Characterization of Enzymes and Phenolics Involved in the Pericarp Hardening of Mangosteen Fruit Stored at Low Temperature

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Abstract

We investigated chilling injury symptom (pericarp hardening) in mangosteen fruit at two maturities, reddish brown and reddish purple, stored at 6°C (87.0%RH) and 12°C (83.5%RH) for 15 days. Fruit stored at 6°C had greater pericarp firmness than those stored at 12°C and reddish purple fruit had greater pericarp firmness than reddish brown fruit. Fruit stored at 6°C for 9 days and transferred to room temperature (29.5°C, 74.0%RH) for 3 days showed more prominent symptom of pericarp hardening. When pericarp hardening occurred, pericarp firmness and lignin contents increased whilst levels of total phenolics decreased. The main phenolic acids associated with pericarp hardening were identified by HPLC as *p*-coumaric and sinapic acids. *p*-Coumaric acid levels of fruit stored at 6°C decreased whereas in those of fruit stored at 12°C did not significantly change. On the contrary, sinapic acid of fruit stored at both temperatures increased throughout the storage time. A histochemical study of lignified cells in mangosteen pericarp using safranin O and toluidine blue stains showed that lignin accumulation increased with pericarp hardening. Reddish purple fruit held in 0.25% O_2 during storage at 6°C did not reduce pericarp hardening and showed no significant differences in firmness, lignin and total free phenolics levels when compared with fruit stored in normal air conditions. Fruit still increased in firmness and lignin contents, while total free phenolics decreased under low O_2. Enzymes in the lignin biosynthetic pathway, namely phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD), were also determined. PAL and POD activities in pericarp of fruit stored at 6°C increased with increasing storage time. Upon transfer of fruit to room temperature, both PAL and POD activities in the pericarp were higher than those in fruit stored at 6°C, and activities declined at the end of storage. CAD activity in fruit pericarp did not change. The key enzymes of lignification in pericarp hardening of mangosteen fruit stored at low temperature are likely to be PAL and POD, and expression of PAL and lignin peroxidase (LgPOD) genes in fruit pericarp was also determined using semi-quantitative RT-PCR, real-time PCR and northern blot techniques. Expression of PAL and POD genes increased with storage time at low temperature and their enzyme activities. However, low O_2 treatment slightly affected on the expression of PAL and POD genes.

These results suggested that the increase in pericarp firmness of mangosteen fruit resulted from induction of lignin synthesis associated with an increase in PAL and POD activities and genes expression.

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