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Polyphenoloxidase Activity of Pear (cv. Rocha) Reduced by Controlled Atmosphere Storage

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The enzymatic browning of fruits during storage may cause undesirable quality changes, specially of colour. During storage of fruits, polyphenoloxidase (PPO) activity and browning were reported by several authors to vary in different ways, depending on species, cultivar, picking maturity, storage conditions, etc (Amiot *et al.*, 1992). PPO soluble content and browning were, in most cases, found to increase during normal atmosphere. According to Burton (1982), since phenolases have a low oxygen-affinity, oxidation of phenolic substrates can be avoided or at least reduced at O₂ levels of 5% or less. Therefore, the use of controlled atmosphere (CA) storage may succeed to reduce these processes.

Although there are studies in literature on PPO of pears (Espín *et al.*, 1997; Halim and Montgomery, 1978; Smith and Montgomery, 1985; Zhou and Feng, 1992), very scarce, if none, information is available on PPO of 'Rocha' pear.

The objective of this work was to evaluate the effects of controlled atmosphere storage (low oxygen and carbon dioxide contents) on PPO activity of 'Rocha' pear, in comparison to storage in normal atmosphere.

Material and Methods

Plant material. Pears (cv. Rocha) were grown at West region of Portugal, harvested in August 28, 1996 and stored at 0°C (relative humidity 85-90%) either in normal atmosphere or controlled atmosphere (4.21 %O₂ and 1.01 %CO₂). The fruits were removed from the cold on February 16, 1997 and stored in normal atmosphere. The pears were supplied by Estação Nacional de Fruticultura Vieira Natividade (ENFVN) for this research work and on February 19 the fruits were transported from the ENFVN in Alcobaça to the laboratory in Porto within approximately 4 hours. They were stored at 2°C and 80 % of relative humidity.

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Treatment and storage conditions. Pears from each atmosphere storage were divided randomly into two samples. On February 21 the fruits of the first sample were cut into pieces, immediately frozen with liquid nitrogen and stored at -80°C. Two weeks later, the same procedure was followed for the second sample. Pear samples were stored for five months until used in the enzymatic assays.

Extraction of PPO. The method was based on the procedure described by Galeazzi *et al.* (1981). Pear samples were homogenized in the extraction buffer (0.2 M sodium phosphate buffer, pH 6.5) with 2% insoluble PVP and 0.25% Triton X₁₀₀ in an external ice bath for 3 min in 1 min intervals. The ratio buffer / pear sample used was 3 (mL/ g). The homogenate was centrifuged at 4°C for 30 min at 14, 000 x g (Sorvall RC-5C, Instruments Dupont). The supernatant was filtered through cheesecloth and its volume determined. Each extraction was performed in triplicate.

Assay for PPO activity. Enzymatic activity was assayed by measuring the rate of increase in absorbance at 420 nm and 28°C in a double beam model UV/VIS spectrophotometer. The substrate solution contained 2.8 mL of substrate solution (0.16 M catechol prepared in 0.05 sodium phosphate buffer, pH 6.5) and 20 µL of enzyme extract. The reference cuvette contained only substrate solution. The unit of the enzyme activity was defined as the change of 0.001 in the absorbance value. All determinations were performed in triplicate.

Results

Table 1. Polyphenoloxidase activity (U/ g of fruit/ min) of 'Rocha' pear after storage in different atmospheres

Storage atmosphere	Date of freezing	Polyphenoloxidase activity (U/ g of fruit/ min)
Normal	February 21	7200a ^z
Controlled	February 21	4534b
Normal	March 7	8058a
Controlled	March 7	3892b

^zData are means of 10 fruits

^yMean separation in columns by Duncan's multiple range test, $P=0.05$

Polyphenoloxidase activity of CA (4.21 %O₂ and 1.01 %CO₂) stored pears was found to be significantly lower than in the fruits stored in air. With the enzymatic assay performed in this study, no significant difference was detected between the samples frozen 5 days after being removed from cold storage and those frozen 20 days after.

Conclusion

There was a reduction of polyphenoloxidase activity by controlled atmosphere storage (4.21 %O₂ and 1.01 %CO₂).

Information regarding the response of PPO activity of pears (cv. Rocha) to different CA conditions would be very useful in order to help understand colour changes during storage, and to select the best CA composition for this cultivar preservation. Therefore, further research is required concerning the enzyme activity of the fruits stored under different sets (oxygen and carbon dioxide levels) of CA composition.

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References

- Amiot, M. J. , Tacchini, M., Aubert, S., Nicolas, J. 1992. Phenolic composition and browning susceptibility of various apple and pear cultivars at maturity. *Acta Horticulturae* **343**, 67-71.
- Burton, W. G. 1982. Postharvest physiology of food crops, Longman, London.
- Espín, J. C., Morales, M., Varón, R., Tudela, J., García-Cánovas, F. 1997. Monophenolase activity of polyphenol oxidase from *Blanquilla* pear. *Phytochemistry* **44**, 17-22.
- Galeazzi, M. A. M., Sagarbieri, V. C. J., Constantidines, S. M. 1981. Isolation, purification and physicochemical characterization of polyphenoloxidase from a dwarf variety of banana (*Musa Cavendishii*, L.). *J. Food Sci.* **46**, 150-155.
- Halim, D. H. and Montgomery, M. W. 1978. Polyphenol Oxidase of *d'Anjou* pears (*Pyrus communis* L.). *J. Food Sci.* **43**, 603-608.
- Smith, D. M. and Montgomery, M. W. 1985. Improved methods for the extraction of polyphenol oxidase from *d'Anjou* pears. *Phytochemistry*, **24**, 910-904.
- Zhou, H. and Feng, X. 1991. Polyphenol Oxidase from *Yali* Pear (*Pyrus bretschneideri*). *J. Sci. Food Agric.* **57**, 307-313.