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Effects of modified atmospheric cool storage conditions on the quality of sweet cherries

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Effects of modified atmospheres during cold storage (2.5 to 3 °C) were monitored for 28 days with the two sweet cherry varieties 'Vanda' and 'Kordia'. The three atmospheric variants were CA (controlled atmosphere: 7 % CO_2 with 2 % O_2), ULO (ultra low oxygen: 0.2 to 0.5 % CO_2 with 1.0 to 1.2 % O_2) and RA (regular atmosphere: 0.03 % CO_2 with 21 % O_2). At harvest the acetaldehyde concentrations in the pulp were 4.6 ± 0.3 mg/l for 'Vanda' and 4.2 ± 0.35 mg/l for 'Kordia', respectively. Acetaldehyde levels showed little response to the different atmospheric conditions, thus they are no useful means for the determination of physiological changes. The ethanol contents of the fruit at harvest were 13.4 ± 1.8 mg/l for 'Kordia' and 6.4 ± 1.2 mg/l for 'Vanda', respectively. Under CA conditions the slow increases of the ethanol contents - up to levels comparable to those of the ULO and RA variant - were similar with both varieties. With all three storage variants no off-flavours were detected in the fruit. Skin firmness of fruit stored at RA increased during the second half of the storage period, fruit stored at CA and ULO were fresh and showed no signs of wilting. Significant decay reduction was observed with ULO and CA, while RA storage was less effective. Soluble solids content (SSC) of fruit was not significantly affected by the different modified atmospheres. After the end of the storage under ULO and CA conditions a slower decrease in the levels of organic acids in the pulp was most obvious with 'Vanda'. 'Vanda' maintained a green stem throughout the whole storage period but the stems of 'Kordia' shrivelled and turned brown their full length, although the fruit remained visually attractive. Weight losses during the subsequent shelf-life storage were higher in fruit stored at RA than in ULO and CA storage.

Keywords: sweet cherries, controlled atmosphere (CA), ultra low oxygen storage (ULO), ethanol, acetaldehyde, organic acids

***Auswirkungen atmosphärisch modifizierter Kühllagerung auf die Qualität von Süßkirschen**. Die Auswirkungen unterschiedlicher atmosphärischer Bedingungen während der Kühllagerung (2,5 bis 3 °C) auf Süßkirschen der Sorten 'Vanda' und 'Kordia' wurden über 28 Tage aufgezeichnet. Es wurden drei Varianten untersucht: CA (7 % CO_2, 2 % O_2), ULO (0,2 bis 0,5 % CO_2, 1,0 bis 1,2 % O_2) und RA (Normalatmosphäre: 0,03 % CO_2, 21 % O_2). Zum Zeitpunkt der Ernte betrug die Acetaldehydkonzentration im Fruchtfleisch 4,6 ± 0,3 mg/l bei 'Vanda' bzw. 4,2 ± 0,35 mg/l bei 'Kordia'. Die Acetaldehydkonzentrationen reagierten aber nur geringfügig auf die unterschiedlichen atmosphärischen Lagerbedingungen, und sind daher kein geeignetes Maß zur Bestimmung physiologischer Veränderungen. Die Ethanolgehalte der Früchte betrugen bei 'Kordia' zur Ernte 13,4 ± 1,8 mg/l und bei 'Vanda'6.4 ± 1.2 mg/l. Unter CA-Bedingungen stiegen die Ethanolgehalte bei beiden Sorten ähnlich langsam an - bis sie vergleichbare Konzentrationen wie die ULO- und RA-Varianten erreichten. Bei keiner der drei Lagerungsvarianten wurden in den Früchten Geschmacksfehler festgestellt. Bei der RA-Variante nahm die Schalenfestigkeit während der zweiten Hälfte der Lagerungsdauer zu, Früchte aus CA- und ULO-Lagerung waren frisch und zeigten keine Welkeerscheinungen. ULO- und CA-Lagerung bewirkten eine signifikante Reduktion der Fäule, während die RA-Variante weniger Wirkung zeigte. Die Trockensubstanzgehalte (SSC) der Früchte wurden durch die unterschiedlichen Varianten nicht signifikant beeinflusst. Am Ende der Lagerung unter ULO- and CA-Bedingungen war die langsamere Abnahme der Gehalte organischer Säuren im Fruchtfleisch bei 'Vanda' am deutlichsten er-*

kennbar. Bei 'Vanda' blieben die Stiele während der gesamten Lagerdauer grün, bei 'Kordia' trockneten sie jedoch ein und wurden über ihre ganze Länge braun,obwohl die Früchte optisch weiterhin attraktiv erschienen. Während der anschließenden Shelf-life-Phase zeigten Früchte aus RA-Lagerung höhere Gewichtsverluste als jene der ULO- und CA-Varianten.
Schlagwörter: Süßkirschen, CA-Lagerung, ULO-Lagerung, Ethanol, Acetaldehyd, organische Säuren

Les effets du stockage au frais sous atmosphère modifiée sur la qualité de cerises douces. *Les effets de conditions atmosphériques différentes au cours du stockage au frais (de 2,5 à 3 °C) sur les cerises douces des variétés 'Vanda' et 'Kordia' ont été enregistrés pendant 28 jours. Trois variantes ont été étudiées: CA (controlled atmosphere: 7 % de CO_2 avec 2 % d'O_2), ULO (ultra low oxygen : de 0,2 à 0,5 % de CO_2 avec 1,0 à 1,2 % d'O_2) et RA (regular atmosphere : 0,03 % de CO_2 avec 21 % d'O_2). Au moment de la récolte, les concentrations d'acét- aldéhyde dans la pulpe étaient de 4.6 ± 0.3 mg/l pour 'Vanda' et de 4.2 ± 0.35 mg/l pour 'Kordia'. Les concentrations d'acétaldéhyde n'ont cependant réagi que faiblement aux différentes conditions atmosphériques du stockage, elles ne sont donc pas très utiles pour la détermination des modifications physiologiques. Au moment de la récolte, les teneurs des fruits en éthanol s'élevaient à 3.4 ± 1.8 mg/l pour 'Kordia' et à 6.4 ± 1.2 mg/l pour 'Vanda'. Sous les conditions CA, les augmentations lentes des teneurs en éthanol - jusqu'aux niveaux comparables à ceux des variantes ULO et RA - étaient similaires pour les deux variétés. Pour les trois variantes de stockage, on n'a pas constaté la présence d'«off-flavours» dans les fruits. La fermeté de la peau des fruits stockés sous RA a augmenté au cours de la seconde moitié de la période de stockage; les fruits stockés sous CA et ULO étaient frais et ne présentaient aucun signe de flétrissement. Une réduction significative de la pourriture a été constatée sous ULO et CA, tandis que la variante RA était moins efficace. La teneur en matière solide soluble (TMS) des fruits n'a pas été influencée de façon significative par les différentes variantes. Après la fin du stockage sous les conditions ULO et CA, la baisse lente des teneurs en acides organiques dans la pulpe a été la plus évidente chez 'Vanda'. 'Vanda' a conservé une tige verte pendant toute la durée du stockage; en revanche, les tiges de 'Kordia' ont flétri et bruni sur toute leur longueur, bien que le fruit ait conservé son aspect attrayant. Les pertes de poids au cours du stockage shelf-life subséquent ont été plus importantes pour les fruits stockés sous RA que pour ceux stockés sous ULO et CA.*
Mots clés : cerises douces; stockage sous CA; stockage sous ULO, éthanol, acétaldéhyde; acides organiques

The shelf-life of fresh sweet cherries is limited to about 14 days, if handled carefully and temperatures do not exceed 2 °C. Post-harvest losses of quality are essentially due to degeneration and browning of the stem, softening, loss of acidity and texture, off-flavours, and reduction of the nutritive value (Vidrih et al., 1998; Crisosto et al., 1993; Kappel et al., 2002; Petracek et al., 2002; Crisosto et al., 2003; Tian et al., 2004). An edible coating based on Aloe vera gel has beneficial effects in terms of delaying stem browning, dehydration, and deterioration of the visual aspects of the fruit, without any detrimental effect on taste, aroma or flavours (Martínez-Romero et al., 2006). The effectiveness of post-harvest treatments using CO_2 enriched atmospheres to control fungal decay in fruit has been demonstrated by many researchers (Ceponis and Cappellini, 1985; Ke et al., 1991). Wilson et al. (1987) showed that high CO_2 levels significantly inhibited disease development in fruit and considered the resistance of fruit to rot under high CO_2 storage conditions to be due to the production of high levels of acetaldehyde and ethyl acetate. But the two standard physiological responses of sweet cherries to increased CO_2 and decreased O_2 levels, that have received considerable attention, are the maintenance of firmness and development of off-flavours, associated with acetaldehyde, ethanol and ethyl acetate production (Goliáš and Böttcher, 2004).

The objective of this study was to investigate the effects of different storage atmospheres (ULO and CA) on the physiological properties, quality characteristics and storability of sweet cherry fruit, and to evaluate the relationship between ethanol and acetaldehyde content on external and internal fruit quality.

Materials and Methods

Degree of ripeness and preparation of fruit prior to storage

Fruit of two sweet cherry varieties ('Vanda' and 'Kordia') were harvested from an orchard belonging to the Agrosad Stošíkovice Company in South Moravia during the two or three days of optimum ripeness determined by visual evaluation. Fruit were transported (ap-

prox. two hours) to the post-harvest laboratory of the Mendel University in Lednice. The fruit were then sorted quickly to remove any underripe, overripe, or damaged fruit and then transferred into hermetically closed chambers of about 450 litres in volume, for cooling at 2.5 to 3 °C for three hours before the atmospheres were modified. All chambers were equipped with inlet and outlet ports, through which they were flushed with N_2 and CO_2 to achieve the required atmospheres, as follows:
CA (controlled atmosphere): 2.0 to 2.2 % O_2 + 6.8 to 7.0 % CO_2
ULO (ultra-low oxygen): 1.0 to 1.2 % O_2 + 0.2 to 0.5 % CO_2
RA (regular atmosphere): 21 % O_2 + 0,03 % CO_2
The gas mixtures were maintained for 28 to 31 days. The CO_2 and O_2 levels in the chambers were monitored with a precision of 0.1 % (Dual gas analyser O_2/CO_2, model TS12E; Arelco ARC, Fontanay, France) every few minutes initially, and every half day thereafter.

Determination of ethanol and acetaldehyde contents

The ethanol levels in the liquified pulp were determined by directly injecting a 1-µl-sample of the filtered juice into a gas chromatogram (Chrom 5, Laboratory Equipment, Praque) equipped with a flame ionization detector (FID) and a metal column (3 mm I.D. x 1.2 m) filled with Porapak P (Waters Ass. Inc., Framingham, Mass. USA). The experimental conditions were: 85 °C oven temperature, 120 °C injector temperature and 100 °C detector temperature, carrier gas He 12 ml/min . The components were identified individually by comparing retention times against standards, concentrations being estimated by external calibration using a regression equation based on four samples of standard concentrations.

Soluble solids content and acidity determination

After firmness measurement fruit were crushed, and frozen to a temperature of -25 °C. Immediately before analysis, samples were thawed and filtered (25 mm diameter syringe filter, 0.45 µm nylon with glass Alltech Associates Inc., Belgium).
A soluble solids concentration (SSC) was determined in the juice from five individual fruit from each variant with a digital refractometer (Toruni, Italy) at 20 °C and results were expressed as the means ± SE of °Brix. The pH-value of the juice was recorded and then titratable acidity (TA) was determined by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 10 ml of diluted juice in 25 ml distilled H_2O and results were the means ± SE expressed as grams of malic acid equivalent per 100 g of fresh weight.

Microbiological decay

A scale of five categories, from 5 (sound fruit) to 1 (diseased fruit), was used to evaluate fruit decay, which was assessed as the percentage of fruit (sample: 2 kg) showing any type of decay (decay organisms not identified). Assessments were made after the end of storage at different atmospheres and gradually until the definitive end of storage.

Firmness determination and mass loss

For each fruit, firmness was determined using a penetrometer (Texan 2000, manufacturer: Mendel University Brno) interfaced to a PC. Three different firmness parameters were evaluated: firmness of skin, firmness of pulp and toughness of pulp. All three parameters of fruit firmness were measured using a cylindrical punch (diameter: 5 mm, tapering to 3 mm), which was inserted to a depth of 3 mm and the resulting force deformation curve was plotted. The break in the curve indicates the puncture point when the plunger breaks the skin (a measure of skin firmness) and the sudden decrease in force observed provides a measure of flesh firmness. The area under the deformation curve measures toughness (the work of compression done by loading to the rupture point). For both varieties, results are expressed as the means ± SE of determinations made for individual fruit (n = 12, two measurements for each fruit).
The weight of individual lots was recorded at the beginning (day 0) and again at several sampling dates. Cumulative weight losses were expressed as the percentage loss from the original weight.

Fruit quality

For stems and berries, symptoms of dehydration and browning were rated on a scale from 5 to 1 (5 = absence of symptoms, 4 = slight occurrence, 3 = moderate, 2 = severe, 1 = extremely severe browning and dehydration). Taste, off-flavour and market value were evaluated on a scale from 5 to 1 (5 = zero defects, 1 = unsaleable). A panel of tasters were offered an ample number of samples (n = 100) after storage at different atmospheres.

Statistical analysis

Results were processed by analysis of variance as a three-factor linear model (ANOVA). Sources of variation were time of storage, storage atmospheres and the interaction 'storage atmospheres × storage'. The results were separated using the least significant difference method. Differences at p = 0.01 were considered to be significant.

Results and Discussion

Changes in levels of metabolic compounds

In sound fresh fruit and under regular atmosphere storage conditions, a low-level anaerobic respiration produces small quantities of ethanol and acetaldehyde. Acetaldehyde concentrations in the pulp at harvest were 4.6 ± 0.3 mg/l for 'Vanda' and 4.2 ± 0.35 mg/l for 'Kordia', respectively. During storage these concentrations were identical for both varieties in ULO, whereas in CA and RA they consistently showed a slightly higher value (Fig.1 and 2). Acetaldehyde concentrations showed little response to the different modified atmospheres and thus are no useful parameters for the determination of physiological changes. The ethanol content of the fruit at harvest was 13.4 mg/l for 'Kordia' and 6.4 mg/l for 'Vanda', respectively. There was no significant difference in ethanol levels between the two varieties (Fig. 3 and 4), although the levels were higher in 'Kordia' compared to 'Vanda'. Normal metabolism stress, e.g. insufficient O_2 and/or higher concentrations of CO_2 promotes anaerobic processes. In CA and ULO fruit, ethanol did not increase over time, and showed the same tendency as in RA (Fig. 3, 4 and 5). The higher levels of CO_2 in the CA variant did not promote the formation of ethanol, thus confirming that 7.0 % CO_2 had no negative effect on the fruit. Perhaps this moderate level of CO_2 inhibited formation of ethanol and acetaldehyde in the pulp (Fig. 1, 2 and 5), and was comparable to the effects of ULO on the two varieties. Off-flavours were detected neither in fruit stored in CA nor in fruit stored in ULO, probably because the ethanol concentration in the pulp did not reach the threshold level for sensory perception. The concentrations of ethanol which are necessary to cause detectable off-flavours vary widely between different fruit. For example, ethanol levels of 300 mg/l in apricots caused off-flavours (Folchi et al., 1995), whereas Tian (2000) found an accepta-

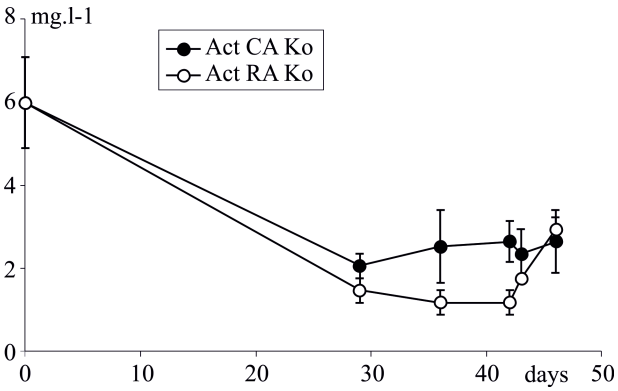

Fig. 1: Changes over time of acetaldehyde (Act) concentration in pulp of cv. 'Kordia' sweet cherries exposed to ULO and RA at 2.5 to 3 °C (each point represents the average of 12 repetitions and vertical bars indicate the standard errors)

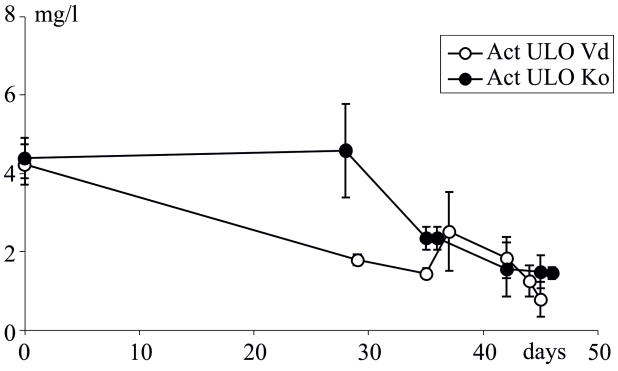

Fig. 2: Changes over time of acetaldehyde (Act) concentration in pulp of cv. 'Kordia' and cv. 'Vanda'exposed to ULO

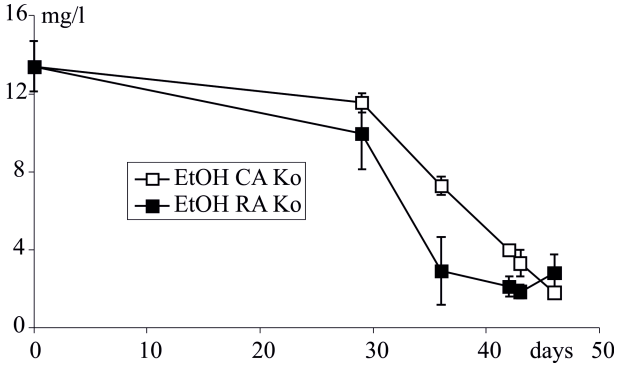

Fig. 3: Changes over time of ethanol (EtOH) concentration in pulp of cv. 'Kordia'exposed to CA and RA

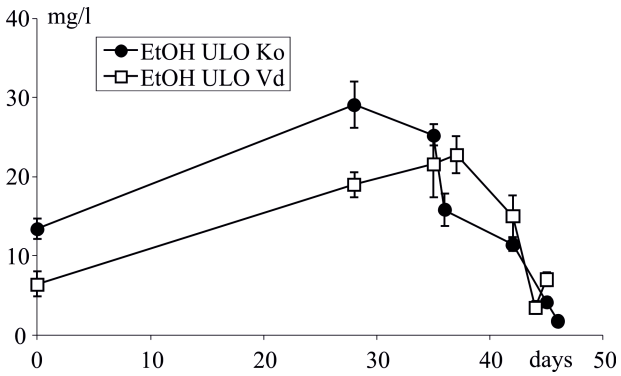

Fig. 4: Changes over time of ethanol (EtOH) concentration in pulp of cv. 'Kordia' and cv. 'Vanda'exposed to ULO

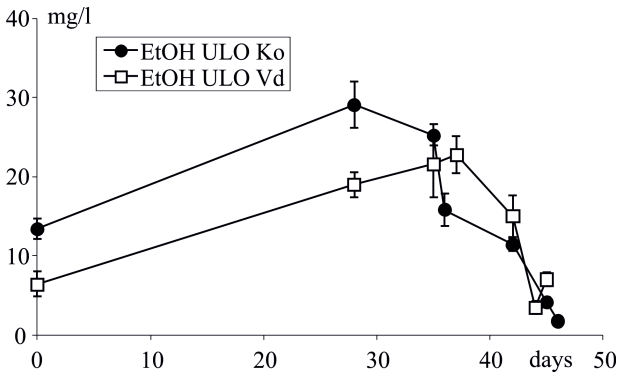

Fig. 5: Changes over time of ethanol (EtOH) concentration in pulp of cv. 'Kordia' and cv. 'Vanda'exposed to CA

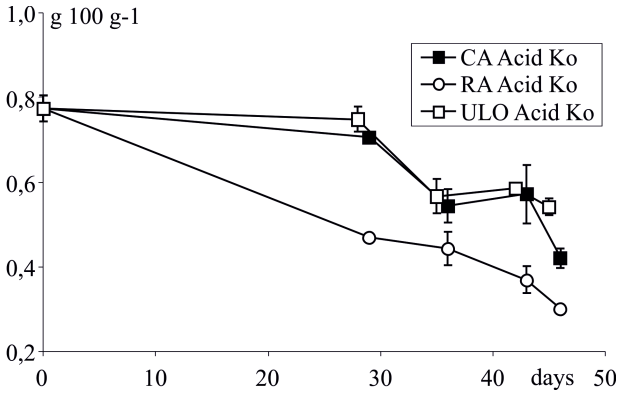

Fig. 6: Changes over time of titratable acid (g/100 g) in pulp of cv. 'Kordia' exposed to ULO, RA and CA (each point represents the average of 6 repetitions and vertical bars indicate standard errors)

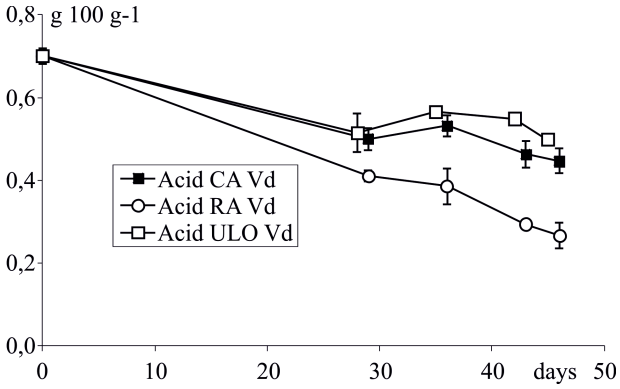

Fig. 7: Changes over time of titratable acid (g/100 g) in pulp of cv. 'Vanda' exposed to ULO, RA and CA

ble taste in sweet cherry fruit stored in anaerobiosis and containing ethanol levels as high as 5 g/l. Other important parameters, such as SSC, play a role in the absorption of off-flavours (Ke et al., 1991).

Firmness of sweet cherries

The softening process in sweet cherries has been reported to be dependent on an increase in polygalacturonase, β-galactosidase and pectinmethylesterase activity (Batisse et al., 1996; Gerardi et al., 2001), and this is responsible for the loss in fruit quality. But the maintenance of firmness could also be related to the lower weight losses. Skin firmness of sweet cherries stored at RA increased in the second half of the storage period (Fig. 10 and 11), where the mass losses were higher and the reduction of fruit turgor lead to an increase in skin deformation compared to fruit of ULO and CA storage. There were no significant differences observed between the two varieties regarding skin firmness (Table l). Nevertheless, by means of discriminant analysis, the features of fruit stored under RA created a separate cluster (Fig.12 and 13). On this basis, other parameters, such as pulp firmness and toughness of fruit seem to be important. This was found by comparing the investigated storage variants. The skin firmnesses of CA and ULO fruit were more similar to each other than to that of RA fruit (Fig. 10 and 11). Again, as shown by Watkins et al. (1999), the differences in firmness observed in strawberry fruit was associated with the stronger adhesion between cells of CO_2-treated fruit compared to RA fruit.

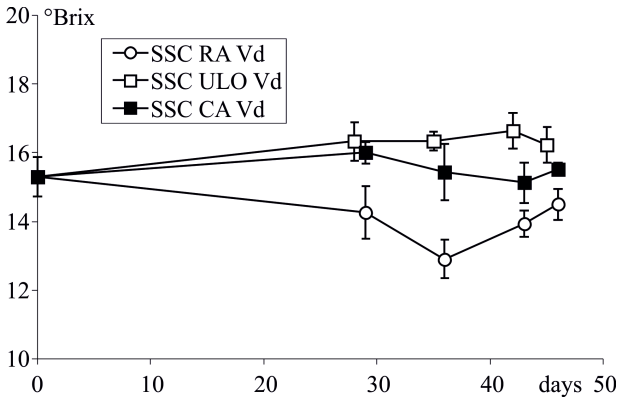

Fig. 8: Changes over time of soluble solid concentration (SSC in °Brix) in pulp of cv. 'Vanda' exposed to ULO, RA and controlled atmosphere CA

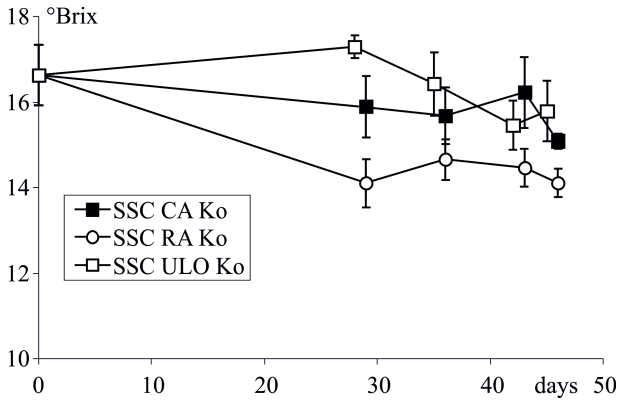

Fig. 9: Changes over time of soluble solid concentration (SSC in °Brix) in pulp of cv. 'Kordia' exposed to ULO, RA and CA

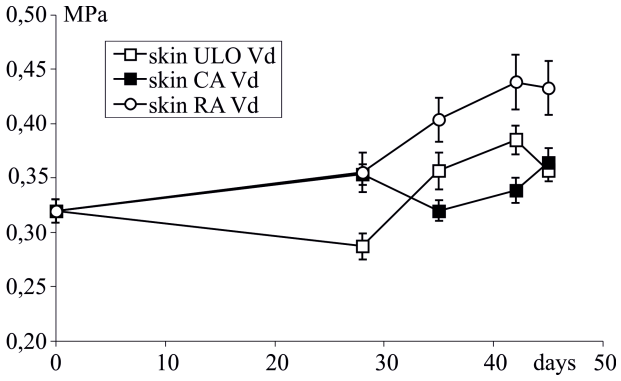

Fig. 10: Changes over time of firmness of skin (MPa) in pulp of cv.'Vanda' exposed to ULO, RA and CA (each point represents the average of 12 repetitions and vertical bars indicate standard errors)

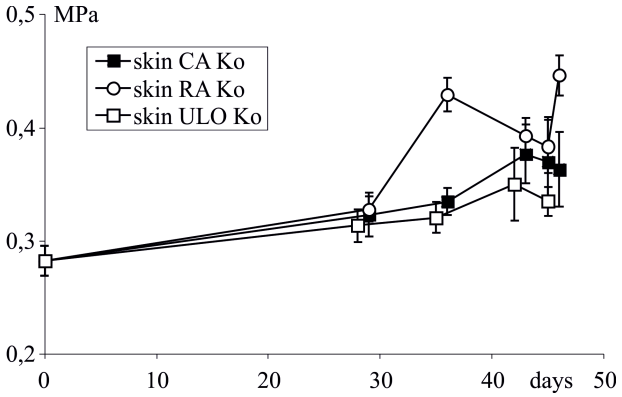

Fig. 11: Changes over time of firmness of skin (MPa) in pulp of cv.'Kordia' exposed to ULO, RA and CA

Microbiological decay

Storage under ULO respectively CA conditions was effective to reduce post-harvest rots of sweet cherries, while storage at RA was not effective (Table 3). ULO storage delayed ripening of fruit and reduced its susceptibility to pathogens. In contrast, the ULO storage with a CO_2 concentration not higher than 0.5 % did not completely prevent microbiological decay, in spite of the very low oxygen content CA storage of 'Vanda' showed very little microbiological decay over the whole storage period, but the fruit of the variety 'Kordia' were more susceptible to superficial microorganisms. However the observed differences in susceptibility to microbial colonization and spoilage may be explained by possible differences in the microbial concentrations on the surface of the fruit (Venturini et al., 2002). Other mechanisms may also be involved in the reduction of decay, for it is known that slight stress conditions in plants can induce resistance to invasion by microbes (Lurie, 1998; Feng et al., 2004).

SSC and acidity during storage

The reduction of the ripening index during storage was related to the reduction of TA while the content of SSC was unchanged during storage, irrespective of treatments. Soluble solids contents were not significantly affected by different atmosphere treatments (Fig. 8 and 9). It is widely accepted that the most important parameters determining consumer acceptance for sweet cherry are a bright red colour, firmness, and the SSC/TA ratio (Crisosto et al., 2003). The commonly used index of cherry quality at harvest, the

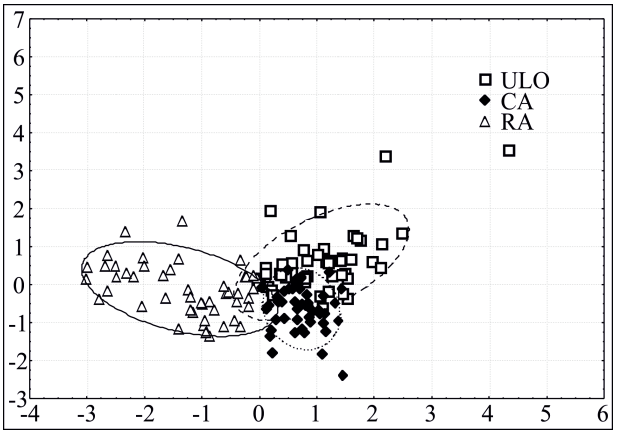

Fig. 12: Discriminant analysis of observed skin and flesh firmness and toughness for cv. 'Vanda' stored at ULO, CA and RA conditions (each point represents the average of 48 observations on individual fruit)

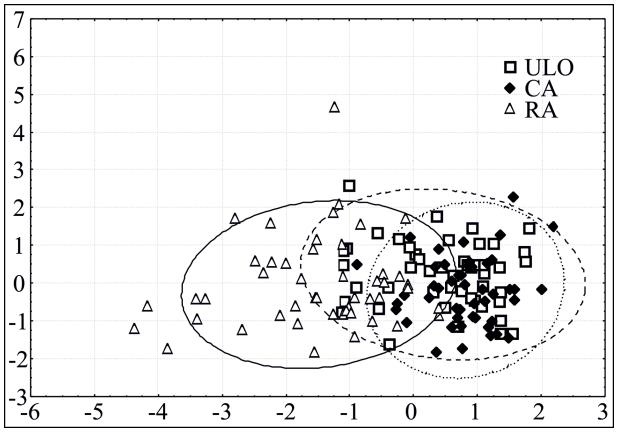

Fig. 13: Discriminant analysis of observed skin and flesh firmness and toughness for cv. 'Kordia' for ULO,CA and RA treatments (each point represents the average of 48 observations)

Table 1: Textural value and content of SSC and organic acids in two cultivars ('Kordia' and 'Vanda') under different storage conditions

	SSC	Acids	Skin firmness	Flesh firmness	Toughness
Cultivar (cv)	ns	**	ns	**	**
Treatment	**	**	**	ns	n.s.
Time	*	**	**	**	**
Cv x treatment	ns	**	*	*	n.s.
Treatment x time	ns	**	**	**	n.s.
Time x cv	**	**	*	ns	*
Cv x treatment x time	ns	**	ns	ns	*

Two-way ANOVA: ** P < 0.01, * P < 0.05, n.s. = not significant

Table 2: Mass loss (%) of the sweet cherry cultivars 'Kordia' and 'Vanda' after the opening of storage containers (on the 28th resp. 29th day) followed by a 46 days long storage at RA

Time (days)	Treatment	Kordia	Vanda
28	ULO	0.1	1.2
35		2.5	7.8
42		4.5	10.0
45		6.9	10.5
29	CA	1.9	0.9
36		4.3	2.7
43		7.8	5.2
46		9.4	7.6
29	RA	1.5	2.2
36		5.2	5.1
43		9.4	6.5
46		11.5	8.9

Table 3: Microbiological decay (%) of fruit of the sweet cherry cultivars 'Kordia' and 'Vanda' after the opening of gas mixtures followed by a 46 days long storage at RA

Time (days)	Treatment	Kordia	Vanda
28	ULO	1	0
35		2	6
42		4	7
45		4	7
29	CA	2	0
36		4	1
43		5	1
46		6	1
29	RA	2	1
36		4	2
43		6	4
46		7	5

SSC/TA ratio, is not useful for monitoring quality changes during storage, because there is a significant decrease in TA over time while the SSC levels remain relatively stable. During storage at RA the course of degradation of TA was nearly linear for both varieties. During shelf-life after ULO and CA storage, the retardation of the degradation in organic acids in the pulp was more obvious with 'Vanda' than with 'Kordia' (Fig. 6 and 7).

Overall quality

Visual appearance was rated the best after 28 days for fruit from CA storage. Stem color was the most sensitive of all the quality characteristics evaluated, and browning of the stem was a crucial factor in determining which storage conditions were more successful. 'Vanda' maintained a green stem throughout the whole storage period, but the stems of 'Kordia' shrivelled and turned brown their full length, although they remained very attractive visually. Like stem browning, dehydration is another typical consequence of storing sweet cherries (Clayton et al., 2003; Schick and Toivonen, 2002), and the loss in quality starts in the stems before any visible changes can be determined on the fruit. The main decline in economic value comes towards the end of the shelf-life, when visible wilting becomes apparent at the base of the stems where they join. Weight losses were the lowest at the end of the storage period, i.e. after 28 and 29 days (Table 2), with CA and ULO storage. Varying storage behaviour of fruit from different storage conditions during shelf-life was not investigated.

Conclusion

Quality characteristics of 'Vanda' and 'Kordia' cherries were influenced by modified storage atmospheres at constant temperatures. During storage ethanol and acetaldehyde contents differentiated little between the variants ULO, CA and RA. Higher CO_2 concentrations (7 %) with a sufficient supply of oxygen did not physiologically damage the fruit by forming anaerobic metabolites. No differences ($p = 0.01$) were found between the variants in acidity, firmness of flesh and SSC. At the end of CA and ULO storage no signs of decay were found in the fruit samples.

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