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On the involvement of adenine nucleotides in the development of brown heart in 'Conference' pears during delayed controlled atmosphere storage

Über die Beteiligung von Adeninnukleotiden bei der Entstehung von Fruchtfleischverbräunungen in 'Conference' Birnen während der CA-Lagerung

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Summary

It is supposed that the development of CA-induced browning disorders in 'Conference' pears is related to the "energy charge" of the tissues which decreased during storage period (SAQUET et al. 2000). It is known that the delay in the establishment of the CA conditions can reduce the incidence of flesh browning in pears (HÖHN et al. 1996). This research was carried out to test the effectiveness of a delayed CA storage treatment (21 days at 0 °C and ambient atmosphere before CA storage) in order to adapt fruits to CA. Thereafter, fruits were stored for up to six months in 3% CO_2 + 1% O_2 at 0 °C. Fruits without delay were immediately stored under CA and developed 80%, 90% and 72% higher incidence of flesh browning, core browning and cavities, respectively, compared to delay-treated fruits. Fruits subjected to delayed CA showed higher ethylene production and higher respiration activity during the first two months of storage, and was associated with higher concentrations of ATP in the fruit tissue. Higher ATP levels remained until the fourth month of storage. A higher ATP:ADP ratio of delay-treated fruits was also found indicating a higher energy charge of tissue during this period. The results suggest that 'Conference' pears can become adapted to CA during the early stages of the storage period making them more resistant against brown heart. The possible role of ATP in maintaining membrane cell integrity under CA conditions and thus reducing browning disorders is discussed.

Zusammenfassung

Die Entstehung von Fleischverbräunungen in 'Conference' Birnen während der CA-Lagerung hängt vermutlich mit der Energieladung der Zellen zusammen, die wie schon berichtet, im Verlaufe der CA-Lagerung stark abnimmt (SAQUET et al. 2000). Es ist bekannt, dass eine verzögerte CA-Lagerung das Auftreten von Fruchtfleischverbräunungen bei Birnen mindern kann (HÖHN et al. 1996). Die vorliegende Untersuchung wurde durchgeführt mit dem Ziel, die Wirkung der verzögerten CA-Einstellung hinsichtlich Atmungsverhalten und Energiestoffwechsel zu untersuchen, um dadurch die verbesserte CA-Anpassung der Früchte zu erklären. Nach einer verzögerten CA-Einstellung (21 Tage bei 0 °C und Luft vor der CA-Einlagerung) wurden die Früchte während sechs Monaten unter 3% CO_2 + 1% O_2 bei 0 °C gelagert. Die Kontrollfrüchte wurden sofort unter CA gelagert. Diese entwickelten am Ende der Lagersaison im Vergleich zu den verzögert eingelagerten Birnen 80%, 90% bzw. 72% höheren Befall an Fleischbräune, Kernhausbräune bzw. Kavernen. Mit Verzögerung der CA-Einstellung zeigten die Birnen höhere Ethylenbildung und Atmungsintensität während der ersten zwei Monate Lagerung, was in Verbindung mit den höheren ATP-Konzentrationen im Gewebe gebracht wurde. Diese höheren ATP-Konzentrationen blieben bis zum vierten Monat Lagerung erhalten. Höhere ATP:ADP-Verhältnisse bei den verzögert eingelagerten Birnen wiesen auf einen höheren Energiezustand der Gewebe während dieser Zeit hin. Die vorhandenen Ergebnisse zeigen, dass sich 'Conference' Birnen an die CA-Lagerung während der beginnenden Stadien der Lagerung anpassen können, so dass sie toleranter auf die Verbräunungen reagierten als die sofort eingelagerten Früchte. Die mögliche Rolle von ATP bezogen auf die Erhaltung der Integrität der Zellmembranen unter CA-Bedingungen verbunden mit der Verminderung des Vorkommens von Verbräunungen wird diskutiert.

Introduction

Brown heart is a physiological disorder of 'Conference' pear that is frequently observed when they are kept in low O_2 and/or high CO_2 levels during CA storage (GARCIA and STREIF 1993, SAQUET et al. 2000). The tolerance of fruits to elevated CO_2 and low O_2 levels is influenced by a number of factors such as genotype, pre- and postharvest conditions, physiological status, rate of cooling, rate of establishment of atmosphere conditions and the duration of storage (LIDSTER et al. 1990). The delay in the establishment of CA conditions is one promising prestorage treatment for reducing browning disorders as shown for some apple cultivars like 'Braeburn' (ELGAR et al. 1997) and 'Bramley's Seedling' (COLGAN et al. 1999). Also for 'Conference' pears, the delay in starting CA storage was efficient in reducing the incidence of brown heart without significant adverse effects on fruit quality (HÖHN et al. 1996, ROELOFS and DE JAGER 1997). However, for apple culti-

vars like 'Gala' the rapid cooling and rapid establishment of CA conditions maintened better fruit quality during storage (BRACKMANN and SAQUET 1999). For pears little information is available on the effectiveness of this treatment on quality maintenance.

Internal browning disorders might develop when the applied CA conditions result in lower energy production than is needed to maintain cell integrity. Early results (SAQUET et al. 2000) indicated that in the tissue of 'Conference' pears kept under various CA conditions ATP is reduced resulting in very low ATP:ADP ratios and consequently in a lower energy charge of the affected fruits.

The aim of this research was to evaluate the effects of delayed CA storage on the incidence of brown heart in 'Conference' pears and to relate it to the energy generation and metabolism of fruits expressed by their ATP and ADP levels. Possible relationships of the energy status of the fruit to their respiration and ethylene production was also investigated.

Material and Methods

Fruit material and storage conditions

Preclimacteric 'Conference' pears were obtained from the orchards of the Research Station of Fruit Science Bavendorf, University of Hohenheim. Fruits were picked on 15. september 1999 at the optimum harvest date for long-term storage, selected for uniformity and immediately stored at 0 °C. For the delayed CA treatment, fruits were held for 21 days in air at 0 °C before establishment of CA conditions. Fruits in the non delayed CA treatment were stored immediately under CA at 0 °C. Two separate containers (560 liter each) were used as replicates for each treatment. Storage conditions were 3 % CO_2 + 1 % O_2. The CA conditions used in these experiments were much higher in CO_2- and lower in O_2-levels compared with those which are normally applied for storage of 'Conference' pears. But such extreme conditions were used in order to provoke fruit damage and to get more pronounced differences in the energy metabolism of fruits during storage period. For more details of storage procedures see Saquet *et al*. (2000). During the 6 months storage period samples of 12 fruits per replicate were removed at several intervals and used for ATP and ADP analysis. For the monitoring of physiological disorders 50 fruits were cut and evaluated. Shelf life following six months of storage was also evaluated during 8 days at 20 °C.

Measurement of ATP and ADP concentrations

ATP and ADP concentrations were analysed by the bioluminescence (luciferin-luciferase assay) technique as described by SAQUET et al. (2000). Three extractions were performed at each sampling date and each extract was assayed twice.

Respiration and ethylene production measurements

For measurement of respiration rate (expressed as CO_2 release), two replicates of 5 fruits each were removed from the storage containers at different sampling dates during storage and placed in separate gas-tight glass jars (4.2 litre volume) at 20 °C for 10 days. The same fruits were also used for ethylene analyses. Fruits were ventilated continuously with air at a rate of 18 L/h and the CO_2 concentration of the air leaving the jars was measured each day using an infrared CO_2 analyser (URAS-2, Hartmann and Braun). Ethylene concentrations were measured by taking gas samples from the headspace of the jars and injecting 1 mL into a gas chromatograph (Carlo Erba Instruments) fitted with a flame ionisation detector (FID), with an injector temperature of 150 °C and a packed aluminium oxide column at 100 °C.

Evaluation of physiological disorders

For the monitoring of physiological disorders, fruits were cut at the equatorial region, longitudinally and transversely, and scored for three types of disorders. Flesh browning was characterized by chocolate-coloured tissue in the cortex of fruits. Cavities were found in the area near the core and often in the cortex. Brown heart or core browning was a disorder of the core and often advanced to the cortex region. The disorders were expressed by an index from 0–100 according to the severity of the symptoms.

Results and Discussion

Effectiveness of delayed CA in reducing incidence of browning disorders

When pears were stored immediately in CA conditions following harvest, significantly more flesh browning, core browning and cavities were found compared to delayed CA storage (Fig. 1). The effectiveness of delayed CA in reducing these disorders in 'Conference' pears was considerable. Fruits subjected to delayed CA showed 80 %, 90 % and 72 % lower severity of flesh browning, core browning and cavities, respectively, compared with those stored immediately in CA. Such benefits of delayed CA in reducing the incidence of brown heart in 'Conference' pears has been previously reported (HÖHN et al. 1996, ROELOFS and DE JAGER 1997). HÖHN et al. (1996) found similar pronounced reduction in the incidence of cavities varying from 50 to 90 % in 'Conference' fruits which had a cooling period of 10 days before starting CA.

No significant differences in quality losses and ripening parameters such as flesh firmness, acidity, skin colour and total soluble solids were observed (data not shown). The prestorage treatment was very effective in reducing browning disorders without affecting other quality attributes important in the market place. These results are in agreement with those found in 'Anjou' (DRAKE 1994) and 'Conference' pears (ROELOFS and DE JAGER 1997, HÖHN et al. 1996), who reported no significant quality losses due delayed CA.

Respiration and the involvement of ATP/ADP in the development of browning disorders

Higher ethylene and CO_2 production occurred in fruits subjected to delayed CA at the storage beginning (Fig. 2) and during the whole storage time (Fig. 3) than

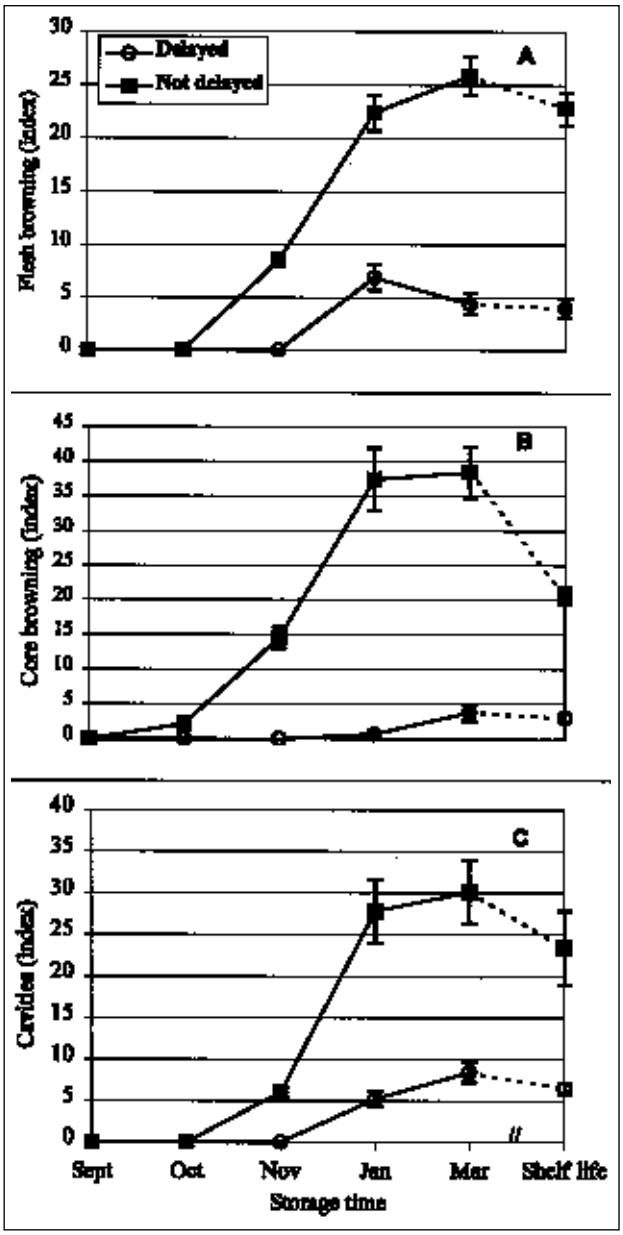


Fig. 1. Effect of delayed CA on the severity index (max. 100) of flesh browning (A), core browning (B) and cavities (C) in 'Conference' pears during storage in 3 % CO_2 + 1 % O_2 at 0 °C. Vertical bars indicate SD between treatment means.
Auswirkung einer verzögerten CA-Einstellung auf den Befall (Befallsindex max. 100) von 'Conference' Birnen mit Fleischbräune (A), Kernhausbräune (B) und Kavernen (C) während der Lagerung in 3 % CO_2 + 1 % O_2 bei 0 °C. Senkrechte Balken zeigen die Standardabweichung.

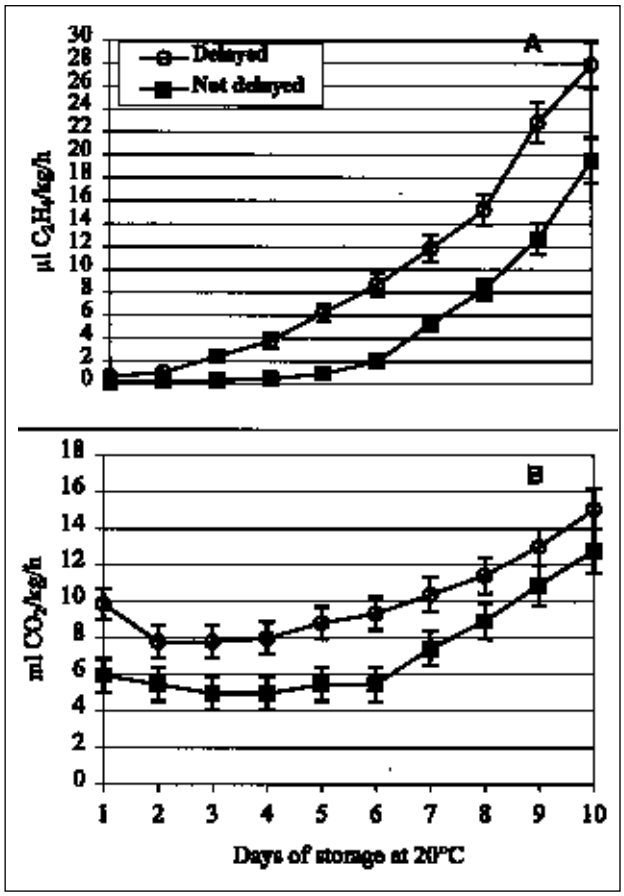


Fig. 2. Effect of delayed CA on ethylene production (A) and respiration rate (B) of 'Conference' pears removed from CA storage after 25 days. Vertical bars indicate SD between treatment means.
Auswirkung einer verzögerten CA-Einstellung auf die Ethylenproduktion (A) und Respirationsrate (B) von 'Conference' Birnen bei der Auslagerung am 25 Tag. Senkrechte Balken zeigen die Standardabweichung.

those not delayed. This directly reflected in elevated ATP levels and ATP:ADP ratios in delayed fruits (Fig. 4). The effects of delayed CA treatment on ethylene production and respiration were maintened for 2 months whereas effects on ATP and ATP:ADP ratios continued for four months of storage.

In plants, many injuries under low O_2 concentrations are related to changes in the energy metabolism of cells (PFISTER-SIEBER and BRÄNDLE 1994). The hypothesis (SAQUET et al. 2000) that ATP could be involved in the development of brown heart in 'Conference' pears seems to be confirmed by the above results. In the previous investigation a close relationship between the incidence of brown heart and a decrease in ATP levels under CA could be shown, which was confirmed by the present results. The higher concentration of ATP and lower concentration of ADP in fruits subjected to delayed CA are likely to have resulted from elevated respiration.

RAWYLER et al. (1999) investigating the behaviour of potato cells under anoxia found a threshold in ATP synthesis, below which potato cells become committed to hydrolysis of their membrane lipids. Lipid synthesis was shown to diminish and desaturation of acyl chains stopped because of their respective ATP and O_2 requirements (VARTAPETIAN et al. 1978, BROWN and BEEVERS 1987). During deprivation of O_2 the adenylate levels and the energy charge decreased both in delayed and not delayed fruits but were stronger in not delayed fruits (Fig. 4). Probably the ATP production rate became too low to sustain the basic metabolic requirements for maintenance of membrane functioning needed at the beginning of storage. During the first

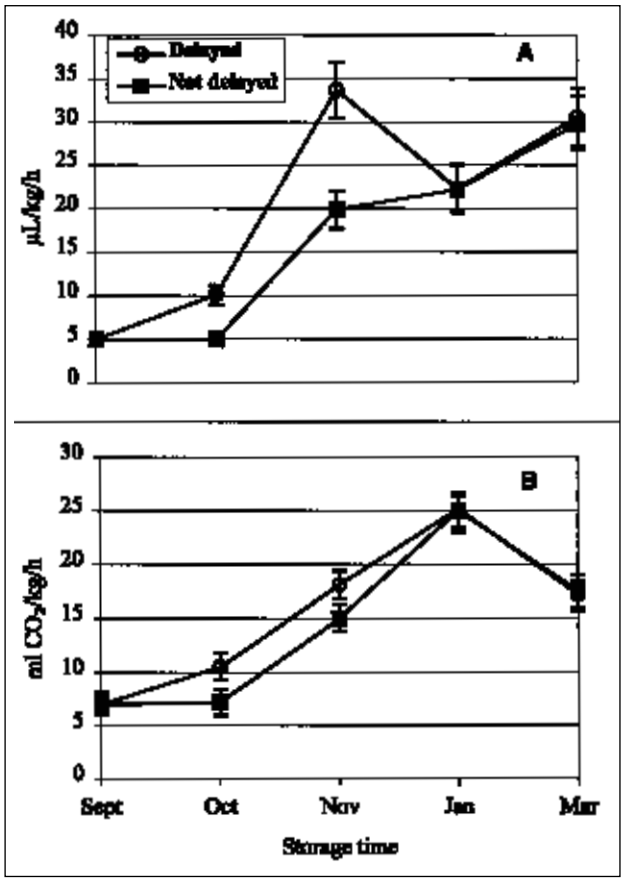


Fig. 3. Effect of delayed CA on ethylene production (A) and respiration rate (B) of 'Conference' pears during six months of storage in 3% CO_2 + 1% O_2 at 0°C. Vertical bars indicate SD between treatment means.
Auswirkung einer verzögerten CA-Einstellung auf die Ethylenproduktion (A) und Respirationsrate (B) von 'Conference' Birnen während sechs Monaten Lagerung in 3% CO_2 + 1% O_2 bei 0°C. Senkrechte Balken zeigen die Standardabweichung.

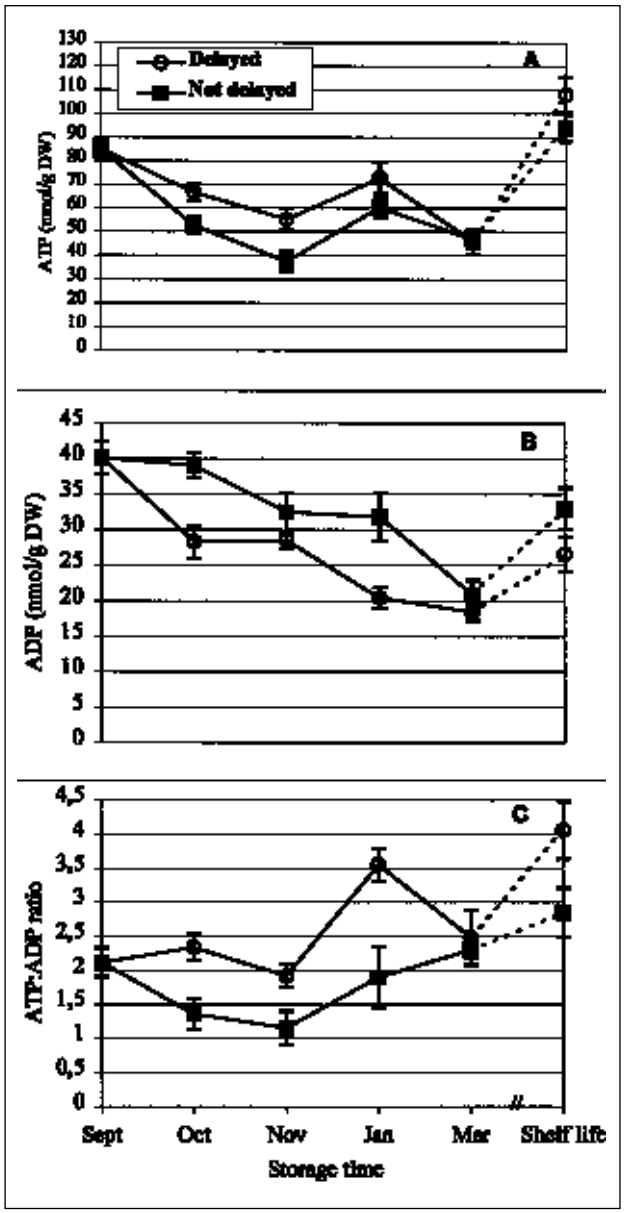


Fig. 4. Effect of delayed CA on ATP (A) and ADP (B) levels and ATP:ADP ratio (C) during six months of storage in 3% CO_2 + 1% O_2 at 0°C. Vertical bars indicate SD between treatment means.
Auswirkung einer verzögerten CA-Einstellung auf die Konzentration von ATP (A) und ADP (B) und auf das ATP:ADP-Verhältnis (C) während sechs Monaten Lagerung in 3% CO_2 + 1% O_2 bei 0°C. Senkrechte Balken zeigen die Standardabweichung.

month of storage some browning could be detected at a time which may be critical for further development of the injuries. Membrane damage could be induced by ATP deprivation, which is suggested by the correlation between the leakage of ions from the cells (SAQUET et al. 2000) and the release of free fatty acids (RAWYLER et al. 1999).

Further investigations are in progress to better understand the metabolism of fatty acids as it relates to the energy metabolism of 'Conference' pears kept under CA.

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