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# SCANNING ELECTRON MICROSCOPY STRUCTURE AND FIRMNESS OF PAPAIN TREATED APPLE SLICES

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## Abstract

'McIntosh' apple *(Malus domestica* Borkh.) slices were treated with papain. Textural changes were recorded with an Instron Universal Testing Machine. Structural changes and distribution of microorganisms in apple tissues after treatment were observed with a scanning electron microscope (SEM). Apple slices submerged in a 1% papain solution were significantly firmer than apple slices submerged in the distilled water control for a 24 hour period ($P < 0.05$). Three and four days after slicing, a significantly smaller decay index was observed on the apple slices submerged in papain solution than on the control slices. Under SEM, less severe cell wall breakdown was observed on the apple tissues treated with papain than on apple tissues without treatment. Less spores were also observed on the papain treated apple slices than apple slices without treatment. Apple tissues treated with papain solution and distilled water also demonstrated noticeable structural differences. The apple tissues treated with papain solution for 18 hours retained the original cell structure while the cells in the apple tissues treated with distilled water collapsed.

**Key Words**: Apple, fruit, slice, firmness, softening, scanning electron microscope, ultrastructure, papain, enzyme, texture.

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## Introduction

Fruit color and texture are important quality criteria for consumers. Discoloration and softening of apple tissues during processing and storage can be a source of serious losses to apple growers and processors. Sodium bisulfite has been widely used in the apple processing industry and resulted in an effective control of browning (Taylor and Bush, 1983). Buffered sodium bisulfite also maintained an acceptable texture of apple slices (Bolin *et al.*, 1964). However, due to the adverse effect of sulfite on health, the use of sodium bisulfite on fresh fruits and vegetables is restricted (Anonymous, 1986). In an effort to search for anti-browning agents, a series of plant proteinases (papain, bromelain and ficin) were tested for anti-browning potential during the past two years in the Horticultural Postharvest Laboratory at Washington State University. Papain proved to be an excellent anti-browning agent. Further, papain also maintained acceptable surface texture of the apple slices.

Papain is a proteinase derived from *Carica papaya* L. crude latex (Poulter and Caygill, 1985) and is generally recognized as safe (GRAS) as a direct human food ingredient (FDA, 1991). Papain is currently used in the food industry as a meat tenderizer and beer chill-proof agent (Poulter and Caygill, 1985). The use of papain as a preservative in the fruit industry has not been previously reported. The dual effect of papain on the color and texture of apple tissues promotes control of discoloration and softening on the surface of sliced apples.

Softening in apples occurs largely as the result of the breakdown of cell wall constituents, namely pectin, cellulose and hemicellulose (Kertesz *et al.*, 1964; Bartley and Knee, 1982). Polygalacturase and cellulase are the major enzyme groups that catalyze the degradation of cell wall polymers and are closely related to tissue softening (Huber, 1983). The content and the movement of calcium in the cell walls also play an important role during tissue softening (Fuller, 1976; Glenn and Poovaiah, 1990). Under scanning electron microscope (SEM), cell separation and intercellular space enlargement were observed in mature (Trakoontivakorn *et al.*, 1988) or soft apples (Mohr, 1979). Cellular failure in the cell wall at different stage of ripening was also

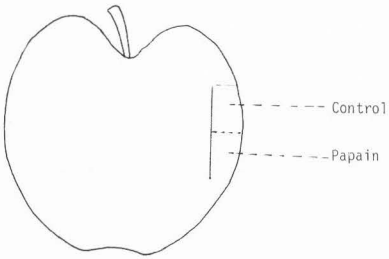



**Figure 1**. Sampling from apple fruit.

**Table 1**. The distribution of fungal spores on the papain treated and untreated apple slices 3 days and 4 days after treatments. Values followed by the same letter at the same incubation time are not significantly different, $P < 0.05$.

| Treatments | Incubation Time | |
|---|---|---|
| | 3 days | 4 days |
| Control | 0.6 ± 0.0325 a | 3.35 ± 0.24 a |
| Papain | 0.012 ± 0.005 b | 0.43 ± 0.017 b |

reported (Diehl and Hamann, 1979). Bolin and Huxsoll (1987) reported a measurable effect of 2% moisture loss on the ratio of the cell length and breadth and the cell roundness index using SEM and image analysis. The objectives of this study were to investigate the effect of papain treatment on the surface textural change of apple slices and to study the related ultrastructural changes with SEM.

## Materials and Methods

### Treating apple slices with papain

'McIntosh' Apples *(Malus domestica* Borkh.) were harvested in the Washington State University Fairway orchard in September, 1990 and stored in a regular storage room at 0 °C for 2 months. Crude papain (E.C. 3.4.22.2) was purchased from Sigma Chemical Company (St. Louis, MO). One percent (weight/volume, W/V) papain solution was prepared by dissolving 1.0 g papain powder in 100 ml distilled water.

Apple slices were obtained from the equatorial sector of apple fruit with a cork borer. Slices (10 mm diameter X 10 mm thick) were submerged in 1% papain solution or distilled water for 10 minutes. The slices were drained and placed in plastic boxes with four small vent holes over water saturated paper towels covered with plastic film (Saran® wrap) to prevent moisture loss. The apple slices were incubated at room temperature (22-25 °C).

### Firmness measurement

The firmness of the surface of apple slices was measured with an Instron Universal Testing Machine (Instron Corporation, Canton, MA) at 0, 12 and 24 hours after treatment. The firmness test was conducted with two parallel flat plates. The force at a cross-head speed of 1 mm/sec required to compress a 10 mm section of the apple slice 2.0 mm was recorded. The stress over strain was calculated to express the firmness of apple slices. The set up of the experiment was according to the model of the completely randomized design with repeated measures. ANOVA and Fisher's Protected LSD test were performed with Statistic Analysis System (SAS) at the significance level of $\alpha = 0.05$.

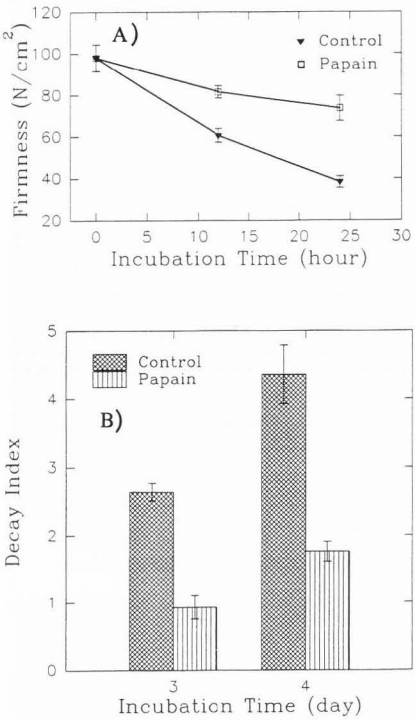
### Decay index assessment

The severity of decay was assessed on apple slices incubated at room temperature 3 and 4 days after treatment. Apple slices were marked and divided into five categories based on the size of decayed areas (spots) and the growth of molds in that area. The marked apple slices were placed in one of five categories: category 0: 0 decay; category 1: 1-25% decay; category 2: 26-50% decay; category 3: 51-75% decay; and category 4: 76-100% decay. The number of slices in each category was multiplied by the value of that category i.e. 0, 1, 2, 3, or 4. The multiplied values were summarized and divided by the total number of apple slices of the treatment to give the decay index. Statistical significance of difference between treatments was analyzed with ANOVA and LSD test at the significance level of $\alpha = 0.05$.

### Apple Preparation for SEM

Experimental pieces were taken by making two parallel cuts, at the equator, across the long axis of the fruit (Fig. 1). The core area was cut away and the remaining truncated sector was cut into two halves parallel to the first two cuts. Matching cut surfaces of each half section were marked by cutting a notch in the right hand corner towards the skin for identification.

Apple sections were submerged in 1% papain solution or distilled water for 10 minutes. Sections were drained and kept moist. After incubation at room temperature for 18 hours or 24 hours or 3-4 days, the apple sections for SEM were cut into 2 x 2 x 1 mm pieces with one original cut surface kept intact for viewing. The apple tissues were fixed overnight in 3.0% glutaraldehyde in a 0.025 M Cacodylate buffer at pH 7.3 at room temperature. Fixation was followed by rinsing in 0.025 M Cacodylate buffer, pH 7.3. Apple tissues were subsequently dehydrated in a graded ethanol series in 10% increments from 10% to 70%, for 10 minutes each; 80%, 90% and 95% for 15 minutes each, and 100% with three changes at 15 minutes each. The dehydrated apple tissues were critical point dried with liquid carbon dioxide as the transitional fluid (Trakoontivakorn et al., 1988).




**Figures 2A and 2B**. Firmness (**Fig. 2A, top**) and decay index (**Fig. 2B, bottom**) of apple slices after papain or distilled water (control) treatments.

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The dried apple tissues were sputter coated by a Hummer V Sputtering Device (Hummer-Technics) with 30 nm gold. Apple tissues were examined with a Hitachi S-570 scanning electron microscope operated at 20 kV. The distribution of the spores on the treated and non-treated apple tissues at 4 days were examined at 2500 X magnification. Spores were counted on each screen for 10 screens of each apple tissue with triplications

## Results and Discussion

### Firmness and decay index

A gradual decrease in firmness indicated that the resistance of tissues to an external deformation force decreased gradually (Fig. 2A). The loss of firmness is attributed to the loss of binding capacity among cell walls caused by the hydrolysis of cell wall polymers after mechanical injury. A significant difference in the firmness between apple slices submerged in papain solution and apple slices submerged in distilled water was observed, indicating that the loss of firmness in apple slices was significantly reduced or retarded by the papain treatment. This result was confirmed by sensory evaluation. By touching, untreated apple slices gave a gradual increase in slimy and sticky feeling over time. Papain treated apple slices remained firm and gave a slightly dry feeling.

In addition to the reduction of firmness loss, a significant retardation of decay by papain was also observed. As presented in Figure 2B, a significantly smaller decay index was assessed after the apple slices were treated with papain than after the apple slices were treated with distilled water. The discovery of the effect of papain on tissue texture and deterioration of apple slices was further confirmed by SEM observations.

### Scanning electron microscopy observations

The cells in the fresh cut apple tissues examined with SEM, were multilateral figures, well arranged in a honey comb pattern (Fig. 3). At higher magnification, fresh cut apple cell walls appeared wavy (Fig. 4).

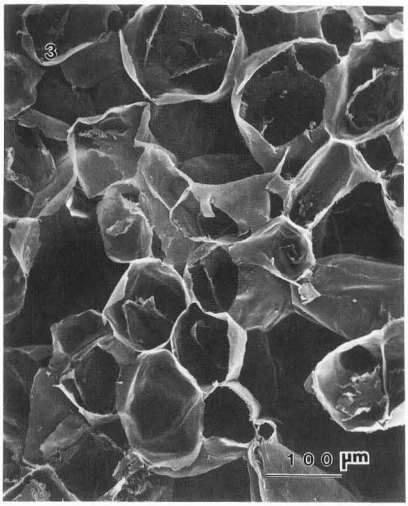
Eighteen hours after cutting, the apple tissues treated with papain solution retained the original cell shapes and arrangements, whereas apple tissues treated with distilled water displayed a collapse of the cells and loss of original cellular arrangement (Figs. 5A and 5B). The differences in cell structure and cell arrangements between treated and untreated apple tissues became more distinct when the samples were fixed 4 days after cutting. The untreated apple tissues exhibited more severe cell collapse than the apple tissues treated with papain. More spores and bacteria were observed on the apple tissues without papain treatment than on the apple tissues treated with papain (Figs. 6A and 6B; and Table 1).

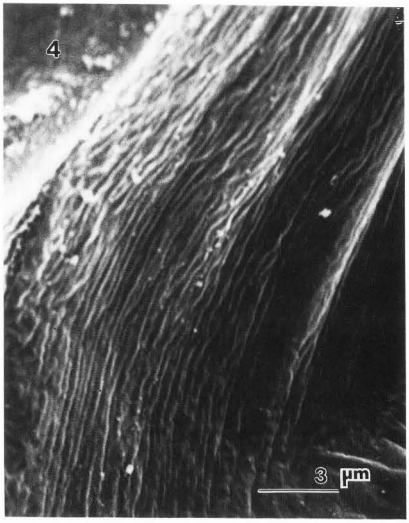
## Conclusion

A significant reduction and retardation of the loss of surface firmness of apple slices was obtained by papain treatment. Cellular shape and cell structure of apple tissues were maintained by papain treatment. As a proteinase, papain may interfere with enzyme systems naturally associated with cell wall degradation. Further evaluation of the responses of cell wall degrading enzymes, such as polygalacturonase, pectinesterase, etc., to papain is pertinent to elucidate the mechanisms of quality retention in fresh fruits and vegetables.

## Acknowledgments

The authors acknowledge the assistance of the faculty and staff in the Electron Microscope Center, Washington State University, and financial support from the Auvil Fruit Company.




**Figures 3-6**. Scanning electron microsgraphs **(Figs. 3 and 4 above; Figs 5 and 6 on the facing page).**

**Figure 3 (top left).** Cellular shapes and arrangements of fresh cut McIntosh apple.

**Figure 4 (top right).** Cell walls of fresh cut McIntosh apple.

**Figure 5 (facing page, top).** Cellular shapes and arrangements of McIntosh apple: **A)** slices treated with papain solution for 18 hours; **B)** slices treated with distilled water for 18 hours.

**Figure 6 (facing page, bottom).** Cell walls of apple tissue 4 days after cutting: **A)** slices treated with 1% papain solution; **B)** slices treated with distilled water. S: fungal spores.

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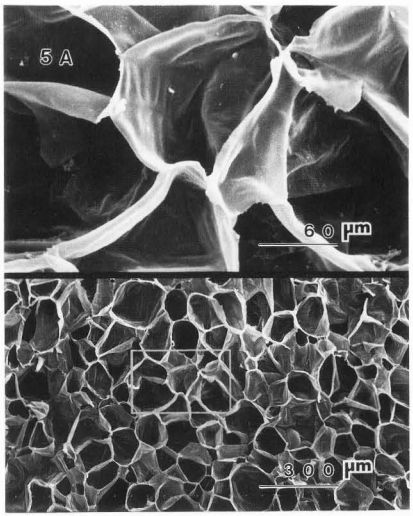
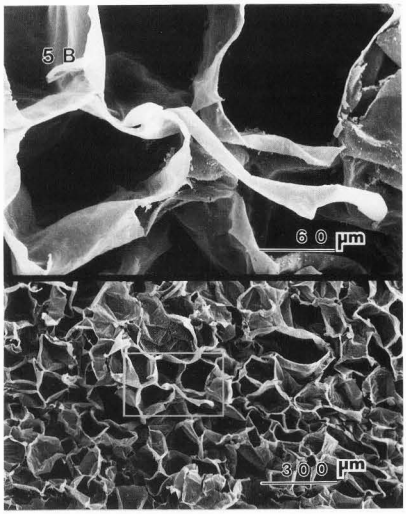
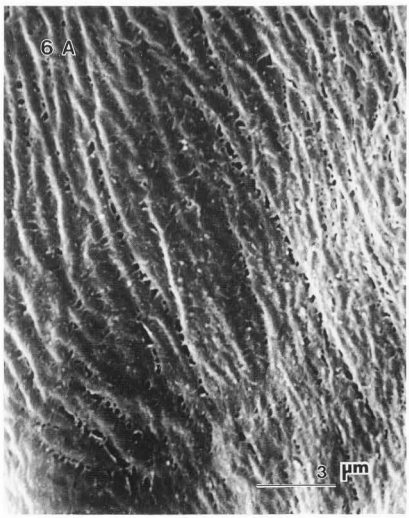
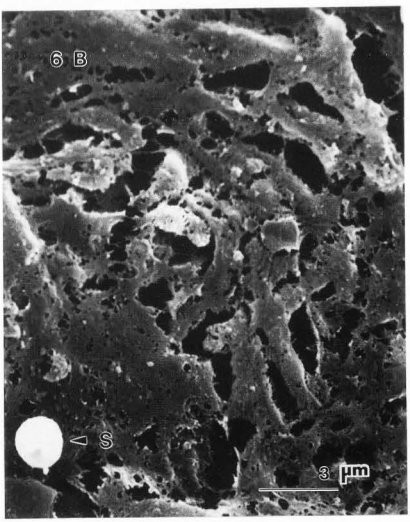
SEM: Papain treated apple slices



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### Discussion with Reviewers

**F.E. Escher**: Is there any indication whether the proteinase activity is restricted to the surface, or whether the enzyme penetrates the tissue?
**Authors**: Papain is restricted to the surface of apple slices due to limited submersion time in this experiment. After submerging, if we cut off the surface tissues of apple slices, apple slices will brown, indicating the loss of the inhibition of browning and softening by papain. The papain appears to be most reactive and inhibitory at the cut surface of apple cells. We have no evidence of papain penetration into apple tissues.

**A. Keresztes**: Apart from a direct study of the papain effect on cell wall degrading enzymes (as mentioned in the **Conclusion**), I would also recommend a transmission electron microscopy study to assess how deeply in the tissue, the cell walls and cell contents are affected by different periods of treatment.
**Authors**: We concur with your recommendation for such a study in the future.

**H.R. Bolin**: Since cut and peeled apples are **always** held under cold-storage conditions, how would this effect final results?
**Authors**: We tend to avoid absolutes as **always**, but agree that refrigeration temperatures will also retard enzyme discoloration. The ambient temperatures were selected to accelerate undesirable color and texture changes and observe inhibition under extremely poor circumstances.

**F.E. Escher**: This reviewer wonders whether there is any literature reference around on the influence of proteinase activity of plant cell tissue. It would be interesting to mention such references, even if they are not related to apples.
**Authors**: No literature reference to influence of proteinase activity on plant cell tissues were available.

**F.E. Escher**: Relating to Figures 1 and 2, why were the firmness readings and the decay observations not carried out at the same time. Also, why no firmness readings are available after 4 days.
**Authors**: Firmness and decay observations were performed at different times because rates were quite different. Firmness was unacceptable after 48 hours. Decay index was unacceptable, but still comparable for 4 days.

**F.E. Escher**: With the present experimental setup the question remains open whether the tissue breakdown is caused by endogenous or microbial enzymes. In the intact apple fruit, breakdown is obviously caused by endogenous enzymes. Therefore, would it not be preferable and possible to carry out such a study under sterile conditions?
**Authors**: We agree that it is difficult to distinguish between tissue breakdown by endogenous or microbial enzymes, however, the rapid tissue softening and discoloration suggest endogenous enzymes are responsible. Similar degradation may appear as microorganisms proliferate on the fresh cut apple slices. Sterile conditions would resolve question, but is not practical.