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CHANGES IN GLYCOSIDASE ACTIVITIES WITH ETHYLENE PRODUCTION DURING FRUIT SOFTENING IN AVOCADO

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Abstract

(α-Arabinofuranosidase and β-galactosidase activities were assayed during fruit development and ripening in avocado (*Persea americana* Mill. cv. Fuerte) fruit. (α-Arabinofuranosidase activity increased drastically with ethylene production at 4 days after harvest, although weak activity was detected during fruit development. This suggests this enzyme is relating to fruit softening. On the other hand β-galactosidase activity was detected during fruit development and changed slightly with ripening.

Additional index words

cell wall, ripening, α-arabinoftu-anosidase, β-galactosidase

1. Introduction

Fruit softening is closely connected with cell wall modifications caused by some cell wall degrading enzymes (Fischer and Bennett, 1991). Polygalacturonase is one of the important enzymes in relation to tomato fruit softening. Recently it was suggested that polygalacturonase was not sole determinant of fruit softening (Smith et al., 1988, Giovannoni et al., 1989). In avocado, cellulase which is synthesized at the onset of ripening (Christoffersen et al, 1984) is well discussed and was suggested to closely relate to the fruit softening. Cellulase purified from avocado fruit degraded the substrates containing (1-4)-β-glycosyl linkages although it did not hydrolyze the cellulose polymer from mature avocado cell wall (Hatfield and Nevins, 1986). Further, it released arabinose and galactose in incubation with the cell wall from unripe fruit (Hatfield and Nevins, 1986).

The release of galactose and arabinose from pectic side chains caused by glycosidases during softening was detected in many kinds of fruit and is an important event in fruit softening (Redgwell et al., 1992, Dawson et al., 1992). β-Galactosidases were also purified from avocado fruit and its role on pectin solubility was discussed (Ian De Veau et al., 1993). However, there are little information of other glycosidases. Therefore, we investigated the alteration of β-galactosidase and α-arabinofuranosidase activities during fruit development and ripening to elucidate the role of those glycosidases in avocado fruit softening.

2. Material & Methods

2.1 Plant material

Avocado fruits (cv. Fuerte) were harvested periodically (August to March) in Numazu, Shizuoka prefecture in Japan. Mesocarp tissue of fruit was sliced and freezed before using. The fruits harvested in November were stored at 25 °C and then mesocarp tissue was sliced and freezed before using.

2.2 Measurement of ethylene production and fruit firmness

Ethylene production was measured by gas chromatography and fruit firmness was measured by Handy HIT (Fujihira Co. Ltd. Japan; nondestructive handy hardness meter, range 500-800gf).

2.3 Assay of glycosidase activities

Enzyme extraction procedure was carried out at 4°C. Tissue was homogenized in 0.1 M K-phosphate buffer (pH 6.5) containing 30 mM 2-mercaptoethanol and 0.1% (w/v) sodium-L-ascorbate. The homogenate was centrifuged at 10,000*g for 20 min. The precipitate was resuspended in 10 mM K-phosphate buffer (pH 6.5) containing 5 mM 2-mercaptoethanol and centrifuged at 10,000*g for 20 min. This step was repeated three times and the precipitation was suspended in the same buffer. The supernatant and precipitate were dialyzed separately against the same buffer and defined as buffer soluble and cell wall bound fractions, respectively. α-Arabinoftiranosidase and β-galactosidase activities were assayed using p-nitrophenylglycosides as substrate.

3. Results and discussion

3.1 Ethylene production and fruit softening

Ethylene production was detected at 4 days after harvest (figure 1), then the fruits reached the best soft to eat at 7 days (figure 2).

3.2 α-Arabinofuranosidase activity

Cell wall bound α-arabinofuranosidase and buffer soluble enzyme activities increased drastically after the peak of ethylene production. The fruit reached to edible soft after their α-arabinofuranosidase activities began to increase (figure 3). There were slight changes in the activities of both forms during fruit development (figure 5). It was reported that (α-arabinofuranosidase activity in apple increased during softening and degraded the pectin from apple (Yoshioka et al., 1995). In Japanese pear, α-arabinofuranosidase activity also increased to 15-fold with ripening (Tateishi et al., in press). Thus, it was suggested that α-arabinofuranosidase was induced specifically for fruit ripening and played an important role in fruit softening.

3.3 β-Galactosidase activity

There was little changes in the activity of β-galactosidase during ripening compared with α-arabinofuranosidase activity although slight increase in activities of both form was detected at 3 days after harvest (figure 4). The high activity of buffer soluble β-galactosidase during fruit development may be related in the release of glycoside from aglycon in cytoplasm. As it was reported that there were isoforms of β-galactosidase in some kinds of fruit, and one of isoforms might play an important role of fruit softening (Kitagawa et al., 1995), further studies are required to elucidate the physiological role of this enzyme in fruit softening.

4. Acknowledgements

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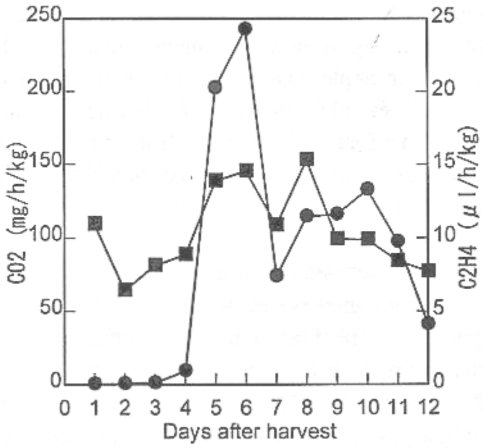

Figure 1. Respiration (■)and ethylene production (●) during ripening.

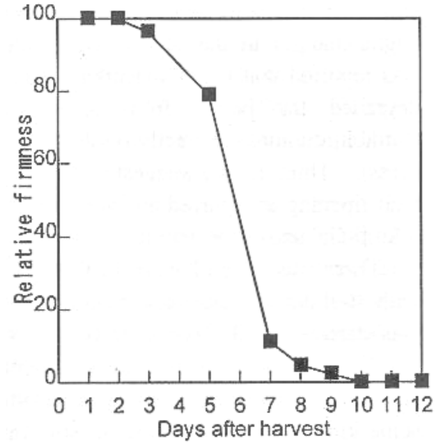

Figure 2. Changes in fruit firmness.

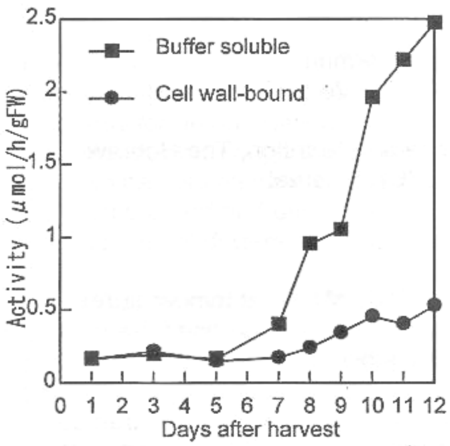

Figure 3. Changes in α-arabinofuranosidase activities during ripening.

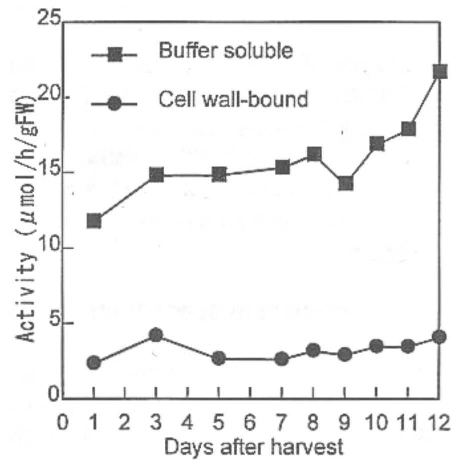

Figure 4. Changes in β-galactosidase activities during ripening.

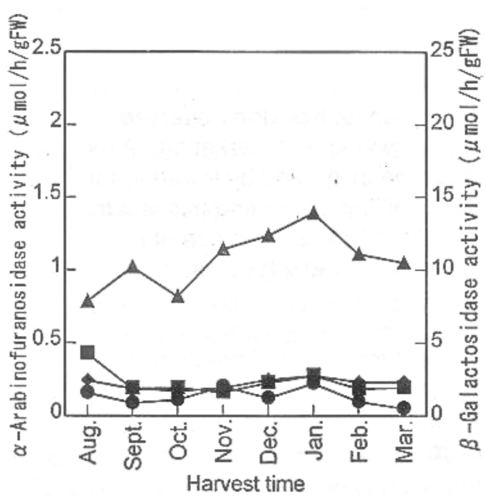

Figure 5. Changes in α-arabinofuranosidase activities(soluble■; bound ●) and β-galactosidase activities (soluble▲; bound ◆) during fruit development.