Tropical Agricultural Research Vol. 10 1998, 214-224

Effect of Pre-harvest Calcium Treatment on Post-harvest Quality of Pineapple

S. Selvarajah, H.M.W. Herath[1], D.C. Bandara[1] and D.M.G. Abeysinghe Banda[1]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT. *Pineapple Ananas comosus L. continues to play a dominant role in the Sri Lankan fruit market and is exported to other countries. Internal browning (IB) disorder in a pineapple is a common problem encountered by exporters as a result of cold storage during shipment.*

A study was carried out on the effect of calcium as a pre harvest treatment on IB development and peroxidase activity in pineapples variety Mauritius. Field experiments were carried out at three different locations, and in each site of the experiment, the recommended fertilizer application was kept constant for all treatments. The treatments consisted of three different levels of calcium (CaO) applied as basal dressing in a randomized complete block design.

Immediately after harvesting fruits from all experimental locations were stored in a cold room (15°C and 80-85% RH) for four weeks. Biochemical parameters of fruits were determined immediately after harvest and at weekly intervals followed by three days exposure at room temperature. Polyacrylamide gel electrophoresis (PAGE) was carried out with fruit samples to determine the peroxidase isozyme activity.

Fruits harvested from plants treated with lower level (75 kg ha^{-1}) of calcium (CaO) and stored for 1, 2, 3 and 4 weeks at 15°C followed by three days at room temperature had significantly lower IB intensity than the controls. With higher level of calcium (150 kg ha^{-1}), there was no IB up to fourth week. The fruits affected by IB had low ascorbic acid content and total soluble solids, and higher titratable acidity than the unaffected fruit. The peroxidase activity of treated fruits was significantly lower than the control.

---

1 Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya. Sri Lanka.

INTRODUCTION

Fresh pineapple (*Ananas comosus* L.) is currently air freighted from Sri Lanka to major overseas markets. Sea freight is desired by the industry to reduce costs and expand export volumes, but is not reliable as pineapple is perishable and tissue deterioration occurs during cold storage. This condition, referred to as internal browning (IB) or endogenous brown spot is the most important physiological disorder that limits both storage (Rohrbach and Paull, 1982) and export of pineapple (Akamine, 1976).

Fruits often show various browning symptoms that have been attributed to deficiencies in mineral constituents (Wills *et al.*, 1989). Fertilizer application, either directly on to the plant or to the soil, is the most common cultural intervention recommended when nutrient deficiencies threaten to limit crop yield or quality (Beverly *et al.*, 1993).

Calcium is known to be an essential plant nutrient involved in a number of physiological processes concerning membrane structure and function, and enzyme activities. The strengthening of cell components may prevent or delay the loss of cell compartmentalization and the enzyme reactions that cause such disorder symptoms (Wills *et al.*, 1989).

Long storage times are needed for export of fruits by sea. Knowledge of pre-harvest effects could lead to management for enhanced storage life or segregation of product for local marketing. The present investigation was carried out to examine the effects of calcium fertilizer treatments at pre-harvesting stage on internal browning (IB) and the quality of pineapple fruit.

MATERIALS AND METHODS

The experiment was carried out from August 1996 to February 1998. The pineapple variety Mauritius was planted at three different locations namely Giriulla (Kurunagala district), Narammala (Kurunagala district) and Meerigama (Gampaha district) on red yellow podzolic soils.

Each site of the experiment received fertilizer as recommended by the Department of Agriculture, Sri Lanka (Anon., 1990), which was kept constant for all the treatments. The calcium (CaO) fertilizer treatments (Table 1) were applied as a basal dressing.

Table 1. Calcium fertilizer treatments.

Treatments	Amount of calcium
Twice the recommended level (DD)	150 kg ha^{-1}
Recommended level (SD)	75 kg ha^{-1}
Control	Without calcium

Sample preparation and storage condition

Fruits were harvested at less than a quarter ripe stage, and were graded according to fruit size. Thirty uniform fruits per plot were selected (ten each from the calcium treatments) and placed in a cold room at 80-85% humidity and 15°C temperature. After 0, 7, 14, 21, 28 days of storage, fruits were removed and kept at room temperature for two days. The fruits were cut longitudinally into two halves and the degree of internal browining (IB) was scored 0-5 (0 = free from IB; 5 = 100% of the scale flesh is affected) (Teisson, 1979).

Determination of percentage of weight loss

The weight of each fruit was measured before storage (initial weight) and after storage (final weight) and percentage of weight loss was calculated according to Equation 1.

$$Percentage\ of\ weight\ loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100 \quad (1)$$

Determination of ascorbic acid, total soluble solids, firmness and pH

The peel and core of the fruit were removed and whole flesh was blended using a mechanical blender. Ascorbic acid was determined by titrating with 2,6 dichloro-phenol indophenol, as described by Askar and Treptow (1993). The total soluble solids (TSS) of fruit juice were determined

by a hand refractometer and pH was determined by a pH meter. Fruit firmness was determined as the force required to initiate a crack by using a penetrometer.

Determination of titratable acidity

Titratable acidity was determined using the standard method of the AOAC. Acidity as percentage of citric acid was determined by titrating with 0.1 N NaOH using phenolphthalein as the indicator, and calculated by Equation 2.

$$\text{Percentage of acid} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{meq. wt. of acid}}{\text{Volume of the sample}} \times 100 \quad (2)$$

Determination of peroxidase activity

Pineapple fruits were stored for four weeks at 15°C and the peroxidase activity was examined by polyacrylamide gel electrophoresis (PAGE). One gram of each sample (treated and control) was ground separately in 1.2 ml of extraction buffer. The extracts were centrifuged at 1300 rpm for 3 min, and the upernatant was stored at -20°C for 1 day.

The PAGE was performed in a vertical slab apparatus as described by Torres (1990) in a gel buffered by tris-HCl (pH 8.8) using a discontinuous buffer system. Lithium hydroxide (1.2 g) and boric acid (11.9 g; pH 8.3) were dissolved in 1 litre of distilled water and used as the electrode buffer. Each well was loaded with 10 µl of sample and plates were clamped into the electrophoresis apparatus (SE-600, Hoeffer Scientific) and placed in the gel tank filled with the running buffer. A constant current of 20 mA per gel was applied for three and half-hours. The fixative stain solution used was 0.05 M sodium acetate buffer (pH 5), $CaCl_2$, H_2O_2 and 3-amino-9 ethyl carbazole dissolved in N, N dimethyl formamide and incubated at room temperature until red bands appeared.

The experiments were conducted in a randomized complete block design with three replicates and comprised of 486 plants per replicate. The treatment mean were compared using Duncan's New Multiple Range test.

RESULTS

The ascorbic acid content (Table 2), intensity of browning (Figure 1), and weight loss (Figure 2) of the fruits harvested from plants treated with twice the recommended (DD) level of calcium were significantly different when compared to those of the control treatment during cold storage. The total soluble solids (TSS) and ascorbic acid contents retained in fruits that were treated with higher level of calcium and their weight loss was reduced.

Table 2 shows the changes in the ascorbic acid contents in Mauritius pineapples after being stored for 1 to 4 weeks at 15°C. In all the treatments, the ascorbic acid content decreased progressively as the storage period was prolonged. However, the reduction in ascorbic acid content was greater in the fruits without calcium treatment (control) than treated fruits.

Table 2. Ascorbic acid content (mg/100g flesh) in fruits during cold storage.

Treatments	1st week	2nd week	3rd week	4th week
DD (higher level)	23.5 a	21.6 a	18.2 b	19.0 b
SD (lower level)	19.2 b	20.0 ab	14.2 b	12.0 c
Control	19.4 b	8.4 c	6.5 c	5.8 c

* Within each column, means followed by the same letter are not significantly different by the Duncan's Multiple Range Test at p=0.05. Each value represents the mean of 10 replicates.

Figure 1 illustrates the development of IB in fruits during cold storage. The intensity of IB in fruits treated with the lower level (SD) of calcium was found to be lower than in the control fruits. In addition, with higher level (DD) of calcium IB was not observed up to the fourth week of storage.

The rate of weight loss in fruits treated with the higher level of calcium (DD) was much lower than in the control during storage (Figure 2),

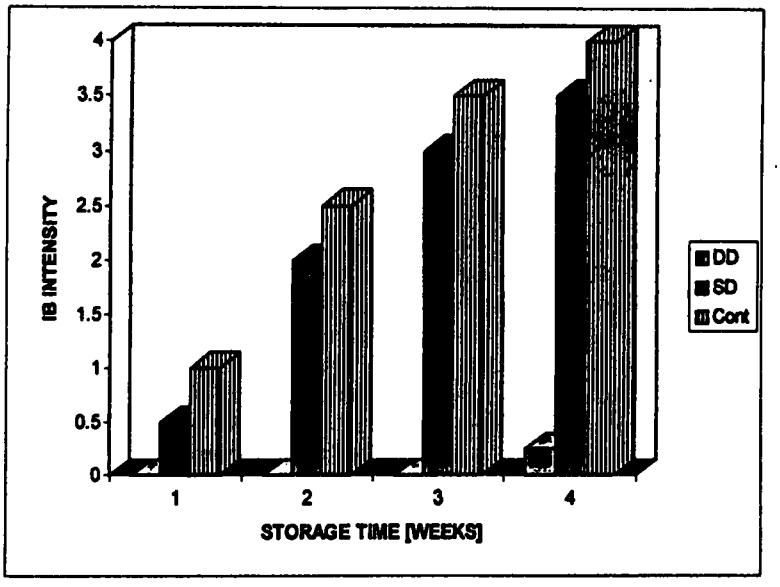

Figure 1. **Effect of calcium on IB intensity (scale 0-5) of Mauritius pineapples during storage at 15°C.**
[Note: DD = Double dose, SD = Single dose, CONT = Control, each value represents the mean of 10 replicates.]

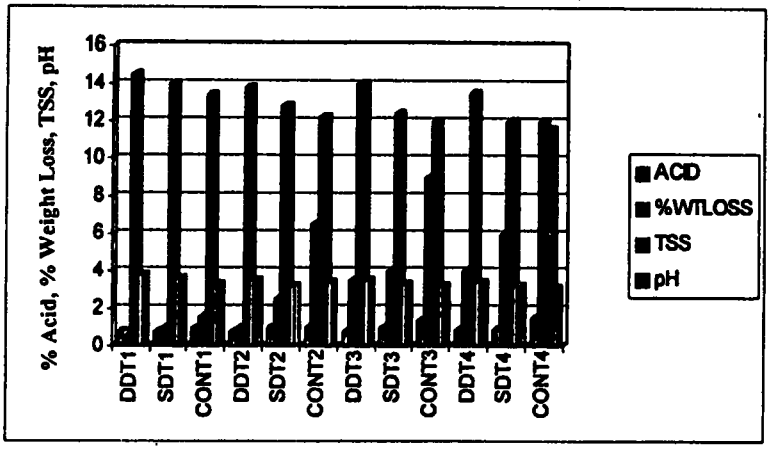

Figure 2. **Effect of calcium on titratable acidity, pH, TSS (brix), weight loss of Mauritius pineapples during storage at 15°C.**
[Note: DD = Double dose, SD = Single dose, CONT = Control, T1, T2, T3, T4 = storage time in weeks, each value represents the mean of 10 replicates.]

delayed the onset of ripening and greenness was maintained. In contrast, fruits from the control plots were completely yellow.

The firmness of the fruit decreased with ripening. Ripe fruits harvested from plants treated with low level of calcium (SD) (2.12±0.13 kg cm^{-2}) and without calcium (control) (1.89± 0.02 kg cm^{-2}) were less firm than those harvested from plants with high level of calcium (DD) (2.97± 0.23 kg cm^{-2}). Statistical analysis revealed a significant difference in firmness of fruits between the fruits in the control treatment and those treated with higher level of calcium. However, firmness was not significantly different between the ripe fruits harvested from plants with low and high levels of calcium.

There was no significant difference in titratable acidity between fruits from treated and control plots. Moreover, there was no correlation between pH and the intensity of IB. Higher TSS (brix) values were obtained in fruits from calcium treated plots than in fruits from untreated control.

Figure 3 shows the polyacrylamide gel electrophoresis of the peroxidase activity in fruits. The results indicate differences in the band intensities between control and treated fruits. Control fruits had a higher peroxidase activity than the calcium treated fruits. The enzyme activity was the lowest in fruits treated with higher level of calcium.

DISCUSSION

This study showed that the presence of calcium in soils has an effect in reducing the incidence of IB in pineapple *cv* Mauritius. The application of calcium as a pre harvest treatment can either reduce IB or totally eliminate the occurrence of this physiological disorder.

Initial responses to lowered temperature usually are considered to be physical in nature and include such phenomena as membrane alterations and protein or enzyme dysfunction. This will result in physiological changes such as cessation of protoplasmic streaming, alterations in respiration rates and changes in ethylene biosynthesis. Wills *et al.* (1989) reported that calcium would suppress respiration and several other metabolic sequences in plant tissues. These include physiological processes such as respiration, transpiration and evaporation leading to changes in the pH, TSS values, titratable acidity, ascorbic acid content and weight of fruit. The structural integrity and overall quality of pineapple fruits can be lost and internal discolouration will occur due to this phenomenon.

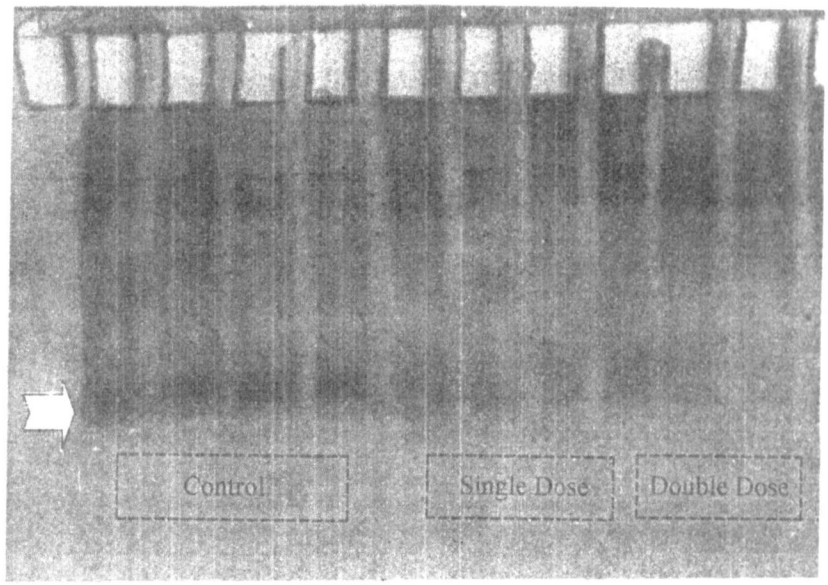

Figure 3. Effect of calcium on peroxidase activity of pineapple fruits during cold storage.
[Note: the arrow indicates the additional band.]

The intensity of IB in fruits treated with calcium was significantly lower than in the control (Figure 1). Zhou *et al.* (1995) showed that the occurrence of IB disorder in pineapple fruit was mainly due to catalyzed oxidation of phenolic compounds by polyphenol-oxidase (PPO) to form brown products. Once cell walls and cellular membranes lose their integrity, enzymatic oxidation proceeds much more rapidly (Martinez and Whitaker, 1995). However, according to Zsoldos and Karvady (1978), the membrane protecting the impact of calcium is most prominent under stress conditions such as low temperature. The development of IB in the control fruits may be due to the differences in the chemical constituents responsible for browning or to the changes in the cellular structure after cold storage.

Ascorbic acid is known to be a browning inhibitor. Reduction in ascorbic acid content was greater in the control fruits than in treated fruits (Table 2). Ascorbic acid levels have been associated with the degree of expression of symptoms of IB caused by chilling (Van Lelyveld and de Bruyn,

1976). The primary cause of loss of ascorbic acid is oxidation under aerobic conditions.

Lagrimini *et al.* (1990) reported that peroxidases are a convenient physiological marker of the plant development, physiology, infection and stress. Figure 3 illustrates the significant change of the peroxidase band intensities with different treatments. A band will appear at the position of the enzyme activity. The control fruits had a higher peroxidase activity than the calcium treated fruits. Hameda and Klein (1990) indicated that peroxidase can combine with hydrogen peroxide to produce an activated complex which can react with a wide range of donor molecules. Some of these reactions cause undesirable changes, such as discolouration in food materials. According to Henshell (1982), both peroxidase and ascorbic acid oxidase may cause high oxidation of ascorbic acid. Therefore, the reason for the reduction of ascorbic acid in control fruits may be due to the higher amount of peroxidase activity.

Fruits with higher level (DD) of calcium treated plants were harder than the other fruits (control). These results are in agreement with Bangerth (1979) and Wills *et al.* (1977) who reported that increasing calcium content of fruits leads to an increase in the firmness of the fruit and delays or even prevents fruit ripening. Low tissue contents of calcium in fleshy fruits also increase the losses caused by enhanced senescence of the tissue. According to Marschner (1995), even a relatively small increase in the calcium level of the fruits can be effective in preventing or at least drastically decreasing the economical losses caused by various storage disorders.

CONCLUSIONS

The results indicate that higher level of calcium treatment may extend the shelf life of Mauritius pineapple fruits by reducing the rate of oxidative metabolism and protein hydrolysis thus preventing the deterioration caused by internal browning. Calcium is able to mitigate cold-induced injury to the cell membrane.

ACKNOWLEDGEMENTS

We acknowledge with thanks to the Council of Agricultural Research Policy (CARP) for providing funds under project number CARP/12/210/182. We also acknowledge the assistance of Mr. K.K. Liyanage when conducting this research and Mr. Asanga Wijetunga for photography.

REFERENCES

Akamine, E.K. (1976). Postharvest control of endogenous brown spot in fresh Australian pineapple with heat. Hort. Sci. II: 586-588.

Anonymous. (1990). Technoguide. Dept. of Agric., Sri Lanka.

Askar, A. and Treptow, H. (1993). Quality Assurance in Tropical Fruit Processing. Berlin Heidelberg, New York.

Bangerth, F. (1979). Calcium-related physiological disorders of plants. Ann. Rev. Phytopathol. 17: 97-122.

Beverly, R.B., Latimer, J.G. and Smittle, D.A. (1993). Preharvest physiological and cultural effects on postharvest quality. pp. 74. *In:* Shewfelt, R.L. and Prussia, S.E. (Eds). Postharvest Handling - A Systems Approach. Academic press, New York.

Hameda, H.M. and Klein, B.P. (1990). Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J. Food Sci. 55: 181-192.

Henshell, J.D. (1982). Ascorbic acid in fruit juices and beverages. pp. 123-136. *In:* Counsell, J.N. and Hornig, D.H. (Eds). Vitamin C. Applied Science Publishers Ltd., New Jersey.

Lagrimini, L.M., Bradford, S. and Rothstein, S. (1990). Peroxidase-induced wilting in transgenic tobacco plants. The Plant Cell. 2: 7-18.

Martinez, M.V. and Whitaker, J.R. (1995). The biochemistry and control of enzymatic browning. Trends in Food Sci. 16: 195-200.

Marschner, H. (1995). Mineral Nutrition of Higher Plants. (2nd Edition). Academic press, London. pp. 1-889.

Rohrbach, K.G. and Pauli, R.E. (1982). Incidence and severity of chilling induced internal browning of waxed (Smooth Cayenne) pineapple. J. Amer. Soc. Hort. Sci. 107: 453-457.

Teisson, C. (1979). Internal browning of pineapples: I. History, II. Materials and Methods. Fruits. 34: 245-261.

Torres, A.M. (1990). Isozyme analysis of tree fruits. pp. 193-205. *In:* Soltis, D.E. and Soltis, S.E. (Eds). Isozymes in Plant Biology. Chapman and Hall Ltd., London.

Van Lelyveld, L.J. and De Bruyn, J.A. (1976). Sugars and organic acids associated with black heart in cayenne pineapple fruit. Agrochemophysica. 7: 9-14.

Wills, R.B.H., Hall, E.G., Lee, T.H., McGlasson, W.B. and Graham. E. (1977). An Introduction to Post Harvest Fruit and Vegetables. Adopt Printing Co., Sydney, Australia. pp. 177.

Wills, R.B.H., McGlasson, W.B., Graham, D., Lee, T.H. and Hall, E.G. (1989). Post harvest: A introduction to the physiology and handling of fruit and vegetables. 3rd Edition. Blackwell Scientific, Boston.

Zhou, Y.C., Tang, Y.L., Tan, X.J. (1995). Mechanism of black heart development induced by low temperature and gibberalic acid in pineapple fruit. Proceeding of 2nd International Ananas Symposium, Martinique (Abs.).

Zsoldos, F. and Karvady, B. (1978). Effects of calcium and temperature on potassium uptake along roots of wheat, rice and cucumber. Physiol. Plant. 43: 326-330.