

Title Regulation of defense and cryoprotective proteins by high levels of CO₂ in *Annona* fruit stored at chilling temperature

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Abstract

This study focuses on how the length of exposure to chilling temperature and atmosphere storage conditions regulate the hydrolytic activity and expression of chitinase (PR-Q) and 1,3- β -glucanase (PR-2) isoenzymes in cherimoyas (*Annona cherimola* Mill.). Storage at 6 °C modified the expression of constitutive isoenzymes and induced the appearance of novel acidic chitinases, AChi26 and AChi24, at the onset of the storage period, and of a basic chitinase, BChi33, after prolonged storage. The induction of this basic isoenzyme was concomitant with the accumulation of basic constitutive 1,3- β -glucanases. These low-temperature-induced chitinases modified the growth inhibition *in vitro* of *Botrytis cinerea*. Short-term high CO₂ treatment activated a coordinated response of acidic chitinases and 1,3- β -glucanases after prolonged storage at chilling temperature. Moreover, the high *in vitro* cryoprotective activity of CO₂-treated protein extracts was associated with the induction of two low molecular mass isoenzymes, AGlu19 and BChi14. Thus, exposure to high concentrations of CO₂ modified the response of fruit to low temperature, inducing the synthesis of cryoprotectant proteins such as specific pathogenesis-related isoenzymes that could be functionally associated with an increase in chilling tolerance *in vivo*.