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SOME BIOCHEMICAL FACTORS UNDERLYING THE DIFFERENTIAL SUSCEPTIBILITY OF TWO PINEAPPLE CULTIVARS TO INTERNAL BROWNING DISORDER

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ABSTRACT

Development of internal browning and some associated biochemical and physicochemical changes were investigated in the pineapple cultivar *Mauritius (syn. Queen)* and compared with those of a lesser susceptible cultivar *Kew (syn. Smooth Cayenne)*. The study was expected to identify the major factors underlying the cultivar resistance to the disorder. Three-week long storage trials were conducted simulating the sea freight export conditions at 10^0C and 85% RH. In *Mauritius*, the internal browning symptoms began within a week of storage at 10^0C initially in the marginal core tissue which subsequently spread to the surrounding flesh, whereas in *Kew*, the symptoms commenced only after 2-3 weeks of storage as isolated, smaller patches in the tissue surrounding the core. There was a clear difference in the time of incidence and the pattern of internal browning symptom development between the two cultivars. The cv. *Mauritius* showed comparatively faster ripening and respiration rates and greater accumulation of acids during cold storage, than the cv. *Kew*. In both cultivars the tissues undergoing browning displayed greater PPO and peroxidase activity and electrolyte leakage than their respective controls. Harvesting fruit early at 100% green stage reduced the incidence and severity of internal browning in both cultivars.

INTRODUCTION

Internal browning of pineapple (also referred to as black heart, endogenous brown spot) is a physiological disorder that develops when the fruit is exposed to low temperature during storage or in the field. The condition limits prolonged storage of fruits following harvest and long distance export (Akamine et al., 1975). This has been reported from many countries including Australia (Smith, 1983), Taiwan (Sun, 1988), Hawaii, Ivory Coast (Teisson et al., 1979), South Africa (Van Lelyveld and Debruyn, 1977) Japan (Mizuno et al., 1982), India and Malaysia (Abdullah and Rohaya, 1983; Abdullah et al., 1986).

The initial symptom characteristic to the disorder is the formation of translucent, water-soaked spots at the base of the fruitlets which later turn black and spread to neighboring tissues. The extent of symptoms may, however, vary with the cultivar, temperature (Stewart *et al*., 2002), fruit size (Hoffman and Smith, 1993), ratio of crown to fruit length (Soler, 1992) and also on the chemical and nutritional composition (Teisson *et al.,* 1979).

Browning of plant tissues is a result of the oxidation of *ortho*-dihydroxy phenolic compounds to *ortho*-quinines by polyphenol oxidase (McEvily *et al*., 1992). Enzymatic browning is one of the major oxidative phenomena inducing the development of undesirable colour, flavour, and loss of nutrients in fruit and vegetables (Martinez and Whitaker, 1995). PPO is related in most cases to discolouration and browning of both fresh (Martinez and Whitaker, 1995; Amiot et al., 1997) and minimally processed fruit and vegetables (Whitaker and Lee, 1995). The involvement of the peroxidases (POD) in the enzymatic browning was found to be limited due to the unavailability of hydrogen peroxide (Amiot et al., 1997). The involvement of peroxidases in the internal browning of pineapples remains possible (Amiot et al., 1997).

The experiments described in this paper were carried out to understand the internal browning development and associated events in two local pineapple cultivars, *Mauritius* and *Kew*, showing differential susceptibility to the disorder. The influence of stage of harvesting maturity on the incidence and severity of the disorder was also investigated. Such a comparative understanding was thought to help identifying the factors underlying the pineapple resistance or susceptibility to the disorder.

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MATERIALS AND METHODS

Pineapple fruit

Pineapple (*Ananas comosus*) fruits were obtained from a two-hectare plantation, consisting of already planted pineapples cvs. *Mauritius* and *Kew* situated at Radawana in Gampaha district (Western Province of Sri Lanka) for all the experiments. Mature (90d after anthesis), fully green pineapples of cultivars *Mauritius* and *Kew*, devoid of any sign of mechanical damage or disease symptoms, were selected at the field. These were hand-harvested, packed in cardboard boxes and transported to the Department of Botany, University of Peradeniya. Immediately after transportation, fruits were cleaned to remove the surface debris and any insect pests present. Fruit stalks were trimmed to about 6 cm length. Sets of 8 fruits, containing 4 replicate fruit from each cultivar were used for experiments.

Internal browning development in the fruit of two pineapple cultivars during cold storage

Six sets of pineapples were used for the experiment. Five sets were labelled (Set 1-5) using paper tags and immediately placed in a cold room at 10^0C and 85% RH. The remaining set (Set 6) was maintained at room temperature (28^0C-30^0C) for 48 h as the control. One set of fruits was withdrawn 7 d after cold storage and allowed to stand for 48 h at room temperature (28^0C -30^0C).

Individual fruits were cut longitudinally into two halves and the intensity of internal browning was assessed in each replicate fruit visually as % area affected in the core tissue and the flesh tissue separately using a 0 – 6 scale described by Teisson (1979) after modification: 0 = good flesh/core with no sign of browning, 1 = appearance of brown spots near the stalk end of the flesh/core, 2 = spots coalesce and cover 10% of the flesh/core, 3 = 25% of flesh/core turned brown colour, 4 = 50% of flesh/core turned brown colour, 5 = 75% of flesh/core turned brown colour, 6 = complete browning of flesh/core. The average intensity of internal browning following 7 d of cold storage was calculated separately for each pineapple cultivar. The second, third, fourth and fifth sets of pineapples were withdrawn from the cold storage 10, 14, 18 and 21 d after cold storage respectively. After allowing the fruits to stand for 48 h at room temperature (28^0C-30^0C), the intensity of internal browning was assessed as described above. The experiment was repeated three times. Flesh tissues were cut from the region surrounding the central core, separately in each replicate fruit withdrawn 0, 7, 10, 14, 18 and 21 d after cold storage, weighed and stored in sealed polythene bags in a freezer (-20^0C). These tissues were used to determine some physical and physicochemical parameters.

Electrolyte leakage in fruit tissue excised following different periods of cold storage

Pieces (1 cm^2 × 5 cm) of tissue cut from the core-flesh interface region of fruit withdrawn different periods after cold storage and the controls were used for the experiment. Four pieces of tissues taken from each replicate fruit were immersed separately in 25 ml portions of deionized water in glass tubes and allowed to remain for 3 h. Conductivity of the external solution was measured using a conductivity meter (WPA CM 35- Linton, Cambridge).

The tissues were then frozen (-20^0C) by keeping the tubes containing tissues in aqueous solution in a freezer for 24 h. The tubes were withdrawn from the freezer and kept at room temperature for 3 h. After stirring gently, the conductivity of external solution was measured again. The relative % electrolyte leakage of tissues was determined by taking the ratio of two measurements and multiplying by hundred.

Total soluble solids (TSS), % titratable acidity (TA) and pH in the pineapple flesh following different days of cold storage

Frozen tissue samples (100 g) taken separately from four replicate fruit of each cultivar were cut in to small pieces and homogenized separately for 3 min in a blender without adding any water. The resulting slurry was squeezed through a muslin cloth to obtain a clear extract. Brix value was taken using a hand refractometer (model Leica 10430) within the range of 0-30 ^{0}Brix. The average value was determined for replicate fruits. The pH of the extracts was measured using a pH meter (TOA Electronic Ltd; Japan HM 205) separately for replicate fruits and the average pH value was determined. Titratable acidity was determined by performing a manual titration to a colour end point (Askar and Trepow, 1993).

Effect of harvesting maturity on the development of internal browning

Fruit maturity was determined by counting the number of d lapsed after anthesis at the time of harvest. Four replicate fruits each from the two cultivars *Mauritius* and *Kew* were harvested at different stages of maturity as follows: Maturity stage 1 - immature fruits harvested 30 d after anthesis, the shell was fully green shell with small pointed eyes; fruit length 9-10 cm, diameter 7-8 cm; Maturity stage 2 - immature fruits 45 d after anthesis, the shell was fully green with small pointed eyes; fruit length 10-11 cm, diameter 9-10 cm; Maturity stage 3 - immature fruits, 60 d after anthesis, the shell was fully green with large pointed eyes; fruit length 12-14 cm, diameter 10-11 cm; Maturity stages 4 - mature fruits, 90 d after anthesis, the shell was fully green with large flat eyes; fruit length 15-18 cm, diameter 10-11 cm; Maturity stage 5 – ripening fruits, 105 d after anthesis, shell with 25% of yellow eyes, fruit length 15-18 cm diameter 10-11 cm.

The fruits after cleaning and trimming their stalks were stored at 10^0 C and 85%RH in a cold room. The fruits withdrawn 21d after the cold storage were allowed to stand for further 48 h at room temperature (28^0C-30^0C) and cut longitudinally into two halves. The intensity of internal browning was assessed visually using a scale separately for core and flesh tissue as described previously. The average internal browning was determined for each maturity stage. Total Soluble Solids, pH, and % titratable acidity of fruit at different maturity stages were also determined as described previously.

Respiration rate of the two cultivars of pineapple

Two sets of pineapples (containing 10 fruit from each cultivar) were weighed and placed separately in sealed glass containers (46 cm X 36 cm X 36 cm). One glass container at a time was connected to a gas analyzer (Fruit Store Analyzer, Type 770, David Bishop instruments, U.K) by an outlet tube. A fresh flow of air (1L/ min.) was passed in to the sealed container through an inlet tube. The respired air from the outlet tube was passed into the gas analyzer and the concentration of CO_2 in the airflow was measured. The respiration rate was calculated using the formula,

$$\text{Respiration rate} = \frac{\text{Change in } CO_2 \text{ concentration} \times \text{Flow rate (ml/h)}}{\text{Fruit weight (Kg)}}$$

Activity of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) during cold storage

Six sets of fruits containing four replicates from each cultivar were used for this experiment. Five sets of fruit were placed in a cold room at 10^0C and 85% RH and one set of fruit allowed to stand for 48 h at room temperature (28^0C -30^0C) as the control. One fruit set each was withdrawn after 7, 10, 14, 18 and 21 d after cold storage and allowed to stand for 48 h at room temperature (28^0C -30^0C).

Tissue samples (5 g) were collected from the area immediately outside the core tissue from each replicate fruit. The tissues (20g) after storing at -20^0C for 24 h were ground into a fine powder using a mortar and pestle in liquid N_2 and the powder was extracted in 150 ml of cold (-20^0C) acetone for 1 minute with constant stirring. The homogenate was filtered through filter paper (Whatman No 2) using a Buchner funnel under vacuum and the residual powder was washed twice with 100 ml portions of fresh cold acetone. The dry powder was stored at -20^0C in sealed polythene bags. Activity of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) was assayed (Riov et al., 1968; Dubery and Schabort, 1986). Each experiment was repeated for three times.

For PAL assay, the enzyme extract (2 ml) was mixed with L-phenylalanine (0.6 ml) and ammonium acetate buffer (3.4 ml, pH 7.7). The absorbance was measured. Using a stock solution (0.001%) of cinnamic acid in 0.1M ammonium acetate buffer (pH 7.7) a concentration gradient containing 10-100 ppm of cinnamic acid was prepared. The absorbance of each solution was measured at 290 nm and a standard curve, the concentration of the cinnamic acid vs. absorbance, was obtained. The concentration of cinnamic acid produced (in ppm) was determined using the standard curve. PAL activity was represented as mμ/h/g fresh weight. Each experiment was repeated for three times.

The non-parametric data were subjected to Kruscal-Wallis test and the parametric data were analysed using a statistical package (SAS Institute, 1988).

Analysis of peroxidase isozymes by polyacrylamide gel electrophoresis (PAGE)

A set of 8 pineapples stored in a cold room for 21 d was used for analysis of isozyme peroxidase. Tissues (3 g) were cut separately from the healthy areas and the areas showing internal browning in each fruit and ground separately with 1.0 ml of extraction buffer. The extracts were centrifuged at 1300 rpm for 3 min, the supernatant was separated and stored at -20^{0}C for 24 h. PAGE was performed in a vertical slab apparatus as described by Torres (1990) in a gel buffered by Tris-HCl (pH 8.8) using discontinuous buffer system. The electrode buffer used was 1.2g lithium hydroxide and 11.9g boric acid (pH 8.3) dissolved and made upto 1 litre with distilled water. Each well was loaded with 20µl of sample and plates clamped into the electrophoresis apparatus (SE-600, Hoeffer scientific) and placed in a gel tank with running buffer. A constant current of 20mA per gel was applied to the gel apparatus for 3 1/2 hours.

The fixative stain solution used contained 0.05M sodium acetate buffer (pH 5), $CaCl_2$, H_2O and 3-amino-9-ethyl cabozole dissolved in N, N, dimethylformamide and the gel was incubated at room temperature until red bands appeared.

RESULTS

Internal browning development in the fruits of two pineapple cultivars during cold storage

Internal browning symptoms first appeared in fruit of cv. *Mauritius* within 7 d of cold storage (Table 1) as small, light brown, translucent and diffused areas (Figure1) in the periphery of the core. These areas enlarged and spread along the periphery of the core and turned brown colour (Figure 1). There was a significant (p=0.05) increase in the intensity of internal browning in the core tissue from the first 7 to 10 d of storage. During this period the brown areas spread both inwards and outwards from the periphery of the core in a uniform manner and in fruit withdrawn 14 d after cold storage a large area of both the core and the flesh tissue were affected. About 75% of the core and flesh tissue was found affected by the disorder, in fruits removed 21 d after cold storage.

Table 1.The development of internal browning in pineapple cvs. Mauritius and Kew during 21 d of cold storage.

Period of storage (days)	*Intensity of internal browning			
	Mauritius		*Kew*	
	Flesh	Core	Flesh	Core
0	0b	0b	0 b	0a
7	0b	1.3b	0 b	0 a
10	0b	2.7a	0b	0 a
14	3.3a	3.3a	1.7a	0.7a
18	4.3a	4.0a	2.0a	0.7a
21	5.0a	5.0a	3.1a	1.3a

Values followed by the same letters within the each column do not differ significantly at P≤0.05 (Kruscal - Wallis test). *Intensity of internal browning (IB) was assessed separately in the core and the flesh tissue using a scale where, 0- no browning, 6- 100% browning.

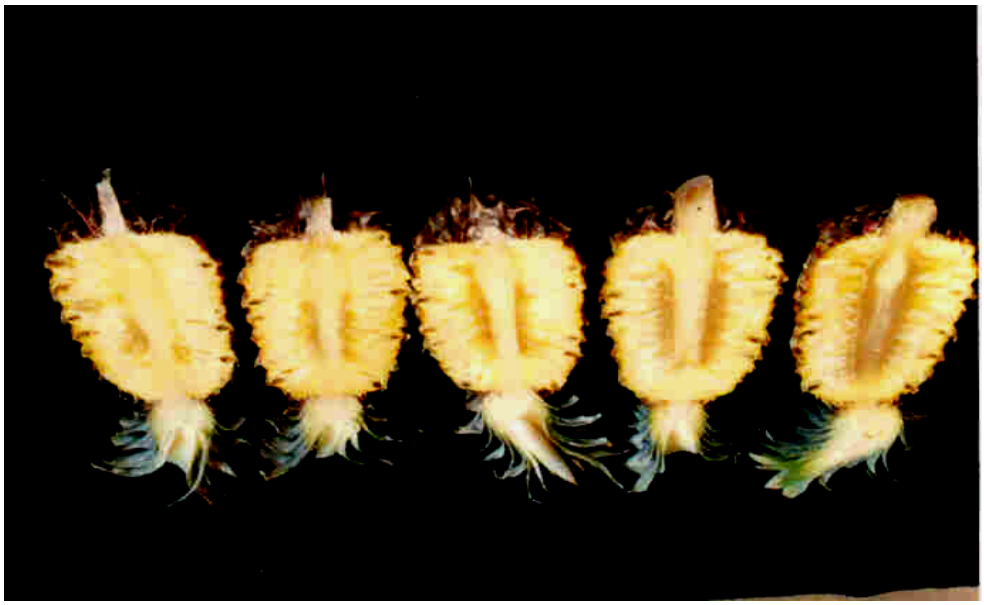

Figure 1. The development of internal browning symptoms in cv. *Mauritius* during cold storage (From left to right: Fruit 1 – 7 d after cold storage with translucent patches; Fruit 2 – 10 d after cold storage with translucent patches spread into the periphery of the core region; Fruit 3, 4 - 14-18 d after cold storage with brown water-soaked areas in the periphery of the core region ; Fruit 5 – 21 d after cold storage with brown water-soaked areas in both the core and flesh tissue.

Figure 2. The development of internal browning symptoms in cv. *Kew* during cold storage (From left to right: Fruit 1 – 7 d of cold storage no visible symptoms; Fruit 2 – 14 d after cold storage showing brown, water-soaked patches in the flesh and in the core; Fruit 3, 4 – 18 d after cold storage with brown, water-soaked patches in the flesh and the core; Fruit 5 – 21 d after cold storage with larger, brown colour, water-soaked areas in core and the flesh tissue)

Internal browning occurred relatively slowly in the fruit of cv. *Kew* compared to cv. *Mauritius* during cold storage. The initial symptoms appeared in fruit cv. *Kew* stored only 14 d after cold storage (Table 1). The symptoms were seen both in the flesh and the core, but were mostly confined to the flesh, as water-soaked, translucent and isolated patches (Figure 2).

These patches enlarged slightly and turned brown colour during the latter stages of storage. About 25% of the flesh was affected 21 d after cold storage. There was a marked difference in symptom development between the two cultivars (Figure 2).Electrolyte leakage in fruit tissue excised following different periods of cold storage.

The % electrolyte leakage of tissues was over 60% in all the tissues sampled irrespective of whether they had internal browning symptoms or not. However, the healthy tissues showed lesser electrolyte leakage than the tissue samples collected from areas with internal browning symptoms in both cultivars. The % electrolyte leakage fluctuated with the increase of cold storage period in cv. *Mauritius* whereas a slight increase was observed in cv. *Kew*. The highest % electrolyte leakage was observed in the affected tissue removed 14 and 18 d after cold storage from cv. *Mauritius* and *Kew* respectively (Table 2).

Total Soluble Solids (TSS), % titratable acidity (TA) and pH in the pineapple flesh during cold storage

Pineapple cv. *Mauritius* maintained higher % titratable acidity and TSS during the entire storage regime compared to cv. *Kew* (Table 3). The % titratable acidity increased progressively in cv. *Mauritius* during storage reaching the highest at 21 d after cold storage, which was about 43% more than the original level at day zero. There was no significant alteration in titratable acidity in the cv. *Kew*. There was a gradual reduction in the pH of the extracts taken from cv. *Mauritius*, the lowest being at 18 d and 21 d after cold storage. In cv. *Kew* the fluctuation of pH was observed. The TSS of cv. *Mauritius* was higher than that of cv. *Kew* at all stages during storage. However, there was no significant change in the TSS value in either cultivar during cold storage.

Table 2: Percentage electrolyte leakage of tissues taken from fruits stored for different time intervals at 10^0C

Period of storage (days)	% Electrolyte leakage			
	Mauritius		*Kew*	
	Affected tissue	Healthy tissue	Affected tissue	Healthy tissue
0	*	65.4	*	62.4
7	80.5a	65.6b	*	63.6
10	86.4a	72.2b	*	66.8
14	91.2a	62.5b	83.3a	66.6b
18	86.2a	73.5b	86.4a	70.3b
21	81.0	**	84.2	**

Values followed by the same letters within the each row are not significantly different (P≤0.05) by t-test. * affected tissues were not found in cvs. *Mauritius* and *Kew* stored for 0 days and stored for 0,7 and 10 d. ** Healthy tissues were not found in tissue samples taken 21 d after cold storage.

Table 3: Changes in % titratable acidity, pH and Total Soluble Solids in the two cultivars of pineapple fruit during cold storage.

Days of storage	%TA		pH		TSS	
	Mauritius	*Kew*	*Mauritius*	*Kew*	*Mauritius*	*Kew*
0	0.7c	0.6a	3.8a	3.7ab	15.2a	9.7a
7	0.8bc	0.7a	3.9a	3.8a	14.6 a	8.4a
10	0.8 bc	0.7a	3.7ab	3.6ab	14.9 a	8.1a
14	0.9ba	0.6a	3.5bc	3.6ab	14.5 a	9.1a
18	0.9a	0.6a	3.4c	3.6ab	14.4 a	9.6a
21	1.0a	0.6a	3.4c	3.6b	15.6 a	9.8a

Values followed by the same letters within the each column are not significantly different (P≤0.05) by Duncans Multiple Range Test.

Effect of harvesting maturity on the development of internal browning

Pineapples cvs. *Mauritius* and *Kew* harvested at different maturity stages were stored at 10^0C and the intensity of internal browning was assessed. In both cultivars, an increased intensity of internal browning was observed during cold storage when the fruits were harvested late. The fruit of cv. *Mauritius* showed greater internal browning both in the core tissue and the flesh compared to those of cv. *Kew* at all maturity stages (Table 4). In both cultivars, the fruit harvested 30 to 45 d after anthesis developed the least internal browning with symptoms only in the core tissue, where the flesh remained unaffected. The intensity of internal browning in the core tissue of cv. *Mauritius* was significantly greater in fruit harvested 90 d after anthesis, compared to fruit harvested at 30, 45 or 60 d after anthesis. The highest intensity was observed in fruit harvested 105 d after anthesis when the fruit were 25% ripe.

There was an increase in TSS, % titratable acid levels and a corresponding decrease of pH with the increase of the stage of harvesting maturity in both pineapple cvs. *Mauritius* and *Kew*. The fruit harvested at 25% ripe stage had the highest TSS and % titratable acidity. Pineapple cv. *Mauritius* recorded higher TSS values compared to cv. *Kew* at all maturity stages (Table 6). The fruit harvested 105 d after anthesis showed the highest % titratable acidity and the fruit harvested 30 d after anthesis had the lowest % titratable acidity in both cultivars.

Ripening and respiration rate of the two cultivars of pineapple

The degreening of shell in cv. *Mauritius* took place faster than that of cv. *Kew*. The shell of fruit cv. *Kew* remained green even after 21 d of cold storage where as total yellowing of shell was observed in cv. *Mauritius*. The respiration rate of pineapple in two cultivars was measured over a period of 14 d at room temperature. The pattern of respiration was similar in both cultivars during the first 14 d period but the rate of respiration in cv. *Mauritius* was greater than that of cv. *Kew*. The respiration rate was highest in cv. *Mauritius* and *Kew* 12 and 10 d respectively after harvest (Figure 3).

Activity of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) during cold storage

The tissues of pineapple cv. *Mauritius* displayed greater polyphenol oxidase activity than cv. *Kew* at all stages during cold storage (Table 6) and in both cultivars the PPO activity increased

during cold storage. PPO activity in pineapple cv. *Mauritius* was low during the first week and increased five fold during the period from 7 – 10 d of cold storage. This activity increased further reaching the highest level of 22.2 units/min/g fresh wt 21 d after storage (Table 6). The PPO activity of fruit tissue of cv. *Kew* taken 21 d after cold storage was ten times greater than that of at day 0. The activity of PPO increased in cv. *Kew* during cold storage at a slower rate than that in cv. *Mauritius* (Table 6).

Table 4. Internal browning in pineapple cvs. Mauritius and Kew harvested at different stage of maturity, following cold storage.

Harvesting maturity (days)	**§ Shell colour**	Intensity of internal browning			
		Mauritius		*Kew*	
		Flesh	**Core**	Flesh	Core
30	1	0c	1.3b	0c	0d
45	1	0c	2.0b	0c	0d
60	1	2.0b	2.7b	2.0b	2.0c
90	4	4.7a	4.3a	3.2a	2.5b
105	5	5.0a	5.0a	3.2a	3.0a

Values followed by the same letters within the each column are not significantly different (P≤0.05) by Duncans Multiple Range Test. §Shell colour scale: 0-green, 5-yellow

Table 5. Total Soluble Solids (TSS), pH and % titratable acidity (%TA) in pineapple cvs. Mauritius and Kew harvested at different maturity stages, following cold storage.

Harvesting maturity (days from anthesis)	TSS (^{0}Brix)		pH		%TA	
	uritius	*Kew*	*Mauritius*	*Kew*	*Mauritius*	***Kew***
30	7.0b	6.3c	4.2a	4.2a	0.4c	0.4b
45	7.2b	6.0c	4.3a	4.1ba	0.6b	0.5b
60	8.3b	7.2bc	4.1a	3.9bc	0.6b	0.5b
90	13.7a	8.8ab	4.0a	3.8dc	0.7b	0.6a
105	15.7a	9.8a	3.5b	3.7d	0.9a	0.6a

Values followed by the same letters within the each column are not significantly different (P≤0.05) by Duncans Multiple Range Test.

Table 6: Polyphenol oxidase activity (PPO) and peroxidase (POD) in pineapple cvs. Mauritius and Kew during cold storage.

Days of storage	*PPO activity (units/min/g fresh weight)*		POD activity (units/min/g fresh weight)		PAL activity (mμ/h/g fresh weight)	
	Mauritius	*Kew*	*Mauritius*	*Kew*	*Mauritius*	*Kew*
0	1.8b	1.3b	1.8b	1.6d	9.0	6.0
7	2.0b	1.7b	1.8b	2.0dc	9.1	6.5
10	11.1ba	5.5ba	3.7b	2.6dc	9.2	6.0
14	13.3ba	8.3ba	3.7b	4.4c	6.6	7.0
18	19.8ba	13.3a	4.4b	7.8b	8.7	7.9

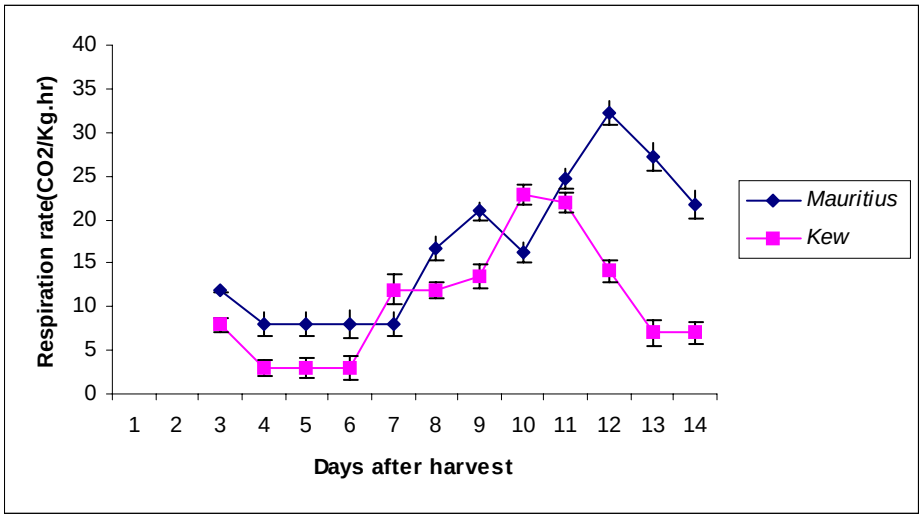

Figure 3. Respiration rate of two cultivars of pineapple at room temperature

There was a gradual increase of peroxidase activity in both cultivars during cold storage reaching the highest at 21 d of cold storage (Table 6). A relatively higher peroxidase activity was observed in the cv. *Mauritius,* 10 d after cold storage compared to the cv. *Kew.* The highest peroxidase activity was shown by the fruit stored for 21 d. In cv. *Mauritius,* the PAL activity was almost constant during the first 10 d, remained around 9 units/min/g fresh weight. The highest PAL activity (10.5 units/min/g fresh weight) was observed in fruit stored for 21 d. A similar pattern of PAL activity was observed in cv. *Kew.*

When soluble peroxidases were extracted and subjected to electrophoresed, the extract from tissue of both cvs. *Mauritius* and *Kew* showing internal browning displayed three soluble peroxidase isozyme bands. However, the extracts of healthy tissues had only two peroxidase isozyme bands.

DISCUSSION

The process of internal browning development in the cultivar *Mauritius* during cold storage was investigated and compared with that of cv. *Kew,* which is believed to be relatively less susceptible to the disorder. Certain physicochemical and biochemical processes associated with the development of internal browning were also determined in the two cultivars. Such information was thought to provide clues to the factors underlying pineapple fruit resistance or susceptibility to internal browning which could help develop meaningful measures to control the disorder.

Internal browning symptoms could be observed in fruits of both pineapple cultivars with prolonged low temperature storage. However, the two cultivars exhibited a marked difference in the nature, location and progression of symptoms and in the susceptibility to internal browning.

The cv. *Mauritius* developed browning within the very first week of cold storage uniformly over the outer core and the inner flesh region. Whereas, in cv. *Kew,* the browning took place about two weeks after cold storage as isolated, small patches in the flesh tissue of the fruit. Although the primary cause of internal browning is low temperature in both, the response of the two cultivars was very distinct reflecting the inherited differences in the two cultivars. The internal browning symptoms of the Malaysian pineapple cultivars *Gandol* and *Mauritius* (Abdullah, 1997; Abdullah and Rohaya, 1983) and *Queen* pineapple harvested from the fields at Nelspruit, South Africa (Van Lelyveld *et al.,* 1991) were similar to that of cv. *Mauritius* observed in this study. On the other hand the nature of internal browning symptoms of cv. *Kew* appeared similar to that of *Smooth Cayenne* pineapple grown in Queensland, Australia (Sanewski and Giles, 1997; Smith and Glennie, 1986).

The electrolyte leakage of tissues showing internal browning symptoms was higher than that of unaffected tissues in both cultivars of pineapple confirming that the internal browning is associated with membrane damage. Wijerathnam *et al*. (1996) also observed a higher electrolyte leakage of core and the flesh tissue in cv. *Mauritius* after cold storage. However, an increase of electrolyte leakage was not always observed in the flesh tissue with the progression of the symptoms. Increased electrolyte leakage after storage might be due to alteration of the membrane structure which could result in changes in cellular permeability and subsequent biochemical activities in the tissue (Wijerathnam *et al.,* 1996). The leakage of the cellular content in plant tissue could be used as an index of altered membrane permeability, ripening, senescing or cell injury (King and Ludford, 1983). Once cell walls and cellular membrane loose their integrity, enzymatic oxidation proceeds much more rapidly and finally resulted in browning of tissues. Martinez and Whitaker (1995) also pointed out that the enzymatic browning in plant tissues usually impairs the sensory properties and softening of tissues.

The pineapple cv. *Mauritius* has maintained a higher PPO activity than that of cv. *Kew* during the three-week period of cold storage. In *Mauritius*, the PPO activity increased over ten times of its initial activity during cold storage and this increase coincided with the onset of internal browning symptoms. In cv. *Kew*, PPO activity was lower compared to that of cv. *Mauritius* and this may be related to its lesser incidence of internal browning in cv. *Kew.* PPO has been implicated to pulp discoloration during internal browning (Paull and Rohrbach, 1985). Miller and Heiman (1952) suggested that browning of pineapple is caused by oxidation of phenolic compounds to quinones, which is brown or black in colour. POD activity, however, remained unaltered during the induction of internal browning (Graham *et al.,* 1998). Browning always results from a loss of sub-cellular compartmentalization following physical modifications to plastid and vacuolar membranes leading to enzyme substrate contact.

The present study indicated a gradual increase of the peroxidase activity during cold storage. A notable increase of peroxidase activity was observed on the 10^{th} day of cold storage and this coincided with the onset of internal browning development in cv. *Mauritius*. The fruit which exhibited higher intensity of internal browning also exhibited the higher peroxidase activity in its tissues showing a positive correlation between the peroxidase activity and the internal browning development. Amiot *et al.* (1997) also found a possible involvement of peroxidases in the internal browning of pineapple. Higher activity of

peroxidase was found in fruit stored at >10 ^{0}C than those stored at <10 ^{0}C (Van Lelyveld and De Bruyn, 1977). Peroxidases could combine with hydrogen peroxide to produce an activated complex, which can react with a wide range of donor molecules (Hameda and Klein, 1990). The peroxidase isozyme assay on polyacrylamide gel revealed that the tissues undergoing internal browning in both pineapple cultivars contained one additional isozyme indicating possible involvement of peroxidase in internal browning. Zhou *et al*. (2003) obtained contrasting evidence regarding involvement of peroxidases, however, there is a positive correlation between the PAL activity and the internal browning disorder. Our present study did not reveal a correlation between tissue browning and phenyalanine ammonia-lyase activity in the two pineapple cultivars.

The cultivar *Mauritius* showed a higher TSS at all stages compared to the cv. *Kew*. The TSS in fruit declined during the first 18 days of storage, but increased subsequently resulting higher values in fruit stored for 21 d. Paull (1997) reported a gradual increase of TSS during early days of storage in cv. *Mauritius* and more rapid increase in the final six days. Storage for two weeks at 10^{0}C had a little effect on TSS or individual sugars (Chen and Paull, 1995). A decrease of TSS was also observed in stored pineapple after 3-4 weeks (Nanayakkara *et al.,* 1990).A gradual buildup of acid levels was observed in cv. *Mauritius* during cold storage, whereas only a slight fluctuation of acid level was observed in the cv. *Kew* while the overall acid levels were relatively lower. The increased acid levels in cv. *Mauritius* might be an important factor in the development of increased internal browning compared to *Kew*. The acids present in pineapples are citric and malic. It was previously reported that the citric acid content increased slightly with little change in malic acid during storage at 8^{0}C. However, there was no apparent change in malic acid or citric acid in fruit held at 22^{0}C. Fruit previously stored at 8^{0}C and then removed to 22^{0}C show a continuous decline in titratable acidity from 13.5 m.eq l^{-1} to 8.5 m.eq l^{-1} (Paull, 1997).

The shell colour development in the cv. *Mauritius* occurred much faster than that of cv. *Kew* showing that the cv. *Kew* ripens at a slower rate at low temperature. The respiratory rate (% CO_2 kg/hr) of cv. *Kew* was also lower compared to that of cv. *Mauritius*. This indicates an inverse relationship between ripening and the susceptibility to the disorder, the slower ripening cv. *Kew* being less susceptible.

Fruits of both cultivars have shown increased susceptibility to internal browning when they were harvested at fully mature-green or ripe stage. Advancing the harvesting maturity stage from 25% ripe stage to fully mature-green, only delayed the onset of internal browning development. Harvesting fruits very early results in little or no internal browning but these fruits do not attain the desired ripeness or develop acceptable flavors. Brief heat treatment prior to cold storage has also been found to reduce internal browning by about 70% (Weerahewa and Adikaram, 2005).

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