

Title Ripening-related defense proteins in *Annona* fruit
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Abstract

In order to obtain a better understanding of the active defense strategy of cherimoya (*Annona cherimola* Mill.) fruit, hydrolytic and antifungal activity, as well as expression of proteins functionally and immunogenically related to the pathogenesis-related proteins chitinase (PR-Q) and 1,3- β -glucanase (PR-2), were estimated in fruit at different ripening stages. Increase in expression of the 27 kDa constitutive chitinase and the induction of two new proteins, a 26 kDa chitinase and a 51 kDa 1,3- β -glucanase were associated with enhanced *in vitro* hydrolytic and antifungal activity of the acidic protein extract in ripe fruit. Ripening modified the expression of constitutive basic isoenzymes, with a sharp decrease in both relative accumulation and hydrolytic activity. Likewise, a new basic 33 kDa chitinase was induced in the over-ripe fruit, concomitant with accumulation of a basic constitutive 76 kDa 1,3- β -glucanase. At this stage, the basic protein extract modified *in vitro* growth inhibition of *Botrytis cinerea*. Short-term high CO₂ treatment delayed fruit ripening and maintained a similar distribution of activity and isoenzymatic pattern in both protein fractions to that in unripe fruit. These results indicate that the changes in the pattern of defense proteins and hydrolytic activity in cherimoyas appear to be associated with ripening. Moreover, unlike the constitutively expressed isoenzymes, only the transitorily induced chitinases and 1,3- β -glucanases were associated with an active defense-related response.