Ascorbic Acid Concentration in Cv. Conference Pears during Fruit Development and Postharvest Storage

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L-Ascorbic acid (L-AA) concentration changes during the development of cv. Conference pears and the influence of postharvest handlings (gas condition, cooling rate, cooling duration) on L-AA breakdown were studied. L-AA concentration fluctuates in young fruits, remains stable during fruit maturation, and starts to decline 1 week before commercial harvest. The most rapid decrease in L-AA concentration was found during immediate controlled atmosphere. During short-term storage, only the gas condition was found to influence L-AA breakdown; no significant difference between gradually or immediately cooled pears was determined. Under air conditions, both cooling strategies did not differ from the L-AA breakdown in pears allowed to ripen on the tree up until 3 weeks after the optimal harvest date. During long-term storage, the cooling duration (1−3 weeks) had no effect whereas both O_2 and CO_2 had a significant effect on L-AA retention. After 7 months of storage, no difference was found in dehydroascorbic acid concentration; the L-AA and total L-AA concentrations, in contrast, were significantly lower in the 5% CO_2 conditions.

KEYWORDS: Ascorbic acid (L-AA); browning; core breakdown; DHAA; HPLC

1. INTRODUCTION

In moderate climate areas, where fruit production is only seasonal, long-term storage of fruit in controlled atmosphere (CA) cooling rooms is needed to allow consumption of fresh fruit throughout the whole year. *Pyrus communis* L. cv. Conference is one of the most important pear cultivars in Europe with a yearly production of more than 500 000 tons. After harvest during the first weeks of September, the pears are immediately cooled for 3 weeks (delayed controlled atmosphere, DCA) before being stored under CA conditions. A reduced O_2 concentration (1.5−2.5%) and a slightly elevated CO_2 concentration (0.7−1%) in combination with a low temperature (−0.5 to −1 °C) are believed to be the optimal CA conditions to prolong the storage life of pears, by reducing the respiration and other metabolic reactions and by preventing microbial growth (*1*). However, suboptimal CA storage, especially of intrinsically inferior fruit, may result in quality loss and/or storage disorders. Brown heart or core breakdown is such a disorder, typical for Conference pears and characterized by brown tissue in the center surrounded by sound tissue. During storage, the intensity of the brown discoloration increases and finally cavities may arise in the inner core (*2*, *3*). It is considered to be CO_2-related (*4*), although it is still unclear how elevated CO_2 concentrations trigger the onset of this disorder. The crucial step in browning

of fruit and vegetable tissue is the enzymatic oxidation of polyphenol compounds by polyphenoloxidase (PPO) to o-quinones, which are very reactive and form brown-colored polymers (*5*, *6*). Because PPO and its substrate are located in different cell compartments, enzymatic browning only occurs after cellular decompartmentation, which follows from membrane disintegration. Therefore, enzymatic browning is only an indirect consequence of the disorder, whose cause should be sought in other oxidative and senescence-related processes (*7*). Oxidative processes involve the presence of reactive oxygen species, produced as alarm-signaling components (*8*) and antioxidants such as L-ascorbic acid (L-AA). L-AA is one of the most important free radical scavengers, which is present in all cell compartments, including the cell wall (*9*, *10*), and protects plants against oxidative damage (*11*, *12*). Besides its antioxidant function, it has an indispensable role in modulating the cell division (*13*) and elongation (*14*, *15*). Cell elongation is closely related to ascorbate oxidase activity and L-AA concentration in the cell wall (*16*).

Recent research ascribes brown core development to a decrease in L-AA concentration (*17−19*). Both Veltman et al. (*18*) and Zerbini et al. (*19*) support the hypothesis that core breakdown appears when the L-AA concentration drops below a certain threshold value. These latter authors found that the rate of L-AA loss differs from year to year and that the L-AA concentration decreased during storage according to an exponential model. This is in contrast with Larrigaudière et al. (*20*) who found that after a rapid decrease of the L-AA concentration
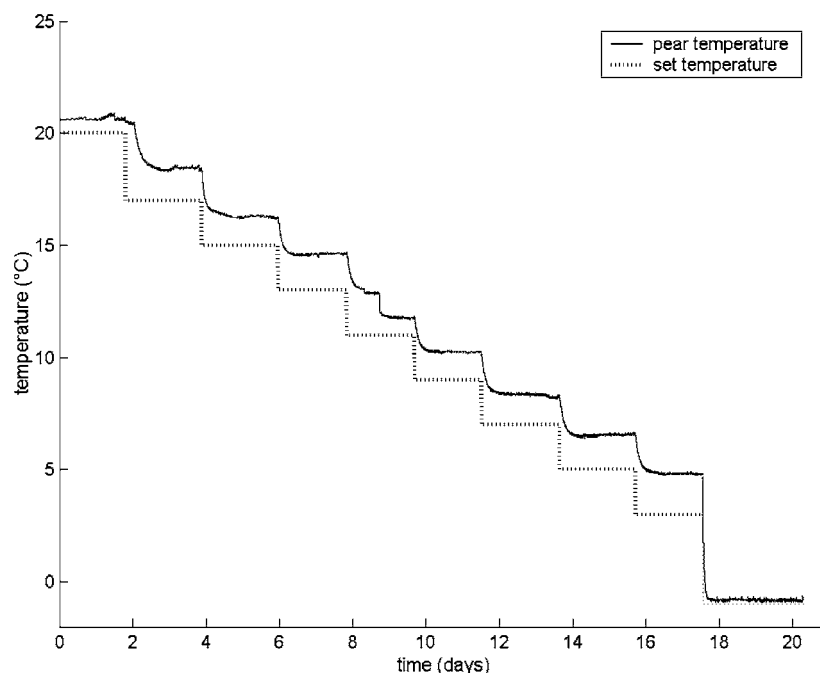
Figure 1. Registration of the temperature profile in a pear in comparison with the set value of the water bath during the gradual cooling from 20 to −1 °C in 21 days.

during the first 22 days of storage, the L-AA concentration was completely or partially regenerated depending on the gas concentrations. Pears stored in air had the smallest loss during the first 22 days and had a significantly higher L-AA concentration throughout the whole storage in comparison with the pears stored under CA conditions. Even though recent research stresses the influence of gas conditions on L-AA metabolism, other papers refer to the temperature management as the most important factor to maintain L-AA (*21* and references therein).

The objective of this research was to investigate the rate of L-AA breakdown after harvest in comparison with the natural variation and evolution of L-AA during fruit development and ripening on the tree. Because higher fruit quality is beneficial for both fruit growers (better resistance to disorders) and consumers (higher nutritional value), the influence of different postharvest strategies on L-AA concentrations was studied. More specifically, we wanted (i) to investigate in which period after harvest the fastest L-AA breakdown occurs, (ii) to investigate whether different cooling strategies and different cooling durations have any effects on the L-AA concentration, and (iii) to study the effect of gas atmosphere conditions on L-AA concentrations during long-term storage.

2. MATERIAL AND METHODS

2.1. Fruit Material. For the experiments 2.2, 2.3.1, and 2.3.3, pears (*P. communis* cv. Conference) were harvested from the west side of one row of trees in the orchard of the Center for Fruit Culture in Rillaar (Belgium). For experiment 2.3.2, pears were picked in a commercial orchard in Meensel (Belgium). Only pears from one side of the trees were used in order to reduce the huge biological variation on ascorbic acid concentration in fruits, since we experienced that the position of the fruit on the tree with respect to the sun has a strong influence on its ascorbic acid concentration (unpublished results). We arbitrarily chose the west side.

2.2. Preharvest Analyses. To follow the L-AA changes during fruit development on the tree, 14 pears were weakly analyzed as described in section 2.4 starting at 11 weeks before harvest until 3 weeks after the commercial picking date (season 2002). From the same pears, sugar analysis was carried out as described in section 2.5.

2.3. Storage Conditions. *2.3.1. Study of DCA Duration.* Pears were picked at the commercial picking date (10−13 September 2001), cooled in air to −1 °C, and transferred to optimal CA conditions (2.5% O_2, 0.7% CO_2) after, respectively, 1, 2, and 3 weeks of cooling (delayed CA, DCA). L-AA analysis was carried out after the DCA period, after, respectively, 6, 8, and 10 weeks of CA storage and, additionally, at 9 and 20 weeks after harvest. Each time, L-AA was determined in 10 pears from each condition (1, 2, or 3 weeks DCA) as described in section 2.4.

2.3.2. Study of DCA Management. The pears were picked on 24 September 2001 and used to investigate different cooling strategies (gradual and immediate) in combination with different air conditions (2.5% O_2, 0.7% CO_2) and air. To study the effect of a gradual cooling in comparison with the traditional, immediate cooling, a system was constructed that allowed us to cool the pears gradually from 20 to −1 °C over 21 days (**Figure 1**). This gradual cooling was carried out for pears stored both in air and under CA conditions (2.5% O_2, 0.7% CO_2). For each gas condition, five airtight glass recipients (1.8 L) were placed in series in expanded polystyrene boxes. These recipients were surrounded by a water jacket and connected to a water bath (C25, Haake, Karslruhe, Germany). The lids contained a gas inlet, a gas outlet, and a septum. The recipients were flushed with a gas mixture prepared from pure gases by means of mass flow controllers (5850 S, Brooks Instruments, Veenendaal, The Netherlands). Before flowing through the recipients, the gas was led through a temperature-controlled humidifier. Five thermocouples (Thermo Electric, Warmond, The Netherlands) were used to register the temperature in the recipients and inside pears. The thermocouples were connected to a data acquisition system (Agilent Technologies, Palo Alto, CA). Four different cooling strategies were carried out as follows: a gradual cooling from 20 to −1 °C during 21 days under CA conditions (strategy 1) and under air (strategy 2) and an immediate cooling under CA conditions (strategy 3) and under air conditions (strategy 4). Five pears of equal size were used for each strategy. Pears analyzed directly after harvest were considered as the control group. After 21 days, the L-AA concentration was determined in all pears as described in section 2.4.

In harvest season 2002, strategies 2 (gradual cooling, air) and 4 (immediate cooling, air) were repeated on a larger scale, using an incubator to apply a temperature profile instead of the experimental set up with recipients connected to a water bath. After harvest at the commercial picking date (3 September 2002), 100 pears were cooled immediately in a cooling room at −1 °C, whereas 100 other pears were

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stored in a cooled incubator in which the temperature was gradually decreased from 20 to -1 °C. At harvest time and at six sampling times during the three week cooling period, L-AA was measured on eight pears of each cooling strategy.

2.3.3. Study of Different CA Conditions. To study the effect of different CA conditions, pears harvested at $10-13$ September 2001 were cooled for 3 weeks in air and subsequently stored under the following conditions: 2.5% O_2, 0.7% CO_2 (optimal CA conditions); 2.5% O_2, 5% CO_2; 15% O_2, 0.7% CO_2; 15% O_2, 5% CO_2. L-AA analysis was carried out at 5, 8, 11, 20, and 28 weeks after harvest, and DHAA analysis was only carried out at 28 weeks after harvest as described in section 2.4.

2.4. L-AA and Dehydroascorbic Acid (DHAA) Determination. *2.4.1. Extraction of L-AA and DHAA in Pear Slices.* Pear slices (0.7 cm thickness) were cut perpendicularly to the length axis at 5 cm from the bottom of a pear and immediately frozen in liquid nitrogen. The frozen pear slice was ground under liquid nitrogen using a mortar and pestle. Approximately 0.2 g of tissue was transferred to a 1.5 mL reaction tube followed by addition of 1 mL of extraction buffer containing 3% metaphosphoric acid and 1 mM ethylenediaminetetraacetic acid (EDTA) (*22*). The tubes were thoroughly vortexed and stored on ice until centrifugation for 10 min at 10 000*g* in a precooled microcentrifuge (Hawk 15/05, Sanyo, Watford, U.K.). The supernatant was filtered through a PVDP filter with 0.45 μm pore size (Millipore, Brussels, Belgium) and analyzed immediately with high-performance liquid chromatography (HPLC). The exact tissue amount was measured by weighing the used reaction tubes before and after filling.

DHAA concentrations were determined as described in Davey et al. (*23*). After they were incubated for 30 min at room temperature, the samples were centrifuged and filtered as described above. This extract contained the total AA concentration (L-AA + DHAA). By subtracting the AA concentration from the total AA concentration, the DHAA concentration was calculated.

2.4.2. HPLC Analysis. Analyses were carried out on a HP 1100 system (Agilent Technologies) using a Lichrospher RP-18 column (150 mm × 4.6 mm ID, 3 μm particle diameter) (Alltech, Lokeren, Belgium) and based on the protocol as described by Davey et al. (*23*). The mobile phases (A: 0.5% MeOH, 1 mM EDTA, and 400 μL/L phosphoric acid; B: 30% CH_3CN, 70% A) were, after they were filtered and degassed, pumped through the column at a rate of 0.8 mL/min. The applied gradient elution time profile was as follows (in % B): 0 min, 0% B; 3.5 min, 0%; 6.5 min, 100%; 8.5 min, 100%; 10.5 min, 0%; 15 min, 0%. The column temperature was maintained at 25 °C, and L-AA was detected with a diode array detector at 242 nm. The L-AA concentrations were calculated in mg/100 g fresh weight.

2.5. Sugar Determination. *2.5.1. Extraction of Sugars.* Approximately 50 mg of homogenized pear tissue from the same pears as used in section 2.4.1 was transferred to a 1.5 mL reaction tube. The exact tissue amount was measured by weighing the used reaction tubes before and after filling. The tissue was extracted with 500 μL of 80% EtOH for 10 min at 78 °C. After they were centrifuged, the supernatants were decanted and the remaining pellet was extracted twice with 500 μL of 50% EtOH under the same incubation conditions. The supernatants were collected (1500 μL) and stored at 4 °C until analysis.

2.5.2. Spectrophotometric Assay. The sugar analysis was carried out as described in detail by Stitt et al. (*24*). Briefly, 2 μL of EtOH extract was added to 200 μL of HEPES buffer (pH 7) containing 20 μL of glucose-6-P dehydrogenase, 20 mg of ATP, and 12 mg of NADP per 20 mL of buffer. Glucose, fructose, and sucrose were measured by registering the increase in absorption at 340 nm after addition of, respectively, 2 μL of hexokinase, 4 μL of phophogluco-isomerase, and 2 μL of invertase. The measurement was carried out using a Multiskan Spectrum Spectrophotometer (ThermoLabsystems, Finland).

2.6. Data Processing. The SAS/STAT software, version 8.2 (SAS Institute Inc., Cary, NC) was used to carry out the analysis of variances (ANOVA or GLM procedure). Significant differences were found using a Tukey test (95% level of significance).

3. RESULTS AND DISCUSSION

3.1. Preharvest Analyses. The changes in L-AA and DHAA concentration in developing fruits were measured starting from
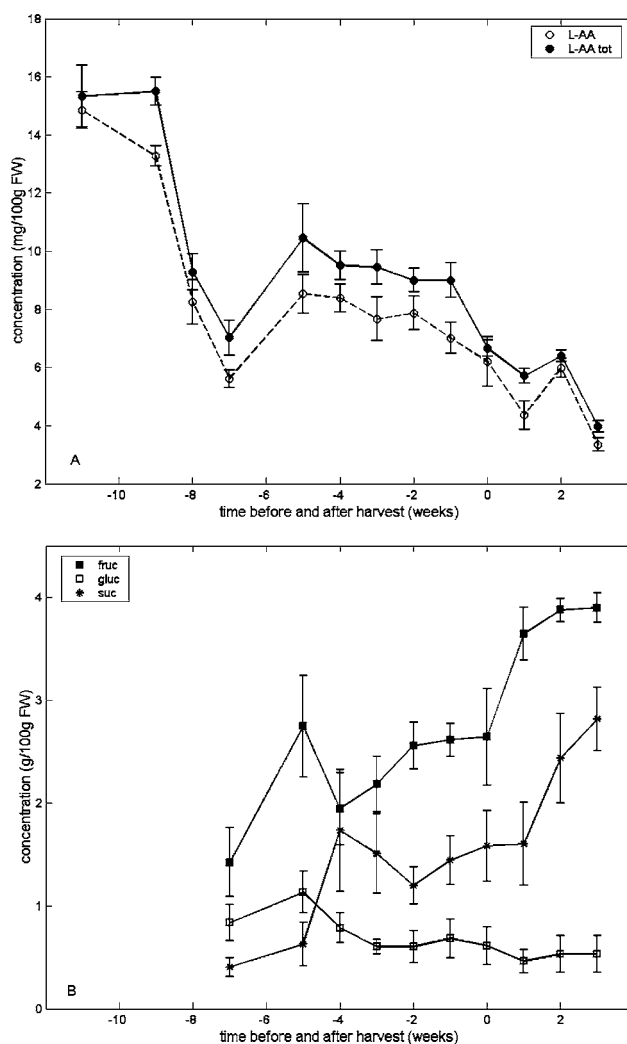

Figure 2. (**A**) Change in L-AA and total L-AA concentration during fruit development and maturation on the tree. Each value is the mean of 14 replicates ± standard error of the mean. (**B**) Change in fructose, glucose, and sucrose concentration. Each value is the mean of eight replicates ± standard deviation. Week 0 is the optimal harvest period (3–5 September 2002).

mid June (11 weeks before harvest) until 3 weeks after harvest. High L-AA concentrations were found in young fruits up to three times the concentration at harvest (**Figure 2A**). Very suddenly, the L-AA concentration was halved in the beginning of July, followed by an increase reaching a L-AA concentration of 8 mg/100 g. Afterwards, the concentration remained stable while the pear weight kept on increasing linearly (data not shown). One week before harvest, a significant drop in L-AA and total L-AA was measured and from that point on, the L-AA concentration decreased reasonably fast. In contrast with the decreasing L-AA concentration around harvest time, the sugar concentration, especially fructose and sucrose, increased fast in the beginning of September (**Figure 2B**). Moreover, around week 6, the L-AA rise coincided with increasing sugar concentrations, indicating an enhanced nutrient influx in the first half of July.

Development, maturation, and ripening of fruits have received considerable experimental attention, primarily due to the nutritional importance of fruit for men. From bloom to maturity, fruits pass through three different stages: (i) fruit set, (ii) cell division, and (iii) cell elongation (*25*). Between stages two and three, an intermediate stage can be defined in which pit

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hardening and embryo maturation start (*26*). The L-AA evolution was registered over a period of 14 weeks starting from the 17th of June, which was 10 weeks after blossoming; hence, it can be assumed that no cell division occurred during the measuring period since cell division can last in pears up till 8−9 weeks after fruit set (*27*, *28*). It is unclear what triggered the sudden drop in L-AA in the beginning of June. Possible explanations could be (i) an unbalanced source−sink relationship due to, e.g., vegetative shoot growth or the increasing sink activity of the seeds, which start to harden and compete with the fruit flesh for assimilates; (ii) the start of cell expanding, which coincides with a high ascorbate oxidase activity (*16*); (iii) a consequence of the June drop, which might cause differences in the pear sampling population (before the June drop, a higher percentage of pears might experience an enhanced stress level, hence, contain more L-AA); (iv) the chemical treatment 2 days before measuring with dimilin and mantrac, an insecticide, respectively, a Mn foliar fertilizer, which were not used on the trees earlier during the growth season. Even though it is hard to verify the factors summed up above, the sudden drop in vitamin C reveals that something is changing considerably, related to the fruit's growth and maturation or elicited by an unknown external (stress) factor, such as light, temperature, salt, drought, atmospheric pollutants, metals, and herbicides (*29*). Source−sink relationships play a key role in fruit and seed development (*27*). The sink strength, in particular, influences the levels of sugars, and the intracellular carbohydrate pool is closely related to the ascorbic acid transport and metabolism (*15*). This fact is illustrated by the correspondence between the sudden rise in both L-AA and fructose around week 6 (**Figure 2A**,**B**). After the increase in L-AA, its concentration remains more or less stable. This means that there is still L-AA biosynthesis in the fruit and/or transport to the fruit from the leaves since the fruit size keeps on increasing. As in pears, the L-AA concentration tends to decrease during ripening in most fruit commodities such as citrus fruits (*31*), kiwis (*32*), apple, and mango (*21*). The drop in L-AA concentration at week 1 precedes the rise in fructose, which starts around the harvest date. From these data, it is impossible to point out the beginning of ripening. However, it is clear that in the beginning of September metabolic rates are changing drastically.

3.2. Comparison of Different Cooling Strategies. To investigate a possible storage strategy to reduce the fast breakdown rate of L-AA, the influence of the cooling rate in combination with the gas atmosphere was examined. The results of the L-AA analyses of pear slices are summarized in **Figure 3**. The L-AA breakdown during the first 21 days after harvest is very high, irrespective of the cooling strategy. Starting with an average L-AA concentration of 6.32 mg/100 g FW after harvest, hardly 50% is left over in the best case (strategies 2 and 4). The cooling strategy consists of two factors: the cooling rate (gradual or immediate) and the gas atmosphere (air or CA). The gas atmosphere appeared to be the most important factor influencing L-AA concentrations. No significant effect of the cooling rate was found. Storage under CA is significantly worse than storage under air conditions, which supports the hypothesis that low O_2 and/or elevated CO_2 concentrations negatively affect the L-AA metabolism. A larger-scale experiment carried out under air conditions with intermediate measurements revealed differences in L-AA loss and partial regeneration, but finally, after 3 weeks, no significant difference was found between gradual and immediate cooling (**Figure 4**). Three weeks after optimal harvest date, mean L-AA concentrations of both
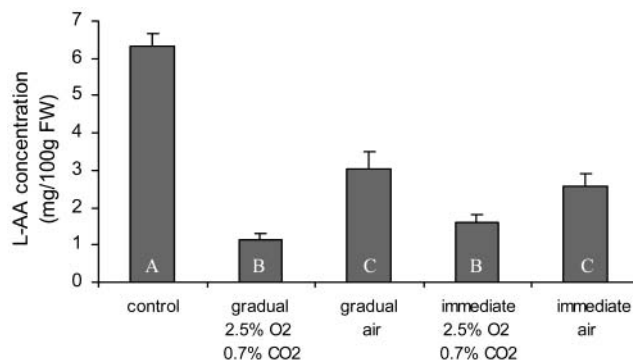
Figure 3. L-AA concentration after 3 weeks according to the four different cooling strategies as compared to the initial L-AA concentration at harvest (control) (harvest season 2001). The bars represent the average of 10 pears for the control group and five pears for the others. Error bars indicate the standard error of the mean. Significantly different groups ($\alpha = 0.05$) are indicated by different letters.
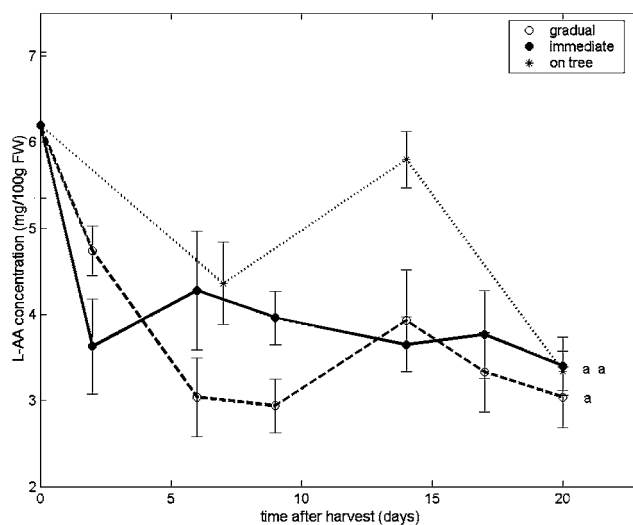
Figure 4. Evolution of L-AA during ripening on the tree (∗) and after harvest during a gradual (○) and immediate (●) cooling (harvest season 2002). Letters at 20 days indicate the absence of a significant difference ($\alpha = 0.05$).

strategies did not differ from the L-AA concentration measured in pears, which were allowed to ripen on the tree.

The relationship between elevated CO_2 concentrations and enhanced L-AA breakdown has often been reported in the literature (*19*, *33*, *34*), and the results of this experiment are compatible with their statements. Larrigaudière et al. (*35*) showed a similar short-term effect between air and CA storage. They measured a difference of 1 mg/100 g FW between CA (2% O_2, 5% CO_2) and air after 21 days; the same difference was noticed in our experiment at lower CO_2 concentrations (CA: 2.5% O_2, 0.7% CO_2) with immediate cooling. This can indicate that Mediterranean grown pears are less sensitive to CO_2 as compared to the more northern grown ones and may explain the lower susceptibility of the Mediterranean ones to core breakdown development. The low L-AA concentrations in strategies 1 and 3 can be assigned to a combined CO_2/O_2 effect. In strategies 2 and 4, the 50% L-AA breakdown over 3 weeks cannot be assigned to a gas effect. However, because of a higher O_2 concentration (higher respiration rate), the CO_2 production is stimulated in the pears stored in strategies 2 and 4, which on turn may influence the L-AA metabolism.

Because pears remain highly metabolically active as long as they remain at ambient temperature, it is recommended to cool

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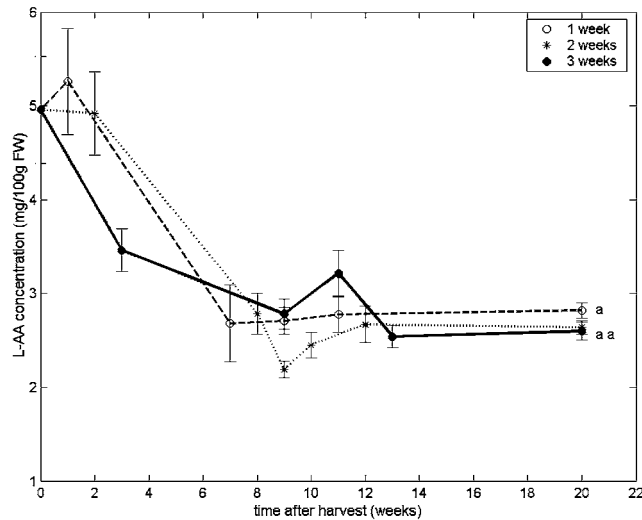
Figure 5. Change of L-AA concentration in pears stored under CA condition (2.5% O_2, 0.7% CO_2) after 1, 2, and 3 weeks of cooling (harvest season 2001). Each value is the mean of 10 replicates ± standard error of the mean. Letters at 20 weeks indicate the absence of significant differences.
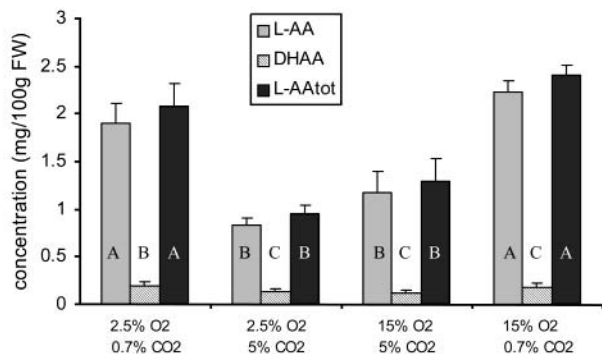

Figure 6. Change of L-AA concentration in pears stored in different CA conditions after a DCA of 3 weeks (harvest season 2001). Each value is the mean of 10 replicates ± standard error of the mean. Different letters indicate significant differences between the four conditions at a certain storage time (11, 20, and 28 weeks).

them down before CA storage. It is known that an immediate cooling has many benefits improving fruit quality parameters (*36*). Van Schaik (*37*) stated that each delay of cooling implies a shortening of the storage duration. In tomatoes (Kader and Morris, 1978), leafy vegetables (Zepplin and Elvehjein, 1944), spinach, cabbage and snap beans (Ezell and Wilcox, 1959), and citrus fruits (Nagy, 1980) (*21*), any delay between harvesting and cooling resulted in direct L-AA losses. Pears cooled gradually experienced temperatures above 10 °C for 10 days, and in contrast with other fruits and vegetables, a different temperature management had no effect on the L-AA concentration in pears. **Figure 1** shows the temperature profile during the gradual cooling of pears over 21 days from 20 to −1 °C. The time needed to cool an individual pear over the same temperature range was 4 h (graph not shown). In commercial fruit auctions, pears are cooled in bins filled with ±350 kg of pears; hence, the real cooling profile lies somewhere between the profile of the immediate cooling of an individual pear and the profile as shown in **Figure 1**. The relative slow cooling rate in commercial auctions is no real drawback with respect to L-AA maintenance since this experiment proved that abrupt temperature changes after harvest are probably not enhancing the L-AA breakdown process since gradual and immediate cooling do not differ significantly in their effect on the L-AA concentration in pears. Moreover, both strategies do not differ from the L-AA breakdown during ripening on the tree: all pears contained about 3.5 mg/100 g FW 3 weeks after optimal harvest date (**Figure 4**).

3.3. Influence of the DCA Period on the L-AA Concentration during Long-Term Storage. It is known that the length of DCA has an effect on the incidence of core breakdown in Conference pears: the longer the cooling treatment is applied, the less susceptible the pears are to this disorder (*38*). Therefore, it was investigated whether this difference in susceptibility could be explained by differences in L-AA concentrations. Optimally, picked pears were brought under optimal CA conditions after, respectively, 1, 2, and 3 weeks of DCA. The change in L-AA concentration during the cooling period and the subsequent CA storage is illustrated in **Figure 5**. The fastest breakdown occurred in the period directly after harvest with losses up to 30% after 3 weeks. Further losses during subsequent CA storage
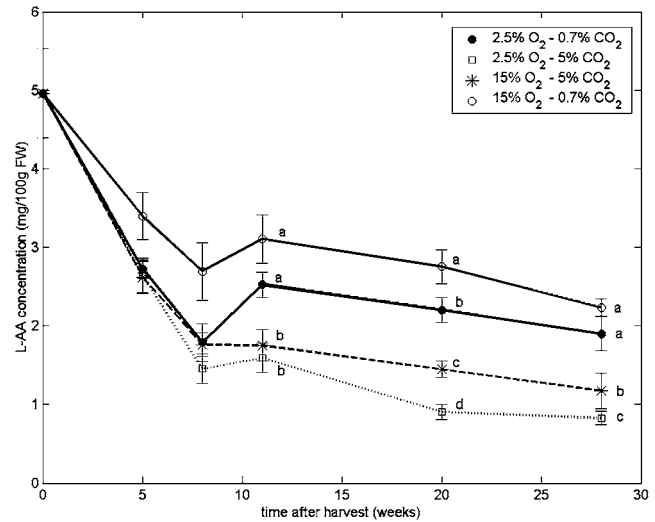
were smaller and resulted in a total loss of 45% after 20 weeks. Lack of measurement points in the first weeks of CA storage for the pears, which were cooled for 1 and 2 weeks, gives the impression that the breakdown rate under CA condition is slower than under air. However, the previous experiment showed a significantly higher L-AA loss for pears stored under CA than those stored under air. Even though the breakdown rate is different in the three groups due to the fact that pears experienced CA conditions at a different starting point and with a different duration, after a long-term storage of 20 weeks, no significant differences in L-AA concentration were measured.

Core breakdown susceptibility is reduced with longer DCA periods; the L-AA concentration, in contrast, is not influenced by different lengths of DCA. This conclusion seems paradoxical, but this experiment illustrates that the effect of DCA on core breakdown occurrence is not reflected by the L-AA concentration. Hence, assuming that the hypothesis of a relationship between L-AA and this disorder is valid, L-AA is probably not the only limiting and determining factor. The biochemical effect of the DCA duration should be sought in other metabolic processes.

3.4. Influence of Storage Condition on L-AA Concentration. The O_2 concentration, the CO_2 concentration as well as the storage duration have a significant effect on the L-AA concentration in pears: a high CO_2, a low O_2 concentration, and a long storage time decrease the L-AA concentration (**Figure 6**). The most beneficial condition for maximal L-AA retention was the one with 15% O_2 and 0.7% CO_2 and resulted in 55% L-AA loss after 28 weeks (25 weeks CA storage); however, this high O_2 condition was after 28 weeks not significantly different any more than the optimal storage condition (2.5% O_2, 0.7% CO_2). The first 11 weeks after harvest seem to be the most dynamical ones: after a fast decrease that lasts 8 weeks, the L-AA concentration seems to stabilize and further losses are relatively small. From 11 weeks on, pears stored under low CO_2 conditions are significantly higher in L-AA concentration than the ones stored under high CO_2 conditions. Pears stored in the low CO_2 conditions seem to be able to regenerate L-AA; however, only for the condition with 2.5% O_2 and 0.7% CO_2, a significant increase was noticed. Analysis of the reduced and oxidized form of L-AA (L-AA,

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Figure 7. Comparison of L-AA, DHAA, and total L-AA (L-AA + DHAA) concentration in pears stored for 7 months under four different CA conditions. Each value is the mean of six replicates ± standard error of the mean. Significantly different groups ($\alpha = 0.05$) are indicated by different letters.

respectively, DHAA) after 7 months of CA storage revealed that DHAA concentrations were the same in all conditions, while the L-AA and the total L-AA concentration (L-AA + DHAA) were significantly higher for the low CO_2 conditions (**Figure 7**). However, no significant difference in DHAA/AA ratios was found, and the DHAA/AA ratios of the 5% CO_2 conditions tended to be higher than the ratios for the 0.7% CO_2 conditions, indicating a change in redox status toward the oxidized form under high CO_2 conditions.

The negative effect of CO_2 on the L-AA concentration has also been observed for berries (*33*) and kiwi slices (*39*). The effect of O_2 on the L-AA metabolism is apparently not the same for different fruits, even not for fruits within the same family, which indicates that the regulation and activity of biosynthetic and/or regeneration enzymes are different. A high O_2 concentration is beneficial for L-AA retention in pears (*18*) but not for apples (*21*) and kiwi slices (*39*).

The mechanism responsible for the sudden L-AA increase around week 11, which is also visible in **Figure 5** in the same period, is still unclear. Larrigaudière et al. (*20*) noticed also a partial regeneration in pears already after 22 days stored in 0.7% CO_2, and Veltman et al. (*18*) described an increase in L-AA concentrations after switching the storage condition from 0 to 10% CO_2.

The long storage time (7 months) may explain why gas effects are not visible anymore in the partition of the oxidized and reduced forms of L-AA. However, similar DHAA concentrations in different storage conditions were also found by Zerbini et al. (*19*). Larrigaudière et al. (*35*) found a higher activity of ascorbate peroxidase under 5% CO_2. Because this enzyme catalyzes the oxidation of AA to DHAA, this can explain the higher DHAA/AA ratios in high CO_2 conditions. To the author's knowledge, no literature is available about the direct effect of CO_2 on enzymes involved in the biosynthetic pathway, the ascorbate−glutathione cycle, and the degeneration of DHAA to 2,3-diketogluconic acid. An indirect effect of CO_2 via its influence on the respiration chain (*40*) and, resulting from this, a changing production of energy and signaling molecules cannot be excluded.

4. CONCLUSION

The biochemical composition of fruits changes throughout its growth and maturation, and source−sink relationships probably play therein an important role. From 10 weeks after blossoming until 3 weeks after harvest, the L-AA concentrations are continuously changing. Starting with a concentration of 15

mg/100 g FW, only one-third is left over at the harvest period. A significant drop was registered in the beginning of July without having a clear explanation. During maturation, the L-AA concentration remains stable and starts to decrease just before the commercial harvest. The fructose concentration profile shows a similar pattern before harvest, suggesting a close relationship between sugar influx and L-AA metabolism. The influence of postharvest factors (storage time, DCA, O_2 and CO_2 concentration) on the L-AA concentration was investigated. The fastest breakdown occurs immediately postharvest. Three weeks after optimal harvest date, no difference was found between gradual and immediate cooling under air conditions and the L-AA breakdown naturally occurring during ripening of pears on the tree. The cooling rate had no influence on the rate of L-AA breakdown while the gas concentration had a clear effect. Application of CA conditions immediately postharvest is known to enhance the susceptibility for core breakdown and appears to have a detrimental effect on the L-AA metabolism. On long-term storage, the duration of the cooling period had no effect on L-AA concentrations. It is known that the occurrence of core breakdown, in contrast, is positively correlated with the length of DCA, and it was concluded that the effect of DCA on core breakdown occurrence is not reflected by the L-AA concentration. L-AA is probably not the only limiting and determining factor in the development of core breakdown, and the biochemical effect of the DCA duration should be sought in other metabolic processes. The gas conditions during long-term storage had a clear influence. The best storage condition with respect to L-AA retention consists of high O_2 and low CO_2 concentrations, although this condition was, from 28 weeks after harvest on, not significantly different from the optimal CA conditions as commercially applied. However, the importance of the CA condition must be put into perspective since major losses occur immediately after harvest, in the cooling period before CA storage, and are probably linked to ripening processes.

LITERATURE CITED

(1) Roelofs, F. P.; de Jager, A. Reduction of Brownheart in Conference Pears. *Controlled Atmosphere Research Conference*; Mitcham, E., Ed.; July 13−18, University of California, Davis, 1997; pp 138−144.

(2) Lammertyn, J.; Dresselaers, T.; Van Hecke, P.; Jancsók, P.; Wevers, M.; Nicolaï, B. M. *Magn. Reson. Imaging* **2003**, in press.

(3) Lammertyn, J.; Dresselaers, T.; Van Hecke, P.; Jancsók, P.; Wevers, M.; Nicolaï, B. M. *Postharvest Biol. Technol.* **2003**, *29*, 19−28.

(4) Kadam, S. S.; Dhumal, A.; Shinde, N. N. Pear. In *Handbook of Fruit Science and Technology*; Salunkhe, D. K., Kadam, S. S., Eds.; Marcel Dekker Inc.: New York, 1995; pp 183−202.

(5) Mathew A. G.; Parpia, H. A. B. *Adv. Food Res.* **1971**, *19*, 75−145.

(6) Mayer, A. M. *Phytochemistry* **1987**, *26*, 11−20.

(7) Larrigaudière, C.; Lentheric, I.; Vendrell, M. *J. Sci. Food Agric.* **1998**, *78*, 232−236.

(8) Foyer, C. H.; Lelandais, M.; Kunert, K. J. *Physiol. Plant.* **1994**, *92*, 696−717.

(9) Noctor, G.; Foyer, C. H. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 249−279.

(10) Smirnoff, N.; Wheeler, G. L. *Crit. Rev. Plant Sci.* **2000**, *1*, 267−290.

(11) Asada, K. *Physiol. Plant.* **1992**, *85*, 235−241.

Ascorbic Acid Concentration in Cv. Conference Pears

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(12) Gille, G.; Sigler, K. *Folia Microbiol.* **1995**, *40*, 131−152.

(13) De Gara, L.; Paciolla, C.; De Tullio, M. C.; Motto, M.; Arrigoni, O. *Physiol. Plant.* **1991**, *109*, 7−13.

(14) Arrigoni, O. *J. Bioenerg. Biomembr.* **1994**, *26*, 407−419.

(15) Cordoba, F.; Gonzalez-Reyes, J. A. *J. Bioenerg. Biomembr.* **1994**, *26*, 399−405.

(16) Smirnoff, N. *Ann. Bot.* **1996**, *78*, 661−669.

(17) Veltman, R. H.; Sanders, M. G.; Persijn, S. T.; Peppelenbos, H. W.; Oosterhaven, J. *Physiol. Plant.* **1999**, *107*, 39−45.

(18) Veltman, R. H.; Kho, R. M.; van Schaik, A. C. R.; Sanders, M. G.; Oosterhaven, J. *Postharvest Biol. Technol.* **2000**, *19*, 129−137.

(19) Zerbini, P. E.; Rizzolo, A.; Brambilla, A.; Grassi, M. *J. Sci. Food Agric.* **2002**, *82*, 1−7.

(20) Larrigaudière, C.; Pinto, E.; Lentheric, I.; Vendrell, M. *J. Hortic. Sci. Biotechnol.* **2001a**, *76*, 157−162.

(21) Lee, S. K.; Kader, A. A. *Postharvest Biol. Technol.* **2000**, *20*, 207−220.

(22) Davey, M. W.; Bauw, G.; Van Montagu, M. *J. Chromatogr. B* **1997**, *697*, 269−276.

(23) Davey, M. W.; Dekempeneer, E.; Keulemans, W. *Anal. Biochem.* **2003**, *316*, 75−81.

(24) Stitt, M.; Lilley, R. McC.; Gerhardt, R.; Heldt, H. W. *Methods Enzymol.* **1989**, *174*, 518−522.

(25) Downie, B. *Fruit Development*. Lecture course 2002; http://www.ca.uky.edu/agripedia/classes/pls622/lec08.htm.

(26) Chalmers, D. J.; Van den Ende, B. *Ann. Bot.* **1975**, *39*, 423−433.

(27) Wertheim, S. J. De ontwikkeling van de vrucht. In *Grondslagen van de Fruitteelt*; Tromp, J. J., Jonkers, H., Wertheim, S. J., Eds.; Staatsuitgeverij's: Gravenhage, 1976; pp 233−239.

(28) Crassweller, R. M. *Fruit and Shoot Growth*. Lecture course 2002; http://hortweb.cas.psu.edu/courses/hort432/lecturenotes/grwth.html.

(29) Davey, M. W.; Van Montagu, M.; Inzé, D.; Sanmartin, M.; Kanellis, A.; Smirnoff, N.; Benzie, I. J. J.; Strain, J. J.; Favell, D.; Fletcher, J. *J. Sci. Food Agric.* **2000**, *80*, 825−860.

(30) Zhou, L.; Paull, R. E. *J. Am. Soc. Hortic. Sci.* **2001**, *12*, 351−357.

(31) Nagy, S. *J. Agric. Food Chem.* **1980**, *28*, 8−18.

(32) Rinallo, C.; Mori, B. *Ital. J. Food Sci.* **2000**, *12*, 435−442.

(33) Agar, I. T.; Streif, J.; Bangerth, F. *Postharvest Biol. Technol.* **1997**, *1*, 47−55.

(34) Pinto, E.; Lentheric, I.; Vendrell, M.; Larrigaudière, C. *J. Sci. Food Agric.* **2001**, *81*, 364−370.

(35) Larrigaudière, C.; Lentheric, I.; Pinto, E.; Vendrell, M. *J. Plant Physiol.* **2001b**, *158*, 1015−1022.

(36) Ding, C.-K.; Chachin, K.; Hamauzu, Y.; Ueda, Y.; Imahori, Y. *Postharvest Biol. Technol.* **1998**, *14*, 309−315.

(37) van Schaik, A. C. R. *De Peer*; Wertheim, S. J., Ed.; Proefstation voor de Fruitteelt: Wilhelminadorp, The Netherlands, 1990.

(38) Verlinden, B. M.; de Jager, A.; Lammertyn, J.; Schotsmans, W.; Nicolaï, B. M. *Biosystems Eng.* **2002**, *88*, 339−347.

(39) Agar, I. T.; Massantini, R.; Hess-Pierce, B.; Kader, A. A. *J. Food Sci.* **1999**, *64*, 433−440.

(40) Mathooko, F. M. *Postharvest Biol. Technol.* **1996**, *9*, 247−264.