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Respiration rate and ethylene metabolism of 'Jonagold' apple and 'Conference' pear under regular air and controlled atmosphere

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ABSTRACT: The 1-aminocyclopropane-1-carboxylate (ACC) oxidase activity, ethylene production, CO_2 release and O_2 uptake of 'Jonagold' apple and "Conference' pear were investigated during regular air and controlled atmosphere storage. Storage conditions tested at 0 °C during 6 months, with a further 10 d shelf-life in air at 20 °C were: 0.5 kPa O_2 + 6.0 kPa CO_2; 3 kPa O_2 + 6 kPa CO_2; 1 kPa O_2 + 3 kPa CO_2; 1.5 kPa O_2 + 1.5 kPa CO_2; 0.5 kPa O_2 + 0.5 kPa CO_2 and 2 kPa O_2 + 1 kPa CO_2. Apple and pear kept in regular air showed higher ACC-oxidase activity, ethylene production and respiration rates. Under controlled atmosphere, lower O_2 and/or higher CO_2 partial pressures strongly inhibited ACC-oxidase activity and ethylene production of apple and pear. Agreeing with ACC-oxidase activity and ethylene production, respiration rate was affected by the controlled atmosphere in a similar manner. The controlled atmosphere condition of 0.5 kPa O_2 + 6.0 kPa CO_2 showed the strongest suppression in ethylene production, respiration and generated higher values in the respiratory quotient. However, fruit metabolism was strongly suppressed in 'Jonagold' than in 'Conference', persisting a strong residual effect of controlled atmosphere during the full 10 d shelf-life in apple.

Key words: *Malus domestica* Borkh., *Pyrus communis* L., ACC-oxidase activity, respiration, respiratory quotient.

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INTRODUCTION

In addition to the low temperature used in regular air (RA), the controlled atmosphere (CA) storage is characterized by a reduction in O_2 and an increase in CO_2 partial pressures depending on the tolerance of the fruit cultivar (Thompson 2013). CA set points for long-term fruit storage should be better studied, mainly for 'Conference' pear, which is very sensitive to low O_2 and/or high CO_2 partial pressures (Saquet et al. 2000; Saquet et al. 2003; Pedreschi et al. 2009).

During the postharvest storage of typical climacteric fruits, like pear and apple, some effects of ethylene are desirable. Most pome fruit cultivars, with some exceptions, produce large amounts of ethylene during ripening. Fruit are harvested at a mature pre-climacteric stage and stored for several months at low temperature. Mainly in pear, cooling induces, after rewarming, a uniform ripening process with the development of satisfactory aroma and fruit texture via ethylene biosynthesis (Fonseca et al. 2005).

On the other hand, ethylene also triggers fruit ripening and senescence and reduces storage life (Streif 1992; Thompson 2013), e.g. in 'Barttlet' pear with yellowing, softening and induction of internal disorders and superficial scald (Bower et al. 2003) as also in 'Conference' (De Wild et al. 1999) and 'Rocha' pears (Fonseca et al. 2005). Normally apples are more sensitive to ethylene than pears. Johnston et al. (2009) have shown that anti-sense suppression of 1-aminocyclopropane-1-carboxylate (ACC)-oxidase resulted in apples with an ethylene production sufficiently low to be able to assess ripening in the absence of ethylene.

The cell respiration is one of the most important processes in stored fruit. It generates ATP and intermediate compounds needed for cell maintenance (Fernie et al. 2004). During storage, pears, like apples, preferentially use organic acids as a respiration substrate, and depending on the conditions, the organic acid content can be drastically reduced. Fruit respiration uses sugars only after the initial levels of organic acids are reduced. Since cell respiration strongly depends on temperature and gas conditions during storage, fruit respiration rates can normally be used as an indicator of storage potential (Streif 1992).

Aerobic cell respiration predominates when enough O_2 is present, and anaerobic respiration begins under conditions of low O_2. Normally, the 2 processes occur together with varying intensities depending on the environmental conditions. When anaerobic respiration is predominant, ethanol and acetaldehyde accumulate in pear (Saquet and Streif 2006) and apple tissues (Saquet and Streif 2008). These substances are toxic to cells and can induce formation of off-flavour and alcoholic aroma (Meheriuk et al. 1994). 'Conference' pear (Saquet and Streif 2006) and 'Jonagold' apple (Saquet and Streif 2008) also produce lactate as a fermentation product. The respiratory quotient (RQ) describes the molar ratio between the quantity of released CO_2 and the uptake O_2 during respiration and gives information about the fermentation processes in CA. Under fermentation conditions, the RQ can increase continuously reaching high values depending on the species and cultivar (Weber et al. 2015).

This investigation evaluates the CO_2 release, O_2 uptake, RQ, ACC-oxidase activity and ethylene production of 'Jonagold' apple and 'Conference' pear stored under various CA-conditions and in RA at 0 °C, as well as during a 10 d shelf-life at 20 °C.

MATERIAL AND METHODS

At-harvest, pre-climacteric 'Conference' pear and 'Jonagold' apple were selected for maturity, size, colour and freedom from damage and/or defects. Each experimental treatment used 30 fruits in 3 replicates.

Over 2 consecutive years, fruits were stored for 6 months under various CA-conditions and RA at 0 °C followed a 10 d shelf-life at 20 °C. The CA-conditions were: 0.5 kPa O_2 + 6.0 kPa CO_2; 3 kPa O_2 + 6 kPa CO_2; 1 kPa O_2 + 3 kPa CO_2; 1.5 kPa O_2 + 1.5 kPa CO_2; 0.5 kPa O_2 + 0.5 kPa CO_2 and 2 kPa O_2 + 1 kPa CO_2 as described by Saquet et al. (2000).

Measurements of respiration under CA or RA at 0 °C were carried out as described by Saquet (2001). At-harvest and every 2 months of storage after, fruit samples were removed from storage rooms and placed under CA-conditions in a laboratory device composed basically by an indirect hydro cooling and a board gas mixing system in order to reproduce the CA-conditions used in CA. For these measurements, 3 replicate samples of 3 fruits each were placed in 2.15 L sealed glass jars with flow-through CA atmospheres. After 24 h, the CO_2 release and O_2 uptake were measured at 0 °C. A 'Chrompack-Varian' micro gas chromatograph (CP 2002P) measured the CO_2 and O_2 partial pressures with a thermal conductivity detector at 45 °C; a molsieve capillary column for O_2 (4 m × 0.15 mm) at 40 °C; a packed hayesep column for CO_2 (25 cm) at 45 °C; carrier gas helium with flow →

of 2.5 mL·min^{-1}. CO_2 release and O_2 uptake were calculated and expressed in mL·kg^{-1}·h^{-1} and the RQs calculated.

For measurements of respiration in air at 20 °C, after storage, 4 fruits per treatment (3 reps) were placed in sealed glass jars with continuous air stream for 10 d at 20 °C as described by Saquet (2001). After 1 d fruits reached 20 °C, and the CO_2 release was determined with an infrared gas analyser (URAS-2, Fa. Mannesmann, Düsseldorf, Germany).

ACC-oxidase activity was determined after Choi et al. (1994). Skin samples (1.5 cm diameter and thickness) were taken from the equatorial region, and 4 g were incubated during 40 min at 20 °C in a 5 mL solution containing 50 mmol·L^{-1} MES-buffer (pH 6.0), 2 mmol·L^{-1} ACC and 0.5 mmol·L^{-1} cycloheximide. After incubation, samples were carefully dried and placed for 40 min in 50 mL sealed syringes containing 1 mL CO_2. The ethylene production was measured and the activity of ACC-oxidase, expressed in nL C_2H_4·g^{-1}·h^{-1}.

Ethylene production of whole fruits was measured in the same samples used for CO_2 release in air at 20 °C as described before. Ethylene of both, whole fruits and skin samples for ACC-oxidase activity were measured as follows: 1 mL headspace was injected into the Varian GC Series 2700 with an activated aluminium oxide packed column (0.9 m); injector temperature at 150 °C; flame ionization detector and oven temperature at 100 °C. The ethylene production of whole fruits was calculated using ethylene standard and the results are given in µL C_2H_4·kg^{-1}·h^{-1}.

For all analyses investigated, at least 3 true replicates were used as described early in each parameter analysed. For statistical comparison it was calculated the standard deviation (SD) of replicates.

RESULTS AND DISCUSSION

No ACC-oxidase activity was detected in 'Conference' at-harvest (Figure 1). In RA stored pears, ACC-oxidase activity increased during the first 4 months of storage reaching 300 nL C_2H_4 g^{-1}·h^{-1} and then decreased. CA conditions inhibited ACC-oxidase activity according to the gas compositions. Pears under 2 kPa O_2 + 1 kPa CO_2 showed ACC-oxidase activity very similar to RA fruits. ACC-oxidase activity of pear fruits under 0.5 kPa O_2 + 6.0 kPa CO_2 was particularly reduced.

No ACC-oxidase activity was detected in 'Jonagold' apple at-harvest (Figure 2). However, in general, apple responded more sensitively to the CA conditions than pears. RA apple showed the highest enzyme activity and significantly different compared to 2 kPa O_2 + 1 kPa CO_2; 1 kPa O_2 + 3 kPa CO_2 and 0.5 kPa O_2 + 6.0 kPa CO_2 treatments. The latter CA treatment showed the strongest inhibition of ACC-oxidase activity in 'Jonagold'.

ACC-oxidase and ACC-synthase are the rate limiting enzymes of ethylene biosynthesis in apple and pear. At-harvest, ACC-oxidase showed very low activity and correspondingly very low ethylene production. Similar results were reported

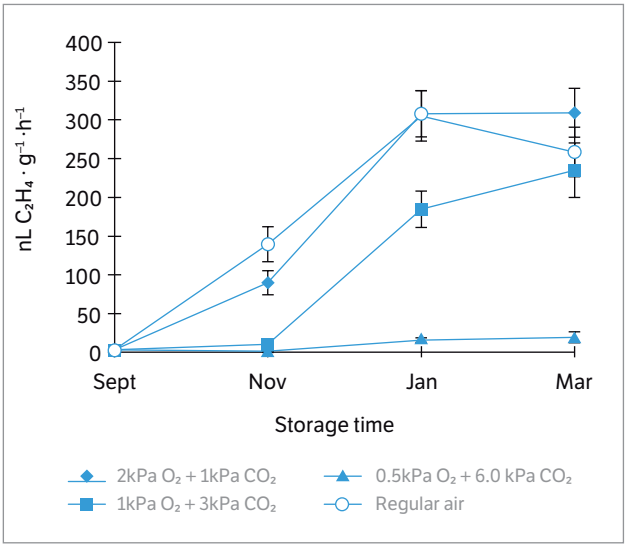

Figure 1. ACC-oxidase activity of 'Conference' pear under various storage conditions. Vertical bars indicate standard deviation of the replicates.

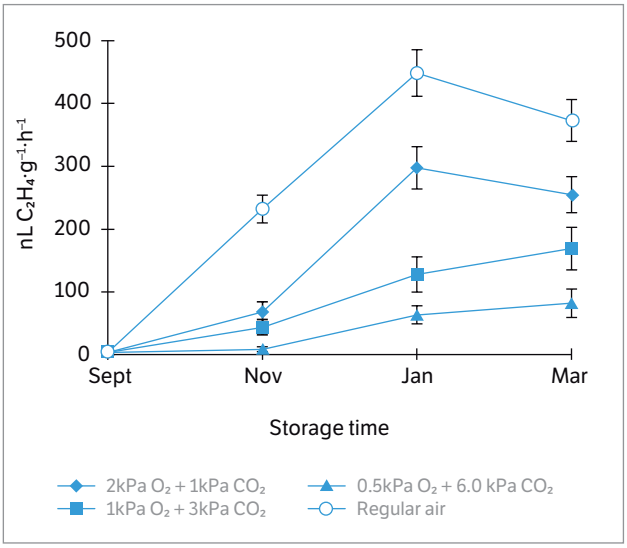

Figure 2. ACC-oxidase activity of 'Jonagold' apples under various storage conditions. Vertical bars indicate standard deviation of the replicates.

by Brackmann et al. (1993) in 'Golden Delicious' apple, Gerasopoulos and Richardson (1997) with 'Anjou' pear and Agar et al. (2000) in 'Bartlett' pear. ACC-oxidase activity increased during the first 4 months of storage and then decreased slowly until the end of storage as shown by Gerasopoulos and Richardson (1997) for 'Anjou' pear.

'Jonagold' showed a clearly inhibition of ACC-oxidase induced by the different CA conditions with a corresponding decrease in ethylene production. A residual effect of CA on ethylene production has also been measured by Brackmann et al. (1993) and Saquet et al. (2003).

The ethylene production of 'Conference' pear was very low at-harvest and increased gradually during storage (data not shown). The RA pears reached their maximum in ethylene levels at 4 months, while the CA stored fruits, only after 6 months of storage. RA fruits always showed the highest productions followed by the CA treatments of 2 kPa O_2 + 1 kPa CO_2; 1 kPa O_2 + 3 kPa CO_2 and 0.5 kPa O_2 + 6.0 kPa CO_2. As shown by the CO_2 release, the ethylene production was most inhibited in fruits held under 0.5 kPa O_2 + 6.0 kPa CO_2. During shelf-life at 20 °C ethylene production in pear increased strongly from d 3, especially for the RA treatment, and then decreased sharply later (Figure 3).

Ethylene production of 'Jonagold' apple during storage behaved similarly to 'Conference' pear (data not shown). However, for apple, the different CA conditions induced a stronger inhibition in ethylene production as well as parallel behaviour of ethylene production and CO_2 release. The ethylene levels under 1 kPa O_2 + 3 kPa CO_2 and 0.5 kPa O_2 + 6.0 kPa CO_2 were substantially reduced. Low O_2 conditions reduce oxidative metabolism and enhance fermentation (Imahori et al. 2013) and ripening may also be delayed by ethanol (Asoda et al. 2009), especially by decreasing ACC-oxidase activity and ethylene production (Liu et al. 2012). Thewes et al. (2015) also observed lower ACC-oxidase activity in 'Royal Gala' apples when stored under O_2 partial pressures lower than 0.6 kPa.

The residual effect of CA-storage on ethylene production, especially in apple fruits under 1 kPa O_2 + 3 kPa CO_2 and 0.5 kPa O_2 + 6.0 kPa CO_2, persisted during the full 10 d shelf-life at 20 °C (Figure 4). However, the RA and the moderate CA treatment like 2 kPa O_2 + 1 kPa CO_2 proportionated high rates in ethylene production. Residual CA-effect during shelf-life are frequently verified in apples in 'Cox's Orange Pippin' and 'Royal Gala' (Johnston et al. 2006).

Lowering O_2 and/or increasing CO_2 partial pressures during CA storage can affect ethylene biosynthesis and its action on fruits. Early investigations decreasing O_2 from 3 to 1 kPa reduced ethylene biosynthesis by ~ 50% in apple (Burg and Thimann 1959). A further challenge was that O_2 is a co-substrate of ACC-oxidase and that the *Km* of this enzyme for O_2 *in vivo* ranges between 0.3 and 6.0 kPa in apple. Lelièvre et al. (1997) showed that low O_2 partial pressures decreased the activity of ACC-oxidase. However, investigations of Gorny and Kader (1996) showed that, apart from ACC-oxidase, the →

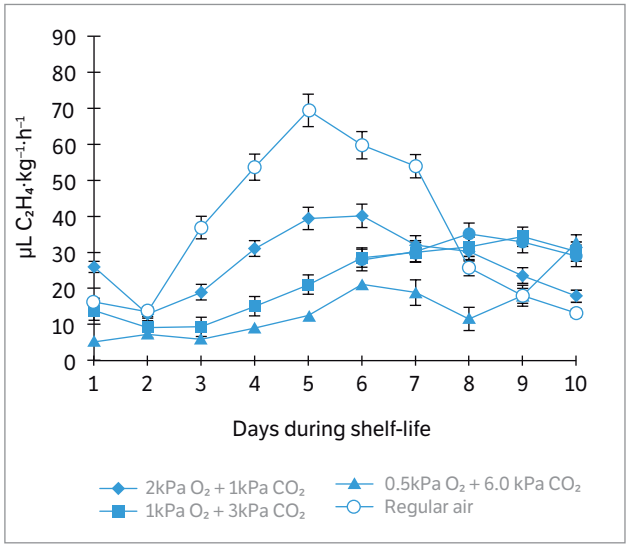

Figure 3. Ethylene production of 'Conference' pear during a 10 d shelf-life at 20 °C after 6 months of storage. Vertical bars indicate standard deviation of the replicates.

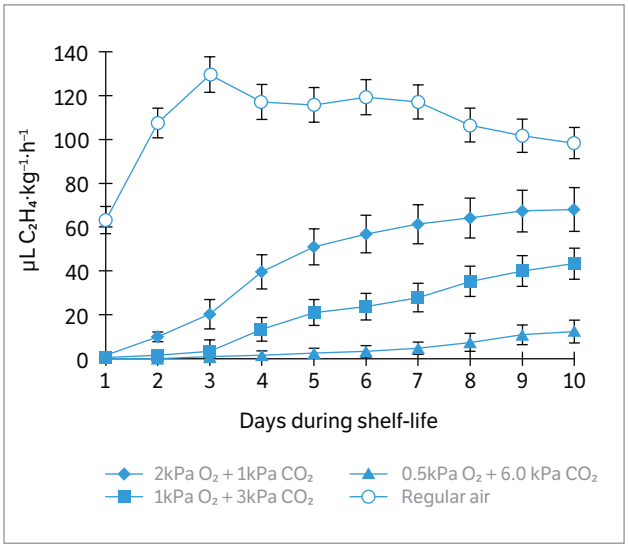

Figure 4. Ethylene production of 'Jonagold' apples during a 10 d shelf-life at 20 °C after 6 months of storage. Vertical bars indicate standard deviation of the replicates.

activity of ACC-synthase was also reduced by lowering O_2 in CA storage.

The presence of CO_2 in CA affects ethylene biosynthesis by different mechanisms and biochemical pathways. Indeed CO_2 stimulates ACC-oxidase activity *in vitro* with an optimal in the range of 2 kPa (John 1997). However, at higher partial pressures, CO_2 acts as a competitive inhibitor of ethylene action (Burg and Burg 1967). In accordance with these observations, there are other results such as the use of CO_2 partial pressures up to 5 kPa to inhibit ACC-oxidase and ACC-synthase (Bufler 1986) as well as the induction of *m*RNA (Gorny and Kader 1996). Detailed review on CO_2 effects and mode of action of CO_2 in ethylene metabolism are available in Pech et al. (2012).

Figure 5 shows the respiration rate of 'Conference' pear during 6 months of storage. From the low respiration at-harvest, it increased reaching a maximum at the fourth month. Pear fruit respiration was lowered as the O_2 decreased and the CO_2 increased partial pressures.

The CO_2 release of pear during the 10 d shelf-life at 20 °C, after 6 months storage, is given in Figure 6. During the first 5 d a residual effect of CA conditions on pear respiration was observed; however, in the last 5 d, this effect practically disappeared.

'Jonagold' apple behaved similarly (Figure 7); however, apple showed a stronger residual effect of CA on respiration, especially when stored under 1 kPa O_2 + 3 kPa CO_2 or 0.5 kPa O_2 + 6.0 kPa CO_2. During the full storage time, apples kept in RA showed the highest production of CO_2. Figure 8 shows results for CO_2 release during shelf-life of 'Jonagold' apple. A pronounced inhibition of fruit metabolism even after 7 d in air at 20 °C can be seen.

Pear respiration measured at-harvest decreased in all treatments according to the CA conditions (Figure 9). The CA treatment 3 kPa O_2 + 6 kPa CO_2 induced an intermediary effect on respiration, with the strongest inhibition caused by 0.5 kPa O_2 + 6.0 kPa CO_2 followed by 0.5 kPa O_2 + 0.5 kPa CO_2. Furthermore, pears in 0.5 kPa O_2 + 6.0 kPa CO_2 →

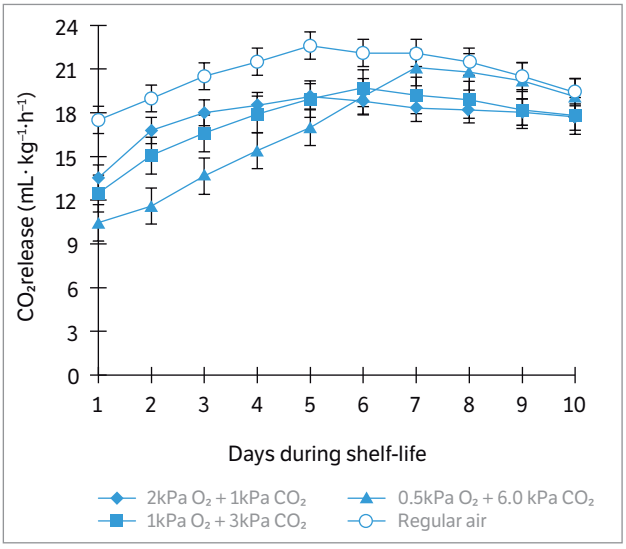

Figure 6. Respiration of 'Conference' pear during 10 d of shelf-life at 20 °C after 6 months of storage. Vertical bars indicate standard deviation of the replicates.

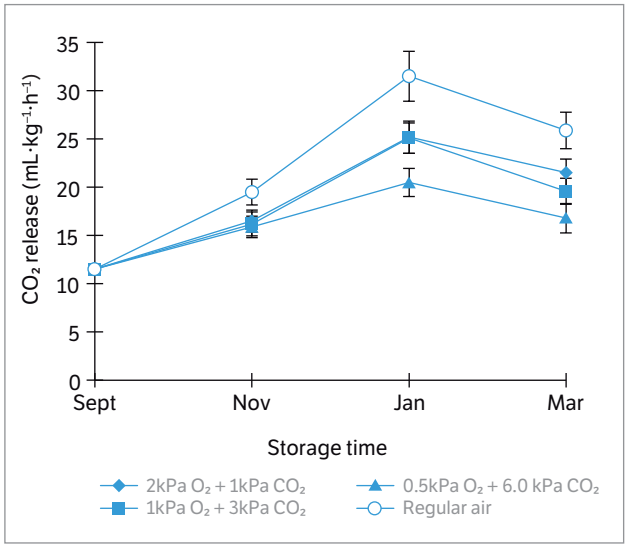

Figure 5. Respiration of 'Conference' pear during 6 months of storage. CO_2 measurements were carried out every 2 months and during shelf-life at 20 °C. Vertical bars indicate standard deviation of the replicates.

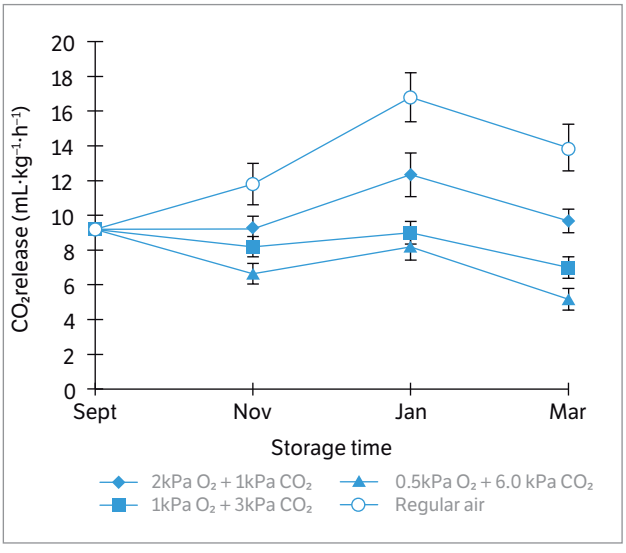

Figure 7. Respiration of 'Jonagold' apple during 6 months storage. CO_2 measurements were carried out every 2 months and during shelf-life at 20 °C. Vertical bars indicate standard deviation of the replicates.

showed an increase in CO_2 release from the second month indicating the triggering of anaerobic respiration prior to the other treatments.

The CO_2 release of 'Jonagold' under CA storage at 0 °C is presented in Figure 10. The RA stored apples released higher amounts of CO_2 with maximum levels at the fourth month of storage. As observed in pear, the 0.5 kPa O_2 + 6.0 kPa CO_2 treatment caused the strongest inhibition in apple respiration. The 1.5 kPa O_2 + 1.5 kPa CO_2 and 0.5 kPa O_2 + 0.5 kPa CO_2 apple treatments gave a similar inhibition of CO_2 release.

Figure 9b shows the O_2 uptake of 'Conference' pear during storage at 0 °C. RA stored pears took up more O_2 than the other storage treatments, and the lowest values were measured in fruits kept in 0.5 kPa O_2 + 6.0 kPa CO_2 and 0.5 kPa O_2 + 0.5 kPa CO_2. Both CA conditions proportionated very similar results indicating that the O_2 uptake was more dependent on the O_2 rather than on CO_2 partial pressures. The strongest reduction in O_2 uptake was detected after the first 2 months of storage, then O_2 uptake remained low and stable up to the end of storage.

The O_2 uptake of 'Jonagold' apple at 0 °C is shown in Figure 10b. 'Jonagold' after harvest up to the second month of storage showed a marked inhibition in respiration rates. After this initial period, the O_2 uptake remained constant at low levels. Apple fruits kept in RA showed a maximum O_2 uptake at the fourth month of storage concomitantly with a maximum CO_2 release. The lowest O_2 uptake was measured under 0.5 kPa O_2 + 6.0 kPa CO_2 and 0.5 kPa O_2 + 0.5 kPa CO_2 with practically no difference between these treatments.

In 'Conference' pear (Figure 9c) and 'Jonagold' apple (Figure 10c) the extreme CA treatment with 0.5 kPa O_2 + 6.0 kPa CO_2 induced a continuous and very high increase in RQ during the full storage period. 'Conference' reached →

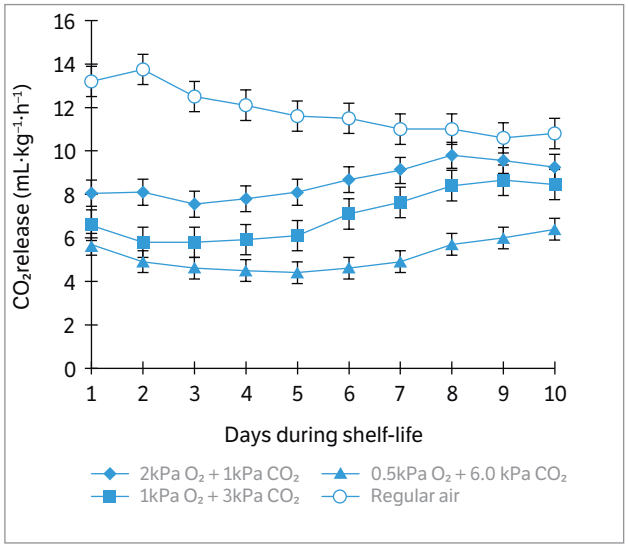

Figure 8. Respiration of 'Jonagold' apple during 10 d of shelf-life at 20 °C after 6 months of storage. Vertical bars indicate standard deviation of the replicates.

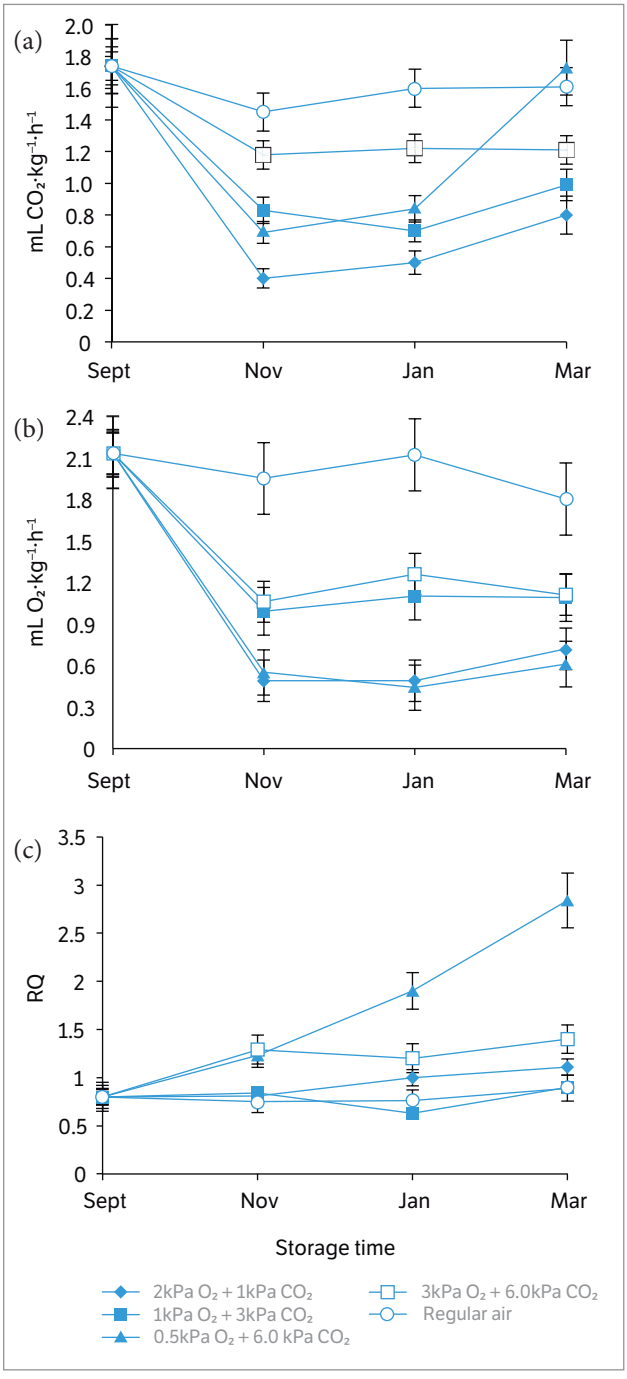

Figure 9. CO_2 release (a), O_2 uptake (b) and respiratory quotient (c) of stored 'Conference' pear. Vertical bars indicate standard deviation of replicates.

the highest RQ values of 2.8 at the end of storage, while 'Jonagold' apple increased up to 3.6. RQ values for fruits in RA and those under moderate CA conditions remained in the range of 1.0.

Results of fruit respiration showed a very good match with ethylene production during storage at 0 °C, and these trends were very similar to the measurements during shelf-life at 20 °C; the only difference was the temperature effect on fruit metabolism.

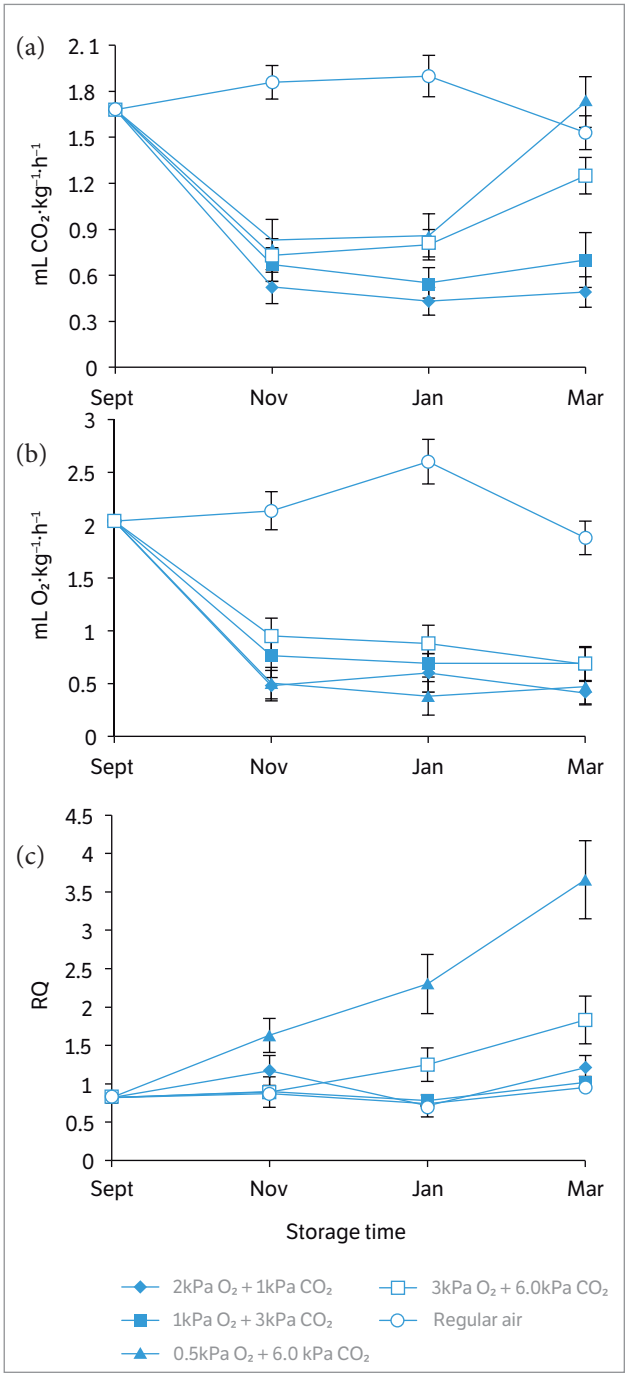

Figure 10. CO_2 release (a), O_2 uptake (b) and respiratory quotient (c) of stored 'Jonagold' apple. Vertical bars indicate standard deviation of replicates.

CA treatments, which strongly inhibited fruit respiration, resulted in a corresponding lower CO_2 release from fruit after storage. A significant inhibition could be measured in 'Jonagold' apple even after 10 d of shelf-life. On the other hand, 'Conference' pear responded faster than apples to the restoration of aerobic conditions at 20 °C.

The increase in the RQ in these trials confirms that, under the more extreme CA conditions with 0.5 kPa O_2 + 6.0 kPa CO_2 and 3 kPa O_2 + 6 kPa CO_2, fermentation started and at the same time increased ethanol and acetaldehyde production (data not shown). Gran and Beaudry (1993) reported about increased RQ values with a concomitant accumulation of ethanol in apple cultivars when O_2 partial pressures were below 2 kPa, agreeing then with Gasser et al. (2008).

Apart from the effects of CA storage on ethylene metabolism, low O_2 and/or high CO_2 partial pressures affect the activity of the various fruit respiratory enzymes. According to Kader (1986), high CO_2 inhibits the activity of different enzymes and can uncouple the oxidative phosphorylation. As reported by Knee (1973) and Monning (1983) the activity of succinate dehydrogenase is especially inhibited by CO_2. Investigations of Lange (1997) on 'Bartlett' pear showed that the activity of cytochrome oxidase was reduced by high CO_2 partial pressures. Furthermore, Ke et al. (1995) reported an inhibition effect of high CO_2 combined with low O_2 partial pressures on the activity of pyruvate dehydrogenase in avocado fruit.

Investigations on the mode of action of CO_2 on some glycolytic enzymes are controversy. While Kerbel et al. (1988) measured a reduction in the activity of ATP- and PPi-phosphofructokinase in 'Bartlett' pear, Hess et al. (1993) could not observe this effect in avocado fruit.

Low O_2 partial pressures reduce the activity of cytochrome oxidase, but this, according to Solomos (1997), is improbable because the affinity of cytochrome oxidase for O_2 is very high. The mode of action of low O_2 partial pressures on the glycolytic enzymes or on the tricarboxylic acid cycle enzymes is not fully understood and, despite the advances in research technologies, not so much has been studied about this effect. According to McGlasson and Wills (1972) low O_2 inhibits enzymatic steps in the TCA cycle between either oxaloacetate or pyruvate and citrate or between 2-oxoglutarate and succinate. Results of Ke et al. (1995) investigating the

metabolism of avocado fruit show an inhibitory effect on the activity of pyruvate dehydrogenase. Many reviews discuss about the possible perception mechanisms and regulation of respiration in plant organs under hypoxia or anoxia, but not much in fruits (Zabalza et al. 2009; Gupta et al. 2009).

CONCLUSION

'Jonagold' apple and 'Conference' pear stored for 6 months under regular air showed higher ACC-oxidase activity, ethylene production and respiration rates. Under CA, the lower O_2 and/or the higher CO_2 partial pressures strongly inhibited the ACC-oxidase activity and ethylene production of apple and pear fruits. The CA condition 0.5 kPa O_2 + 6.0 kPa CO_2 caused the strongest suppression in ACC-oxidase activity, ethylene production and fruit respiration and, consequently, higher RQ values in both apple and pear fruits. Fruit metabolism was strongly suppressed in 'Jonagold' apple than in 'Conference' pear, including the persistence of a marked residual effect of CA in 'Jonagold' apple during the full 10 d shelf-life at 20 °C.

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