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Effect of low oxygen and anaerobic conditions as post-harvest treatment on the quality of peach fruit[1)]

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*The effects of different controlled atmospheres on peach fruit of the cultivar 'Red Haven' (*Prunus persica, L. Batsch*) during storage were examined. Fruit were kept for 21 days under ultra-low oxygen storage (ULO = 0.9 % O_2 and 0.3 % CO2), anaerobic (AN = 0.2 % O_2 and 0.3 % CO2) and regular atmosphere (RA = 21 % O_2 and 0.03 % CO_2). Major changes of chemical composition and physical characteristics as well as formation of ethanol and ethanal according to the different oxygen regimes were evaluated. Ethanol concentration increased during storage time with slight differences in velocity between the variants. It was found that exposure to ULO and RA conditions suppressed the formation of ethanol (5 to 9 mg/l and 5 to 17 mg/l) while under anaerobic conditions highest values of ethanol (600 mg/l) were reached after 15 to 21 days. Formation of ethanal coincides with ULO and RA conditions. No oxygen stress occured under ULO condition. After transferring the peaches into normal atmosphere the concentration of ethanol declined but still after 36 days the ethanol value of fruit that had been stored under anaerobic conditions was higher than that in fruit stored under ULO and RA conditions. Concentration of sugars such as sucrose, glucose and fructose showed marked differences after storage at different oxygen levels, under anaerobic conditions sugars as well as firmness of skin and flesh remained at high levels, while under ULO and RA conditions comparable reductions of sugars and firmness were observed.*
Key words: Peach *(Prunus persica*, L. Batsch), CA-storage, ULO-storage, flesh firmness, sugars

Einfluss von sauerstoffreduzierten und anaeroben Lagerbedingungen auf die Qualität von Pfirsich. *Der Einfluss unterschiedlicher CA-Lagerbedingungen auf Pfirsiche der Sorte 'Red haven' (*Prunus persica, L. Batsch*) wurde untersucht. Die Früchte wurden für 21 Tage unter ULO-Bedingungen (0,9 % O_2 und 0,3 % CO_2), anaeroben (O,2 % O_2 und 0,3 % CO_2) und normalen Bedingungen (21 % O_2 und 0,03 % CO_2) gelagert. Die wichtigsten Veränderungen der chemischen Zusammensetzung und der physikalischen Eigenschaften wie auch die Bildung von Ethanol bzw. Ethanal unter den jeweiligen Zusammensetzungen der Lageratmosphäre wurden ermittelt. Die Ethanol-Konzentration nahm während der Lagerung zu, wobei die Geschwindigkeit dieser Zunahme zwischen den Varianten leicht variierte. Es wurde festgestellt, dass ULO- und RA-Bedingungen die Bildung von Ethanol einschränkten (5 bis 9 mg/l bzw. 5 bis 17 mg/l), während unter anaeroben Bedingungen die höchsten Ethanolgehalte (600 mg/l) nach 15 bis 21 Tagen erreicht wurden. Unter ULO- und RA-Bedingungen verläuft die Bildung von Ethanal gleich; Sauerstoffstress war bei ULO-Lagerung nicht vorhanden. Nachdem die Früchte in Normalatmosphäre zurückgebracht wurden, nahm die Ethanolkonzentration ab. Aber noch nach 36 Tagen war der Ethanolgehalt von Früchten aus den anaeroben Lagerbedingungen höher als der von Früchten aus ULO- und Normalatmosphäre. Die Gehalte von Zuckern (Saccharose, Glucose, Fructose) zeigten deutliche Unterschiede nach der Lagerung bei unterschiedlichen Sauerstoffkonzentrationen. Bei Früchten aus anaeroben Lagerbedingungen waren die Zuckergehalte wie auch die Fruchtfleischfestigkeit hoch, während unter ULO- und Normalatmosphäre vergleichbare Verminderungen von Zuckergehalten und Fruchtfleischfestigkeit beobachtet wurden.*
Schlagwörter: Pfirsich *(Prunus persica*, L. Batsch), CA-Lagerung, ULO-Lagerung, Fruchtfleischfestigkeit, Zucker

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Influence des conditions de stockage réduites en oxygène et anaérobies sur la qualité des pêches.
*L'influence des différentes conditions de stockage en atmosphère contrôlée (AC) sur les pêches de la variété «Red haven» (*Prunus persica, L. Batsch*) a été examinée. Les fruits ont été stockés pendant 21 jours dans des conditions de typeá ULO (Ultra Low Oxygen) (0,9 % O_2 et 0,3 % CO_2), anaérobies (O,2 % O_2 et 0,3 % CO_2) etá normales (21 % O_2 et 0,03 % CO_2). Les modifications majeures de la constitution chimique et des propriétés physiques ainsi que la formation d'éthanol et/ou d'éthanal dans différentes atmosphères de stockage ont été déterminées. La concentration d'éthanol a augmenté au cours du stockage, la vitesse de cette augmentation variant légèrement entre les variétés. Il a été constaté que les conditions ULO et normales limitaient la formation d'éthanol (de 5 à 9 mg/l ou de 5 à 17 mg/l), tandis que les teneurs les plus élevées en éthanol (600 mg/l) ont été atteintes dans des conditions anaérobies au bout de 15 à 21 jours. Dans des conditions de type ULO et normales, la formation d'éthanal se déroule de la même manière ; sous ULO, les fruits ne présentaient pas de stress oxydatif. La concentration d'éthanol baissait une fois que les fruits avaient été ramenés en atmosphère normale. Au bout de 36 jours encore, la teneur en éthanol des fruits stockés dans des conditions anaérobies était pourtant plus élevée. Les teneurs en sucres (saccharose, glucose, fructose) présentaient des différences significatives après le stockage dans l'oxygène avec des concentrations différentes. Les teneurs en sucres ainsi que la fermeté de la pulpe des fruits ayant été stockés dans des conditions anaérobies étaient élevées, tandis qu'une diminution comparable des teneurs en sucres et de la fermeté de la pulpe a été observée après stockage sous atmosphères ULO et normale.*
Mots clés: pêche *(Prunus persica, L. Batsch*), stockage en AC, stockage sous ULO, fermeté de la pulpe, sucre

Peach fruit are limited in their shelf-life by a high rate of softening. This rapid ripening can be slowed down by fast cooling and storage at a low temperature, which, however, should not cause chilling injuries. For example chilling injuries may disrupt the complex physiological reactions in the tissue and may cause irreversible damage (Zhiguo et al., 2000; Luza et al., 1992; Fernández-Trujillo et al., 1998; Artéz et al., 1996; Beckman and Krewer, 1999). Additionally the storage period can be extended by selection of special gas mixtures with reduced oxygen and increased CO_2 contents, but the formation of anaerobic metabolites like ethanol or ethanal has to be avoided. Ethanol occurs naturally in fruit and its physiological properties have been thoroughly studied (Ke and Kader, 1992; Toivonen, 1997; Bonghi et al., 1999). Ke et al. (1991) showed that the lowering of the oxygen content to 1 % for several days and an increase of the CO_2 content up to 3.5 % reduced the rate of fruit softening and extended the postharvest life of peach fruit. High CO_2 levels during storage, especially in combination with low O_2, significantly delayed fruit ripening, kept the fruit firmer and prevented the development of wooliness, internal browning and reddish discoloration in fruit flesh during the shelf life (Streif et al., 1992). Anaerobic conditions of treatment with ethanal vapour inhibited softening of peaches for several days, increased production of volatile substances and decreased ethylene formation (Lurie and Pesis, 1992; Ritenour et al., 1997). Fruits which were treated with ethanol kept firm for a longer period of time, but soluble solid contents were not significantly influenced (Margosan et al., 1997).

The objective of the present study was to examine the tolerance of peach to low oxygen and slightly increased CO_2 levels. Furthermore it was planned to describe the effects of storage under different controlled atmospheres on the concentration of anaerobic metabolites like ethanol and ethanal. In addition analysis of main chemical components of peach fruit (sugars, acids) and texture were carried out. The objective was to get an accurate assay which can describe the onset and development of the ripening process.

Material and methods

Description of fruit and storage conditions

Peach fruit (cultivar: 'Red Haven') were obtained from the experimental orchards of the University of Brno in Lednice. Peaches were sorted into uniform stage of ripeness after visual evaluation according to green coloration of the skin. Fruit (95 kg in total, 40 kg for ULO-container, 10 kg for AN-container and 45 kg for RA conditions) were weighed, cooled to 3 °C and placed into 400 l metal containers (for AN conditions) and a 1200 l steel metal container (for ULO condition) in one repetition. The containers were immediately sealed

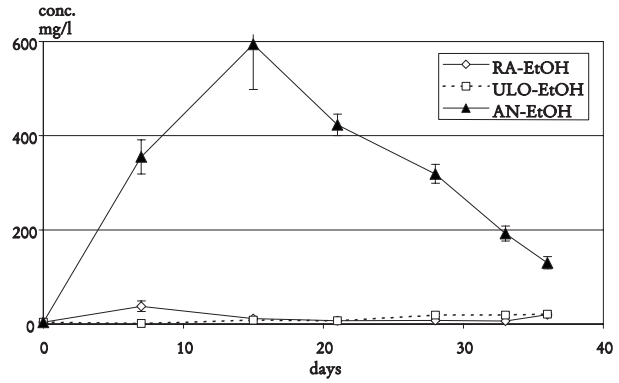

Fig. 1: Ethanol concentration in peaches during storage at three different oxygen levels at 3 °C for 36 days. After 21 days, the fruit were transferred into air. Each point is the mean of 6 replicates. Vertical bars indicate standard error SE, ($P < 0.05$).

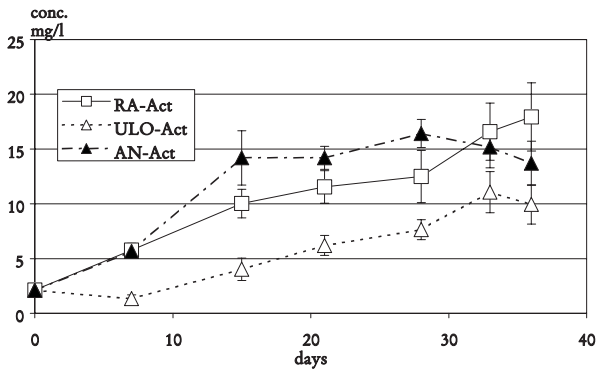

Fig. 2: Ethanal concentration in peaches during storage at three different oxygen levels at 3 °C for 36 days. (AN = 0.2 % O_2, 0.03 % CO_2; ULO = 0.9 % O_2, 1.0 % CO_2 and RA = 21 % O_2, 0.03 % CO_2). After 21 days, the fruit were transferred into air. Each point is the mean of 6 replicates. Vertical bars indicate standard error SE, ($P < 0.05$).

and experimental gas compositions were formed by flushing with N_2. The experiment consisted of three levels of oxygen (0.2 % O_2 = anaerobic, AN), (0.9 % O_2 = ultra-low oxygen, ULO, (21 % O_2 = regular atmosphere, RA), each concentration maintained to $\pm$ 0.1 % O_2. Concentration of CO_2 was set at 0.3 % ($\pm$ 0.1 %) or 0,03 % RA. Concentrations of CO_2 and O_2 were monitored every hour by gas analysis (Arelco, ARC, France) connected to a processor. The storage temperature was kept at 3 °C. After a storage time of six and twelve days, resp., six peaches were removed from each container (AN, ULO, RA) and analysed. After a storage time of 21 days each chamber was opened, six fruit were used for chemical and physical analysis and the remaining fruit were kept at normal atmosphere and analysed again after 36 days.

Fruit analysis

One µl filtered sample of the frozen and rethawed pulp were injected into a GC system (Chrom 5, Laboratory Equipment, Prague, Czech Republik) consisting of a sample block fitted with Teflon and a packed column with Porapak P. Concentration of ethanal and ethanol were quantified with external standards and expressed in mg/l.

Six fruit from each experiment were individually analysed for firmness (Multipurpose Equipment for mechanical properties,TU Brno, Czech Republik), 8 mm plunger with four measurements at opposite positions for each fruit. In the flesh of the fruit titratable acidity (TA) and soluble solids (SS) were measured as juice extracted from the flesh. Concentration of sugars (sucrose, glucose, fructose) were determined by HPLC of aqueous extract from pulp by isocratic elution with water as a mobile phase. Separation was achieved on an ion exchange column (Polymer IEX H^+ form, 250 x 8 mm, Watrex) at 50 °C and at a flow rate of 0.7 ml/min and with refractometric index detection. Each sugar was quantified by external standard. Organic acids (malic and citric acid) were analysed by identical procedures except the mobile phase (20 mMol/l methansulphonic acid), ambient temperature of column and UV detection at 210 nm. Standard error, differences of mean values ($P = 0.05$) and variance analysis were calculated.

Results and discussion

Concentration of ethanol and ethanal during and after storage

Ethanol and ethanal are products of anaerobic respiration under limited O_2 supply. The internal O_2 concentration for aerobic/anaerobic transition is the critical point. Ethanol production is primarily associated with glycolytic activity in tissue, which is enhanced by anaerobic conditions. The knowledge of the low oxygen-limit is a critical presumption of the optimal control of the gas composition of storage atmosphere.

Table 1:
Sugar concentration (g/l) at the beginning of storage and after 21 days in different oxygen regimes*

Treatment	Time (days)	Sucrose	Glucose	Fructose
IN	0	72.5 ± 2.5a	12.9 ± 0.5a	14.5 ± 0.4a
ULO	21	52.9 ± 1.6b	12.0 ± 0.3a	14.2 ± 0.5a
AN	21	89.6 ± 8.7a	19.6 ± 1.9b	23.8 ± 2.4b
RA	21	62.4 ± 6.3ab	18.6 ± 1.3b	22.3 ± 1.6b
ULO	36	59.6 ± 6.2a	27.2 ± 2.2b	33.1 ± 2.5b
AN	36	58.1 ± 8.1a	25.3 ± 2.1b	31.2 ± 2.5b
RA	36	70.5 ± 7.7a	24.5 ± 2.4b	30.8 ± 2.7b
Two-way ANOVA : ** P< 0.01, * P< 0.05, ns = not significant				
Treatment		*	**	**
	Time	ns	**	**
Treatment x time		**	**	**

*Values are means and standard errors calculated from six peaches subjected to treatment. Variants with no statistical difference are indicated by common letters (a, b, c)

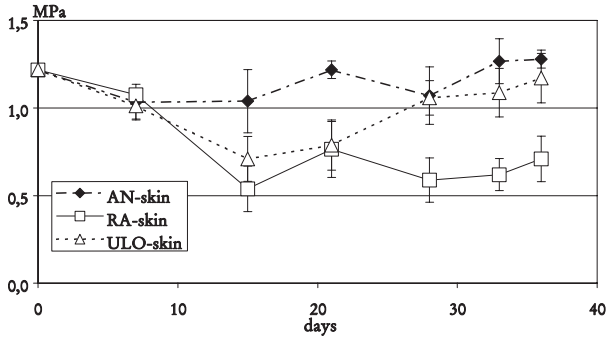

Fig. 3: Firmness of skin of peach fruit. Puncture tests were performed with a plunger (diameter: 8 mm) and recorded as deformation curve. Rupture point of skin coincides with these values. Firmness of skin of peach fruit stored in ULO (0.9 % O_2 plus 0.3% CO_2,), AN (0.2 % O_2 plus 0.3 % CO_2) and RA-condition (21 % O_2 plus 0.03 %CO_2)., respectively. ULO-and AN condition lasted 21 days, then the fruit were kept at cold-storage in air. Each point is the mean of 6 fruit with four punctures for each fruit. Vertical bars indicate SE, (P < 0.05)

Traces of ethanol are identified inside the fruit at the beginning of storage of peach fruit under aerobic conditions. They are at the level of few mg/l (Fig. 1). The gas mixture of the AN-treatment induced a strong accumulation of ethanol. Ethanol concentration depended on time, showing a linear increase to more than 600 mg/l approximately after 15 days of storage (Fig. 1). Only slight increases in the ethanol concentration were found in ULO- and RA-treatment.

Ethanal formation during storage is shown in Figure 2. In all experimental conditions an comparable increase of ethanal concentration is detectable, lowest levels are generally found in the AN-treatment.

After transferring the fruit to normal atmosphere, the concentration of ethanol decreased, but it seems that ethanol degradation started already during storage at controlled atmopshere (Fig. 1). These findings are in a slight contrast to BONGHI et al. (1999), who detected a slight increase in ethanol content a few days after picking. After storage under normal conditions (36 days) the final concentration of ethanol is still higher in fruit of the AN-treatment than in such of the ULO- and RA-treatment. Contrary to that, TONUTTI et al. (1998) described, that ULO-storage conditions caused a strong stimulation of ethanol and off-flavour formation.

In contrast to ethanol, ethanal concentrations reached their maximum at a late ripening stage after transferring to air (approx. after 30 days) without apparently competitive decrease of ethanol content at the same time. Stepwise accumulation of ethanal may be considered part of the ripening syndrome, but the differences between all treatments are not significant (Fig. 2). It is assumed that it is reduced to ethanol and/or reacts further to form ethyl acetate. Our results show tentatively that it is the rest of ethanol in the tissue which is subsequently degraded by oxidative metabolism.

Firmnes of peach fruit

Flesh firmness is an important parameter of fruit quality and the firmness patterns appear to differ among cultivars with internal variation in firmness for middle and outer regions of the mesocarp (MANESS et al., 1992). Mesocarp firmness delineates harvest maturity and directly influences bruise susceptibility and post-

Table 2:
Concentration of organic acids (g/l) at the beginning of storage and after 21 days in different oxygen regimes*

Treatment	Time days	Citric acid	Malic acid
IN	0	4.10 ± 0.14a	5.39 ± 0.23a
ULO	21	2.23 ± 0,12b	3.26 ± 0.10b
AN	21	4.91 ± 0.68a	5.61 ± 0.45a
RA	21	4.39 ± 0.32b	4.65 ± 0.32ab
ULO	36	3.64 ± 0.43ab	4.27 ± 0.43ab
AN	36	4.74 ± 0.67a	4.77 ± 0.49a
RA	36	3.88 ± 0.42b	4.38 ± 0.37ab
Two-way ANOVA : ** P< 0.01, * P< 0.05, ns = not significant			
Treatment		**	**
	Time	ns	ns
Treatment x time		ns	**

*Values are means and standard errors calculated from six peaches subjected to treatment.
Variants with no statistical difference are indicated by common letters (a, b, c)

harvest storability. On the basis of the deformation curve obtained by stressing peach fruit with a 8 mm plunger after storage we made following observations. The firmness of skin is affected by the oxygen content in the storage atmosphere (Fig. 3), lowest values were measured for fruit stored under ULO-conditions. Statistically significant losses of firmness were observed in the flesh of the fruit stored under RA-conditions (Fig. 4).

Concentration of sugars and organic acids

The concentrations of the main sugars (sucrose, glucose and fructose) and acids (citric and malic acid) were determined by HPLC-analysis of six replicate samples from each of the three storage variants. The ratio of sucrose, glucose and fructose was quite constant with the investigated cultivar, sucrose being prevalent (Table 1). During the whole experiment the disaccharid sucrose was hydrolysed enzymatically into monosaccha-

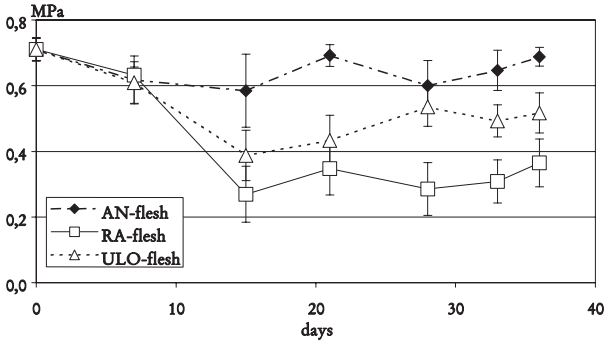

Fig. 4: Firmness of flesh of peach fruit at ULO, AN- and RA-conditions ULO (0.9 % O_2 plus 0.3% CO_2,), AN (0.2 % O_2 plus 0.3 % CO_2) and RA-condition (21 % O_2 plus 0.03 %CO_2), respectively. ULO-and AN condition lasted 21 days, then the fruit were kept at cold-storage in air. Each point is the mean of 6 fruit with four punctures for each fruit. Vertical bars indicate SE, ($P < 0.05$)

Table 3:
Content of titratable acidity (TA) and soluble solids (SS) at the beginning of storage and after 21 days in different oxygen regimes

Treatment	Time days	Titrable acid (g.100 g-1)	Soluble solids (°Rf)
IN	0	0.64 ± 0.02a	8.72 ± 0.26a
ULO	21	0.48 ± 0.02b	9.53 ± 0.13a
AN	21	0.48 ± 0.02b	9.20 ± 0.34a
RA	21	0.55 ± 0.04ab	9.43 ± 0.58a
ULO	36	0.35 ± 0.02c	9.42 ± 0.38a
AN	36	0.43 ± 0.02b	9.10 ± 0.18a
RA	36	0.40 ± 0.02bc	9,78 ± 0.44a
Two-way ANOVA : ** P< 0.01, * P< 0.05, ns = not significant			
Treatment		*	ns
	Time	**	ns
Treatment x time		ns	ns

*Values are means and standard errors calculated from six peaches subjected to treatment.
Variants with no statistical difference are indicated by common letters (a, b, c)

rids and therefore not consumed. Significant differences were found in glucose and fructose during storage under different oxygen regimes (Table 1). Highest concentrations of measured sugars were detected after AN-treatment. The concentration of organic acids in this cultivar was an equal proportion of citric and malic acids and this ratio was not changed markedly during storage (Table 2). Metabolic consumption during storage was slowest under anaerobic conditions with a good retention of acid content. Interestingly enough the final concentration of citric acid and malic acid was higher than the initial concentration. Under ULO- and RA- conditions significant decreases of acid concentrations took place.

Storage conditions had a statistically significant influence on losses of titrable acidity and concentration of malic and citric acid (Table 3).

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