[IMP2 02] Chilling injury alleviation of papaya fruit: changes related to softening and cell wall ultrastructure

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Introduction

Short storage life, susceptibility to diseases and chilling injury (CI) are the major factors that limit export of papaya fruits to long distant markets (Biglete *et al*., 1994; Ali & Lazan, 1998). Some of the CI symptoms of papaya are uneven ripening, sunken spots, skin blister and development off-flavor. Postharvest technology need to be established to overcome these problems. Heat treatment and the used of edible polymers as coating managed to lengthen storage life and the fruit became more resistant to fungal attack.

Heat treatment able to reduce fruit sensitivity towards low temperature (Paull & Chen, 2000) while coating are used on fruits and vegetables to improve appearance, to mimic modified atmosphere packaging effects as barriers to moisture loss and gaseous exchange and to reduce fungal infections (El Ghaouth *et al*., 1992). This present study reports the effects of postharvest treatment on chilling injury alleviation and on changes related to softening as well as cell wall ultrastructure of papaya fruit.

Materials and Methods

Postharvest treatment

Papaya (*Carica papaya* L. Exotica) at 5% yellow ripening stage were selected and divided into 5 groups. The first group was the control fruits (AT) which left to ripen at ambient temperature without further treatment. Fruits in second group (LT) were considered as control in low temperature. The third group (HT) was given heat treatment, 38°C for 6 hours. The forth group (HTC) was heated before treated with coating formulation contained carbohydrate polymer and the last group (NHTC) was treated with coating only. Before subjected to 10°C, LT, HT, HTC and NHTC were wrapped in polyethylene film. After 4 weeks, all 4 groups were unpackaged and transferred to ambient and the effect of treatments on cell, cell wall ultrastructure, softening enzymes activities and other ripening changes were studied. Tissue firmness was measured using a texture meter (Bishop, model FT327).

Light microscopy and electron microscopy

Samples for light and electron microscopy were prepared from fresh cut tissue based on modified method of Glauert (1975). The tissue was fixed in 2.5% glutaraldehyde and 1% osmium tetroxide before dehydrated through graded series of ethanol. The samples were then infiltrated in Epoxy resin. For light microscopy, toluidine blue was used for staining while uranyl acetate and lead citrate were used for electron microscopy (Philip CM12) staining.

Extraction and assay for enzymes activities

Enzymes α- and β-galactosidase were extracted using method in Ali *et al*. (1995). α-Galactosidase was assayed using method in Soh *et al*. (1997) while β-galactosidase was assayed based on modified method of Pressey (1983).

Results and Discussion

One of the most obvious changes in ripening of papaya was a drastic decrease in firmness (FIGURE 1) as shown by AT. Low temperature was very successful in suppressed fruit softening. The fruit softened rapidly when transferred to ambient temperature. Every group exhibited similar pattern to AT. At 75% yellow ripening stage, HTC appeared to be firmer than other groups with different treatment.

In cell structure study, at 5% yellow (Day 0), mesocarp tissue was found to be composed of large, isodimetric and tightly packed parenchymous cells (data not shown). These results were also reported in avocado (Platt-Aloia & Thomson, 1982). After 14 days at

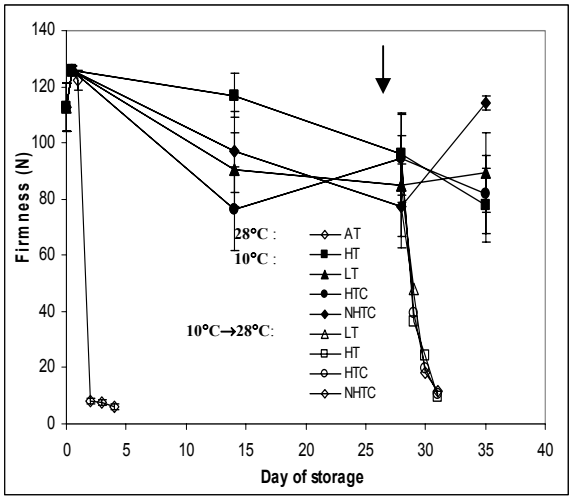

FIGURE 1 Changes in firmness during normal ripening and in fruits stored at 10°C for 4 weeks. Arrow indicates time of transfer to ambient (28°C). Vertical bars represent standard error of mean of 6 fruits.

low temperature, no conspicuous differences were seen between cells of every group.

However, differences were only observed after transferred the fruit to ambient. When the fruit reached 25% yellow, tissues of every group relatively soft and displayed sharply reduced staining intensity of cellular structures. Stainability reduction also occurred in soft tissues of apple and pear. These represent the disruption of cell wall where fibrillar material began to dissolve and the walls loss their intense stainability (Ben-Arie *et al*., 1979). Cellular structure of HTC was more intact almost similar to AT. Other groups, structure of the cells began to disrupt and adjacent cells were started to diffuse.

At 50% yellow ripening stage, every group displayed loosely attached cells; adjacent cells were diffused and created expansion of intercellular space. Structures of control fruits were more intact compared to treated fruits (data not shown).

The ultrastructure of control and treated fruits at 5% ripening stage (data not shown) appeared to have undisrupted cell walls and middle lamellas. Similar results were detected in tomato (Crookes & Grierson, 1983), apple and pear (Ben-Arie *et al*., 1979). Cell walls of the firm papaya fruits consist of tightly packed and darkly stained fibrillar materials and a conspicuous middle lamella in between as noted in every group. As ripening began, fruits started to soften and changes in ultrastructure occur. At 25% yellow stage, some of the fibrillar materials from the wall had undergone dissolution and appeared to be dispersed in every group. The most obvious destruction was in the middle lamella area as seen by the disintegration of the remaining middle lamella. Staining was also reduced in softer papaya (data not shown), apple and pear (Ben-Arie *et al*., 1979). The number of mitochondria and chromoplast was higher at this stage. These were also reported in tomato (Crookes & Grierson, 1983). When the fruit reached 50% yellow, middle lamella and fibrillar materials were totally dissolved in most of the groups except for control fruit. As a result, the cell wall ultrastructure was hardly stained. Mitochondria and chromoplast were markedly increased. Control fruits exhibited the best quality in this study while HTC showed the best result among the treated fruits.

Activities of softening enzymes, α- and β-galactosidases were suppressed when stored at low temperature. After transferred to ambient, activities of β-galactosidase increased and correlated with firmness which decreased as well as disrupted cell wall structure. As for HTC, activities of β-galactosidases were less increased compared to other groups (data not shown). This may result a firmer tissue and less disrupted cell wall in HTC compared to others. α-Galactosidase, on the other hand, seems irrelevant to the observed dramatic softening; the enzyme appeared susceptible to a prolonged storage under 10°C because its activity failed to recover when the fruits were transferred to ambient (data not shown). Interestingly, both enzymes, including α-galactosidase, correlated strongly with increased softening during normal ripening at ambient (FIGURE 1). It thus appears that mechanisms of papaya fruit softening during normal ripening and in fruits ripened after extended storage under low temperature might not necessarily be similar.

The result of this study suggested that combination of heat treatment and coating able to delay ripening, alleviates chilling injury, increase fruit's resistant to fungal infection and at the same time, maintains better storability quality of papaya fruits after a prolonged storage under low temperature.

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