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Controlled Atmosphere Storage of Highbush Blueberries cv. 'Duke'

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Summary

The highbush blueberry cultivar 'Duke' was subjected to controlled atmosphere storage up to seven weeks under following CO_2 + O_2 conditions (kPa CO_2 + kPa O_2): 0.03+21 (=air, control); 6+18; 12+18; 18+18; 24+18; 12+2 and 18+2 at 0–1 °C storage temperature. The changes of ripening and quality parameters of the berries were monitored in 2–3 weeks intervals. In contrast to the experience with other fruits, blueberry fruits reacted to increasing CO_2 partial pressure (>12 kPa) with loss of firmness, regardless of the height of O_2 pressure. Only berries stored in 6–12 kPa CO_2 maintained their firmness on acceptable values. The titratable acidity concentration of the fruits from the different storage treatments reacted in a similar way: Increasing CO_2 partial pressure from 12 to 24 kPa resulted in an increasing loss of acidity. However, total soluble solids (TSS) of fruits did not differ significantly between all treatments in most sampling dates. Moreover, the respiration rate of fruits stored under increasing CO_2 levels was significantly higher than that of air-stored fruits. Respiration quotient (RQ) values by these fruits reflected an enhanced consumption of organic acids as respiration substrate. Incidence of fungal diseases, mainly *Botrytis cinerea*, could be efficiently controlled by higher CO_2 levels. In this respect, 6 kPa CO_2 proved to be not high enough to retard the fungal growth. Weight loss of berries was minimized by all storage conditions. The overall judgment of the effect of storage conditions on fruit quality through the consumer sensory test revealed that blueberry fruits could be kept under air conditions at 0–1 °C up to three weeks in acceptable condition. However, for a longer storage period up to six weeks, it is highly recommended to store highbush blueberries cv. 'Duke' under high CO_2 levels up to 12 kPa without O_2 reduction. Higher CO_2 level more than 12 kPa can not be recommended due to negative impact on flavour, firmness, and acidity content.

Zusammenfassung

Lagerung der Kulturheidelbeersorte 'Duke' in kontrollierter Atmosphäre. Kulturheidelbeeren der Sorte 'Duke' wurden während sieben Wochen bei folgenden CA-Bedingungen gelagert (kPa CO_2 + kPa O_2): 0.03+21 (=Luft, Kontrolle); 6+18; 12+18; 18+18; 24+18; 12+2 und 18+2 bei 0–1 °C Lagertemperatur. Die Änderung der Reife und Qualitätsmerkmale der Beeren wurde im Abstand von 2–3 Wochen untersucht. Im Gegensatz zu den Erfahrungen bei der CA-Lagerung mit anderen Früchten zeigten Heidelbeeren einen Festigkeitsverlust mit steigendem CO_2-Partialdruck (>12 kPa) in der Lageratmosphäre, unabhängig von der Höhe des Sauerstoffgehalts. Nur bei Beeren, die bei CO_2-Werten von 6 bzw. 12 kPa gelagert wurden, blieb die Festigkeit bei akzeptablen Werten. Die Konzentration an titrierbarer Säure in den Beeren verhielt sich ähnlich: Über 12 kPa steigende CO_2-Partialdrücke in der Lageratmosphäre führten zu einem zunehmenden Säureverlust. Dagegen ergaben sich im Gehalt der Beeren an löslicher Trockensubstanz zwischen den verschiedenen Lagervarianten an den meisten Untersuchungsterminen keine signifikanten Unterschiede. Die Atmungsrate der Beeren, die bei steigenden CO_2-Gehalten gelagert wurden, nahm ebenfalls im Vergleich zu den Kontrollfrüchten in Luft deutlich zu. Der Atmungsquotient (RQ) deutete auf einen verstärkten Säureverbrauch als Atmungssubstrat hin. Der Pilzbefall, hauptsächlich durch *Botrytis cinerea*, konnte mit steigenden CO_2-Werten wirksam kontrolliert werden, wobei 6 kPa CO_2 allerdings in der pilzhemmenden Wirkung noch zu gering war. Der Gewichtsverlust der Beeren konnte bei allen Lagervarianten minimal gehalten werden. Durch Verkostung der Beeren in einem Geschmackstest wurde eine Gesamtbeurteilung der Wirkung der Lagerbedingungen auf die Beerenqualität vorgenommen. Dabei wurde festgestellt, dass die Luft/1 °C-Kontrollfrüchte bis drei Wochen bei akzeptabler Qualität gelagert werden können. Eine längere Lagerung von Kulturheidelbeeren der Sorte 'Duke' bis zu 6 Wochen Dauer ist nur bei höheren CO_2-Werten von 12 kPa und ohne O_2-Absenkung möglich. Bei CO_2-Werten über 12 kPa muss mit zunehmend negativem Einfluss auf den Geschmack, die Festigkeit und den Säuregehalt der Beeren gerechnet werden.

Key words. *Vaccinium corymbosum* – quality parameters – firmness – acidity – TSS– taste

Introduction

Blueberries are appreciated for their content of biologically active substances, mainly flavonoids and phenolic acids (HÄKKINEN and TÖRRÖNEN 2000). Concerning the phenolic profile, it is reported that the main phenolics in blueberries are flavonols, mainly quercetin, hydroxycinnamic acid, and ellagic acid. (HÄKKINEN 2000). Moreover, STÖHR and HERRMANN (1975) found caffeic acid to be the main phenolic acid in blueberries.

Highbush blueberries (*Vaccinium corymbosum*) are cultivated widely in North America. Their cultivation in Europe is increased slowly, mainly due to their highly specific need for acidic soil. New cultivation techniques with special substrates combined with fertigation enable some fruit growers to produce blueberries independent of site conditions. Berries should be picked only when ripe, since unripe fruits remain sour and TSS does not increase during storage (PEANO et al. 2002).

The cultivar 'Duke' was selected in 1972 in Beltsville and is known to be an early one, which produces large, light blue, and firm fruits (VORSA 1998). Furthermore, 'Duke' was described as the best highbush variety offered at this time for fresh market and dessert quality frozen product

Blueberries are marketed mainly fresh, but increasing quantities are being frozen and canned. The sale prices of blueberries drops towards the middle of the harvest period. This encouraged many researchers to widen the harvest period by growing blueberries in greenhouses, tunnels and under rain covers (BAL 1997). Despite these new techniques, storage of blueberries remains the most effective method for stabilizing supply; cold storage is still considered as the most effective method for short-term storage. LOYOLA et al. (1993) stated that fruits of the blueberry cultivars 'Bluecrop' were cold-stored for 21 days without loss of fresh export quality. Moreover, KORON and SIMCIC (1996) concluded that berries cv. 'Bluecrop' could be stored for 28–42 days at 0.5 °C under normal atmosphere conditions. However, decay caused by *Botrytis cinerea,* the main cause of fruit rot, is the limiting factor of cold storage. Various researchers advocate the adoption of controlled atmosphere (CA) storage with increased CO_2-concentrations, particularly for a prolonged storage period (BORECKA et al. 1985). However, ROELOFS (1993) found for the cultivars 'Bluecrop' and 'Dixy' that 20 % CO_2 and 2 % O_2 maintained the quality of fruits for only 21 days, and extension of storage period to 56 days gave a bad quality under all CA-conditions tested. Moreover, KIM et al. (1995) concluded that the optimal storage conditions are 17–18 % CO_2 and 9 % O_2. Further, SONG and YAM (1995) found that initial atmosphere of 15 % CO_2 and 3 % O_2 instead of air generally reduces fruit loss.

The aim of this study was to investigate the storage potential of blueberry fruits cv. 'Duke' grown in Southwest Germany and to evaluate some fruit quality parameters, decay development, and consumer acceptability of CA stored berries.

Materials and Methods

Fruit material and storage conditions

Blueberry fruits (cv. 'Duke') were obtained from an orchard in the Lake of Constance area in Southwest Germany. Fruits were picked and selected for uniformity in size and colour, without decay and external injuries, and stored at the same day of picking. 250 g berries in three replicates for each sampling date were placed in 240 L containers, which were continuously monitored by gas analysers connected to a process computer. The storage temperature was 0–1 °C and the following CA-conditions were tested (kPa CO_2+ kPa O_2): 0.03+21; (air control); 6+18; 12+18; 18+18; 24+18; 12+2 and 18+2. The required gas compositions inside chambers were achieved within 12 hours.

Determination of fruit quality and ripening parameters

At harvest time and at 1–2 weeks intervals, three sub-samples from each storage condition, which served as three replicates, were obtained and analyzed for the following fruit quality parameters:

a) **Fruit firmness** using a nondestructive analyzer (FirmTech2, UP, Germany) especially designed for soft fruits, that measures the maximum weight needed to compress the fruit tissues for a certain distance. Readings obtained are in g/mm.
b) **Total soluble solids % (TSS)** determined in fruit juice by digital refractometer (Atago, Japan). Values obtained represent the total amounts of soluble solids solved in the fruit juice. 80–85 % of it are sugars.
c) **Titratable acidity (TA):** 10 ml of fruit juice were diluted with 100 ml distilled water and titrated with 0.1 N NaOH to pH 8.1 using pH-meter. Titratable acidity was calculated as citric acid and expressed in g L^{-1}.

Respiration measurement

Respiration of blueberry fruits was measured only once during the storage period after 28 days of storage. Fruit samples were removed from the CA-containers and adjusted to air conditions and room temperature. After 24 and 48 hours 120 g of fruits from each treatment were placed in glass jars in two replicates. The jars were tightly closed for eight hours and the respiration rate of CO_2-production and O_2-uptake was calculated from the difference in concentration of the gases before and after the enrichment period. All respiration measurements were conducted by a micro-GC (Chrompack, CP 2002P) with a thermal conductivity detector (TDC). The following columns for gas detection were used: O_2: Molsieve, 20 m, 0.15 mm i.d., 40 °C (isothermal); CO_2: Haysep, 25 cm, 45 °C (isothermal).

Sensory test

A taste panel with five persons on average conducted this test on fruit samples stored for seven weeks under different CA-treatments. The panelists looked for both visual quality criteria, such as fruit colour, and taste criteria, such as sweetness, acidity, crispness, and off-flavour. All tests were performed after a shelf-life period of eight hours at 20 °C in air. The visual properties (appearance, colour, injuries) and organoleptical impression were judged by numerical scores between 1 and 5 as follows: for decay: 1= no decay, and 5= strong infection; for colour: 1= green-red, fresh as at harvest time, and 5= dark red and overripe; for taste: 1= very good, and 5= very bad.

Weight loss and fruit decay

During storage, the weight loss due to transpiration and respiration of fruits was followed by weighing the same sample of fruits both at the beginning of the storage period and at sampling date for each treatment. At each sampling date, three boxes from each chamber were removed and weighted as quickly as possible to eliminate errors due to water condensation on the fruit surface. Furthermore, number of decayed fruits was counted and decay percentage was calculated as a percentage to the total number of fruits from each box.

Statistical analysis

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

Results

Quality parameters

High CO_2 levels (≥18 kPa CO_2) caused a rapid and continuous decrease of berry firmness during the entire storage period, in which the level of O_2 had no influence on firmness loss (Fig. 1). The softening of blueberries as affected by increased CO_2-concentrations in the storage atmosphere is completely different from that what we know from other fruits, where higher CO_2-concentrations reduce loss of firmness. On all sampling dates, fruits stored under 18+2, 18+18 and 24+18 CO_2 + O_2 had the lowest firmness values; even significantly lower than fruits from control treatment or from lower CO_2 levels (≤12 kPa). Fruits stored under 6+18 and under control conditions remained as firm as in the beginning of the storage period with the exception of reduced firmness values of control fruits at the end of storage after 7 weeks. However, blueber-

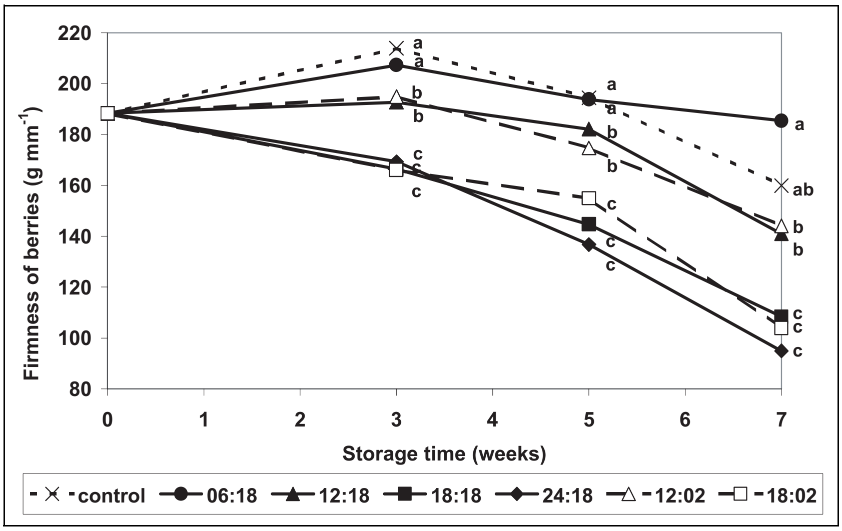

Fig. 1. The influence of storage conditions (kPa CO_2 + kPa O_2) on fruit firmness of highbush bluberry, cv. 'Duke' during a 7 weeks storage period at 0–1 °C. Different letters in column at the sampling dates indicate significant differences between storage treatments at P≤0.05.

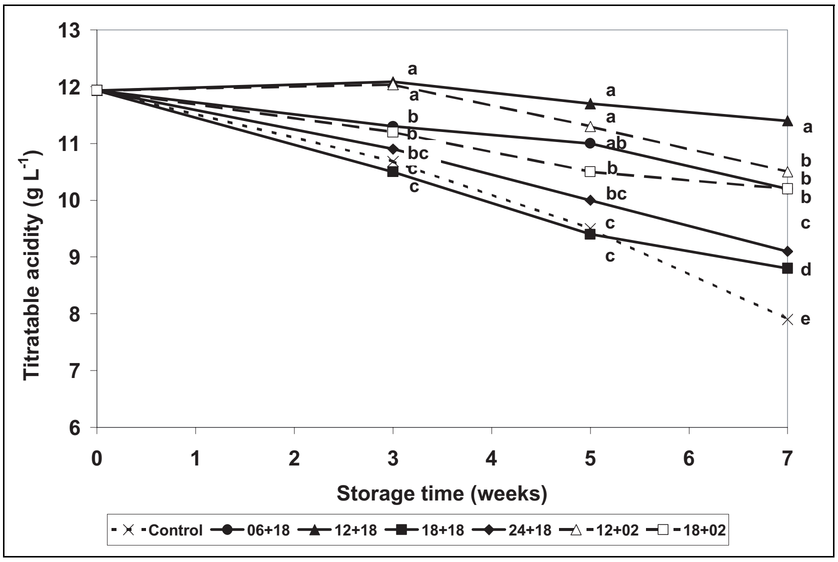

Fig. 2. The influence of storage conditions (kPa CO_2 + kPa O_2) on titratable acidity in fruits of highbush bluberry, cv. 'Duke' during a 7 weeks storage period at 0–1 °C. Different letters for values of the same storage time indicate significant differences between storage treatments at P≤0.05.

ries kept in 12 kPa CO_2 had an intermediate firmness value.

The titratable acidity concentration (Fig. 2) of the blueberries decreased continuously during the storage period. However, fruits from the different storage treatments reacted in different ways: CO_2 levels lower and higher than 12 kPa accelerated the loss of acidity in the fruits. The lowest acidity concentration was registered with fruits stored in air (control), and 18 and 24 kPa CO_2. High CO_2 combined with low O_2 seemed to reduce the loss of acidity. The best acidity retention was found in fruits stored in 12+18 CO_2 + O_2 condition.

Concerning the sugar content (~TSS) of the blueberries (Fig. 3) no significant differences were recorded among all CA storage treatment at all sampling dates, except with lower value of 18+18 CO_2 + O_2 treatment at the end of storage.

Incidence of fruit decay and weight loss

Fig. 4 shows the incidence of diseases, mainly *Botrytis cinerea,* in the blueberry fruits in response to various CA-conditions during storage time. The first decay occurred after 5 weeks in control fruits and those stored under 6+18 CO_2 + O_2 conditions. At the end of the storage period, fruits from all treatments were affected, but under higher CO_2 conditions to a significantly lower extent. Increasing CO_2 level to 12 % retarded the decay development, irrespective of O_2 level. The results indicate that keepability of 'Duke' blueberries is limited to 6 weeks also under CA-conditions, otherwise non-acceptable levels of decay will happen.

Weight losses during the storage period under the various storage conditions were not significantly different and remained within an acceptable range of 5 % till the end of storage time (data not shown).

Respiration rate and respiration quotient (RQ)

In contrast to the experience with other fruits, the respiration of 'Duke' blueberries increased with higher CO_2 and lower O_2 levels (Table 1). The respiration measurements of the fruits were done after 28 storage days, followed by a conditioning period of 24 hours at 20 °C in air. Fruits stored under very high CO_2 levels (18 and 24 kPa) respired at significantly higher rates than fruits at lower CO_2 levels (6 and 12 kPa), irrespective of O_2 level. The lowest respiration rate was recorded with control fruits in air. The calculation of respiratory quotient (RQ) indicates that air stored fruits reached the highest RQ, both after 24 or 48 hours con-

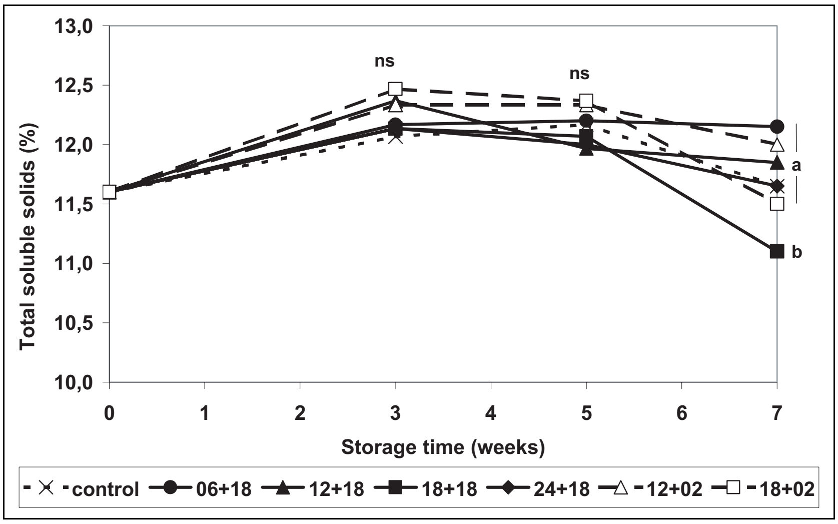

Fig. 3. The influence of storage conditions (kPa CO_2 + kPa O_2) on total soluble solids (TSS) in fruits of highbush bluberry, cv. 'Duke' during a 7 weeks storage period at 0–1 °C. Different letters for values of the same storage time indicate significant differences between storage treatments at P≤0.05; ns: non significant.

Table 1. The influence of storage conditions on respiration rate (mMol CO_2 kg^{-1} h^{-1}) and respiration quotient (RQ) of highbush blueberries, cv. 'Duke' at the 28th day of storage at 0–1 °C. Fruits were conditioned for 24 h and 48 hours at 20 °C before measurements.

Storage conditions	Respiration rate after 24 hours	RQ after 24 hours	Respiration rate after 48 hours	RQ after 48 hours
Control	0.51 c[1]	2.0 a	0.48 c	1.42 a
06+18	0.56 b	1.4 b	0.54 b	0.89 b
12+18	0.58 b	1.5 b	0.55 b	1.22 ab
18+18	0.66 a	1.1 b	0.60 b	1.26 a
24+18	0.67 a	2.2 a	0.60 b	1.02 ab
12+02	0.57 b	1.1 b	0.57 b	1.01 ab
18+02	0.65 a	1.0 b	0.65 a	1.14 ab

[1]Means within each column followed by different letters are significantly different at P≤0.05.

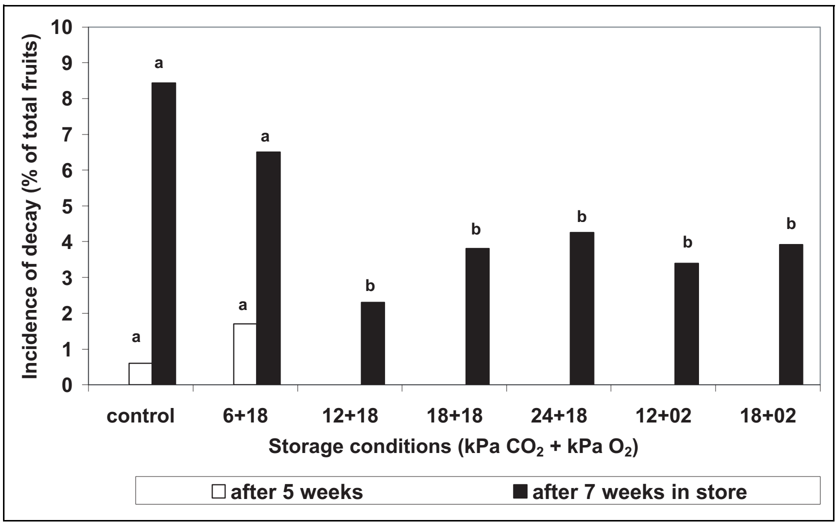

Fig. 4. Incidence of total fruit decay of highbush bluberry, cv. 'Duke' during a 7 weeks storage period at various CA conditions at 0–1 °C. Columns corresponding to the same storage duration, with different letters are significantly different, P≤0.05.

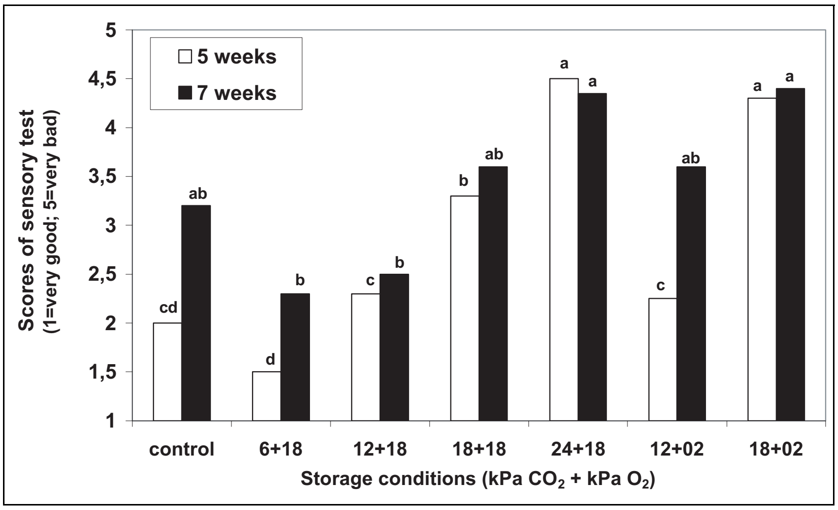

Fig. 5. Result of sensory tests with fruits of highbush bluberry, cv. 'Duke' after 5 and 7 weeks in store under various CA conditions at 0–1 °C. Columns corresponding to the same storage duration, with different letters are significantly different, P≤0.05.

ditioning period at 20 °C. These fruits used obviously their organic acids as the major substrate. High CO_2-conditions also led to the same behaviour, whereas decreasing O_2 down to 2 % led to RQ values close to 1, indicating that sugars served as the main substrate.

Sensory test

The taste of fruits stored for 5 weeks under air conditions (control) was judged as good, tasteful and firm as nearly fresh harvested fruits. However, these berries lost their acceptability with an additional extension of the storage period up to 7 weeks. Fruits stored under 6 kPa CO_2 combined with high O_2 level obtained best scores both after 5 and after 7 weeks in store. These blueberries had more acid taste, even after prolonged storage, and adequate aroma. Similarly good results, mainly after 7 weeks of storage, were obtained when fruits were stored under 12 kPa CO_2 without O_2 reduction. The storage with reduced O_2 levels increased the problem of off-taste. This could be observed especially with increasing CO_2 level (≥18 kPa) combined with high or low O_2. Fruits from these treatments were criticized due to off-taste, an accelerated softening, and mealy taste, already after 5 weeks in store. Data of external quality appearance are not given, since all fruits were judged as acceptable or good and no differences could be observed between various storage treatments.

Discussion

The benefit of CA-conditions on extending fruit life is well known since many years. However, fruit species differ in tolerance thresholds against an elevated CO_2 level and/or O_2 decrease in the storage atmosphere. Critical thresholds are dependent on many factors such as fruit type, stage of maturity, and duration of CA-storage. The described experiments with highbush blueberries cv. 'Duke' show, that the critical CO_2 level should lie between 12 and 18 kPa, whilst the reduced O_2-concentration seems to exert no crucial effect on berry storability. Storing fruits in 12 kPa CO_2-enriched atmospheres maintained fruit firmness and acidity content with a minimum of decay incidence. Also CAMERON et al. (1994) stated that suitable CA-conditions for highbush blueberries range from 5–12 kPa CO_2 and 1.5 to 2.5 kPa O_2 at 0 °C. However, increasing the

CO_2 level to 18 kPa or more resulted in an accelerated softening of fruits and coincided with an increased respiratory activity. This reaction of 'Duke' blueberries to elevated CO_2 levels is not widespread among fruit species or even among blueberry cultivars. SMITTLE and MILLER (1988) achieved the storage of Rabbiteye blueberry cv. 'Climax' for 42 days in 20 % CO_2 with 5 % O_2, and reported that quality of stored fruits were similar to freshly harvested berries. MEBERG (1999) stated that blueberries cv. 'Bluecrop' stored under high CO_2 level (5–20 %) with 10 % O_2, were firmer, but developed off-flavours. It is clear that there are differential responses among blueberry cultivars: 'Duke' may be considered as highly sensitive to high CO_2 levels (above 15 %). Further indication of the sensitivity of 'Duke' to high CO_2 levels is the highly accelerated degradation of organic acids. It is to assume that CO_2 injury leads to decompartmentation of cells, which causes leakage of enzymes from their compartments and a further increase in respiration rate. High respiration means an accelerated consumption of organic acids. WATKINS et al. (1997) reported that CO_2 injury with Bramley's apples occurred within a susceptible period soon after harvest, in which a transitory increase in respiration rate occurred. KREBEL et al. (1988) reported that high CO_2 level highly reduces the activities of ATP-phosphofructokinase and Ppi-phoshofructokinase and the content of fructose 1,6 bisphosphate in pear fruits. Our results clearly show that softening is coincided with increased respiration rate. ATP accumulates in plant tissues, while glycolysis proceeds. Concerning glycolysis, two pathways exist in plant cells: a maintenance pathway catalyzed by ATP-dependent phosphofructokinase that is inhibited by high concentration of ATP; and an adaptive path catalyzed by a pyrophosphate-dependent phosphofructokinase (SUZUKI et al. 2003). The adaptive pathway promotes glycolysis even in the presence of high levels of ATP. Moreover, the same adaptive pathway enables plants (or plant organs) to cope with environmental stresses (PURVIS 1997). Consequently, it is hypothesised that an enhanced glycolysis led to an increased amount of pyruvate that can be decarboxylized to acetaldehyde and reduced further to ethanol, which may reach harmful levels that caused the accelerated softening of fruits. In many studies it is mentioned that very low O_2 and very high CO_2 atmospheres caused accumulation of acetaldehyde and ethanol in tissues of harvested plants (KATO-NOGUCHI and WATADA 1996**).**

Another approach to explain the negative impact of very high CO_2 on fruit may be based on the interaction between elevated CO_2 and the antioxidants status of fruits. FOYER et al. (1997) stated that reactive oxygen species are implicated in most stress responses. In our experiments, very high CO_2 level could be considered as stress condition for the stored berries. However, the intrinsic potential danger of the reactive species is balanced by the presence of an endogenous detoxification system, termed antioxidants. Concerning the impact of a very high CO_2 level, various researchers found that elevated CO_2 has a depressive effect on the activity of oxygen species-scavenging and detoxifying enzymes (THIBAUD et al. 1995; TAUSZ et al. 1996). Moreover, XUAN (2003) stated that the antioxidant status in pear fruits was generally suppressed under elevated CO_2. Further indication of the involvement of antioxidants lies on the highly reduced ascorbic acid-content of highbush blueberries stored under very high CO_2 atmosphere (data not shown). AGAR et al. (1997) suggested that oxidation of ascorbate is stimulated under high CO_2 atmosphere; oxidation of ascorbate may render fruit tissue susceptible to CO_2-injury.

Concerning decay incidence, LANG and TAO (1992) considered decay percentage lower than 20 % as commercially tolerable, which cannot be accepted for European markets. Consequently, the results of the storage experiments clearly indicate that 'Duke' blueberries can sufficiently be stored for 5 weeks in cold storage at 0–1 °C as well as for 7 weeks in CA-storage with CO_2-atmosphere (6–12 %) without O_2 reduction.

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