Effects of controlled atmosphere (CA) storage on pectinmethylesterase (PME) activity and texture of 'Rocha' pears

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Abstract: Firmness and pectinmethylesterase (PME) activity were evaluated in pears (cv Rocha) after 9 months of storage in controlled atmosphere (CA) followed by various periods of exposure to air at room temperature. The free calcium content was also evaluated in tissues. Fruit firmness decreased with increasing time of air exposure for all four different CA storage conditions tested. After 9 days of air exposure, fruits stored in 2% O_2 + 1.5% CO_2 were less firm than control fruits (stored in air) and showed higher PME activity. In spite of normal textural changes being observed with increasing time of exposure to air at room temperature, the underlying metabolism might have been affected by CA storage.

INTRODUCTION

It has been reported that pears can lose their capacity to ripen normally after storage in air for long periods of time.[1,2] Storage in controlled atmosphere (CA) has been used as a way to increase the shelf-life while maintaining the quality of fruits in terms of colour, texture and sensory characteristics.[3,4] The effects of the conditions and time of storage have been evaluated for various fruits, as they can have different responses to CA.[5,6]

The role of enzymes such as pectinmethylesterase (EC 3.1.1.11), *endo*-polygalacturonase (EC 3.2.1.15) and *exo*-polygalacturonase (EC 3.2.1.67) has been studied in order to seek a possible correlation between their activities and the process of softening of fruits.[7] Softening in pre-chilled tomatoes was correlated with a high extracted pectinmethylesterase (PME) activity after storage at 5 °C for 15 days.[8] Avocados stored in CA showed a reduction in initial softening time and this was associated with a decrease in PME activity.[9] Klein *et al*[7] reported an increase in PME activity of heated and unheated apples after 5 months of storage at 0 °C. However, they found no relationship between PME activity and apple textural changes. An increase in PME activity was found during ripening of Bartlett pears.[10]

The objective of this study was to evaluate the PME activity and firmness of 'Rocha' pears after CA storage in order to use them as indicators of the ripening process monitored by textural measurements during subsequent exposure to air at room temperature.

EXPERIMENTAL

Plant material and storage conditions

Pears (cv Rocha) were grown in the western region of Portugal. The fruits were harvested in August 1997 and stored at 0–0.5 °C and 90–95% relative humidity in air (NA) or controlled atmosphere (CA) by the Estação Nacional de Fruticultura Vieira Natividade (ENFVN). Four different CA storage conditions were established: two atmospheres with 2% O_2 and either 0.5 or 1.5% CO_2, and two atmospheres with 4% O_2 and the same levels of CO_2. The pears were removed from storage after 9 months and allowed to ripen in air at room temperature (19–20 °C). After various periods of air exposure (2, 6 and 9 days), corresponding to various stages of ripeness, 10 fruits from each storage composition were assayed for firmness. The fruits were peeled, cut into small pieces, frozen with liquid nitrogen and stored at −20 °C until they were used for the determination of pectinmethylesterase enzyme activity.

Firmness evaluation

The texture measurements were performed with a Universal Testing Machine (model 4501, Instron Corp, Ohio, USA). All tests were performed with the

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skin removed from the test section. The texture was measured as the maximum force (N) to the lateral-radial peeled surface of each fruit. Each fruit was punctured twice on opposite sides using a puncture probe of 8 mm diameter at 10 mm min^{-1}. A load cell of 100 N was used.

Enzymatic assay

Pectinmethylesterase extraction

Frozen pear samples from each CA condition were homogenised in a solution of 1.5 M NaCl in an external ice bath for 6 min. The pear/NaCl solution ratio was 1:4 (g ml^{-1}). The homogenate was centrifuged at 20 000 × *g* for 30 min at 4 °C (Sorvall RC-5C, Instruments Dupont, France). The supernatant was filtered with sixfold cheesecloth, collected and adjusted to pH 7.5 with 0.1 M NaOH. Three replicates of 10 pear samples were performed.

Pectinmethylesterase activity

Enzyme activity was determined by a continuous spectrophotometric assay according to the method of Hagerman and Austin.[11] A 2 ml portion of 0.5% (w/v) citrus pectin (pH 7.5) was combined in a cuvette with 0.15 ml of 0.01% (w/v) bromothymol blue in 3 mM potassium phosphate buffer (pH 7.5) and 0.75 ml of distilled water adjusted to pH 7.5. After adding 100 µl of filtered homogenate, the rate of decrease in absorbance at 620 nm was recorded *versus* a water blank for 20 s using a UV/visible recording spectrophotometer (model UV-260, Shimadzu Corporation) at 20 °C. PME activity was expressed as µmol galacturonic acid g^{-1} pear min^{-1}, taking into consideration the total volume of the extract and the volume of extract taken for the determination of PME activity (100 µl) (µmol of galacturonic acid/Δt min × V_{ext} ml/0.1 ml/g of pear). A calibration curve was previously prepared to relate the quantity of galacturonic acid to the variation in absorbance. Triplicate enzyme assays were performed for each extract from the PME extraction.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to detect differences among storage conditions with increasing time of exposure to air at room temperature. Significant differences ($P = 0.05$) among storage conditions were detected using Duncan's multiple-range test. ANOVA and correlation analysis were performed using SPSS 8.0 for Windows 95.

RESULTS AND DISCUSSION

Firmness

There was a significant decrease in the firmness of all samples from the first to the last day of exposure to air at room temperature. This is a consequence of the normal ripening process.[1] After 2 days, pears stored in 4% O_2 were firmer than those from 2% O_2 + 0.5% CO_2 CA storage and from NA storage (Table 1). It has been reported that pears can lose their capacity to ripen normally when they have been stored in air for a long time.[12,13] This phenomenon has been related to a lack of protein synthesis.

Table 1. Firmness (maximum force, N) of 'Rocha' pears after 9 months of CA storage followed by exposure to air at room temperature (19–20 °C)

	Time at 19–20°C (days)		
Storage condition	*2*	*6*	*9*
Air	65.15b	28.40a	22.55a
2% O_2 + 1.5% CO_2	66.55ab	24.95a	13.65c
2% O_2 + 0.5% CO_2	65.55b	30.25a	18.90ab
4% O_2 + 0.5% CO_2	70.70a	25.85a	17.50bc
4% O_2 + 1.5% CO_2	71.65a	25.50a	15.15bc

Data are means of three replicates of 10 pears.
Means separation in columns by Duncan's multiple-range test ($P = 0.05$); different means are followed by different letters.

No significant difference was found at day 6. Finally, at day 9, pears stored in air were firmer than those stored in 2% O_2 + 1.5% CO_2 and in 4% O_2. They were at the same level of firmness as those stored in 2% O_2 + 0.5% CO_2. Together with samples stored in 4% O_2, pears stored in 2% O_2 + 1.5% CO_2 were the softest after 9 days of exposure to air at room temperature.

Pectinmethylesterase activity

After 2 days of exposure to air at room temperature, fruits stored in 2% O_2 + 1.5% CO_2 showed the lowest PME activity, although at the same level of significance as NA-stored fruits (Table 2). Samples stored in 4% O_2 had the highest PME activity and fruits stored in 2% O_2 + 0.5% CO_2 were at the same level of significance. After 6 days of air exposure the enzyme activity of fruits stored in 4% O_2 decreased to the same level as that of NA-stored fruits. PME activity was highest for fruits stored in 2% O_2 + 1.5% CO_2. After 9 days, NA-stored fruits and fruits stored in 4% O_2 continued to show the lowest PME enzyme activity, and those stored in 2% O_2 + 0.5% CO_2 were at the same level of significance. Fruits stored in 2%

Table 2. Pectinmethylesterase activity (µmol galacturonic acid g^{-1} pear min^{-1}) of 'Rocha' pears after 9 months of CA storage followed by exposure to air at room temperature (19–20 °C)

	Time at 19–20°C (days)		
Storage condition	*2*	*6*	*9*
Air	70.90bc	56.77c	69.17b
2% O_2 + 1.5% CO_2	61.50c	126.11a	90.78a
2% O_2 + 0.5% CO_2	80.82ab	82.11b	77.26ab
4% O_2 + 0.5% CO_2	93.99a	47.42c	64.68b
4% O_2 + 1.5% CO_2	86.03a	48.80c	62.97b

Data are means of three replicates of 10 pears.
Means separation in columns by Duncan's multiple-range test ($P = 0.05$); different means are followed by different letters.

O_2+1.5% CO_2 showed the highest PME activity (Table 2).

The enzyme activity did not correlate with the softening of fruits from the different storage conditions. Klein *et al*[7] found a correlation between the decrease in firmness of heated and unheated apples and the increase in PME activity after 5 months of storage at 0 °C.

CONCLUSION

The texture results indicated that storage under CA permitted 'Rocha' pears to show normal textural changes as a consequence of the normal ripening process. It was expected that, once transferred to room temperature, pears would follow the normal ripening process and therefore their metabolic process would be re-established. However, this did not happen for some of the CA-stored fruits. Further biochemical studies are recommended on the characterisation of the enzyme PME used in this study.

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