sweet cherries is also attributed to decay caused by both *Botrytis cinerea* and *Monilinia* spp., which are the main rot pathogens (Borecka and Wojtas, 1986). Highly elevated CO₂ concentrations (>10%) are usually considered to be fungistatic (Petracek *et al.*, 2002). Streif *et al.* (1998) reported that *in vitro* mycelial growth of *Botrytis cinerea* was significantly retarded with increasing CO₂ concentrations, whereas O₂ reduction to 1% had no effect. With sweet cherries, Shiping *et al.* (2001) found no CO₂ injury or off-flavours after 18 d of storage at 0°C at any CO₂ concentration tested (15–30%). Kader and Morris (1977) further set the tolerance concentrations for sweet cherries to be 3% O₂ and 10% CO₂.

The objective of this study was to investigate the impact of controlled atmosphere (CA)-conditions that combine various CO₂ concentrations with low or ambient O₂ concentrations, on quality parameters and consumer acceptability of the sweet cherries cultivar Regina cultivated under rain covers. Further, some physiological aspects of CA-treatments on fruit physiology such as respiration and energy metabolism were investigated.

MATERIAL AND METHODS

Plant material and CA-conditions

In two successive years, fruits were obtained from a rain-protected orchard located in the Lake Constance area of Southwest Germany. Cherry fruits were picked at commercial harvest date, selected for uniformity and freedom for external injuries, immediately cooled to 1°C, then transferred to different CA-containers (0.240 m³) representing eight treatments of the following %CO₂ + %O₂-combinations: 0.03 + 21 (=air), 6 + 18, 12 + 18, 18 + 18, 6 + 2, 12 + 2, 18 + 2 and 24 + 2 at 1°C. The gas concentrations inside the chambers were measured and regulated automatically and the desired composition was achieved within 12 h. Three subsamples consisting of 3 × 500 g fruits in carton trays were taken for each treatment and sampling date. The same experimental design was repeated in the second year with slight modifications, namely: the combination 24% CO₂ + 2% O₂ was eliminated. At harvest time and at 10–13 d intervals samples with three replicates of each treatment were analysed for various quality parameters. A completely randomized design was used in both years with three replications.

Measurement of firmness, soluble solids, titratable acidity and colour

Fruit firmness was measured by a nondestructive instrument (FirmTech2, UP, Germany) especially designed for soft fruits. The maximum force needed to compress fruit tissues a unit distance was measured. Fruit juice total soluble solids was measured with a digital refractometer (Atago, Japan), and titratable acidity by titration of 10 ml juice with 0.1 N NaOH to pH 8.1 and expressed as % (W/V) citric acid. Ten fruits

*Author for correspondence

TABLE I

The influence of CA-storage conditions (%CO₂ + %O₂) on firmness, total soluble solids, and titratable acidity of sweet cherries 'Regina' after seven weeks in store at 1°C. First value represents the initial reading at harvest time

CA-condition (%CO ₂ + %O ₂)	Fruit firmness (g mm ⁻¹)		Total soluble solids (%)		Titratable acids (g 1000ml ⁻¹)	
	2001	2002	2001	2002	2001	2002
At harvest	315.1	245.9	16.1	16.5	7.1	9.7
Air	343.4 a ¹	278.1 b	15.7 a	15.8 a	4.7 b	4.0 a
06 + 18	344.1 a	336.5 a	16.1 a	14.9 a	5.5 a	3.9 a
12 + 18	348.3 a	328.8 a	16.3 a	15.7 a	5.8 a	3.9 a
18 + 18	365.4 a	356.8 a	15.3 a	15.6 a	5.4 a	4.3 a
06 + 02	358.2 a	336.3 a	16.7 a	15.3 a	5.9 a	4.2 a
12 + 02	356.9 a	306.4 ab	15.9 a	16.0 a	5.6 a	4.2 a
18 + 02	345.8 a	342.7 a	16.2 a	15.9 a	5.4 a	4.2 a
24 + 02	349.1 a	—	15.9 a	—	5.7 a	—

¹Means within each column followed by different letters indicate significant differences between treatments at P≤0.05, Student-Newman-Keuls range test.

from each replicate were used for colour determination using a Minolta Chroma Meter (model CR-200). The parameters a* and b* were measured and the final results were expressed as hue angle (h°) according to McGuire (1992).

Determination of respiration rate

Respiration of cherry fruits was measured under storage conditions comparable with that in the CA-containers. For this purpose, 500 g of fruits were enclosed in 2.14 dm³ ml airtight jars kept at 1°C and flushed with the same gas mixture as used in the CA-containers. At 10 d intervals, glass jars were detached from the gas supply and tightly closed for 8 h. The increase in CO₂ concentrations and the decrease in O₂ concentrations during this period was measured by injection into gas chromatograph with TDC detector (Micro GC CP2002P, Chrompack, The Netherlands). Fruits from the last sampling date were conditioned for 36 h at 20°C and subsequently subjected to respiration measurements as above.

ATP/ADP extraction and assay

The extraction and assay of ATP and ADP were carried out according to Tan (1999). The concentrations of ATP and ADP were determined by a bioluminescence technique using the ATP detection kit from Bio-Orbit Oy, Turku, Finland. One gram of powdered and dried sample was homogenized in 10 ml of 5%-TCA solution plus EDTA (2 mM) and incubated in ice for 30 min. Samples were then centrifuged at 18000 g at 4°C for 15 min. An aliquot (0.1 ml) of the supernatant was diluted (1:40) with EDTA-buffer (2 mM, pH 7.75) and assayed with a luminometer (model 1250, Firma LKB-Wallac, Finland) at 25°C. For the ADP determination, the samples were incubated with pyruvate kinase at 25°C for 30 min, during which ADP was converted into ATP. An internal ATP-standard was used for calculating ATP and ADP concentrations.

Sensory test

A taste panel, with a minimum of three people, evaluated the sensory quality of the cherry fruits from the different CA-treatments after four and seven weeks. The panelists looked for both visual quality criteria, such as stem freshness (green colour and dryness) and fruit colour, and taste criteria, such as sweetness, acidity, crispness, and off-flavour. All tests were performed after a shelf-life period of 24 h at 20°C in air. The visual

properties (appearance, colour, stalk condition, injuries) and organoleptical impression were judged by numerical scores between 1 and 5 as follows: for decay: 1 = no decay, and 5 = strong infection; for colour: 1 = fresh as at harvest time, and 5 = very dark; for stalk condition: 1 = fresh as at harvest time and 5 = dry and brown colour; for taste: 1 = very good, and 5 = very bad.

Weight loss and internal injury evaluation

During storage, the weight loss due to transpiration and respiration of the fruits was followed by weighing the same sample of fruits both at the beginning of storage period and at each sampling date for each treatment. At the end of the storage period (after seven weeks) 20 fruits from each treatment and replicate were halved and estimated visually for decay and storage disorders. Decay was recorded as a percentage of damaged fruits.

Statistical analysis

Analysis of variance (ANOVA) was performed by CoStat-software (CoHort Software, Monterey, USA). Mean separations were calculated by Student-Newman-Keuls range test at P≤0.05.

RESULTS

Fruit quality parameters

The influence of different CA-conditions on various quality parameters is shown in Table I for both years. No significant differences in fruit firmness were registered between treatments after seven weeks in store at 0°C, in the first year, whereas control fruits showed significantly lower values in the second year. In both seasons, it was obvious that fruits became firmer with time, although firmness declined slightly in some treatments at the end of the storage period.

During storage of the fruits, there was little change in total soluble solids, but an appreciable decrease of titratable acidity was observed compared with values obtained immediately after harvest time. In both years, the titratable acidity of fruits declined steadily with time under all CA-storage conditions (data not shown). After seven weeks of storage, sweet cherries stored in air had the lowest value of titratable acidity, but significant differences between the treatments were noticed only in the first year. The results show that storing fruits either under low oxygen or high carbon dioxide conditions, separately or in combination, preserved the acidity of fruits.

TABLE II

The influence of CA-storage conditions (%CO₂ + %O₂) on various colour attributes (CIE L*, a* and hue°) of sweet cherries 'Regina' after five weeks in store at 1°C. First value represents the initial reading at harvest time

CA-condition (%CO ₂ + %O ₂)	Colour attributes					
	a*	a*	L*	L*	h°	h°
	2001	2002	2001	2002	2001	2002
At harvest	15.5	18.9	27.9	29.0	10.3	12.2
Air	12.0 a ¹	10.2 a	27.5 ab	27.5 b	10.4 a	08.1 a
06 + 18	11.9 a	10.3 a	27.4 ab	27.3 b	09.5 ab	08.7 a
12 + 18	13.5 a	12.5 a	27.2 ab	28.0 a	09.8 ab	09.7 a
18 + 18	13.2 a	13.5 a	27.5 ab	28.0 a	10.7 a	09.6 a
06 + 02	11.7 a	11.8 a	26.5 b	28.3 a	09.3 b	09.2 a
12 + 02	12.3 a	11.4 a	27.1 b	27.2 b	09.0 b	08.8 a
18 + 02	13.3 a	10.9 a	28.4 a	27.2 b	09.6 ab	09.0 a
24 + 02	13.5 a	—	27.6 ab	—	09.7 ab	—

¹Means within each column followed by different letters indicate significant differences between treatments at P≤0.05, Student-Newman-Keuls range test.

The impact of storage conditions on colour attributes is shown in Table II. Concerning the brightness (L*-value) the results showed that fruits used as starting material in the first year was already darker (lower L*-value) at harvest, than those used in the second year. Consequently, the differential response of fruits to various CA-storage conditions between the two years may be related to this difference. In both seasons, air-stored fruits became darker at the end of storage period, whereas fruit stored under very high CO₂ concentration (12–18%) or under low O₂ concentration combined with high CO₂ concentration (6 + 2) remained lighter (results of the second year). Moreover, it seems that combining low O₂ concentration with very high CO₂ concentrations (12–18%) resulted in darker fruits, which may be a result of CO₂-injury. The h° angle of fruits at harvest time differed in the two seasons, fruits in the second year had a lighter red colour (higher h°); no significant differences were registered among all treatments. In the first year, however, results showed clearly that increasing CO₂ concentration retarded partially the change towards a more dark red colour.

Respiration rate

The CO₂-production of fruits measured at 0°C for the various CO₂ and O₂ treatments during the entire storage period is shown in Figure 1. Sweet cherries stored under air (control) respiration significantly faster on all measurement dates when compared with CA treatments. Increasing CO₂ concentration while maintaining O₂ concentration at 18% resulted in significant lower respiration. The lowest respiration rates were measured

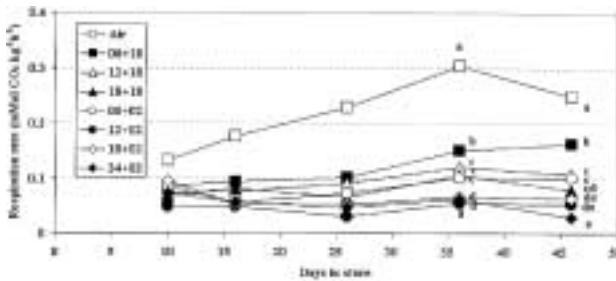


FIG. 1

Influence of storage conditions (%CO₂ + %O₂) on the CO₂ production of sweet cherries 'Regina' at 1°C over the entire storage period (seven weeks). Means followed by different letters indicate significant differences between treatments at P≤0.05, Student-Newman-Keuls range test.

when fruits were stored under low O₂ concentrations combined with high concentration of CO₂. With the extension of storage period, respiration rate increased mainly in air-stored fruits where the magnitude of respiration increased two or three fold above that of CA-treatments (e.g. 24 + 2 and 18 + 2). Further, fruits from the last sampling date (after seven weeks of storage) were kept for 36 h at 20°C shelf-life conditions to assess any post-storage effects on respiration (Figure 2). The respiration of 36 h-conditioned fruits corresponded very well with their respiration behaviour under storage conditions; which means that the respiration of cherries from various CA conditions remained significantly lower than that of air-stored sweet cherries. Moreover, it was obvious that fruits stored under low O₂ concentration combined with 6% CO₂ respired almost at the same rate as those stored at high O₂ and higher CO₂ concentrations (12–18%). The respiration quotient CO₂/O₂ did not differ significantly, but a higher RQ was evident with higher CO₂ and lower O₂ concentrations.

Changes in ATP and ADP concentrations and ATP:ADP ratios

Table III shows the changes in ATP and ADP concentrations in sweet cherries at harvest time, and after four and seven weeks in store. At harvest time, fruits contained the minimum concentrations of ATP

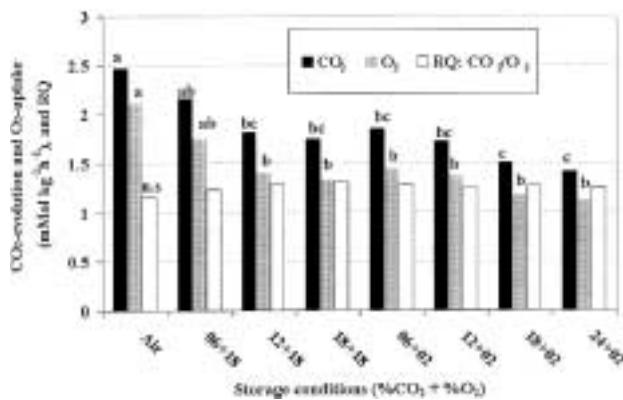


FIG. 2

Influence of storage conditions (%CO₂ + %O₂) on subsequent respiration behaviour (CO₂-evolution, O₂-uptake, and respiration quotient) of sweet cherries 'Regina' stored for seven weeks after 36 h shelf life at 20°C. Means followed by different letters indicate significant differences between treatments at P≤0.05, Student-Newman-Keuls range test.

TABLE III

Changes in ATP, ADP concentrations (nmol g^{-1} dry weight) and ATP/ADP ratio of sweet cherries 'Regina' stored under various CA-conditions (%CO₂ + %O₂) and 1°C after four and seven weeks in store

CA-condition %CO ₂ + %O ₂	ATP		ADP		ATP/ADP	
	4 weeks	7 weeks	4 weeks	7 weeks	4 weeks	7 weeks
At harvest	55.3 g ¹	—	16.9 b	—	3.3 b	—
Air	196.6 a	194.6 a	37.4 a	46.4 a	5.3 a	4.2 a
06 + 18	108.2 d	—	35.5 a	—	3.0 b	—
12 + 18	106.4 de	107.2 c	27.0 a	33.4 bc	3.9 b	3.2 b
18 + 18	119.0 c	85.7 d	36.4 a	30.8 c	3.3 b	2.8 b
06 + 02	138.3 b	137.3 b	34.7 a	41.2 ab	4.0 b	3.3 b
12 + 02	90.4 f	—	30.0 a	—	3.0 b	—
18 + 02	101.9 e	72.7 e	29.4 a	39.2 b	3.5 b	1.9 c
24 + 02	85.6 f	68.8 f	29.4 a	35.3 bc	2.9 b	1.9 c

¹Means within each column followed by different letters indicate significant differences between treatments at $P \leq 0.05$, Student-Newman-Keuls range test.

compared with fruits stored for four and seven weeks, independent of the storage conditions. However, significant differences occurred between treatments: decreasing O₂ and/or increasing CO₂ concentrations resulted in a reduced amount of ATP in fruits compared with air-stored fruits at the corresponding sampling dates. The ADP concentrations seem to be less affected by changes in storage atmosphere, although higher CO₂ concentration and lower O₂-concentration, particularly when combined with high CO₂, slightly but significantly reduced the ADP concentration. Concerning ATP:ADP ratios, control fruits showed the highest ratio, which indicates a higher turnover rate. On the other hand, increasing CO₂ and/or decreasing O₂ concentrations resulted in significantly reduced ratios and consequently, a lower energy charge of the fruit tissue.

Weight loss

Sweet cherries stored for seven weeks under various CA-conditions and in air lost less than 2% of their initial weight; the differences between various treatments were only small and not significant (data not shown).

Stem appearance

Stem appearance is a very critical quality parameter in consumer perception of sweet cherries. The data indicate that increased humidity inside the CA-chambers which could be achieved in the second year by enclosure of fruits in perforated plastic bags, retained better stem freshness. In the first year, fruit stems from all

treatments were generally scored as brown and dry looking especially when stored under very high CO₂ concentrations, which may indicate an additional CO₂-injury. In the second year, and probably due to higher humidity inside the CA-chambers, fruit stems of all treatments were evaluated as good or adequate by visual evaluation of fruits stored for four weeks (Figure 3). No significant differences occurred between all treatments. However, fruits stored for seven weeks under high O₂ concentrations received poor evaluation, which may indicate an accelerated oxidation of phenol compounds.

External and internal injuries

At the end of the storage period of seven weeks, internal and external decay and storage disorders were estimated. Sweet cherries stored under different conditions showed no symptoms of external injuries (data not shown), although fruits did not receive any chemical treatment after harvest. Although no symptoms of internal browning could be observed in control fruits, fruits stored under CA-conditions exhibited increasing problems with internal browning, depending upon the concentration of CO₂ and O₂ in the storage atmosphere (see Table IV). Cherries stored under 18% CO₂ + 2% O₂ showed the highest percentage of losses, almost 40% of fruits.

Consumer perception

It can be seen from the taste panel scores in Figure 4 that fruits stored under air conditions received a bad score for taste after four and seven weeks of storage. Air-stored fruits were criticized as bitter tasting, too sweet, little acidity, and that aroma was not detectable. Fruits stored at low O₂ combined with increased CO₂ received good scores for taste when tested four weeks after storage. However, this acceptance of taste declined

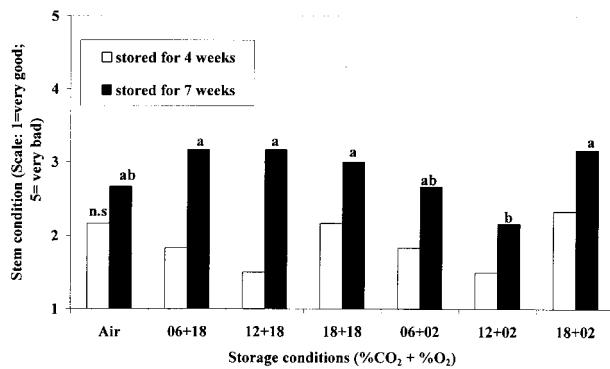


FIG. 3

Stem appearance of sweet cherries 'Regina' stored for four and seven weeks under various storage conditions (%CO₂ + %O₂). Tests were performed after a shelf-life period of 24 h at 20°C in air. Means followed by different letters indicate significant differences between treatments at $P \leq 0.05$, Student-Newman-Keuls range test.

TABLE IV
Internal browning of sweet cherries 'Regina' stored under various CA-conditions (%CO₂ + %O₂) after seven weeks in store at 1°C. Fruits were inspected visually and percentages represent all fruits showed mild to severe injuries

CA-Conditions (%CO ₂ + %O ₂)	Internal injuries (%)
Air	0 d ¹
06 + 18	16.6 bc
12 + 18	20.0 b
18 + 18	15.0 bc
06 + 02	10.0 cd
12 + 02	15.0 bc
18 + 02	36.4 a

¹Means within each column followed by different letters indicate significant differences between treatments at $P \leq 0.05$, Student-Newman-Keuls range test.

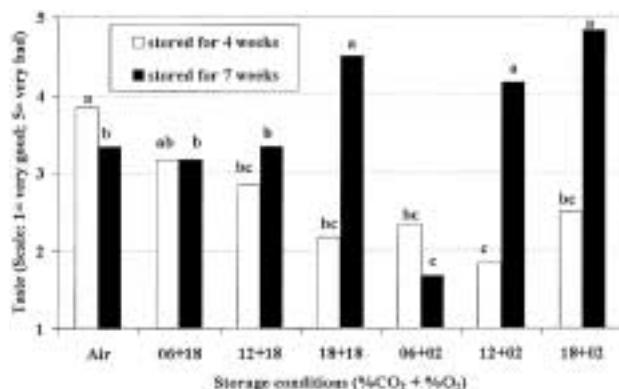


FIG. 4

Taste score of sweet cherries 'Regina' stored for four and seven weeks under various storage conditions (%CO₂ + %O₂). Tests were performed after a shelf-life period of 24 h at 20°C in air. Means followed by different letters indicate significant differences between treatments at $P \leq 0.05$, Student-Newman-Keuls range test.

drastically with extension of storage up to seven weeks, especially for the 12 + 18 and 12 + 2 stored fruits, mainly due to off-taste. Cherries stored under 6% CO₂ and 2% O₂ received the best acceptance, even after seven-weeks storage period.

DISCUSSION

The aim of this study was to extend the storage period of sweet cherries 'Regina' for up to seven weeks. Previous studies showed the difficulty in achieving this goal. Sive and Resnizky (1988) achieved a storage period of four weeks for cultivars Rainier and Bing and only two weeks for 'Sam' at 0°C. Grazianetti *et al.* (1998) reported that sweet cherries stored unwrapped under CA-conditions (5% O₂, 15% CO₂) at 0°C for 25 d had higher soluble solids, titratable acidity and firmness and lower weight loss than air-stored fruits. However, Lurie *et al.* (1998) succeeded in storing 'Bing' cherries for two months at 0°C in 'Xtend' film at 7.5% CO₂ and 16% O₂. The main benefit of this film on cherry quality was the reduction of weight loss and maintenance of green stems.

The results of this study show that various CA-storage conditions resulted in an acceptable preservation of the quality of sweet cherries, particularly when stored not longer than four weeks; the most interesting CA-condition for long-term storage was 2% O₂ + 6% CO₂. Furthermore, our results indicate clearly that neither firmness nor acidity level was the determining factor in the overall judgment of the fruits; it is much more the balance between sugars and organic acids and the absence of off-flavour. Our results with firmness are in agreement with Kappel *et al.* (2002), who found that modified atmosphere packaged-fruits had higher fruit firmness than at harvest time or when stored in air. Remon *et al.* (2000) noticed also with 'Burlat' cherries that firmness increased at first and decreased at the end of the storage period relative to the initial values. In respect of the acid content, a comparison of initial acidity values (at harvest) between the two years indicates that the acid content of sweet cherries was clearly lower in the samples of the second year, which could be influenced by climatic conditions and/or ripening stage at harvest. However, a general trend stated by Naichenko and Skrypnik (1987) who found a

negative correlation between respiration rate and content of organic acids, is partly confirmed by our results. Furthermore, it seems that increased CO₂ and decreased O₂ have a negative and cumulative impact on the respiration of sweet cherry fruits. In this sense, acidity in fruits was highly preserved under low oxygen and/or under high CO₂ conditions (results of first year), mainly due to the reduced respiration rate. This is in agreement with the findings of Stanley (1991) and Kader (1985). However, various researchers found differential effects of low O₂ and high CO₂ concentrations on fruit cell metabolism stored under stressful CA-conditions. Kerbel *et al.* (1988) reported a significant reduction in the activities of ATP: phosphofructokinase and PPi: phosphofructokinase and in the content of fructose 1,6 bisphosphate in pear fruits stored under a high CO₂ atmosphere. Kato-Noguchi and Watada (1996) also found that fructose 1,6-bisphosphate accumulates with a decreased O₂ concentration. Lange and Kader (1997a) reported that climacteric 'Hass' avocados treated with 20% CO₂ at 10°C showed increased pyruvate dehydrogenase activity and decreased cytochrome oxidase activity. Moreover, Lange and Kader (1997b) showed that mitochondrial respiration of avocados was affected in response to elevated CO₂ treatment, with a shift to the alternative pathway respiration. In our experiments, the allosteric inhibition of phosphofructokinase under high CO₂ and low O₂ atmospheres may have resulted in the production of various off-flavour compounds that adversely affected the perception of the taste by panelists.

It is obvious that development of off-taste and/or off-flavour in sweet cherries under extreme conditions (e.g. 24% CO₂) was the main factor resulting in poor consumer acceptance. The development of off-flavour is associated with ethanol (Ke and Kader, 1992) and acetaldehyde accumulation (Folchi *et al.*, 1994), which is the most common and detrimental effect that limits fruit tolerance to low O₂ storage. Using modified atmosphere packages (MAP), Lurie *et al.* (1998) achieved a gas composition of 7.5% for CO₂ and 16% for O₂ inside packages, whilst in 'E-fresh' films Grazianetti *et al.* (1998) found 10.5% and 7%, respectively. They noticed that cherries packed in 'E-fresh' maintained their firmness better but tasted different in comparison to the other treatments. The impact of the storage conditions on aroma volatiles is already in progress (data not shown) and early results indicate the presence of large amounts of alcohols and aldehydes in fruits stored under very high CO₂ concentrations.

Another problem encountered in this study at the end of storage period was the browning of mesocarp under CA-conditions. Folchi *et al.* (1994) found that storing various stone fruits under ultra-low oxygen concentration (<0.1%, CO₂ + 0.3% O₂) resulted in discoloration and browning of the mesocarp of both apricots and plums. The damage appeared initially after 11 d in apricots and after 15 d in plums and progressively increased to 100% of fruits. Consequently, browning and discoloration of fruits noticed in this study may be due to prolonged storage of sweet cherries under highly stressful conditions, namely low O₂ and/or very high CO₂ concentrations.

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