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Effects of antibrowning agents on the shelf life of fresh-cut green jackfruit (*Artocarpus heterophyllus* Lam.)

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Abstract

Green mature jackfruits were minimally processed into cubes, dipped in solution of citric acid (0 and 1%) and ascorbic acid (0, 1 and 2%), vacuum packed at 550 mbar atmospheric pressure in 80 µm laminated low density polyethylene vacuum pouches and stored at 2-4°C for 15 days. A control was prepared, using water. Quality parameters like colour, firmness, pH, titratable acidity and total soluble solids were determined during storage. Colour parameters indicated increase in browning during storage. A significant increase ($P<0.05$) in titratable acidity and significant decrease ($P<0.05$) in pH were observed in all treatments. Texture significantly decreased ($P<0.05$) in all treatments during storage. Combinations of the browning inhibitors were more effective than when applied individually. Citric acid and ascorbic acid when applied together resulted in non-significant change ($P>0.05$) in microbial counts, browning, and colour lightness. Treatment of 1% citric acid and 2% ascorbic acid in combination with moderate vacuum packaging and low temperature storage was found most effective in inhibiting browning and deterioration of fresh-cut green jackfruit for up to 15 days.

Key words: Antibrowning agents, citric acid, ascorbic acid, *Artocarpus heterophyllus*, minimal processing, green jackfruit, moderate vacuum packaging.

Introduction

Fresh-cut products are defined as fruits or vegetables that have been freshly cut, washed and packaged that offer consumers a nutritious, convenient and fresh-like product (Gimenez *et al.,* 2003). There is significant growth in the market for these products worldwide. However, although minimally processed products satisfy consumer demand towards fresh, healthy and convenient products, minimal processing such as peeling, cutting, shredding and grating renders the product highly perishable (Laurila and Ahvenainen, 2002). Fresh-cut processing operations result in a wound response that leads to chemical reactions such as increased respiration rates, cut surface browning, increased ethylene production, water loss, and off-flavour development, which in turn results in texture loss and reduces product quality and shelf-life (Escalona *et al.,* 2005; Gonzalez-Aguilar *et al.,* 2000; Agar *et al.,* 1999). Apart from biochemical causes, spoilage of fresh-cut products is also caused by the growth of micro-organisms, the growth of which is enhanced in the processing process due to the availability of cell nutrients and increased surface area for growth.

Fresh-cut fruits and vegetables are normally packaged in film bags to reduce the respiration rate and slow the growth of anaerobic spoilage micro-organisms on the product. Creation of a modified atmosphere of low oxygen and elevated carbon dioxide levels inside the package may retard browning, spoilage and maintain the fresh appearance (Gonzalez-Aguilar *et al.,* 2000; Farver and Dodds, 1995). Modified atmosphere packaging is used as a supplement to low temperature storage to reduce the rate of enzyme activity. Moderate vacuum packaging of shredded lettuce with polyethylene (80µm) resulted in a shelf life greater than 10 days at 5°C (Heimdall *et al.,* 1995).

Green jackfruit, as a cooked vegetable, is an appreciated dish by the Asian community. Therefore, presentation of jackfruit as a fresh-cut product would be very convenient to reduce preparation time in the kitchen and would be a product welcomed by consumers. However, enzymatic browning was found to be the major limiting factor in fresh-cut jackfruit commercialisation. Enzymatic browning occurs when phenolic compounds are oxidised by the copper-containing enzyme, polyphenol oxidase (PPO) (Paul and Palmer, 1972). During fresh-cut preparation, cells are ruptured causing the intermixing of PPO with phenolic substrates, which are normally compartmentalised, resulting in an undesirable brown colour (Dong *et al.,* 2000; Sapers *et al.,* 2002).

Browning and decay in fresh-cut fruits and vegetables can be reduced by the use of natural antibrowning agents (Gonzalez-Aguilar *et al.,* 2000). As an alternative to sulphites, which has been found to cause allergic reactions in asthmatics (Sapers, 1993), the most studied alternative has been ascorbic acid (Laurila and Ahvenainen, 2002). Since ascorbic acid eventually oxidises to dehydro-ascorbic acid, it is best used in combination with other antibrowning agents like citric acid. Ascorbic acid and citric acid have been found to be effective enzymatic browning inhibitors. Langdon (1987) found that immersion of peeled and sliced potatoes in solutions of ascorbic and citric acid followed by vacuum packaging, resulted in product shelf life of greater than 14 days.

The objective of this study was to investigate the effects of ascorbic acid and citric acid as antibrowning agents on the quality and microbial load of moderately vacuum packed fresh-cut green jackfruit stored at 2-4°C.

Materials and methods

Plant materials: Disease free jackfruits of the same maturity were hand-harvested from registered growers in Mauritius. Harvested

jackfruits were immediately transported from the field to the University of Mauritius.

Sample preparation: Jackfruits were manually cut at the base and the latex allowed to flow. The fruits were then cut into rings, peeled, cored and finally cut into cubes of 25 mm. The jackfruit cubes were dipped in the test solutions, which included different concentrations of ascorbic acid and citric acid (Table 1). These cubes were drained and blotted dry with paper towels, placed in laminated polyethylene vacuum bags of thickness 80 µm (oxygen transmission rate: 35 cm^3/m^2/24h at 23°C; Linpac Ltd., Pontivy, France) and vacuum packaged at 550 mbar atmospheric pressure using the Multivac vacuum packaging machine (Multivac, Wolfertschwenden, Germany). Each pack contained about 100-125 g cut fruit. The packaged fresh-cut fruits were stored at 2-4°C for 15 days. All utensils, containers and work surfaces were sanitized with 3% Oxonia® solution.

Table 1. Composition of antibrowning dip solutions used on jackfruit slices

Treatment	Concentration
Control (T1)	Water
Citric acid (T2)	1 %
Ascorbic acid (T3)	1 %
Ascorbic acid + Citric acid (T4)	1 % + 1 %
Ascorbic acid (T5)	2 %
Ascorbic acid + Citric acid (T6)	2 % + 1 %

Physico-chemical determinations: Destructive analysis was carried out in duplicate on days 0, 3, 7, 10, 13 and 15. Two packages selected at random were subjected to physical and chemical analyses. Diced jackfruits selected randomly from each package were blended and then squeezed manually through cheesecloth. The homogenate was used for titratable acidity (TA) determination and the juice analyzed for total soluble solids content (TSS) and pH. The TSS was measured using a standard hand refractometer, corrected at 20°C, and expressed as °Brix (Askar and Treptow, 1993). The pH was determined using a pH meter as per AOAC method (1995). For TA determination, 10g of pulp homogenate was boiled with 50 ml hot water for 5 minutes. The mixture was filtered and the filtrate titrated with 0.1 N NaOH (Askar and Treptow, 1993) in triplicate. The mean titre value obtained was used to calculate TA, which was expressed in g of citric acid/ 100 g of sample.

Colour measurement: Browning of the jackfruit cubes was measured as L*, a*, b* using a Minolta CR-300 colorimeter (Minolta Company Ltd, Osaka, Japan). Measurements were taken at six different points on each of the cut surface of five dices of the green jackfruit. The L* values for colour indicate lightness, whereby an increase in lightness is indicated by an increase in the L* values. Positive a* values indicate redness and negative a* values indicate greenness. Positive b* values indicate yellowness and negative values blueness.

Determination of firmness: Firmness of five jackfruit dices chosen randomly from each package was determined using a hand-held penetrometer at six different places on each dice (HP-Fff; Tracer 0.25 cm^2; Bareiss Prufgeratebau GmbH, Oberdischingen, Germany) as per Askar and Treptow (1993).

Microbiological analysis: Microbiological analysis was carried out in triplicate for each treatment on days 0, 6 and 12. A sample of 10g of jackfruit was taken from each replicate of the six treatments. This was added to 270 ml sterile (0.1 %) peptone water and blended in a stomacher for at least 2 minutes. Serial dilutions (10^{-1}-10^{-4}) were carried out using 1 ml of the macerated sample and 9 ml aliquots of peptone water. The pour plate technique was used to inoculate the medium. Plate count agar and potato dextrose agar were used for enumeration of total viable count and yeast and mould count, respectively. The inoculated plates were incubated at 25°C for 3-5 days. The counts were expressed as log colony-forming units per g (Log_{10} CFU/g) of sample.

Statistical design and analysis: The statistical design used was a factorial Randomized Block Design with two levels of citric acid (0 and 1%) and three levels of ascorbic acid (0, 1 and 2%). Days in storage (0, 3, 7, 10, 13 or 15) were used as the blocks. Two replicates per treatment combination were subjected to destructive analysis. Data was analyzed using MINITAB Version 13.1. All Least Significant Differences (LSD) were computed at the 5% level of significance.

Results and discussion

Titratable acidity and pH: Increase in TA (as g citric acid 100 g^{-1} fresh tissue) was associated with a decrease in pH during storage (Fig. 1). High acidity was due to the presence of both the acids. Ascorbic acid as well as citric acid was found to have a significant effect on TA ($P<0.05$). A significant interaction effect ($P<0.05$) was also noted between citric and ascorbic acids, whereby TA was found to be higher in fresh-cut jackfruit treated with both acids (Table 2). This could be due to respiration.

Utilization of organic acids during respiration was found to cause a decrease in TA in fresh-cut apples (Fan *et al.*, 2005). Increase in acidity during storage could be due to ripening of the fruit, where the degradation of pectin in the cell wall results in the formation of galacturonic acids (Eskin, 1990). Another factor contributing to increased acidity could be due to the fixation of carbon dioxide formed during respiration into organic acids (Wang, 1990).

Table 2. Two-way table of treatment means for TA

Citric acid (%)	Ascorbic acid concentration (%)		
	0	1	2
0	0.120	0.097	0.135
1	0.124	0.156	0.160

SED (interaction) = 0.007

Both citric acid and ascorbic acid led to a significant decrease ($P<0.05$) in the pH during storage (Table 3). There was no evidence ($P>0.05$) of any interaction between the two treatments indicating that they were acting independently on the pH during storage.

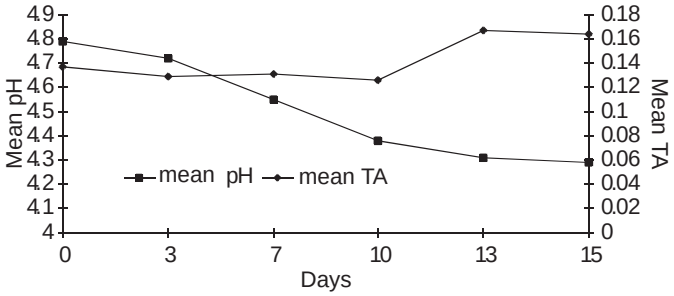

Fig 1. Changes in mean TA and pH during storage of the diced jackfruit
LSD (pH) = 0.2303; LSD (TA) = 0.0138

Table 3. Overall pH of diced jackfruits for citric acid and ascorbic acid treatments

Citric acid (CA)	pH	Ascorbic acid (AA)	pH
0%	4.70	0%	4.65
1%	4.32	1%	4.55
		2%	4.48

SED (CA) = 0.065, SED (AA) = 0.079

Total soluble solids: On day 0, a high °Brix was observed, which decreased on day 3. This could be due to increased respiration as a result of wounding during processing. Sugars and organic acids are the main substrates for respiration in plants (Tovar *et al.*, 2000). Citric acid was found to have a significant effect ($P<0.05$) on the TSS (Table 4). No significant interaction effect ($P>0.05$) between ascorbic and citric acid was noted. Changes in the soluble solids may be attributed to changes occurring during ripening (Nakasone and Paul, 1999). However in the present study, it was found that the TSS remained almost constant throughout storage.

Table 4. Overall TSS of diced jackfruits for citric acid and ascorbic acid treatments

Citric acid (CA)	°Brix	Ascorbic acid (AA)	°Brix
0%	4.09	0%	4.22
1%	4.33	1%	4.14
		2%	4.27

SED (CA) = 0.064, SED (AA) = 0.078

Colour: Changes in a* and L* values have been used to monitor enzymatic browning of cut apples (Soliva-Fortuny *et al.*, 2001). Kim *et al.* (1995) correlated a* and L* values with PPO activity but there was no correlation with b* values. A significant interaction effect ($P<0.05$) was noted for a* value suggesting better browning control when using both acids (Table 5).

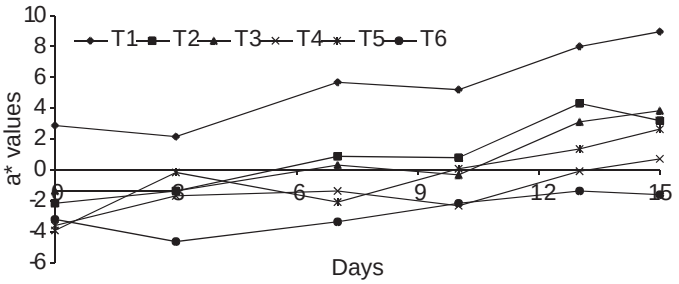

Fig 2. Changes in a* values during storage of the diced jackfruit. LSD (any 2 means) = 2.072

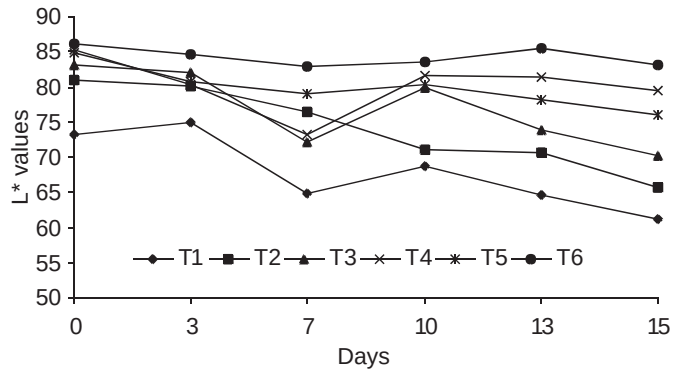

Fig 3. Changes in L* values during storage of the diced jackfruit. LSD (any 2 means) = 5.20

Table 5. Two-way table of treatment means for a* values

Citric acid (%)	Ascorbic acid (%)			Mean
	0	1	2	
0	(+) 5.49	(+) 0.70	(-) 0.22	(+) 1.99
1	(+) 0.97	(-) 1.33	(-) 2.38	(-) 0.91
Mean	(+) 3.23	(-) 0.32	(-) 1.30	

SED (interacion) = 0.581; SED (AA) = 0.411; SED (CA) = 0.335. F-ratios: CA=74.91; AA=67.28; CA*AA=5.85

However, the F-ratio for the interaction effect was much smaller compared to the main effects' sums of squares. This indicates that changes in a* were mostly driven by the main effects. The increase in a* values during storage indicates an increased occurrence of browning (Fig. 2).

L* values were found to decrease during storage (Fig. 3). The control was found to have the lowest L* value. This is due to the fact that no antibrowning agent was used in the control, thereby causing greater extent of browning (Fig. 3). There was no significant interaction between the effects of citric and ascorbic acid ($P>0.05$). Both acids were found to have significant effect ($P<0.05$) on the lightness of the fresh-cut green jackfruit (Table 6). Application of citric acid and ascorbic acid individually were found to inhibit browning moderately. The combination of 1% CA and 2% AA proved to be the most effective treatment in reducing browning for 15 days. Combination of 1% CA and 1% AA was also effective in suppressing browning but to a lesser extent as slight browning was noted on day 15.

Table 6. Mean L* values of diced jackfruit for citric acid and ascorbic acid treatments

Citric acid (CA)	L* value	Ascorbic acid (AA)	L* value
0%	75.2	0%	71.3
1%	79.9	1%	79.6
		2%	81.8

SED (CA) = 0.84, SED (AA) = 1.03

Firmness: Firmness is considered as an important quality criterion for fresh-cut products. Both citric acid and ascorbic acid had significant effects ($P<0.05$) on the firmness of the fresh-cut jackfruit where both acids were found to retain the firmness (Table 7). Retention of firmness could be due to cross-linking between endogenous calcium ions and the freely available carboxyl groups in the cell wall pectin, following acidification (Sapers and Miller, 1995). Percentage loss in firmness for all the treatments is depicted in Fig. 4.

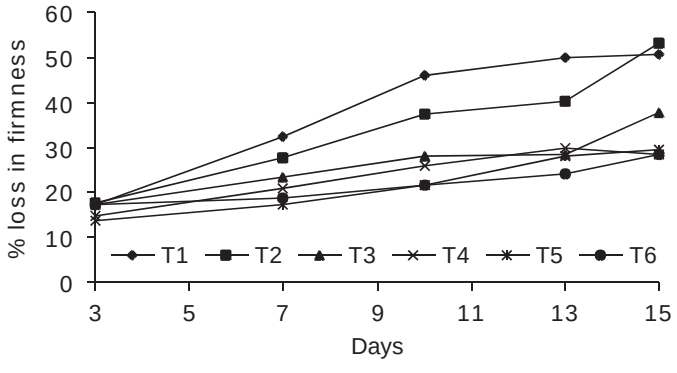

Fig 4. Percent loss in firmness during storage of the diced jackfruit. LSD (any 2 means) = 9.708

Onset of ripening could be a factor responsible for the firmness loss. Fruit pulp loses firmness during ripening, which occurs as a result of the decomposition of the primary cell wall constituents, following the solubilization of pectin by several hydrolytic enzymes. (Eskin, 1990; Riquelme *et al.*, 1999). In addition to ripening, loss of firmness could also be due to decreased turgor due to water loss (Beaulieu and Gorny, 2002).

Table 7. Mean firmness of diced jackfruits for main effect treatments

Citric acid (CA)	Firmness	Ascorbic acid (AA)	Firmness
0%	26.2	0%	24.0
1%	27.1	1%	27.7
		2%	28.3

SED (CA) = 0.70; SED (AA) = 0.86

Microbial growth: An increase was observed in both total viable count (TVC) and yeast and mould (Y:M) count over the storage period (Fig. 5).

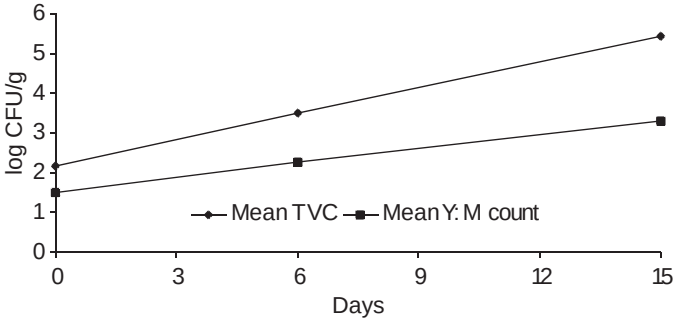

Fig 5. TVC and Y:M count during storage of the diced jackfruit. LSD (TVC) = 0.711; LSD (Y:M count) = 0.3619

Both citric and ascorbic acid significantly ($P<0.05$) decreased the microbial load of the fresh-cut jackfruit (Table 8). No significant interaction effect ($P>0.05$) was noted between the two treatments. Dipping fresh-cut produce in ascorbic acid/citric acid prior to packaging significantly decreased aerobic and anaerobic counts (McLachan and Stark, 1985; O'Beirne and Ballantyne, 1987). These had an impact on the product's shelf life.

Table 8. Mean TVC (in Log_{10} CFU/g) of the jackfruit dices for main effect treatments

Citric acid (CA)	TVC	Ascorbic acid (AA)	TVC
0%	4.04	0%	4.29
1%	3.37	1%	3.64
		2%	3.18

SED (CA) = 0.18, SED (AA) = 0.23

1 % CA was found to be more effective in reducing plate count as compared to 1% AA. However 2% AA reduced the plate count in almost the same way as 1% CA. The antimicrobial effects of the acids can be explained by the fact that they lower the pH resulting in unfavourable conditions for the growth of bacteria (Piagentini *et al.*, 2003). Although the TVC continued to increase during storage, the total count was quite low and below the recommended level of Log_{10} 10^7 CFU/g (7.0) as per Mauritian Food Regulations (1999).

No significant effects ($P>0.05$) on Y:M count were observed when using citric acid and ascorbic acid individually. However a significant interaction effect ($P<0.05$) between ascorbic acid and citric acid was noted (Fig. 9). This showed that combinations of both acids resulted in an increase in Y:M count. Acidic environments were found to be favourable for yeast and mould growth (Beaulieu and Gorny, 2002; Jay, 1992). Although the Y: M count increased during storage, the counts were still low on day 15. According to Debevere (1996), the recommended Y: M count for fresh-cut vegetables should not exceed Log_{10} 10^4 CFU/g (4.0).

Table 9. Two-way table of treatment means for Y:M count in Log_{10} CFU/g

Citric acid (%)	Ascorbic acid concentration (%)		
	0	1	2
0	2.81	2.00	2.14
1	2.05	2.45	2.69

SED (interaction) = 0.162.

The low TVC and Y:M counts can also be explained by the possible development of a high CO_2 atmosphere inside the packages. High CO_2 atmospheres inhibit most aerobic microorganisms, especially gram-negative bacteria that cause off-flavours and off-odours (Martin-Belloso, 2006; Farver and Dodds, 1995). Elevated CO_2 environments are also fungistatic (Gorny, 1997). However lactic acid bacteria are unaffected by high CO_2 and they continue to grow (Farver and Dodds, 1995). Growth of lactic acid bacteria may result in a decrease in pH and may produce antimicrobial compounds, which may suppress the growth of pathogens (Francis *et al.*, 1999).

Slow growth of bacteria, yeasts and moulds was also due to the low storage temperature. Results obtained on shredded chicory salads, and shredded lettuce (Nguyen-The and Carlin, 1994) showed that growth of mesophilic microflora was significantly reduced at low storage temperatures. The Institute of Food Science and Technology recommends a storage temperature in the range of 0-5°C (Francis *et al.*, 1999) for prepared salads to limit the growth of pathogens. In this study, the storage temperature was maintained in the range of 2-4°C, thus explaining the lower plate counts observed. Low temperature storage is crucial for maintaining the microbiological quality of fresh-cut green jackfruit.

Two percent ascorbic acid was found to prolong the shelf life of fresh-cut green jackfruit, but its effect was limited to only 7 days at 2-4°C. The combination of 1% citric acid and 1% ascorbic acid was successful in inhibiting browning up to 13 days at 2-4°C, while the combination of 1% citric acid and 2% ascorbic acid was the most suitable treatment in maintaining the quality attributes of the fresh-cut jackfruit for up to 15 days at 2-4°C. This treatment also prevented microbial growth although the acidic conditions did favour the growth of yeast and mould, which were still less than the permissible amount. The use of the combined preservative factors has greater effectiveness in preserving quality attributes and delaying microbial growth than when used singly.

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