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Fermentative Metabolism in 'Jonagold' Apples under Controlled Atmosphere Storage

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Summary

The fermentative metabolism was studied in cv. 'Jonagold' apples stored at 0 °C in the following controlled atmosphere (CA) conditions: 6 kPa CO_2 + 0.5 kPa O_2; 3 kPa CO_2 + 1 kPa O_2; 1 kPa CO_2 + 2 kPa O_2, and also in regular air (RA) as a control. Apples in RA storage showed very low pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activity, and low concentrations of acetaldehyde and ethanol. The production of lactate, and the activity of lactate dehydrogenase (LDH) in apple fruit were inhibited during storage, but a slight increase was observed during subsequent 8 days shelf-life at 20 °C. During CA-storage, cv. 'Jonagold' apples both the activity of PDC and ADH increased with a corresponding accumulation of acetaldehyde and ethanol, mainly in fruits stored at high CO_2 and/or low O_2 partial pressures. Both enzymes, and their products, decreased to low levels during the 8 days shelf-life at 20 °C after CA-storage. Lactate content and activity of LDH in CA-stored fruit showed a similar behaviour to the RA-stored fruit, however, the reduction of the enzyme activity was greater under CA-conditions. Overall, it is concluded that cv. 'Jonagold' apple fruit synthesize minute amounts of lactate and LDH and play a minor / negligible role in the fermentative metabolism of cv. 'Jonagold' and possibly other apple fruit.

Key words. Acetaldehyde – alcohol dehydrogenase [E.C 1.1.1.1] – enzymes – ethanol – lactate – lactate dehydrogenase [E.C. 1.1.1.27] – pyruvate dehydrogenase [E.C. 1.2.4.1]

Introduction

Controlled atmosphere (CA) storage improves the maintenance of various fruit quality attributes such as flesh firmness, organic acids, vitamins and sugars, when compared to regular cold storage in air (Streif 2002). Furthermore, the use of very low O_2, and/or its combination with high partial pressures of CO_2 during CA assists in insect desinfestation, disease control (Ke et al. 1995) and the alleviation of some physiological disorders such as superficial scald (Schouten 1988).

However, some undesirable physiological changes may also occur in plant tissues under low O_2 and/or high CO_2 storage. Plant cell responses to very low O_2 and/or very high CO_2 partial pressures during storage include the induction of fermentative metabolism pathways, the accumulation of succinate and/or alanine (Perata and Alpi 1993), a decrease in cell pH (Lange and Kader 1997) and decrease in ATP levels (Saquet et al. 2003). The most active pathway of fermentative metabolism in plants results in the accumulation of acetaldehyde and ethanol, catalysed respectively by the enzymes pyruvate decarboxylase (PDC; [E.C. 1.2.4.1]) and alcohol dehydrogenase (ADH; [E.C. 1.1.1.1]) (Ke et al. 1994a). In avocado fruit (Ke et al. 1995) and carrots (Kato-Noguchi 1998) lactate can also accumulate, catalysed by lactate dehydrogenase (LDH; [E.C. 1.1.1.27]). The major function of fermentative metabolism is to use NADH and pyruvate when electron transport and oxidative phosphorylation are inhibited, so that glycolysis can proceed. This enables the production of ATP through substrate phosphorylation, which allows the plant tissues to survive (Davies 1980).

Low O_2 or high CO_2 partial pressures have been shown to result in the accumulation of ethanol and acetaldehyde in 'Fuji' (Volz et al. 1998) and 'Delicious' apples (Nichols and Patterson 1987), in 'Bartlett' pears (Ke et al. 1990), avocados (Ke et al. 1995) and grapes (Tesnière et al. 1994). Gene expression and the activities of ADH, PDC and LDH can be influenced differentially by the partial pressures of O_2 and CO_2 used in CA. The activities of PDC and ADH in 'Bartlett' pears is increased by exposure to high partial pressures of CO_2 (Ke et al. 1994a). Kanellis et al. (1991) found that ADH isozymes could be induced by exposure of avocado fruit to 2.5, 3.5 or 5.5 kPa O_2. Increased activity of PDC in 'Golden Delicious' apples kept in 4 kPa CO_2 + 3 kPa O_2 and low pressure storage was also detected, but no ethanol accumulated in these fruits (Bufler and Bangerth 1982).

At present, the role of LDH and lactate accumulation, and the behaviour of PDC, in stored apples remains relatively unknown. Thus, the objective of this research was to investigate the effects of various storage conditions on ADH, PDC and LDH activity, as well as on the accumulation of ethanol, acetaldehyde and lactate in 'Jonagold' apples.

Materials and Methods

In two consecutive years, apple fruit were harvested at the pre-climacteric ripening stage, selected for uniformity, size and colour and stored at 0 °C in regular air (RA-control), 1 kPa CO_2 + 2 kPa O_2, 3 kPa CO_2 + 1 kPa O_2 and 6 kPa CO_2 + 0.5 kPa O_2 (SAQUET et al. 2000). At 2-month intervals, samples for enzyme activity and lactate analysis were taken, immediately frozen in liquid nitrogen and stored at −30 °C. An aliquot of juice (10 mL) for ethanol and acetaldehyde determinations was also collected, placed in gas-tight sealed 20 mL vials, frozen and stored at −30 °C until analysis. Fruit were also evaluated after a shelf-life period of 8 days at 20 °C, following 6 months of storage.

Ethanol and acetaldehyde concentrations were analysed by the headspace technique. For the measurements, samples were warmed to 65 °C for 30 min and 1 mL of headspace gas was injected into a Fisons 3000 Series gas chromatograph fitted with a Porapaq Q column at 150 °C and a flame ionisation detector (FID) at 180 °C. Concentrations were calculated from the peak area using standard solutions of the respective products. Lactate was extracted according to KATO-NOGUCHI (1998) with some modifications. Frozen apple tissues [10 g fresh weight (FW)] without skin and core were ground to a fine powder in liquid N_2. The powder was homogenised with 15 mL 0.6 M $HClO_4$ and kept below 4 °C for 30 min, with occasional shaking, then centrifuged at 14,000 g for 20 min at 4 °C. The supernatant was neutralised with 2 M KOH and centrifuged again at 14,000 g for 20 min at 4 °C. The last supernatant was used for analysis. Lactate was quantified by monitoring the reduction of NAD^+ at 340 nm for 15 min at 25 °C in a 2.35 mL reaction mixture of glycylglycine buffer (pH 10) containing glutamate (14,66 mg), NAD^+ (7 mg), and ca. 40 units glutamate pyruvate transaminase and ca. 135 units lactate dehydrogenase (Boehringer, Mannheim, Germany).

For extraction of ADH, PDC and LDH from the apples, frozen fruit tissues (20 g FW) without skin and core, were ground to a fine powder as above. The powder was homogenised with 40 mL ice-cold 100 mM 2-(N-morpholino) ethane sulfonic acid (MES) buffer (pH 7.5), 5 mM DTT and 2.5 % (w/v) polyvinylpyrrolidone (PVP) and 0.02 % (w/v) Triton X-100. The homogenate was kept below 4 °C for 20 min and stirred continuously. The samples were then filtered through four layers of cheesecloth and centrifuged at 14,000 g for 20 min at 4 °C. The supernatant was used as a crude enzyme extract.

To assay ADH, a 0.3 mL aliquot of the crude extract was added to a reaction mixture consisting of 0.15 mM NADH solution in MES buffer (pH 6.5) and 80 mM acetaldehyde. The PDC activity was measured in a reaction mixture containing 5 mM thiamine pyrophosphate, with 50 mM $MgCl_2$ in MES buffer (pH 6.5), 0.85 mM NADH and 180 units mg^{-1} ADH, 50 mM pyruvate and 0.3 ml of crude extract. The LDH was assayed according to SUGIYAMA and TANIGUCHI (1997) with a mixture of 2.3 mM methyl pyrazole, 2.3 mM KCN, 0.5 mM NADH, 4.0 mM pyruvate and 0.5 ml of the crude extract. All enzymes were assayed spectrophotometrically at 340 nm. The protein concentration of the crude extracts was determined using the Bio-Rad protein assay.

Results and Discussion

Ethanol and acetaldehyde concentrations and the activity of alcohol dehydrogenase and pyruvate decarboxylase

At harvest, the concentrations of ethanol and acetaldehyde in the cv. 'Jonagold' apples were very low, but accumulated in fruit under the lowest oxygen storage condition of 6 kPa CO_2 + 0.5 kPa O_2 , mainly during the last part of the six month storage period (Fig. 1). Ethanol and acetaldehyde biosynthesis are catalysed by ADH and PDC, respectively. PDC catalyses the decarboxylation of pyruvate to acetaldehyde, which can further re-

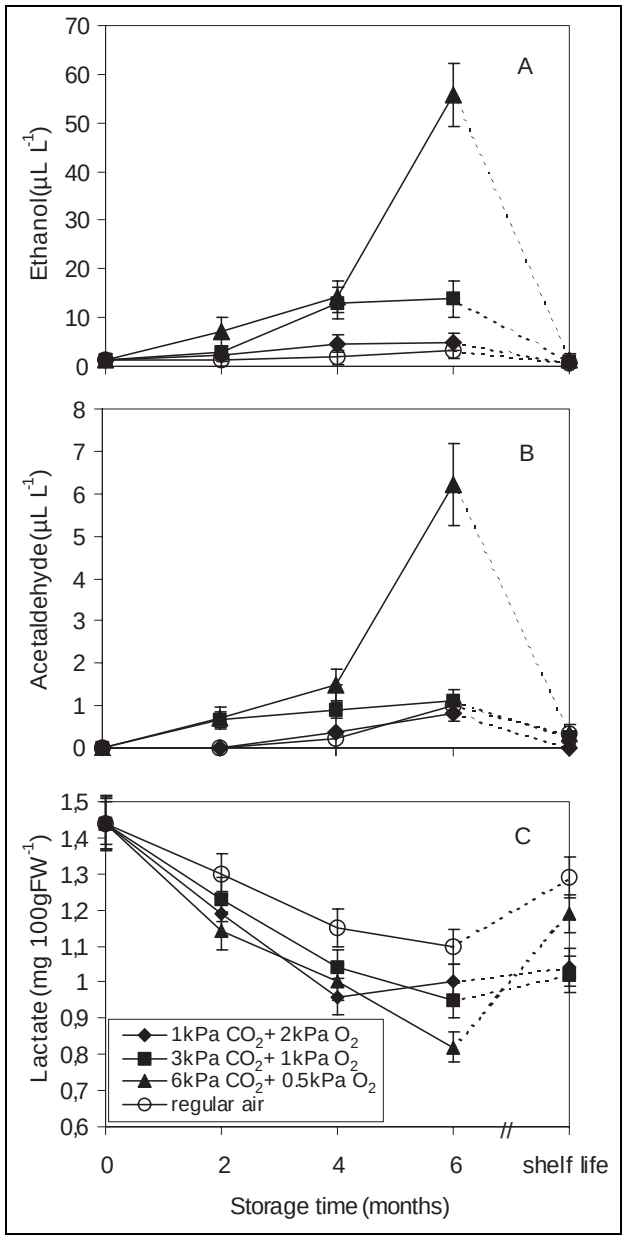


Fig. 1. Changes in ethanol (A), acetaldehyde (B) and lactate (C) concentrations in cv. 'Jonagold' apples after six months of storage, followed by 8 days shelf-life at 20 °C. Vertical bars indicate the standard deviation between treatment means.

duced by ADH to ethanol. In apples stored under the other CA-conditions, as well as in RA, ethanol and acetaldehyde concentrations increased only slightly. Similar results with the accumulation of ethanol and acetaldehyde in apples during CA-storage were published by VOLZ et al. (1998).

During the subsequent 8 days shelf-life at 20 °C, ethanol and acetaldehyde concentrations decreased rapidly to low levels in cv. 'Jonagold' apples , even in fruits kept at 6 kPa CO_2 + 0.5 kPa O_2 (Fig. 1). Similar behaviour was reported by FIDLER and NORTH (1971) for 'Cox Orange Pippin' and by NICHOLS and PATTERSON (1987) for 'Delicious' apples. Ethanol or acetaldehyde can be volatilised or metabolised during aeration. ADH is a reversible enzyme which can transform ethanol into acetaldehyde. Furthermore, acetaldehyde can be transformed to esters and others compounds. On the other hand, ethanol may directly be transformed to esters by the action of alcohol acetyl-CoA transferase (KE et al. 1994b).

The activity of ADH and PDC was closely related to the production of ethanol and acetaldehyde during both storage and shelf-life (Fig. 2). VENNESLAND (1960) reported the involvement of PDC in ethanol and acetaldehyde production during fermentative metabolism of apples. Information about the possible physiological role of PDC during storage of apples is given by BUFLER and BANGERTH (1982). They verified an increase of PDC activity in cv. 'Golden Delicious' apples during CA storage, however, without a corresponding increase of ethanol or acetoin in the fruit tissues. Higher ADH and PDC activity in cv. 'Jonagold' apples, especially in fruit stored under low O_2 and/or high CO_2 partial pressures, can be explained by the sensitivity of these enzyme reactions to changes in cellular pH. PDC and ADH activities are normally induced by lower pHs, which can be caused by low O_2 and/or high CO_2 partial pressures during CA-storage (KE et al. 1995). Others factors, which may also play important roles in the regulation of these enzymes, include the presence of ADH isozymes, and/or an increase in its substrates pyruvate and NADH (KE et al. 1994a).

Lactate concentrations and activity of lactate dehydrogenase

The concentration of lactate in apples decreased during the storage period in all storage conditions, especially in fruit under CA-storage (Fig. 1). LDH activity (Fig. 2) corresponded, in part, with decreased lactate concentrations, although a large decrease at the beginning of the storage period was the followed by a slight increase. During the subsequent 8 days shelf-life of the apples at 20 °C, lactate concentration and LDH activity increased. Literature on lactate and LDH in apples could not be found, but an inhibition in LDH is normally observed due to a decrease in cell pH in other plant organs (DAVIES et al. 1974). Changes in cell pH induced by CA-conditions as previously discussed may be responsible for the inhibition of LDH under CA, because according to DAVIES et al. (1974), the optimal pH of LDH is around 7. The low activity of LDH in RA, however, can not be explained by this theory.

DAVIES et al. (1974) showed that LDH can be inhibited by low cell pH and by high ATP concentrations. In 'Jonagold' apples, high ATP concentrations during RA-storage were found by SAQUET et al. (2000) and this could influence the activity of LDH. However, this can still not explain the further increase of LDH during shelf-life. This could be a simple effect of the high temperature during shelf-life or of the CA-conditions on the respiration rate of apples.

It is concluded that ADH and PDC seem to be rate limiting enzymes in the fermentative metabolism of 'Jonagold' apples. However, the physiological role and function of LDH and lactate accumulation in apples is not clear. Therefore, more research is needed in order to better understand the fermentative metabolism of apples under controlled atmosphere storage.

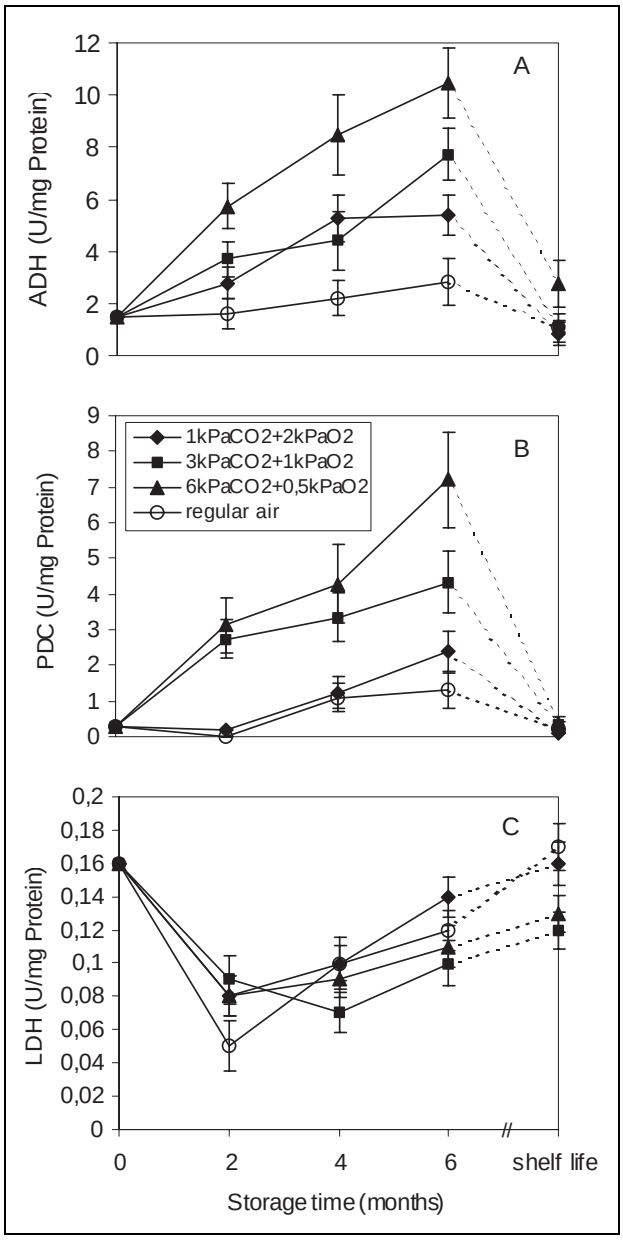


Fig. 2. Changes in alcohol dehydrogenase (A), pyruvate decarboxylase (B) and lactate dehydrogenase (C) of cv. 'Jonagold' apples after six months of storage, followed by 8 days shelf-life at 20 °C. Vertical bars indicate the standard deviation between treatment means

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