Research Article

Suitability of micro-perforated PVC containers for modified atmosphere packaging of minimally processed fruit salad containing pineapple, papaya and mango

K.G.L.R. Jayathunge¹, M.F. Rishana Fazy², D.C.K. Illeperuma³, B.M.K.S. Thilakarathne¹, M.D. Fernando¹ and K.B. Palipane³

¹Institute of Post Harvest Technology, Research & Development Center, Jayanthi Mawatha, Anuradhapura, Sri Lanka.
²Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka.
³Department of Food Science and Technology, Sabaragamuwa University of Sri Lanka.

Email: lasanthijayathunge@yahoo.com

Abstract

Ripe fruit of pineapple, papaya and mango were washed with 5% H₂O₂, peeled and cut into cubes (1"x1"x1"). Suitability of ascorbic acid, citric acid and a combination of citric and ascorbic acid as anti–browning treatments were tested based on the browning index (BI) of fruit salad containing papaya pineapple and mango. A suitable micro–perforation level for the polyvinyl chloride (PVC) containers in modifying the in–package gaseous atmosphere was established based on the concentration of oxygen and carbon dioxide, visual quality rating (VQR), BI and off-odour development of the fruit salad 10 days after storage at refrigerated condition (7 ±1°C and 65±0.5% RH). The acceptability of the minimally processed fruit salad was tested by analyzing physico-chemical and microbiological properties. The sensory attributes of the fruit salad stored for 16 days at refrigerated condition in PVC containers was compared with a fresh fruit salad.

Pre-treatment of 1000 ppm ascorbic acid and 150 ppm citric acid was found to be effective in preventing browning of the minimally processed fruit salad. The concentration of oxygen and carbon dioxide were 5.2% and 3.8% respectively, in PVC packages with 15 micro–perforations and parameters of off-odour development, BI and VQR were found to be better in fruit salad packaged in PVC containers with 15 micro-perforations. The microbial count of minimally processed salad was below the critical level, 10⁴, up to 16 days of storage. However, in the 19th day of storage, microbial count exceeded the critical level.
Immersion of papaya, pineapple and mango cubes in a solution containing 1000 ppm citric acid and 150 ppm ascorbic acid, at 4°C for 2 min and packaged in PVC containers with 15 perforations was effective to extend the shelf-life of minimally processed mango for 16-days under refrigerated condition (7± 1°C and 65± 0.5 %RH).

**Keywords:** ascorbic acid, citric acid, physico-chemical, microbiological properties, Sri Lanka.

**Introduction**

Fruit have an appealing flavour and are nutritious, serving as a source of energy, vitamins, minerals and dietary fibre. Consumers’ renewed interest in fruit as health food with no cholesterol, low to no fat, sodium and more recently as potential anti–oxidants, have increased the consumption of fresh fruit.

Available processing techniques destroy many of the sensory attributes, nutrients and other fruit qualities as high temperatures are employed [1]. Therefore consumer demand for high quality products with fresh like characteristics, simplicity of use, convenience and health benefits is increasing [2]. The demand is from airlines, hotel industry, hospitals, military forces and many other sectors where fast food is important. Hence, an introduction of a suitable processing technique to keep fruit in its fresh form will have a good advantage to extend the postharvest life and reduce the portion of fruit which is otherwise wasted.

Minimal processing of fruit is an appropriate processing technique to meet many of the requirements mentioned above. This technique includes, careful cleaning prior to processing, gentle washing, peeling, cutting, slicing and/or shredding and use of low temperatures and mild chemical treatments to retard physiological activities during processing and packaging [3]. Hence, minimal processing keeps the fruit in its fresh form, saving most of the nutrients with it. Moreover, during minimal processing, peels, seeds and other inedible portions are removed and only the edible portion goes to the consumer. It reduces the unwanted cargo load which is important in export trade to minimize the transport cost. Further, a considerable load of by-products accumulated at a single place, facilitates practicing a method of by-product utilization.

Use of modified atmosphere packaging techniques (MAP) with, low oxygen and high carbon dioxide environment, for minimally processed products, has been successful in slowing down deterioration and growth of microorganisms in fresh fruit and vegetables.

Hence this study was focused on development of minimally processed fruit salad containing pineapple, papaya and mango. The effect of anti-browning treatment on prevention of enzymatic browning and micro-perforation level of PVC packages on fruit quality and shelf-life of the product were also tested.

**Methodology**

**Sample collection and preparation**

Fully matured, ripe, fresh fruit of pineapple (variety “Moritius”), papaya (variety “Rathna”) and mango (variety “Karuthakolomban”) were purchased from the Anuradhapura market and transported to the laboratory. Fruit were washed with clean water and immersed in a 5% H₂O₂ solution at 4°C for 1 min [4], peeled and cut into cubes (1”x1”x1”) with sharp knife.
Preliminary experiment 1: Selection of suitable anti–browning treatment
The fruit cubes of papaya, pineapple and mango were dipped in two anti-browning solutions of citric acid and ascorbic acid in ten combinations, as shown in Table 1, for 2 min., drained and mixed at 1:1:1 ratio and packaged (100±5g) in pouches made of 0.05 mm Poly Vinyl Chloride (PVC).

Table 1. Treatment combination of anti–browning solutions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citric acid ( ppm )</td>
</tr>
<tr>
<td>A1</td>
<td>500</td>
</tr>
<tr>
<td>A2</td>
<td>1000</td>
</tr>
<tr>
<td>A3</td>
<td>1500</td>
</tr>
<tr>
<td>A4</td>
<td>0</td>
</tr>
<tr>
<td>A5</td>
<td>0</td>
</tr>
<tr>
<td>A6</td>
<td>0</td>
</tr>
<tr>
<td>A7</td>
<td>1000</td>
</tr>
<tr>
<td>A8</td>
<td>1000</td>
</tr>
<tr>
<td>A9</td>
<td>1500</td>
</tr>
<tr>
<td>A10 (Control)</td>
<td></td>
</tr>
</tbody>
</table>

The experimental treatment structure was a completely randomized design (CRD), with ten treatments. Three packages were made from each treatment, heat-sealed and stored under refrigerated condition (7±1\(^\circ\)C and 65±0.5% RH) for 10 days. Samples were withdrawn 10 days after storage and suitable anti-browning treatment was selected based on subjective measurement of browning index.

Preliminary experiment 2: Selection of optimum perforation level for the package
The experiment was conducted to select the optimum micro–perforation level for polyvinyl chloride containers (PVC) containers of 15x10x4 cm. The experimental treatment structure was a completely randomized design (CRD), with 6 levels of micro–perforation, namely, 0, 3, 5, 10, 15 and 20, as a treatment. PVC containers were micro–perforated using a needle (diameter – 0.29mm) on the sides of the top lid. Fruit cubes were dipped in 5% \( \text{H}_2\text{O}_2 \) solution 1 min., dipped in the anti–browning treatment solution, selected in the preliminary experiment 1, for 2 min., drained, mixed at 1:1:1 ratio and packaged (100 ±5g). Three samples from each treatment were prepared, self-sealing septa were fixed on top of each container, heat-sealed and stored under refrigerated condition for 10 days. Samples were withdrawn 10 days after storage and in-package gaseous composition (O\(_2\) and CO\(_2\)) was measured using a gas chromatograph (Varian, CP – 3800 USA). Subjective measurement of visual quality rating (VQR) and browning index (BI) and off-odour development were also taken.

Main experiment
The results obtained from preliminary experiment 1 and 2 were used in performing the main experiment. Fresh fruit cubes were immersed in a 5% \( \text{H}_2\text{O}_2 \) solution at 4\(^\circ\)C for 1 min, immersed in a solution containing 1000 ppm citric and 150 ppm ascorbic acids for 2 min, drained and packaged in
PVC containers with 15 micro-perforation levels. Self-sealing septa were fixed on the packages to facilitate gas measurements. The packages were stored at refrigerated condition for 19 days, withdrawn in 3-day intervals and in-package gaseous concentration, total soluble solids, titratable acidity, firmness, pH and microbial count of the fruit cubes were determined. The experimental treatment structure was a completely randomized design (CRD), with days as the treatment.

**Quality analysis**

*Visual quality rating (VQR)*

VQR was determined using a scale of 1-9. 1-poor, 3-fair, 5-good, 7-very good and 9-excellent, with 10 panelists [5].

*Browning index (BI)*

BI was determined using a scale of 1-7. 1-brown, 2-moderately brown, 3-slight brown, 4-neither yellow nor brown, 5-slight yellow, 6-moderate yellow and 7-yellow with 10 panelists [5].

*Odour*

Odour was determined by a scale of 1-4, 1-none, 2-slight, 3-moderate and 4-severe with 10 panelists [5].

*Titratable acidity*

Mango, pineapple and papaya were chopped separately and 5g of each fruit was centrifuged with 20 ml of distilled water separately. Then it was filtered and the filtrate was volumerized to 100 ml with distilled water. Ten millilitres of solution was titrated against 0.1 N NaOH with phenolphthalein as an indicator. Titratable acidity was expressed as percentage citric acid.

*Total soluble solids (TSS)*

TSS was measured using a refractometer (Model: HR-5 A022-1, Japan). A small amount of chopped fruit was taken in a clean piece of cloth and juice was squeezed into the prism surface of the refractometer and reading was taken directly.

*pH*

The pH measurement was performed using a pH meter (420A, USA) with a glass electrode. Portion of the solution prepared to measure the titratable acidity was used to determine the pH value.

*Gaseous composition*

In-package concentrations of oxygen (O₂) carbon dioxide (CO₂) and ethylene were measured by using a gas chromatograph (Varian, CP-3800, and USA). Oxygen was measured by using thermal conductivity detector (TCD) while CO₂ and ethylene were measured using flame ionization detector (FID). Argon was used as the carrier gas at a flow rate of 60 ml/min. column oven, TCD and FID temperatures were of 70, 140 and 300°C, respectively.

*Firmness*

Firmness was measured as penetration force required for a 4 mm cylindrical probe to depress the cube surface, held perpendicular to the probe to a depth of 1cm using a digital firmness tester (CST2, Italy).

*Microbiological analysis*

Microbiological test was done to determine the total microbial count and yeast and mould counts. Total plate count and yeast and mould count were performed for the fruit salad samples at 3-day intervals. Total aerobes were enumerated on plate count agar after 72±1 h incubation at 30±1°C [6].
Yeast and mould counts on yeast extract agar medium were done after 72±1 h incubation at 25±1°C [7]. The results were reported as the number of colony forming units per gram (CFU/g) of the product.

Sensory quality
The acceptability of the stored product after 16 days was evaluated by comparing with the freshly prepared salad by sensory evaluation panel consisting 30 panelists. Five-point Hedonic scale was used to evaluate the colour, odour, taste, texture and overall acceptability. (5- extremely like, 1- extremely dislike).

Statistical analysis
The subjective measurements of VQR, BI and odour results were analyzed by Friedman non-parametric test using the MINITAB statistical package. A suitable micro-perforation level was selected by comparing means at p< 0.05 according to multiple comparison procedure. Objective measurements of firmness, gaseous composition, pH and acidity were subjected to variance analysis (ANOVA) using the SAS package according to the Duncan’s multiple range test procedure. The results of the sensory analysis were analyzed by Friedman non-parametric analysis using the MINITAB Statistical package at p<0.05.

Results and Discussion
Selection of suitable anti–browning treatment
Good sensory quality is a critical factor in maintaining the commercial marketability of fresh-cut fruit products. The greatest phenomena that limit the shelf-life of fresh-cut fruit are excessive tissue softening and cut surface browning [8]. Enzymatic browning is the main reason for dis-colouration of cut surface in minimally processed fruit and vegetables during storage. Therefore, prevention of browning is an important aspect of minimal processing industry. Application of anti-browning agents such as acetic acid, citric acid and ascorbic acid are the commonly used methods to prevent browning in minimal processing industry [9].

The effect of anti-browning treatment on preventing browning of cut fruit as indicated by the rank totals is given in Table 2. Washing with water, citric acid or ascorbic acid alone followed by packaging was not effective in preventing browning in cut fruit. Fruit slices treated with 1000 ppm citric acid and 150 ppm ascorbic acid showed significantly higher results (p< 0.05) for browning than those treated with other combinations of citric and ascorbic acid.

Citric acid acts as a chelating agent and acidulates, reducing pH and chelating copper in the active site of polyphenol oxidase, thereby inactivating polyphenol oxidase. Thus the ascorbic acid and citric acid mixture showed the combined effect on prevention of enzymatic browning [10]. A combination of 1000 ppm citric acid and 200 ppm ascorbic acid was effective in prevention of browning in avocado slices as reported by Nikapitiya and Illaperuma [5]. Soliva-Fortuny et al., [8] have also reported that the use of a dipping treatment of ascorbic acid (10g/l water) and calcium chloride (5g/l water) was effective in preserving the initial colour of fresh-cut golden delicious apple cubes.
Table 2. Effect of anti-browning treatments on brown colour development of fruit salad containing mango, papaya and pineapple packaged in PVC containers and stored at 7±1°C and 65±0.5% RH for 10 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rank total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (500 ppm citric acid)</td>
<td>53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A2 (1000 ppm citric acid)</td>
<td>64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>A3 (1500 ppm citric acid)</td>
<td>73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>A4 (150 ppm ascorbic acid)</td>
<td>43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A5 (200 ppm ascorbic acid)</td>
<td>42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A6 (250 ppm ascorbic acid)</td>
<td>37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A7 (1000 + 150 ppm citric acid and ascorbic acid)</td>
<td>36&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>A8 (1000 + 200 ppm citric acid and ascorbic acid)</td>
<td>89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A9 (1000 + 250 ppm citric acid and ascorbic acid)</td>
<td>42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A10 (water)</td>
<td>76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Selection of optimum perforation level for the package

In general the use of controlled atmosphere is beneficial for minimally processed products. Typically concentration of 5% to 10% CO₂ and 2% to 5% O₂ is applied to extend the shelf-life of minimally processed products [11]. The creation and maintenance of an optimal atmosphere inside a modified atmospheres package depends on the respiration rate of the product and the permeability of the films to O₂ and CO₂ and both of which are affected by temperature. The oxygen concentration at which anaerobic process begins depends on the type of produce, the storage temperature and on the CO₂ concentration. Once anaerobic respiration has been initiated, the O₂ concentration remained constant during subsequent fluctuation cycles regardless of the temperature, presumably due to irreversible membrane damage and reduced mitochondrial activity [12].

Gunes and Lee [10] have also reported that reduction of O₂ and elevation of CO₂, surrounding the minimally processed fresh fruit and vegetables, was effective in reducing their respiration rate by inhibiting some steps in the Kreb cycle though inactivating some enzymes.

Most of the packaging containers which are used to package minimally processed products are made of materials not much permeable to gases. This may result in accumulation of CO₂ inside the package. Micro-perforation is a way to develop a modified atmosphere condition inside the package below the injury level of the product [13].

The effect of micro-perforation level on visual quality rating (VQR), browning index (BI) and odour is shown in Figure 1. The salad packaged in PVC containers with 15 and 20 micro-perforations was better in terms of VQR and BI than the sample packaged in PVC containers with no preparation or less than 15 micro-perforations. The salad packaged in PVC container with 15 micro-perforations was found to be the best in terms of odour.
Figure 1. Estimated medium values of the sensory qualities and gaseous concentration of minimally processed fruit salad packaged in micro-perforated PVC containers and stored at 7±1°C and 65±0.5% RH for 10 days.

[Note: T1-0, T2-3, T3 5, T4 10, T5 15, T6 20 and VQR (1-poor, 3-fair, 5-good, 7-very good and 9-excellent ), BI (1-brown , 2-moderately brown , 3-slight brown , 4-neither yellow nor-brown, 5-slight yellow, 6-moderate yellow and 7-yellow), odour (1-none, 2-slight, 3-moderate and 4-severe)].

The effect of micro-perforation on in-package CO₂ and O₂ concentration is also shown in Figure 1. The average O₂ percentage was above the critical level (2%) for all treatments. However T5 was the only treatment which showed desirable CO₂% within the limit reported for minimally processed products [11]. Therefore, treatment 5 (15-perforation level) was selected as the best perforation level for the PVC containers to package minimally processed fruit salad.

Main experiment
Titratable acidity, pH and total soluble solids
Change of pH, titratable acidity and total soluble solids of the minimally processed fruit salad during storage is shown in Figure 2. The pH of the product increased significantly during the storage period. However, the pH increment of papaya cubes was not significant up to the 5th day. Afterwards, the pH increased significantly. Significant changes in pH were observed for pineapple and mango cubes throughout the storage period. The acidity significantly reduced during the storage period in comparison to pH values. The decrease in acidity and increase in pH during storage may be due to the use of organic acids as respiratory substrates during storage and conversion of acids into sugars [14]. Acidity of minimally processed nectarine and peaches were gradually and
uniformly reduced during storage [15]. The pH of minimally processed pomegranate fruit, packaged in 25 µm poly-propylene bags, was increased during storage at 5°C [16].

Figure 2. Change of titratable acidity, pH and total soluble solids content of minimally processed fruit salad packaged in PVC containers with 15 micro - perforations during storage at 7±1°C and 65±0.5% RH. [Note: Each value represents the mean of triplicate. Different letter denotes statistical difference at p< 0.05].

Total soluble solids significantly increased during storage regardless of the fruit type. Significant change in total soluble solids of papaya was observed after the 9th day of storage while mango and pineapple fruits showed significant difference after the 2nd day. This may be due to breakdown of pectin and conversion of carbohydrates into simple sugars. Rocculi et al., [17] have reported increment of total soluble solid content in modified atmosphere packaged kiwifruit slices.
Firmness
Firmness of minimally processed fruit during the storage period is shown in Figure 3. During the storage, fruit firmness significantly (p<0.05) changed. Firmness of mango slices did not change significantly up to 5th day of storage. Firmness of pineapple slices reduced significantly throughout the storage period. However, firmness of papaya slices reduced significantly after the 5th day of storage and did not change significantly throughout the storage thereafter. Firmness reduction of minimally processed fruit cubes occurs due to dissolution of middle lamella and subsequent cell separation and is a consequence of the activity of pectin esterase, an enzyme that remains active even at low temperatures [18].

![Figure 3. Change of firmness of minimally processed fruit packaged in PVC containers with 15 micro-perforation during storage at 7±1°C and 65±0.5% RH.](image)

Illeperuma and Nikapitiya [19] reported that firmness of minimally processed avocado, stored at 8°C and 90 ± 2% RH, reduced during storage regardless of the packaging material. Reduction of firmness of fresh carnival peach slices packaged in glass jars under controlled atmosphere has also been reported [20]. Sanz et al., [21] showed that firmness of strawberries packaged in perforated 25 μm polypropylene reduced the firmness during storage. However, Soliva-Fortuny et al., [8] have reported that sensory perception of texture is sometimes more suitable than instrumental measurement of firmness.

Microbiological quality
Microbial counts of the fruit salad, as shown in Table 3, were found to be below the critical level of 10^5 reported by Harrigan [22] and Lopez-Rubira [23]. However, the microbial counts increased above this critical level by the 19th day in storage making the salad unsuitable for consumption.
Table 3. Change in microbial count of minimally processed fruit salad during storage at days at 7±1°C and 65±0.5% RH.

<table>
<thead>
<tr>
<th>Time of storage (Days)</th>
<th>Total plate count (CFU/g)</th>
<th>Yeast &amp; module count (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.4 * 10^4</td>
<td>1.0 * 10^4</td>
</tr>
<tr>
<td>5</td>
<td>6.2 * 10^4</td>
<td>2.1 * 10^4</td>
</tr>
<tr>
<td>9</td>
<td>8.8 * 10^4</td>
<td>3.4 * 10^4</td>
</tr>
<tr>
<td>13</td>
<td>9.0 * 10^7</td>
<td>1.2 * 10^2</td>
</tr>
<tr>
<td>16</td>
<td>8.8 * 10^4</td>
<td>5.3 * 10^2</td>
</tr>
<tr>
<td>19</td>
<td>1.9 * 10^5</td>
<td>1.4 * 10^3</td>
</tr>
</tbody>
</table>

Microbiological quality is a common criterion used to determine the acceptability and shelf-life of minimally processed products. Microbial count of minimally processed food depends on handling quality of utensils used during preparation and sanitary practices adopted. Method of slicing too has a severe influence on microbiological quality [3]. Hydrogen peroxide has been used as a disinfectant in minimal processing and it is recognized as a safe chemical for use in food product as a bleaching, oxidizing, reducing and anti-browning agent. It was reported that dipping fresh cut cantaloupe melon in 5% H_2O_2 was effective in improving the microbiological quality and shelf-life [24]. Similarly, dipping of mango, papaya and pineapple cubes in 5% hydrogen peroxide solution was effective in controlling microbial growth during storage.

**Sensory quality**
The fresh product was rated higher than the product stored for 16 days for taste, odour, colour, texture and overall acceptability. However, the estimated medians were above the point “like very much” which corresponds to number 4 of the 5-point Hedonic scale. Moreover, there was no significant difference between the estimated median value of the fresh and the 16 days stored product for all sensory attributes (Figure 4) and indicates that the minimally processed mango product can be packaged in PVC containers with 15 micro-perforation and stored at 7 ± 1°C for 16 days without significant changes in the sensory attributes.
Figure 4. Estimated median values of minimally processed fruit salad after 16 days of storage packaged in micro-perforated polyvinyl chlorite (PVC) containers at 7±1°C and 65±0.5% RH.

[Note: Hedonic scale (5-like very much, 4-like moderately, 3-neither like nor dislike, 2-dislike moderately and 1-dislike very much).

Conclusion

Immersion of papaya, pineapple and mango cubes in a solution containing 1000 ppm citric acid and 150 ppm ascorbic acid, at 4°C for 2 min and packaged in PVC containers with 15 perforations was effective to extend the shelf-life of minimally processed mango for 16-days under refrigerated condition (7± 1°C and 65± 0.5 %RH).

References


