

Storage of sweet cherries in low oxygen and high carbon dioxide atmospheres

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ABSTRACT: Acetaldehyde and ethanol are already present in detectable levels at the time of harvest, under aerobic conditions and in healthy, undamaged fruit. Both metabolites can be detected, at different concentrations, in all cultivars. Several hours after harvest, the levels of acetaldehyde in cultivars Summit, Těchlovan and Kordia were 6.41, 9.78 and 22.00 mg/l, respectively. Both ethanol and acetaldehyde accumulate to significant levels in anaerobically stored cherries, particularly in atmospheres with high levels of CO₂. The highest levels of ethanol observed, after 31 days of exposure to anaerobic conditions, were in the cultivars Těchlovan (1,159 mg/l) and Summit (1,168 mg/l); both concentrations are broadly similar. The metabolites decreased after a return to aerobic conditions, but remained higher than the levels first observed. Sweet cherries stored in anaerobic conditions are also sensitive to the development of off-flavours in the first 24 hours after opening the storage box. The very slow ripening of the fruit under anaerobic conditions was satisfactorily quantified by measuring the higher degree of fruit firmness, when the usual, visual attributes of ripeness were almost undetectable. Stems also remained green, in contrast to the usual browning that occurs under normal atmospheres. Discrimination analysis of various parameters observed gave a good resolution of different cultivars.

Keywords: ethanol; acetaldehyde; firmness; sweet cherry; off-flavour

Sweet cherries are highly perishable, non-climacteric fruits. Their shelf-life is shortened by loss of firmness, discoloration of the stem, desiccation, and moulds such as blue mould (*Penicillium expansum*), gray mould (*Botrytis cinerea*) and Rhizopus rot (*Rhizopus* spp.). Maintaining lower fruit temperatures immediately after harvest results in firmer fruit with reduced decay and greener stems (SCHICK, TOIVONEN 2002). The optimum relative humidity for the storage of sweet cherries was reported to be between 90 and 95% (HEVIA et al. 1998; ALIQUÉ et al. 2005; CHEN et al. 1981). However, stem browning continues to be a problem for sweet cherry marketing. Temperature and humidity are two factors that have been implicated in the stem browning of sweet cherries. Relative humidity in this range is particularly important in maintaining green color of the stem. From a practical viewpoint, a strict control of temperature is the best tool for prolonging the shelf-life of sweet cherries. TIAN et al. (2004) compared controlled atmospheres (CA) with a high oxygen concentration to CA with a high carbon dioxide concentration; the latter better controlled

flesh browning and delayed fruit senescence, as well as reduced fruit decay and extended the storage life of sweet cherries. Low oxygen levels slow the rate of metabolic conversion and maintain fruit quality for longer than normal air storage. Fruit fermentation occurs when ambient oxygen levels fall below some critical level, which is typically shown by an increase in respiratory quotient (RQ: the ratio of mols of CO₂ produced divided by mols of O₂ utilized), ethanol production, or both (PESIS 2005). Undesirable responses include the induction of fermentation, development of disagreeable flavors, reduction in aroma biosynthesis, induction of tissue injury and alteration in the makeup of microbial flora (BEAUDRY 1999; MEHERIUK et al. 1995). High levels of CO₂ are also useful for the prevention of chlorophyll degradation in a number of tissues, but can have a negative effect on other metabolic processes.

The main objectives of this study were to determine the influence of low oxygen and high carbon dioxide atmospheres on sweet cherries in cold storage; specifically on the formation of the volatile by-products of fermentation with respect to determining the po-

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tential of ultra-low oxygen (ULO) storage as a practical technique for extending sweet cherry shelf-life.

MATERIAL AND METHODS

Fruit preparation

Sweet cherries (*Prunus avium* cv. Těchlovan, Summit and Kordia) with stems attached were hand harvested on July 6, two days before the optimal harvest stage, from the commercial orchard Agro Stošíkovice at the Horticultural Faculty in Lednice. The fruits were sorted to exclude those with obvious defects or dissimilar states of maturity, based primarily on their appearance. Selected fruits were then transported into small boxes and during two hours they were chilled to 3°C before placing in the various gas mixtures.

Preparation of the atmospheres

A. Time under special gas mixture conditions:

31 days with 100% relative humidity in hermetically sealed, 450 litre chambers.

Storage atmosphere variants:

Variation 1: ultra low oxygen (ULO) (0.9% O₂, 0.5% CO₂)

Variation 2: anaerobic (AN) (0.3% O₂, 0.5% CO₂)

Variation 3: controlled atmosphere (CA) (1.5% O₂, 11.5–12.0% CO₂)

Variation 4: polyethylene box (PE) (6.0–15.0% O₂, 9.0–12.0% CO₂)

Variation 5: regular (normal) air (RA) (21% O₂, 0.03% CO₂).

B. Subsequent storage period (equating to “shelf-life”):

21 days in 3.0–3.3°C cold storage, with air of regular (normal) composition.

Preparation of atmospheres: Immediately after cooling, the final atmosphere composition was obtained by flushing with nitrogen. To eliminate the possibly excessive influence of CO₂ as a respiration inhibitor, the chambers were connected to absorption tubes filled with solid tablets of KOH, which were mixed with an inert material (polystyrene). Gas mixtures were monitored twice a day, using a dual CO₂/O₂ analyzer (Arelco, ARC, France).

Ethanol and acetaldehyde levels

The cherries were temporarily stored in a freezer and then defrosted prior to analysis. The juice produced was filtered (25 mm diameter syringe filter, 0.2 µm nylon with glass, Alltech Associates Inc.,

Belgium). 1 µl aqueous samples were injected into a sample block fitted with Teflon, and analyzed with a gas chromatograph equipped with FID (Chrom 5, Laboratory Equipment, Prague). Separation was achieved on a packed column (Porapak P, 3 mm i.d., 120 cm length), gas flow rates were 50 ml/min for H₂, 12 ml/min for He and 300 ml/min for air, respectively, and FID was used for analysis. The column was maintained at 92°C. Ethanol and acetaldehyde readings were quantified using commercial standards and expressed in mg/l for each of the compounds.

Firmness measurement

Skin and flesh firmness of the intact fruit were measured using a universal testing machine, Texan 2000 (constructed at the Mendel University of Agriculture and Forestry Brno), which records the degree of compression and the rate of loading. The testing machine was set up with a load of 30 kPa. A steel plunger of 5 mm diameter was pressed into the fruit at a rate of 8 mm per minute, and the resulting force-deformation curve was plotted by an *x-y* recorder. The break in the curve indicates the puncture point when the plunger breaks the skin, and measures skin firmness, and the sudden decrease in force measures flesh firmness.

Evaluation of fruit and off-flavour

The list of sensorial terms included 5 descriptors chosen by the assessors to describe differences between the two varieties, two days after the storage period in the gas mixtures was finished. The fruit was rated for each descriptor on a scale from 1 (low intensity) to 9 (high intensity). As for the term “fermented” (or “alcoholic”), fruits were classified as either “not fermented” and scored 9 points, or “fermented” and scored 1 point, with no intermediate scores allowed. Evaluations were conducted in a panel room equipped with individual stations and white incandescent lighting. At each sensory test, four samples were evaluated at room temperature, in duplicate by a panel of 12 judges. Most panel members had had some training in the sensory evaluation of fruit. Microbiological spoilage was calculated by subtracting the percentage of damaged fruits after the storage in the gas mixture, from that of the subsequent ventilated phase of cold storage, which is effectively their storage life. Cherries exposed to regular air (RA) treatments were rated daily for signs of mould or rot on the fruit surface, or apparent physiological breakdown such as softening, splitting and juice loss. The fruit exposed to the various gas storage mixtures were sealed for 31 days in airtight

Table 1. Anaerobic metabolites and textural parameters at the beginning of storage and after 31 days in different oxygen and carbon dioxide regimes, followed by 52 days in air for the cvs. Těchlovan and Summit

	Act	EtOH	Skin	Flesh	Toughness
Cultivar	**	*	ns	**	**
Treatment	**	**	**	ns	**
Time	**	**	**	**	**
Cultivar × treatment	**	**	*	*	ns
Cultivar × time	*	ns	**	ns	ns
Treatment × time	**	**	*	ns	ns
Cultivar × treatment × time	**	ns	ns	ns	ns

ANOVA: ** $P < 0.01$, * $P < 0.05$, ns – not significant

Values are means and standard errors calculated from five sweet cherry fruits subjected to RA, ULO, CA and AN treatments

Table 2. Loss in weight of sweet cherry cultivars (%) after gas mixture storage (31 days) and further “shelf -life” storage (45 days)

Cultivar	Treatment	31 st day	45 th day
Těchlovan	ULO	0.39	5.52
	AN	1.55	6.77
	CA	1.7	8.01
	RA	8	12.51
Summit	ULO	0.83	3.46
	AN	0.91	3.72
	CA	0.92	3.96
	RA	5.97	8.23
Kordia	PE	2.46	4.92
	RA	7.18	8.6

containers, but then evaluated in the same way. The shelf-life of each treatment was determined as the number of days after opening the containers until the first visible signs of deterioration.

RESULTS AND DISCUSSION

Origin of metabolites in sweet cherries at harvest

Acetaldehyde and ethanol are already present in detectable levels at the time of harvest, under aerobic conditions and in healthy and undamaged fruit. Both metabolites can be detected, at different concentrations, in all cultivars. Several hours after harvest, the levels of acetaldehyde in the cultivars Summit, Těchlovan and Kordia were 6.41, 9.78 and 22.00 mg/l, respectively (Tables 1 to 3). Traces of ethanol inside the fruit at the beginning of storage under aerobic conditions were at a level of 44.71, 30.00 and 36.43 mg/l for the cultivars Summit, Těchlovan and Kordia, respectively (Tables 4 to 6). However, no differences were observed between these three cultivars at the

end of the aerobic “shelf-life” storage period, following the treatments in various gas mixtures. If they are endogenously produced in fruit during ripening, as precursors of natural aroma compounds, they might be important for postharvest fruit quality. The internal concentrations of acetaldehyde and ethanol

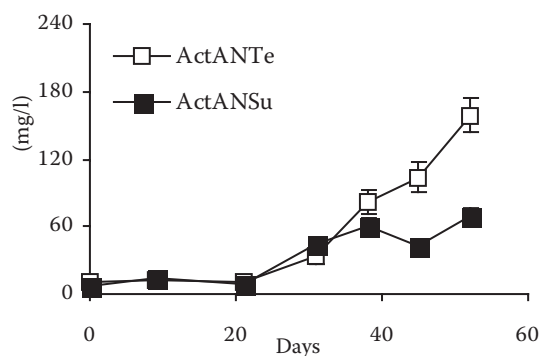


Fig. 1. Changes over time in acetaldehyde (Act.) concentration in pulp of sweet cherry cvs. Summit and Těchlovan, exposed to AN (anaerobic atmosphere 0.1% O₂ and 0.5% CO₂). Each point is the mean of 6 repetitions. Vertical bars indicate standard error

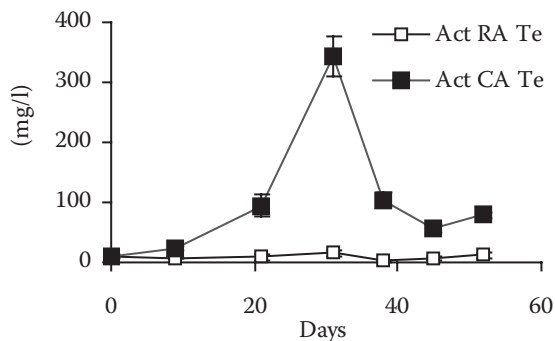


Fig. 2. Effect of O_2 level on the acetaldehyde (Act.) in pulp of cv. Těchlovan by RA (regular atmosphere 21% O_2 and 0.03% CO_2) and CA (controlled atmosphere 1.5% O_2 and 11.5–12.0% CO_2) at 3.0–3.3°C for 52 days. Each point is the mean of 6 repetitions. Vertical bars indicate standard error

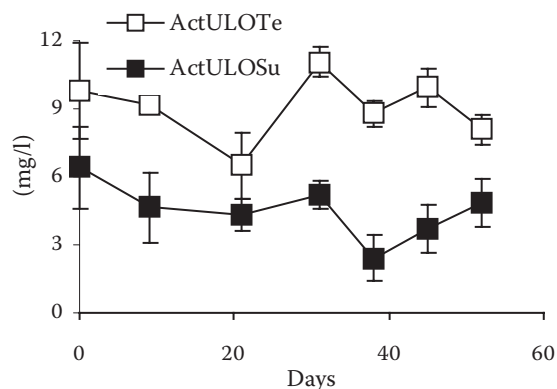


Fig. 3. Changes over time in acetaldehyde (Act.) concentration in pulp of sweet cherry cvs. Těchlovan and Summit exposed to ULO (ultra low oxygen 0.9% O_2 and 0.5% CO_2) at 3.0–3.3°C for 52 days. Each point is the mean of 6 repetitions. Vertical bars indicate standard error

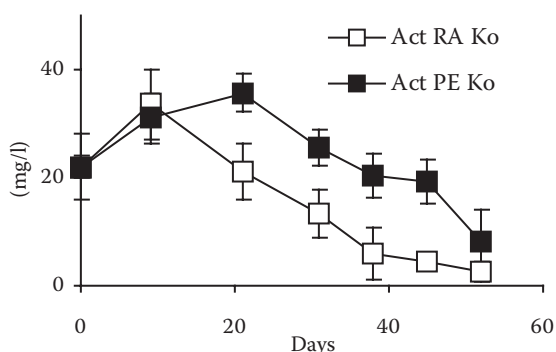


Fig. 4. Changes over time in acetaldehyde (Act.) concentration in pulp of sweet cherry cv. Kordia exposed to RA (regular atmosphere 21% O_2 and 0.03% CO_2) and CA (controlled atmosphere 1.5% O_2 and 11.5–12.0% CO_2) at 3.0–3.3°C for 52 days. Each point is the mean of 6 repetitions. Vertical bars indicate standard error

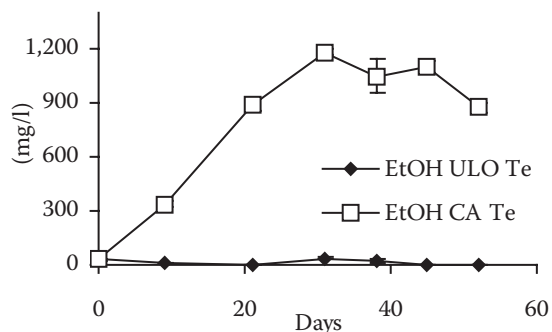


Fig. 5. Changes over time in ethanol (EtOH) of sweet cherry cv. Těchlovan when exposed to CA (controlled atmosphere 1.5% O_2 and 11.5–12.0% CO_2) and ULO (ultra low oxygen 0.8% O_2 and 0.5% CO_2) at 3.0–3.3°C for 52 days. Each point is the mean of 6 repetitions. Vertical bars indicate standard error

in sweet cherries, after 32 days of storage under normal atmosphere of air, were not significantly higher than those at the time of harvesting (GOLIÁŠ et al. 2003).

Levels of metabolites at physiologically undamaging atmospheres

In general, the establishment of the lower O_2 limit for the storage of fruits was established empirically, via a gradual decrease in the partial pressure of O_2 until an intolerable storage damage occurred. For sweet cherries, the practical use of ULO to suppress ethanol production, as a means of extending shelf-life, may be limited, since little or no reduction was accomplished without concurrent fermentation and the accumulation of anaerobic off-flavours related to the production of ethanol and acetaldehyde.

The lower O_2 limit for long-term storage of sweet cherry cultivars was established as 0.9% (Figs. 5 and 6).

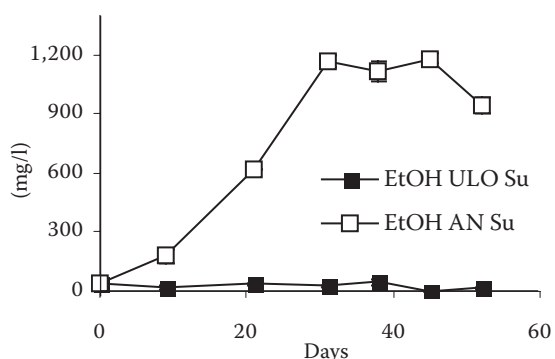


Fig. 6. Changes over time in ethanol (EtOH) of sweet cherry cv. Summit when exposed to AN (anaerobic atmosphere 0.1% O_2 and 0.5% CO_2) and ULO (ultra low oxygen 0.8% O_2 and 0.5% CO_2) at 3.0–3.3°C for 75 days. Each point is the mean of 6 repetitions. Vertical bars indicate standard error

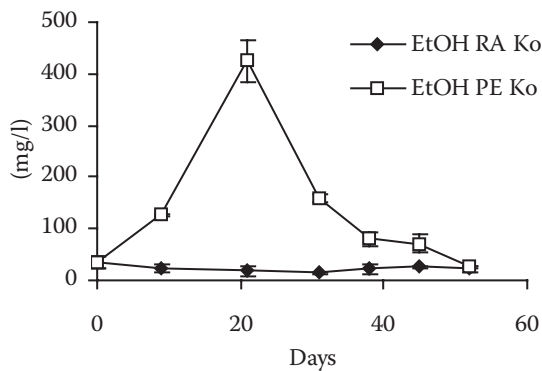


Fig. 7. Changes over time in ethanol (EtOH) of sweet cherry cv. Kordia when exposed to RA (regular atmosphere 21% O₂ and 0.03% CO₂) and PE (polyethylene film box – 8% O₂ and 11% CO₂) at 3.0–3.3°C for 52 days. Each point is the mean of 6 repetitions. Vertical bars indicate standard error

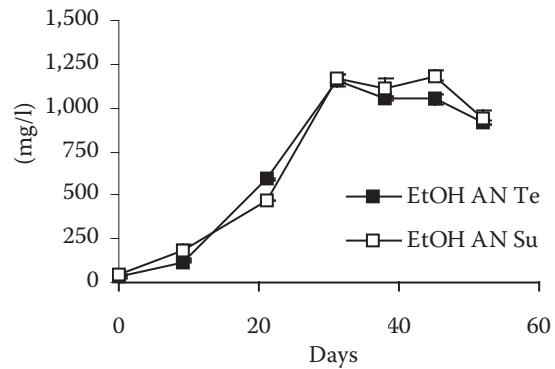


Fig. 8. Changes over time in ethanol (EtOH) of sweet cherry cvs. Summit and Těchlovan when exposed to AN (anaerobic atmosphere 0.1% O₂ and 0.5% CO₂) at 3.0–3.3°C for 52 days. Each point is the mean of 6 repetitions. Vertical bars indicate standard error

The method measures the ethanol concentration in whole fruits under long-term storage conditions at low oxygen levels. The information can be used to establish the lower O₂ limit as O₂ levels become limiting for aerobic respiration. At the same time, it is possible to evaluate the simultaneous addition of CO₂ in a prepared gas mixture. GRAN and BEAUDRY (1993), however describe a method for determining the lower O₂ limit that is rapid, relatively simple, requires a minimal number of fruits and is based on the measurement of physiological responses rather than empirical observations.

The results of the experiments with different cherry cultivars showed very low levels of these metabolites, with no significant difference observed between RA and ULO treatments (Figs. 5, 6 and 7). BEAUDRY (1993) showed a close correlation between the ethanol content in the headspace atmospheres of containers of whole fruits and the ethanol concentrations in their tissues.

Formation of acetaldehyde and ethanol by the fermentation process

Both ethanol and acetaldehyde significantly accumulate in anaerobically stored cherries, particularly at higher CO₂ atmospheres (Figs. 1 to 8). The ethanol concentration is also suitable for estimating the minimum oxygen level. Ethanol levels were dramatically different after anaerobic (AN) storage, compared to ULO and RA storage (Figs. 6 and 7). The concentration of oxygen in anaerobic conditions is a result of anaerobic glycolysis. The O₂ level at which fermentation starts and ethanol tends to accumulate was named the Pasteur point, but more recently has been referred to as the lower oxygen limit (LOL) (BEAUDRY 1993) or fermentation induction point (FIP) (PETRACEK et al. 2002). The response of the cultivars Těchlovan and Summit to the 0.2% oxygen level during 31 days was practically the same, assessed as the rate of accumulation of ethanol in the

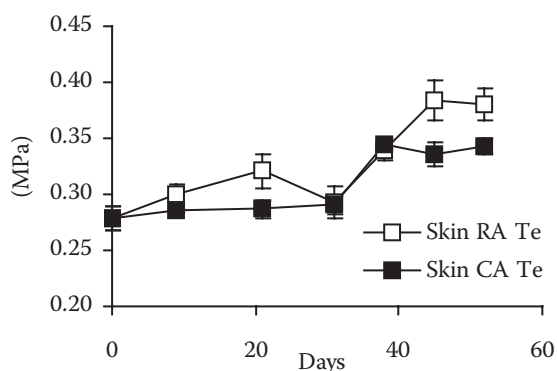


Fig. 9. Changes over time in skin firmness of sweet cherry cv. Těchlovan when exposed to RA (regular atmosphere 21% O₂ and 0.03% CO₂) and CA (controlled atmosphere 1.5% O₂ and 11.5–12.0% CO₂) at 3.0–3.3°C for 52 days. Each point is the mean of 12 repetitions. Vertical bars indicate standard error

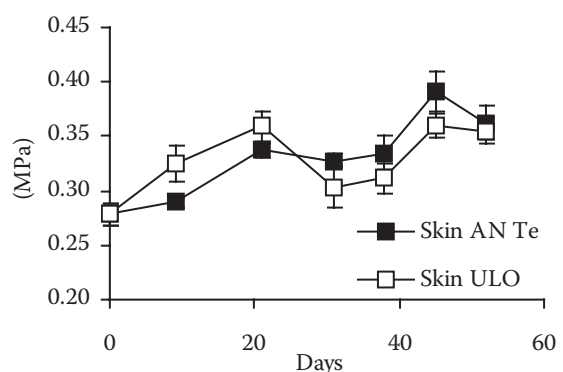


Fig. 10. Changes over time in skin firmness of sweet cherry cv. Těchlovan when exposed to AN (anaerobic atmosphere 0.1% O₂ and 0.5% CO₂) and ULO (ultra low oxygen 0.8% O₂ and 0.5% CO₂) at 3.0–3.3°C for 75 days. Each point is the mean of 12 repetitions. Vertical bars indicate standard error

flesh of the fruit (Fig. 8). The highest levels of ethanol after 31-days' exposure to anaerobic conditions were in Těchlovan (1,159 mg/l) and Summit (1,168 mg/l), which are broadly similar. Ethanol and acetaldehyde decreased under aerobic conditions (21% O₂ and 0.03% CO₂), but remained higher than the original levels observed.

Appearance of off-flavours in fruit with advanced fermentation

The build-up of anaerobic conditions in fruit exposed to very low oxygen levels in the surrounding atmosphere leads to enhanced anaerobic respiration and increased production of off-flavour volatiles, such as ethanol and acetaldehyde. Sweet cherries stored in AN conditions are sensitive to the development of off-flavours in the first 24 hours after opening the storage box (Fig. 15). The fruit released volatiles through the skin because although the pulp contained 1.16 g/l of ethanol and 0.4 g/l acetaldehyde, their concentration did not dramatically fall during the next 10 days. It is presumed that the sharp smell emanating from whole fruit was generated by compounds with low molecular weight. Under these same anaerobic conditions, MATTHEIS et al. (1997) observed that some alcohols, including butanal, 2-butanone and pentyl acetate, were only detected after four weeks of storage. The sensory impact of off-flavours was more pronounced in the CA treatments than in the classical AN storage conditions (Fig. 15).

Effect of high CO₂ concentrations on the levels of acetaldehyde and ethanol

Fermentative metabolism in fruit exposed to high CO₂ concentrations may occur due to the inhibition

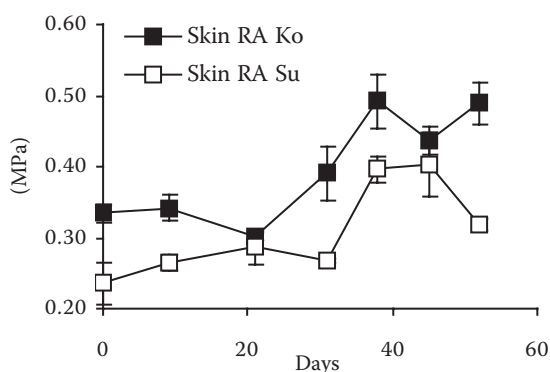


Fig. 11. Changes over time in skin firmness of sweet cherry cvs. Summit and Kordia when exposed to RA (regular atmosphere 21% O₂ and 0.03% CO₂) at 3.0–3.3°C for 52 days. Each point is the mean of 12 repetitions. Vertical bars indicate standard error

of tricarboxylic acid (TCA) cycle enzymes, particularly succinate dehydrogenase, and the subsequent increase in pyruvate decarboxylase and alcohol dehydrogenase activity (OR et al. 2000). Qualitative and quantitative changes in ester production, particularly ethyl acetate, were coincident with the accumulation of ethanol (MATTHEIS et al. 1992, 1997). The gas mixture containing 1.5% O₂ and 11.5–12.0% CO₂ was harmful, but the CO₂ concentrations were too high (Figs. 2 and 5). These findings are in contradiction to previous findings by TIAN et al. (2001).

Storage of sweet cherry in a polyethylene plastic film box (PE treatment)

Comparing PE (6–15% O₂, 9–12% CO₂) and CA (1.5% O₂, 11.5–12.0% CO₂) treatments, the CA treatment, with CO₂ concentrations practically identical to the PE treatment, inhibited ethanol production more significantly, results being one half the PE treatment values (Figs. 4 and 7). Fluctuating O₂ concentrations between 6% and 15% during every 24 hours brought no additional effect on the stability of fruit metabolism, but the effect of high CO₂ levels was attenuated. This has no significance for practical commercial use.

Effects of different atmospheres on quality attributes

The percentage of rots as well as the overall acceptability can be considered as indicators of the shelf-life of cherries. At the same time, detailed studies of the compositional and sensory aspects are necessary to complement and strengthen our understanding of the effect of post-harvest treatments on shelf-life. The percentage of rots and

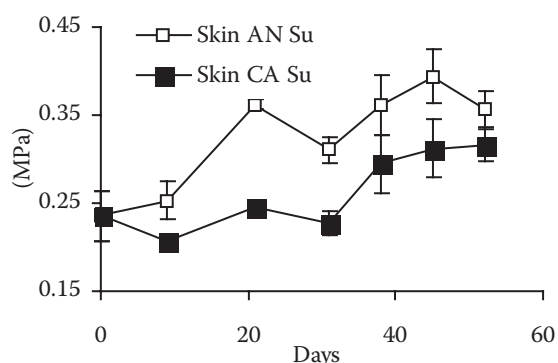


Fig. 12. Changes over time in skin firmness of cv. Summit when exposed to AN (anaerobic atmosphere 0.1% O₂ and 0.5% CO₂) and CA (controlled atmosphere 1.5% O₂ and 11.5–12.0% CO₂) at 3.0–3.3°C for 52 days. Each point is the mean of 12 repetitions. Vertical bars indicate standard error

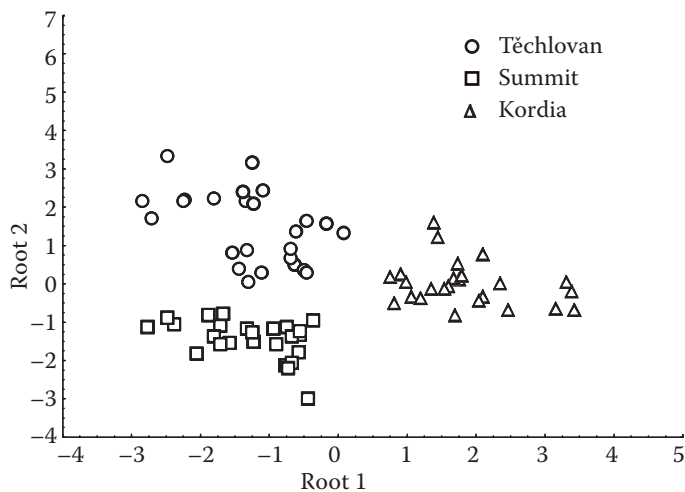


Fig. 13. Discrimination analysis of cultivars for both physiological and physical parameters in fruits in RA (regular atmosphere 21% O₂ and 0.03% CO₂)

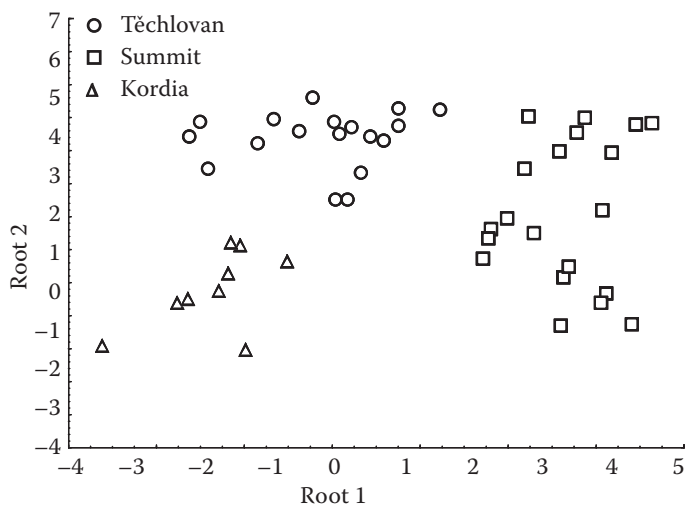


Fig. 14. Discrimination analysis of cultivars for both physiological and physical parameters in fruits in RA (regular atmosphere 21% O₂ and 0.03% CO₂) after 31 days

overall acceptability in terms of taste, were assessed at the end of the 31-day period in the various gas mixtures. There were no detectable signs of rot in any of the treatments. The AN fruits had very good quality, with the exception of off-flavour attributes. Under AN conditions, almost all signs of ripening were inhibited and the stems remained green, in contrast to the RA treatments where stem browning was apparent.

Firmness evaluation in storage atmospheres

The firmness of sweet cherries under all storage treatments slightly decreased or remained the same. The higher values for the RA variant were influenced by the evaporation of water from the fruit, but corresponded with the loss in weight. The fruit RA treatments had fivefold greater loss in weight than the other treatments after 31 days of storage in gas

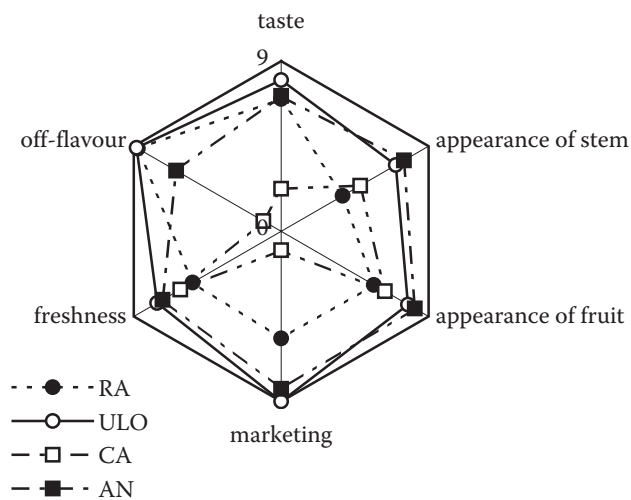


Fig. 15. Sensory evaluation of sweet cherry cv. Summit for all treatments, after 31 days

mixtures. A very slow ripening of the fruit in the AN treatments was reflected by higher firmness (Figs. 10 and 12).

Statistical analysis of cultivars and treatments

Figs. 13 and 14 illustrate the differences between cherry cultivars, four treatments and five parameters. The differences were established using five different parameters, i.e. the levels of acetaldehyde and ethanol, firmness of skin, freshness and toughness of sweet cherries, by forward stepwise discrimination analysis for 31 days of storage in various gas mixtures and normal air. The good results of the three cultivars using various physiological and physical parameters reflect different characteristics of the cultivars under investigation.

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Vliv nízkého O₂ a vysokého CO₂ obsahu v okolní atmosféře skladovaných třešní na vznik anaerobních metabolitů a dalších znaků zrání

ABSTRAKT: V aerobních podmínkách je v době sklizně ve zdravých a nepoškozených plodech stanovitelný obsah acetaldehydu a etanolu. Oba metabolity je možné určit v různých koncentracích u všech odrůd. Několik hodin po

sklizni byl obsah acetaldehydu u odrůd Summit, Těchlovan a Kordia 6,41, 9,78 a 22,00 mg/l. K významnému nahromadění acetaldehydu a etanolu dochází v anaerobních podmínkách, a to především při vysoké koncentraci CO₂. Po otevření skladovacích nádob 31. dne byl nejvyšší detekovaný obsah etanolu u odrůdy Těchlovan (1 159 mg/l) a u odrůdy Summit (1 168 mg/l), přičemž obě koncentrace jsou srovnatelné. Při uložení plodů v aerobních podmínkách obsah etanolu postupně klesal, přesto však jeho hodnoty zůstaly vyšší než hodnoty původní. Třešně skladované v anaerobních podmínkách jsou velmi citlivé na vznik cizí příchutě, která se projevila v prvních 24 hodinách po otevření skladovacích nádob. Zpomalené zrání zavedením anaerobních podmínek se projevilo nejen zpomaleným měknutím plodů, ale byly zastaveny i další procesy zrání, které se prokázaly zelenou barvou stopky ve srovnání se zcela zhnědlou stopkou u plodů skladovaných v atmosféře s normálním obsahem kyslíku. Na základě různých parametrů byly diskriminační analýzou vyhodnoceny jednotlivé skladované odrůdy.

Klíčová slova: etanol; acetaldehyd; pevnost; třešně; cizí příchut'

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