

Original article

Biochemical changes of fresh-cut pineapple slices treated with antibrowning agents

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Summary The effectiveness of ascorbic acid (AA), isoascorbic acid (IAA) and N-acetyl-cysteine (AC) in inhibiting browning of fresh-cut pineapple slices that were stored for up to 14 days at 10 °C, were studied. Slices treated with IAA and AA maintained higher levels of sugars and vitamin C than AC and controls. A low reduction of total phenolic content in treated slices was correlated with a lower polyphenol oxidase activity. IAA and AA slowed the degradation rates of sugars, vitamin C, and phenolic content, whereas AC was less effective in affecting these processes. A higher content of AA and IAA was associated with better compositional quality parameters and appearance of the pineapple slices during the storage period.

Keywords Acetyl cysteine, ascorbic acid, phenols, polyphenol oxidase activity, sugars, vitamin C.

Introduction

Fresh-cut produce (FCP) is rapidly becoming popular with consumers because of its convenience. A major challenge faced by the produce industry is to manipulate the quality of FCP so that the shelf-life is long enough to ensure efficient marketing. FCP deteriorates faster than intact produce because of internal and external browning of the cut surfaces. Browning detracts from the appearance of the slices and reduces their marketability. Physical damage during the peeling and cutting processes also causes an increase in respiration rates, biochemical changes and microbial spoilage, which often result in degradation of colour, texture (flaccidity due to loss of water) and flavour of the produce (Varoquax *et al.*, 1990; Watada *et al.*, 1990; Varoquax & Wiley, 1994; Brecht, 1995; Saltveit, 1997).

Nutrient losses may also be accelerated when plant tissues are wounded (Klein, 1987). Little information is available concerning the retention of vitamins, minerals, sugars and organic acids in FCP during cold storage.

The most important enzyme associated with discoloration and appearance loss of FCP is polyphenol oxidase (PPO) (Varoquax & Wiley, 1994; Garcia & Barrett, 2002). The browning reaction is important in FCP preservation and is generally considered an undesirable reaction because of the deterioration in appearance that it causes and concomitant development of off-flavours. While ascorbic acid (AA) is a widely used natural inhibitor of PPO, other compounds have been reported to be potent inhibitors of this enzyme's activity, these include N-acetyl-cysteine (AC) and hexylresorcinols (Monsalve *et al.*, 1993; Sapers & Miller, 1998; Buta *et al.*, 1999).

Biochemical changes, such as the concentrations changes in vitamin C, sugars, soluble solids and phenols during storage of FCP are very important

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because they are used as primary quantitative parameters of quality (Gorny, 2001). Previous studies, have reported the physicochemical changes of fresh-cut mango and pineapple treated with antibrowning agents (Gonzalez-Aguilar *et al.*, 2000, 2004). There is a lack of reports on the effects of antibrowning agents in the biochemical changes of fresh-cut tropical fruits such as pineapple. This study examined the changes in the content of vitamin C, sugars, total phenols and PPO activity during the storage of pineapple slices after samples were treated with antibrowning agents.

Material and methods

Plant material

Pineapple fruit (*Ananas comosus* L. Merr. Cv Cayena Lisa), obtained from a wholesale market in Hermosillo, Sonora, Mexico, were used for this study. Fruit were sorted to eliminate damaged or defective units, cleaned, the crowns removed, washed in a 10% Clorox solution and air dried. Fruit used for this experiment initially had a firmness of 54–58 N and soluble solids content of 12–13°Brix. Fruit were peeled with a sharp knife, halved and sliced transversely (8 mm thick). After slicing, mixtures of antibrowning agents that had been previously selected were applied. Previous studies revealed that different combinations of antibrowning agents were not as effective as when applied individually (Gonzalez-Aguilar *et al.*, 2004). The concentration ranges were: isoascorbic acid (IAA) (0.05–0.10 M), AC (0.025–0.010 M) and AA (0.05–0.10 M). Preliminary experiments showed that optimal results were obtained using IAA at 0.1 M, AC at 0.05 M and AA at 0.05 M, and so these concentrations were used. Pineapple slices were dipped for 2 min in test solutions, drained and centrifuged for 30 s using a manually operated commercial salad spinner (Essoreuse Model 1642; Metro, Jakarta, Indonesia). Two slices (85 ± 10 g) were placed in a 250 mL polystyrene plastic tray covered with a lid and sealed with Parafilm 'M' laboratory film (Pechiney Plastic Packaging). Control samples were dipped in distilled water, centrifuged and sealed. Sixty trays per treatment were stored at 10 °C for up to 14 days.

Samples for analysis of AA, sugars, phenols and PPO activity were removed every 3 days. Each

sample (six replicates per treatment) was analysed at each sampling time. Samples were taken as follows: two wedges were cut from each slice, and hence nine wedges from eighteen slices from each treatment were used for these analyses.

Ascorbic acid

The AA determinations were based on the method reported by Doner & Hicks (1981). For the extraction, fruit tissue (10 g) was homogenized for 2 min with 50 mL of a mixture of metaphosphoric acid and acetic acid. The homogenate was filtered with Whatman No. 1 filter paper and centrifuged for 15 min at 5000 g (Beckman Coulter centrifuge, Allegra 64R, Walnut Creek, Palo Alto, CA, USA). The supernatant was filtered through filter paper (22 µm). The analysis was performed by HPLC (Varian 9012; Palo Alto, CA, USA), equipment using a Waters-NH₂ type µBondapak, analytical column (3.9 × 300 mm, 10 µm) equipped with a 10 µL loop injector. An isocratic method was used, with acetonitrile:KH₂PO₄ (75:25 w/w) as the mobile phase at a flow rate of 1.5 mL min⁻¹. AA was detected by UV (UV detector, Varian 9050; Palo Alto, CA, USA) at 268 nm. The concentrations of AA were calculated using a standard curve and expressed in terms of fresh weight.

Sugars

Determinations of fructose, glucose and sucrose were based on the method used by Smith *et al.* (1986(L)-1.4pleo

calculated using standards curves and expressed in terms of fresh weight.

Total phenols

Phenolic compounds were determined based on the methods described by Singleton & Rossi (1965) and Kahkonen *et al.* (1999). A 5-g sample of pineapple tissue was homogenized with 25 mL of a solution of 80% aqueous methanol and 0.5% sodium bisulphate using an Ultra-Turrax tissue homogenizer (Takmar, Cincinnati, OH, USA) at moderate speed for 1 min. The homogenate was then centrifuged at 4 °C for 10 min at 5000 rpm (Beckman Coulter Centrifuge, Allegra 64R) and the supernatant was filtered through Whatman No. 1 paper and adjusted to 50 mL. Samples of the diluted extract (100 µL) were centrifuged with 200 µL of Folin-Ciocalteu reagent plus 700 µL of an aqueous solution containing 20 g Na₂CO₃ per 100 mL plus 4 mL water. The contents were mixed and after 2 h the absorbance was read at 740 nm, to determine the total concentration of phenols, in an UV-VIS recording spectrophotometer (UV-VIS; Perkin-Elmer, Lambda 3A, Chicago, IL, USA). The concentrations of the phenols were calculated using standard curve of gallic acid and expressed as gram gallic acid per gram of fresh weight.

Assay for PPO activity

As a crude extract of PPO may contain interfering substances, the enzyme was extracted with acetone according to the procedure described by Flurkey & Jen (1978) and Das *et al.* (1997). Fresh pineapple tissue, 25 g for each sample, was homogenized for 1 min in an Ultra-Turrax homogenizer with 100 mL of cold acetone (−20 °C) and 1% polyvinylpyrrolidone to remove the phenolic compounds occurring naturally in the fruit. The pellet was re-extracted twice with acetone (−20 °C) to give a white powder that was dried overnight at room temperature to remove residual acetone. Two grams of the acetone powder was suspended in 10 mL of 0.01 M phosphate buffer, pH 7, containing 1 M KCl plus 20 µL of phenylmethyl sulphonyl fluoride 0.1 M. The suspension was stirred for 60 min at 4 °C and then centrifuged at 0 °C for 30 min at 10 000 rpm. The supernatant

was filtered through Whatman No. 1 paper and kept for 60 min at 25 °C. The reaction mixture contained 150 µL of the extract plus 850 µL of 0.5 M catechol in sodium citrate buffer, pH 5. The changes in absorbance at 420 nm using a UV-VIS recording spectrophotometer (UV-VIS; Perkin-Elmer, Lambda 3A) were recorded every 10 s up to 60 s. Each sample was run in triplicate. One unit of enzyme activity was defined as a change in absorbance of 0.001 units min^{−1} mg protein^{−1}.

Protein determination

Protein concentration was determined in the extracts according to the dye-binding assay of Bradford (1976) with bovine serum albumin as standard.

Statistical analysis

All data points represent the mean ± SEM of all replicates. Analysis of variance (ANOVA), followed by Tukey's multiple range test for comparison of means. Least significant differences $P < 0.05$, were performed on the data using Statistical Analysis System Software, ver. 8.0 (SAS, 2001).

Results and discussion

Tropical and subtropical fruit like guava and pineapples are important sources of vitamin C, which is one of the more important vitamins for human nutrition. In this regard, vitamin C is a quality parameter of fruits, and should be kept at an appropriate level. In general, the higher the content of vitamin C, the better the quality of the fruit.

Figure 1 shows changes in vitamin C (AA levels) of pineapple slices treated with different antibrowning agents. No significant changes in AA levels were observed in controls or slices treated with AC during the storage period at 10 °C. In contrast, it has been found that dehydrated pineapple and guava pretreated with cysteine hydrochloride had increased AA retention and reduced colour change during storage (Mohamed *et al.*, 1993). As is logical, vitamin C content was significantly increased after the treatments with 0.05 M of AA. However, after 3 days, vitamin C content decreased continuously from 45

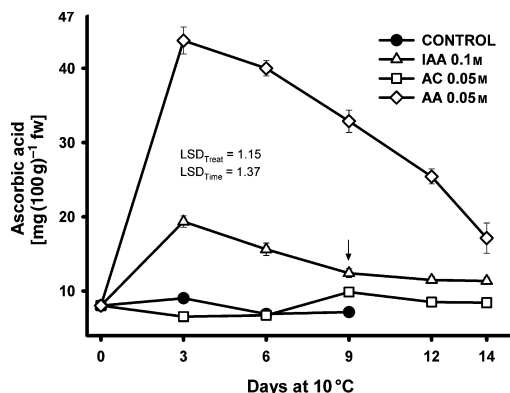


Figure 1 Effect of various antibrowning agents on ascorbic acid concentration of fresh-cut pineapples, during 14 days at 10 °C. Vertical bars represent standard deviation of the mean ($P < 0.05$) ($n = 6$). Arrow indicates the limit of shelf life of controls. LSD, least significant difference.

to 18 mg/100 g FW at the end of the storage period. Gorny *et al.* (2002), found that the AA content of pear slices treated with exogenous AA (2%), dropped to endogenous control levels after 3 days at 0 °C. It appears that AA is most likely converted to dehydroascorbic acid and further degraded to 2,3 diketo-gluconic acid. The Vitamin C content of slices treated with 0.1 M IAA followed a similar pattern, although to a smaller extent, increasing to 20 mg/100 g FW during the first 3 days but decreasing to 12 mg/100 g FW towards the end of storage. IAA treated slices maintained higher endogenous AA content than AC treated slices or controls during the first 9 days of storage. Afterwards, no significant differences were observed among these treatments. It is important to point out that the limit of shelf life of control slices was 9 days while in those treated with AC, AA and IAA it was 13, 15 and 16 days, respectively. In this study we only reported data until 14 days of storage.

Levels of glucose, fructose and sucrose in slices treated with different antibrowning agents followed a similar pattern of change during storage (Fig. 2). An increase in glucose and fructose content was observed in all treatments during the first 6 days of storage. Afterwards, a decline in these sugars was observed in controls and slices treated with AC, reaching the lowest levels at the end of the storage period (Fig. 2a, b). The reduction in sugars can increase the formation

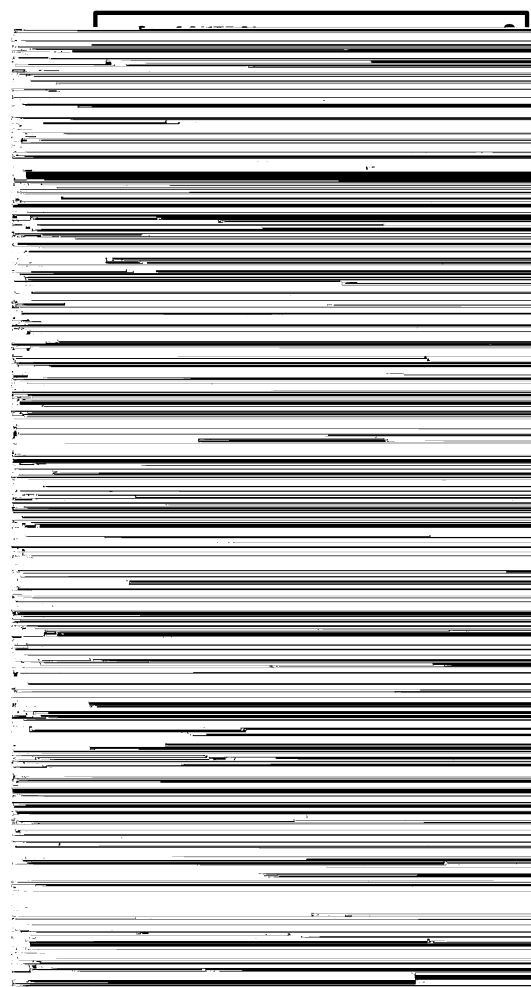


Figure 2 Effect of various antibrowning agents on fructose (a), glucose (b) and sucrose (c) concentrations of fresh-cut pineapples, during 14 days at 10 °C. Vertical bars represent standard error of the mean ($P < 0.05$) ($n = 6$). Arrow indicates the limit of shelf life of controls. LSD, least significant difference.

of esters and other aromatic compounds that increase acceptability of the slices. IAA and AA treatments generally maintained higher sugar levels without significant change after 6 days of storage at 10 °C; probably these treatments suppressed degradation of sugars during this period. Overall, AC treatment did not prevent the degradation of the sugars during storage (Fig. 2). Sucrose content exhibited a slight decrease at day 3 for all treatments. Sucrose content of controls, AC and IAA started to increase after 3 days of storage reaching levels of 4.5, 3.5 and

3.6 g/100 g FW, respectively. Sucrose content increased to a higher extent than fructose and glucose in control and IAA slices during the first 6 days of storage and then dropped to levels similar to those of the initials. Slices treated with AC experienced a sharp decrease after day 6, reaching a value of 2 g/100 g FW of sucrose by end of the storage (Fig. 2c).

One of the most interesting findings in this work was that antibrowning agents containing AA or IAA appeared to slow the degradation rates of the sugars during storage, whereas antibrowning agents containing the amino acid type agent such as N-acetyl cysteine, were less effective in inhibiting these processes. It has been proposed that AC has a somewhat different mode of action to IAA and AA. AC acts by competing with amides and amine-carbonyl interactions that result in browning. An alternative mode of action may be suppression of free radical formation involved in browning reactions. Toledo *et al.* (1999), reported that cysteine could react with both glucose and rhamnose to produce unidentified compounds that increase the visible absorbance. The results in pineapple slices, stored at 10 °C, suggested that in addition to prolonging storage life by inhibiting browning and deterioration, IAA and AA maintained higher levels of sugars and vitamin C, than AC treatment. According to these result, it appears that IAA and AA treatments prevented, to a greater extent than AC, the breakdown and oxidation of sugars. It has been reported that the combination of hexyresorcinol, IAA and potassium sorbate prolonged for 7 days the shelf life of fresh-cut mangoes during storage at 10 °C and also maintained the fructose and glucose at a higher concentration but not sucrose, when compared with controls (Gonzalez-Aguilar *et al.*, 2000).

Figure 3 shows the total phenols content (measured as caffeic acid). A sharp reduction in phenols was observed in control slices after cold storage. However, the antibrowning agents significantly reduced the decline in total phenols. Treatments with AC and IAA were significantly more effective in preventing phenol reduction during cold storage of slices. However, the effectiveness of antibrowning compounds in reducing phenols content diminished with length of storage. These results are in agreement with previous reports where AA

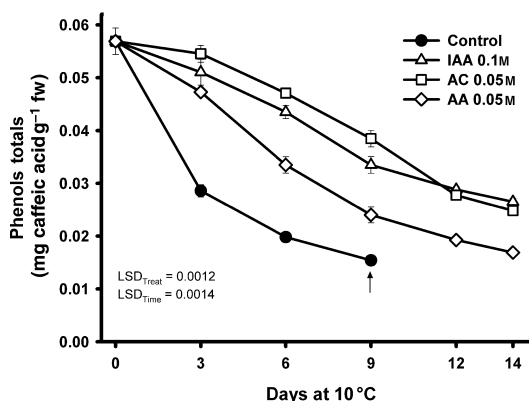


Figure 3 Effect of various antibrowning agents on total phenolic concentration of fresh-cut pineapples, during 14 days at 10 °C. Vertical bars represent standard error of the mean ($P < 0.05$) ($n = 9$). Arrow indicates the limit of shelf life of controls. LSD, least significant difference.

treatments reduced the loss of phenols content in apple (Amiot *et al.*, 1992; Gil *et al.*, 1998). The AA and IAA treatments, acting as efficient reducing agents, prevented a decrease in phenolic content, when combined with low oxygen. However, an individual application of AA softened ‘cv Fuji’ apple slices and promoted mould growth (Pizzocaro *et al.*, 1993). The higher phenolic content in pineapples slices could prevent other undesirable reactions and maintain quality during extended cold storage.

Changes in PPO activity are presented in Fig. 4. No significant differences were found among the slices treated with the antibrowning agents. However, control slices had a higher PPO activity during the same period. After 3 days of storage, PPO activity decreased continuously until the minimum activity was reached at 9 days of storage for the AC treatment. A lower suppression of the PPO activity was observed in slices treated with AA and IAA. No significant differences in PPO activity were observed after 3 days of storage in slices treated with the antibrowning agents. It appears that differences in the reduction of PPO activity by antibrowning agents involve other mechanisms of action, which are related to the prevention of browning of pineapple tissue.

No reports have been found regarding the effect of antibrowning agents on the activity of PPO in pineapple slices. However, effects have been reported in other fresh cut fruits. Andres *et al.* (2002),

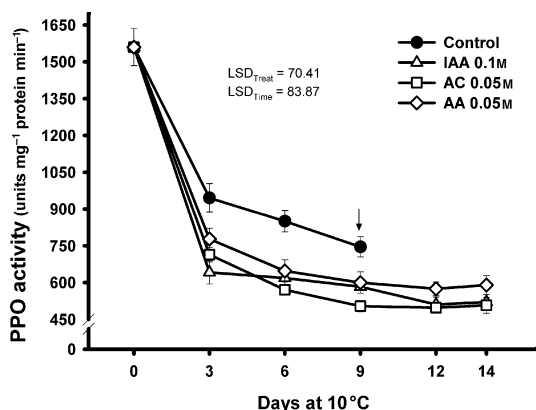


Figure 4 Effect of various antibrowning agents on polyphenol oxidase activity of fresh-cut pineapples, during 14 days storage at 10 °C. Vertical bars represent standard error of the mean ($P < 0.05$) ($n = 6$). Arrow indicates the limit of shelf life of controls. LSD, least significant difference.

reported that the application of AA and citric acid to apple cubes reduced PPO activity by two-thirds. They also found that apple cubes of Red Delicious and Granny Smith cultivars treated with citric acid and AA, and in conjunction with film packaging, maintained acceptable quality attributes, up to 25 days at 10 °C. These results are similar to those obtained in the present study.

Lower total phenols were correlated with higher PPO activity. AA treatment resulted in lower phenol content in slices when compared with IAA and AC treatments. Apparently a greater level of enzymatic activity remained in AA treated slices than other treatments. A high correlation has also been found between total phenols and PPO activity and with the degree of browning in apple (Coseteng & Lee, 1987). Consequently, brown pigmentation has been attributed directly to the enzyme mediated action of PPO, as reported for lychee (Underhill & Critchley, 1993).

The browning reaction in pineapple slices is a complex process involving several factors such as substrate levels, enzyme activity and the presence of different exogenous inhibitors which influence browning reactions to various extents. These seem to exhibit a protective action on the surface tissue of the pineapples slices.

While the highest external quality (reduced browning and better overall appearance) was achieved with the AC treatment, higher levels of

sugars and vitamin C resulted from IAA and AA treatments. Therefore, a consideration of biochemical changes inside the slices may be just as important as appearance in selecting treatments to extend the shelf life of fresh-cut pineapples slices. It is important to mention that the concentrations of antibrowning agents that were used in the present study did not affect the sensorial characteristic of fresh-cut pineapple. Also, for practical purposes, we recommend the use of AA and IAAs to maintain quality of fresh-cut pineapples. Furthermore, another advantage over other antibrowning agents is the low cost of AA.

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