

¹Department of Biology and Biochemistry, Birzeit University, Birzeit, Palestine

²Federal Center of Technological Education, Sao Vicente do Sul, Brazil

³Kompetenzzentrum für Obstbau, Ravensburg, Germany

Quality and biochemical changes of sweet cherries cv. Regina stored in modified atmosphere packaging

J. Harb¹, A.A. Saquet², R. Bisharat¹, J. Streif³

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Summary

Biochemical and quality changes of sweet cherries cv. Regina were assessed over three consecutive years after storage in different modified atmosphere packaging (MAP) liners, with or without hydrocooling, compared to regular atmosphere (RA) storage. All plastic liners used in the experiment resulted in CO₂-enrichment and O₂-reduction inside packages, with the following impact on fruit quality after five weeks of storage: improved retention of fruit firmness and red color of skin, no significant effect on acidity and total soluble solids, and minimal loss of fruit weight. Fruit decay was absent under both storage conditions (RA and MAP), probably due to rain-protected cultivation of cherry trees. Stalks of MAP-fruits remained fresher than control fruits, obviously due to higher relative humidity condition inside packages. MA-packaged cherries were preferred by the taste panel, while cold-stored fruits were criticized due to flat and slightly bitter taste. The ATP concentration in air stored fruits was higher than in MA-packaged fruits, while ADP level was higher in MA-packaged fruits. Further, MA-packed sweet cherries exhibited higher antioxidant potential and ascorbic acid content than air-stored fruits. Moreover, hydrocooling did not cause any significant effect compared to nontreated fruits.

Introduction

MAP refers to the technique of sealing actively respiring produce like fruits in polymeric film packages to modify O₂ and CO₂ partial pressures within the packages (KADER and WATKINS, 2000). In their study on table grapes, ARTÉS-HERNÁNDEZ et al. (2004) used 35 mm thick microperforated polypropylene (PP) to generate a modified atmosphere packaging of 15 kPa O₂ and 10 kPa CO₂. The changes in O₂ and CO₂ concentrations within a package depend on the interaction between respiration of the commodity and the permeability properties of the packaging film and/or microperforations (BEAUDRY et al., 1992). Consequently, researchers stated that it is crucial to use films with suitable gas permeability, and to determine the time needed for the development of the concentrations of the respiratory gases inside the package, to achieve any success with MAP (EVELO, 1993). It was found that MAP can double the shelf-life of tomatoes, citrus, cucumber and apples compared to the non-packaged fruits, when the permeability characteristics of the package matched the respiration rate of the produce (CAMERON et al., 1989; AIT-OUBAHOU et al., 1994). Usually it is desirable to generate an atmosphere low in O₂ and/or high in CO₂ to influence the metabolism of the produce being packaged, and/or to retard the activity of decay-causing organisms, which resulted in most cases in better storability of fruits and an extended shelf life. SKOG et al., (2003) reported that MAP reduced decay by 50% and significantly maintained firmness with 'Hedelfinger' cherries. In addition to atmosphere modification, MAP improves moisture retention, which had greater influence on preserving stalks freshness than O₂ and CO₂ levels (JOBLING, 2001). However, the atmosphere modification inside packages may in-

duce various undesirable effects. Fermentation and off-flavors may develop if decreased O₂ levels cannot sustain aerobic respiration (KAYS, 1997). Similarly, injury will occur if CO₂ exceeds tolerable levels. Production of compounds that contribute to characteristic taste of many fruit, including apple, banana, pear, peaches, strawberries and others, can be adversely affected by low O₂ and elevated CO₂ (MATTHEIS and FELLMAN, 2000). Synthesis of aroma compounds are generally suppressed by high CO₂ and low O₂ concentrations (HARB et al., 2000), in part by their action on ethylene perception, but also via oxidative processes, including respiration, required for substrates production (SAQUET et al., 2003). Further findings by CRISOSTO et al. (2002) indicate that rachis browning was accelerated and a trained panel perceived 'off-flavor' in grapes (cv. 'Redglobe') exposed to > 10 kPa CO₂ partial pressure in the packages.

The aim of this work was to study the influence of plastic liners on the fruit quality and storability of sweet cherries cv. 'Regina' cultivated under rain covers. Rain-protection of sweet cherries during fruit ripening, in particular during harvest period, is essential in various regions in Western-Europe to avoid fruit cracking.

Materials and methods

Over three consecutive years various plastic liners in several MAP-trials were used. In all experiments, cherry fruits were obtained from a rain-protected orchard at the Kompetenzzentrum Obstbau-Bodensee (KOB) located in South-West Germany, in Lake of Constance area. Fruits were picked and selected for uniformity in size and color and absence of decay and external injuries, and packed at the same day.

Experiments in 2001: Fruits were divided into 16 samples, each sample amounts about 2000 g. Eight samples were enclosed in PVC plastic bags that were kept open (control treatment). A second set of eight samples were enclosed in 30 µm Life-Plus[®] gusseted polyethylene bulk liners supplied from Danisco *Flexible*, Bristol-UK (MA-packed treatment).

Analyses of quality parameters were conducted as described for Experiment 2003.

Experiments in 2002: Repetition of the 2001 experiments.

Experiments in 2003: The following treatments were conducted:

- Control treatment: Fruits were stored in cold storage without precooling. The cherries were enclosed in macro-perforated LDPE-liners (50 µm) for the purpose of increasing the relative humidity without altering gas composition around fruits.
- MAP experiment: Fruits were enclosed in two different plastic films (SJ 304 and SJ 604) delivered from a Chilean manufacturer (San Jorge, Santiago, Chile) and designed specifically for sweet cherries. Furthermore, the 30 µm Life-Plus[®] gus-

seted polyethylene bulk liner supplied from Danisco *Flexible* was used also.

- Hydrocooling experiment: Fruits were cooled in ice-water for 10 minutes before enclosure in various liners (304, Life-span, or LDPE). The aim of hydrocooling trial was to investigate the impact of hydrocooling on various quality aspects, in particular on the freshness of stem condition.

All samples were stored at 0 °C for the entire storage period. From each treatment two samples were taken every two weeks, and gas composition inside packages was determined using a micro-GC (Model CP 2002P, Chrompack,); *detector*: thermal conductivity detector (TDC), *column for O₂-determination*: Molsieb at 40 °C, *column for CO₂-determination*: Hayesep at 45 °C.

Fruits were analyzed for the following quality parameters:

Weight loss: Upon storage the initial weights of all samples were recorded, and at each sampling date, the weights of two samples for each treatment were recorded directly after removal from room, to avoid any condensation of water on the fruits.

Fruit firmness: Firmness was measured using a nondestructive instrument (FirmTech2; UP GmbH, Ibbenbüren, Germany) designed for soft fruits, which measured the maximum force needed to compress the fruit tissues. Results are given in g mm⁻¹.

Total soluble solids (TSS): The TSS in the fruit juice was determined with a digital refractometer (Atago, Japan).

Titrateable acidity: 10 ml of fruit juice were diluted with 100 ml distilled water and titrated with 0.1 N NaOH solution to pH 8.1.

Skin color: Fruit color was assessed using a chromameter (Minolta, Japan). Ten fruits per replicate were measured. The color assessment with this instrument is specified by the coordinates L*, a*, b* in a three-dimensional color space. L* represent the brightness, whereas a* is the green (with negative values) and the red component (with positive values), and b* is the blue (with negative values) and the yellow component (with positive values).

Ascorbic acid (AA) concentration: All steps of this determination procedure were carried out under cold and dark condition to minimize the degradation of vitamin C. At each sampling date fruit sections were obtained from at least 15 fruits per replicate, two replicate for each treatment, shock frozen in liquid N₂, and ground to fine powder. Six grams of fruit powder was added to 15 ml of 3 % HPO₃ solution and homogenized for 30 seconds. After centrifugation at 14 000 g for 20 minutes, the supernatant was filtered and 20 µl were used for HPLC determination under the following conditions: Column: ProntoSil 60-5-C18-H; size: 4.0 x 125 mm; particle size: 5.0 µl; eluent: tetra-n-butylammoniumhydrogensulfate (2.5 g) + methanol (55 ml) in 1 L H₂O; flow: 800 µl·min⁻¹; temperature: 25.0 °C; pressure: 16.0 MPa. An ascorbic acid standard solution (20 mg 100 ml⁻¹) was used to calculate the vitamin C concentration of the fruits.

Determination of antioxidants capacity of the water soluble compounds (ACW): Determinations were carried out by Photochem system (Analytik Jena AG) with photo-chemo-luminescence method. Standard kits from Analytik Jena, Germany, were used to measure water soluble antioxidants. ACWs were measured using the same extract used for vitamin C determination after dilution with water at a ratio of 1:10 according to the protocol provided with the ACW determination kit (Analytik Jena, Germany).

ATP/ADP extraction and assay: The extraction and assay of ATP and ADP were carried out according to SAQUET et al. (2003). The

concentrations of ATP and ADP were determined by bioluminescence technique using a kit from Bio-Orbit Oy (Finland). 1 g of the lyophilized fruit powder was homogenized in a 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 of 10 µl of the supernatant was diluted with Tris-EDTA-buffer (pH 7.75) and assayed with a luminometer (model 1250, LKB-Wallac, Finland) at 25 °C. For ADP-determination, 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 four people, evaluated the sensory quality of the cherry fruits from different storage conditions after four and seven weeks. The panelists looked for both visual quality criteria, such as stalks freshness (green color and dryness) and fruit color, and taste criteria, such as sweetness, acidity, crispness, and off-flavour. All tests were performed after a shelf-life period of 24 hours at 20°C in air. The visual properties (appearance, color, stem 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 color: 1 = fresh as at harvest time, and 5 = very dark; for stalks condition: 1 = fresh as at harvest time and 5 = dry and brown color; for taste: 1 = very good, and 5 = very bad. Furthermore, a detailed discussion was conducted at each session to describe the quality of sweet cherries. Therefore, our results will be shown in both numerical as well as a descriptive manner.

Statistical analysis: All results were subjected to analysis of variance (ANOVA) using the CoStat-software (CoHort Software, Monterey, CA, 1998), and mean separations were calculated by Duncan's multiple range test at $P \leq 0.05$.

Results and discussion

Partial pressures of gases inside the MA-packages: Fig. 1 shows changes in gas composition occurred over a storage period of five weeks after using various MAP-liners.

The lifespan liner led to the strongest reduction in CO₂ partial pressure, although it was not low enough to slow the ripening process according to WILLS et al. (1998).

CO₂ partial pressure increased up to 5 kPa in all liner treatments and this influenced some aspects of fruit ripening and quality as shown in Tab. 1. None of the tested liners was clearly superior over the others and all liners affected fruit quality and freshness mainly by increasing the relative humidity around the fruit more than by changing the gas composition inside because stem freshness was clearly better than control.

Quality parameters: Tab. 1 shows the influence of MAP-treatments and hydrocooling on various quality parameters after five weeks of storage in 2003. Fruit firmness decreased significantly upon cold storage (control), but preserved upon storage of fruits in plastic liners, and hydrocooling did not contribute with most treatments to the preservation of firmness. Changes in the total soluble solids, which reflect the amount of sugars in fruits, were minimal and did not differ significantly between control and all MAP treatments. Concerning the titrateable acidity, fruits in all conditions suffered great losses, which possibly reflect that the gas composition developed inside plastic liners was not effective in reducing the respiration rate of fruit, and no significant differences were registered between all treatments. However, significant differences were recorded in color attributes (a* and b* values). The a*-value, which gives the red

component of color decreased with both treatments. However, a significant lower decrease was registered with MAP-fruits, which indicates that increased CO₂-level inside the liner may have preserved the red pigments in MAP stored fruits more than in control fruits. Concerning the b*-values, which reflect the blue/yellow components of color, a significant lower decrease was registered also in MAP-fruits compared to control fruits. The L*-values did not differ significantly between both treatments. Hydrocooling of cherries for

two minutes in ice water soon after harvest did not affect fruit quality, when fruits were analysed after five weeks storage period. Our results are in agreement, although partially, with that of MEHERIUK et al. (1995), who succeeded in maintaining the quality of sweet cherries cv. 'Lapins' through storing in LDPE-bags (30 µm) with an immediate flushing of bags with a gas mixture composed of 5% O₂ + 5% CO₂. ARJONA et al. (1994) also found that yellow passion fruit wrapped with VF-60 plastic film and stored for 15 and 30 days showed less weight loss, while maintaining external appearance. Moreover, KUPFERMAN and SANDERSON (2005) mentioned also good results, but concluding that controlling fruit temperature is more important than using MAP, particularly if sweet cherries are to be stored for less than 10 days. In another experiment, MAP using 80 µm LDPE film retarded fruit softening and inhibited development of peel and flesh disorders of Japanese 'Fuyu' persimmon (BEN-ARIE and ZUTKHI 1992). However, fruit quality deteriorated more rapidly in a 60 µm package, which was attributed to specific physiological effects of the different atmospheric equilibria established due to film thickness.

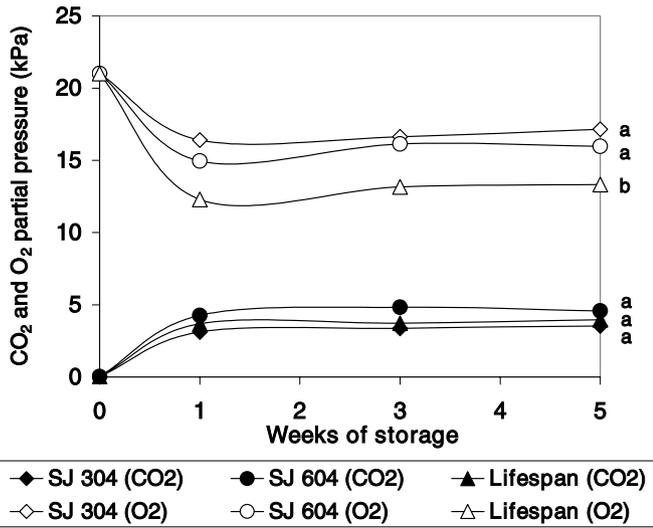


Fig. 1: Changes in partial pressures of CO₂ and O₂ inside various MAP-liners filled with 500 g sweet cherries cv. Regina, and stored at 0 °C, over a storage period of five weeks in 2003. Different letters indicate significant differences between treatments at P ≤ 0.05, Duncan's multiple range test.

Weight loss: Results show that cold-stored fruits without packaging lost more weight than MA-packaged fruits (Data not shown). This was attributed to the buildup of a high relative humidity inside MA-packages. OTHIENO and THOMPSON (1993) reported that sweet corn packed in polypropylene film with no perforations showed a reduced rate of weight loss. ARJONA et al. (1994) found also that yellow passion fruit wrapped with VF-60 plastic film and stored for 15 and 30 days showed less weight loss. It is not expected that any differences in respiration rates between treatments could cause such a difference in weight loss.

Visual inspection and sensory test: Panelists were not able to detect differences between control and MAP stored fruits, in respect to external fruit quality (appearance and color) (Tab. 2). However, significant differences were detected in stalks condition and taste. It

Tab. 1: Influence of various MAP-treatments with or without hydrocooling (+HC) on various quality parameters of sweet cherries cv. Regina. Measurements were taken at harvest time and after storage period of five weeks at 0 °C in 2003.

	Assessment time	Control	SJ 604-liner	SJ 304-liner	SJ 304-liner +HC	Lifespan-liner	Lifespan-liner +HC	Untight enclosure ¹	Untight enclosure +HC
Firmness (g mm ⁻¹)	at harvest				277				
	after 5 weeks	237 d*	343 a	317 abc	311 bc	288 c	327 ab	312 bc	322 ab
TSS (%)	at harvest				17.7				
	after 5 weeks	18.0 a	17.2 a	17.6 a	17.1 a	17.6 a	17.6 a	17.7 a	17.3 a
Acidity (g l ⁻¹)	at harvest				11.4				
	after 5 weeks	9.0 a	8.9 a	8.5 a	8.2 a	8.9 a	8.5 a	8.5 a	7.9 a
Color (L*)	at harvest				27.2				
	after 5 weeks	24.9 a	25.2 a	25.0 a	26.3 a	25.1 a	24.7 a	25.0 a	25.5 a
Color (a*)	at harvest				13.6				
	after 5 weeks	10.3 a	10.6 a	9.1 bc	9.7 ab	9.7 ab	8.9 bc	8.3 c	8.2 c
Color (b*)	at harvest				1.7				
	after 5 weeks	1.6 a	1.4 ab	1.2 cd	1.3 bc	1.3 bc	1.3 bc	1.2 cd	1.0 d

¹ Fruits were enclosed inside a PVC plastic film without tight enclosure with the aim of increasing relative humidity around fruits without changing the gas composition around fruits.

* Means within lines followed by different letters indicate significant differences between treatments at P ≤ 0.05, Duncan's multiple range test.

is well known that stalks condition plays crucial role in the consumer acceptance, and it is obvious from Tab. 2 that stalks of MA-packed fruits remained fresher than control fruits. The high relative humidity inside packages may contribute highly to this positive stalks appearance. Reduced dehydration and better condition of stalks were reported also by HORVITZ et al. (2004) with cherries stored in MA up to 42 days.

Tab. 2: Percentages of weight losses and scores of consumer perception of sweet cherries cv. Regina after six weeks storage period in 2001. Before tasting, fruits were conditioned for 24 hours at room temperature. Scores for fruit color and appearance: 1 = fresh as at harvest time, 5 = very dark; scores for stem condition: 1 = fresh as at harvest time, 5 = dry and brown colored; scores for fruit taste: 1 = very good, 5 = very bad.

	Weight loss (%)	Stem freshness (1-5)	Fruit color (1-5)	Fruit appearance (1-5)	Fruit taste (1-5)
MAP (Life-Plus 30µm)	3.1 b*	1.7 b	2.8 a	5.0 a	2.7 b
Control	7.4 a	3.5 a	2.5 a	5.0 a	4.3 a

*Means within each column followed by different letters indicate significant differences between treatments at $P \leq 0.05$, Duncan's multiple range test.

Moreover, our results with odor volatiles (data are not shown), allow us to believe that CO₂-level created inside packages did not reach the injurious level. LARSEN (1993) found that strawberries packed in LDPE-films developed very strong off-flavor, which is reflected through an increase in the content of acetaldehyde, ethanol, and ethyl acetate, and a decrease in the content of methyl ester, hexanal, and trans-2-hexenol. The current results revealed the preference of taste panel to MA-packed sweet cherries, in particular due to more juicy and crunchy taste. Furthermore, these fruits were judged to be more acidic, although panelists criticized the weak odor of fruits – no off-flavor-, while with control fruits flesh dryness and slight bitter and a non-homogenous taste were perceived. Dryness is partly a result of higher dehydration of cold-stored fruits. Hydrocooling of sweet cherries before MAP (year 2003) did not caused any significant improvement in the consumer acceptance and stalks condition after five weeks of storage (data not shown).

Vitamin C and antioxidants: Tab. 3 shows further aspects related to quality that is highly important for human health. Both ascorbic acid content and the potential of water soluble antioxidants of fruits decreased in sweet cherries stored either in air or inside the MAP-liners, compared to those analyzed at harvest time. This reflects a gradual oxidation of constituents with time, and it is obvious that MAP slows down this degradation.

Tab. 3: Ascorbic acid content and potential of water soluble antioxidants (ACW) of MA- packed sweet cherries cv. Regina at harvest time and after five weeks in storage at 0 °C. Year 2003.

	Ascorbic acid (mg 100g ⁻¹ FW)		ACW. (µg g ⁻¹ FW)	
	Control	MAP	Control	MAP
Harvest time	2.8	1.65
After 5 week	1.9 b*	2.3 a	1.26 b	1.36 a

* Means within rows -for each parameter- followed by different letters indicate significant differences between treatments at $P \leq 0.05$, Duncan's multiple range test.

Tab. 4: Changes in ATP, ADP concentrations and ATP/ADP ratio of sweet cherries cv. Regina stored for five weeks (year 2003).

	ATP (nmol g ⁻¹ DW)	ADP (nmol g ⁻¹ DW)	ATP:ADP ratio
At harvest time	55.3 c*	16.9 c	3.3 a
Control fruit (after 5 weeks)	194.7 a	46.4 b	4.2 b
MAP fruit (after 5 weeks)	168.8 b	51.4 a	3.3 a

* Means within each column followed by different letters indicate significant differences between treatments at $P \leq 0.05$, Duncan's multiple range test.

ATP- and ADP-levels: Tab. 4 shows the changes in ATP and ADP levels in sweet cherries at harvest time, and after five weeks in store. At harvest time, fruits contained the minimal levels compared with fruits stored for five weeks. However, significant differences occurred between control and MA-packaged fruits, by which air stored sweet cherries contained significantly higher ATP-concentrations than MA-packaged fruits, whereas ADP levels show an inverse situation. Significant differences were registered also in ATP-ADP ratios, by which control fruits had significantly the highest ratio. CO₂-enriched atmosphere inside MA-packages may possibly reduced the respiration rate, and consequently the turn over of energy carriers may be slow enough to cause the accumulation of ADP in MAP-fruits; lower respiration rate was registered also with CA-storage of sweet cherries cv. Regina (HARB et al., 2003).

Modified atmosphere packaging lengthened the postharvest life of cherry fruit by retaining firmness, reducing the rate of acidity loss, and by retaining stalks freshness. In conclusion, MAP seems to exert a positive impact on both external and internal quality of fruits, which means better storability of sweet cherries. However, MAP should be seen as a complementary measure, and not as a substitute for cold temperature; cherries held at lower temperature are generally superior to those held at slightly warmer temperatures (KUPFERMAN and SANDERSON, 2005).

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Addresses of the authors:

- Dr. J. Harb, Department of Biology and Biochemistry, Birzeit University, P. O. Box 14, Birzeit, West Bank, Palestine (via Israel)
- R. Bisharat, Department of Biology and Biochemistry, Birzeit University, P. O. Box 14, Birzeit, West Bank, Palestine (via Israel)
- Dr. A. Saquet, Federal Center of Technological Education, Sao Vicente do Sul, Brazil
- Dr. J. Streif, KOB, Schuhmacherhof 6, D-88213 Ravensburg