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Enzymes associated with blackheart development in pineapple fruit

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Abstract

The involvement of browning enzymes, polyphenoloxidase (PPO), peroxidase (POD) and phenylalanine ammonia-lyase (PAL) in blackheart development was investigated in pineapple fruit (*Ananas comosus*, Smooth Cayenne) following low temperature storage. An increase in PPO activity was related to the incidence of blackheart symptoms, both temporally and spatially. Fruit maturity significantly affected blackheart susceptibility; immature and over-mature fruits developed less blackheart injury than mature fruit. The effect of maturity on blackheart susceptibility was highly correlated to the response of PPO activity to chilling. POD showed no significant change after chilling. Enhanced PAL activity was observed during chilling at 6, 13 and 18 °C. Chilling (6, 13 and 18 °C) also inhibited the increase of ascorbate peroxidase activity observed in the fruit stored continuously at 25 °C, but had less effect on catalase activity. The results indicate that the development of blackheart symptoms in pineapple fruit results from the disturbance of a number of metabolic processes that occur at sub-ambient temperatures.

Keywords: Polyphenoloxidase; Peroxidase; Phenylalanine ammonia-lyase; Catalase; Ascorbate peroxidase; Chilling; Blackheart; Pineapple fruit

1. Introduction

Blackheart is a physiological disorder of pineapple (*Ananas comosus*, L. Smooth Cayenne) that is induced by exposure to low temperature, either in field or in postharvest storage and results in severe internal discoloration of pineapple fruit (Paull & Rohrbach, 1985; Smith, 1983). Blackheart has caused heavy losses in pineapple production countries (Rohrbach & Paull, 1982; Smith, 1983). In Australia, blackheart is the major postharvest limitation for the pineapple industry, as fruit rapidly develop blackheart following low temperature, which severely restricts refrigerated seafreight export (Smith, 1983). The development of effective methods to alleviate blackheart has been widely reported, and these include heat treatment, waxing and controlled atmosphere (Rohrbach & Paull, 1982). However, these methods do not completely eliminate blackheart. Transgenic strategies to control the browning process in other crops, such as potato, have proved highly encouraging (Bachem et al., 1994). A similar strategy, based on PPO gene silencing, is being developed for pineapple (Graham et al., 1998), which has the potential to control blackheart injury in pineapple fruit. However, the efficient exploitation of this biotechnology to control blackheart relies on a precise understanding of the biochemical pathway in blackheart development.

The biochemical pathway of blackheart development during low temperature storage has not been clearly documented. In other species, similar internal damage has been widely linked to stress-induced polyphenoloxidase (PPO, EC 1.10.3.2 or 1.14.18.1) and peroxidase (POD, EC. 1.11.1.7; Mayer, 1987). PPO has been proposed to be related to blackheart development (Teisson, 1972; van Lelyveld, 1977). Recently Stewart, Sawyer, Bucheli, and Robinson (2001) indicated that PPO mRNA was induced, by chilling, in pineapple fruit. However, the significance of the involvement of this enzyme to blackheart development has not been adequately documented. While POD has been implicated in discoloration of numerous fruits and vegetables, its significance in blackheart development is unresolved. Both higher and lower POD activity were reported in fruit

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with blackheart symptoms (Botrel & de Carvalho, 1993; Van Lelyveld, Visser, & Swarts 1991). Being a key enzyme in phenolic biosynthesis, phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) has also been considered to be associated with browning and the accumulation of chlorogenic acid and lignin-like materials (Hahlbrock & Scheel, 1989). PAL has been reported to play an important role in the browning process of many fruits and vegetables (Ke & Saltveit, 1989), but the possible relationship of PAL to blackheart injury is still unknown.

On the other hand, evidence is increasing to suggest that chilling injury in other species is mediated by the balance of reactive oxygen species (ROS) production and the cell protective system during low temperature stress or upon release from the stress (Prasad, Anderson, Martin, & Stewart, 1994; Purvis, Shewfelt, & Gegogeine, 1995). The cell protective system comprises a variety of antioxidant enzymes, including ascorbate peroxidase (AsPOD; EC 1.11.1.11) and catalases (CAT; EC 1.11.1.6; Foyer, Lopez-Delgado, Dat, & Scott, 1997). Both enzymes are important H_2O_2-degrading enzymes in inducing chilling-tolerance in maize seedling (Hodges, Andrews, Johnson & Hamilton, 1997; Prasad, Anderson, & Stewart, 1994). A lower level of CAT and AsPOD was related to chilling injury of cold stored mandarin (Sala, 1998). An inhibition of CAT by antisense RNA increased susceptibility to chilling injury in transgenic tomato plants (Kerdnaimongkol & Woodson, 1999). There is little information to correlate pineapple blackheart injury with the cell protective system. Given its significance in other species, it would be of value to examine the relationship of the antioxidant enzymes to blackheart development in pineapple fruit after chilling.

In this paper, the roles of PPO, POD and PAL were investigated, to identify the biochemical pathway of blackheart development in pineapple fruit following low temperature treatment. The activities of AsPOD and CAT were also determined in an attempt to provide information about the relationship of these antioxidant enzymes to blackheart development.

2. Materials and methods

2.1. Enzyme activity during blackheart development

Pineapple fruits (*Ananas comosus*, L. Smooth Cayenne) were harvested at a local farm (latitude 27 °C, 100 km north of Brisbane, Queensland) in early March. Batches of 50 fruit were stored at each of 6, 13 or 18 °C for 3 weeks, then transferred to 25 °C for another weeks storage. A further 50 fruits were stored at 25 °C for 4 weeks as a control. All fruits were held at 95% relative humidity. Determination of blackheart injury and enzyme assay were carried out concurrently. The experiments were replicated three times. At least 10 samples for each treatment at each storage time were used for blackheart assessment, and five samples from each treatment were used for all other enzyme assays.

2.2. Blackheart and polyphenoloxidase activity at different fruit maturities

Harvested fruits (200) were immediately assigned into one of three maturity ranges, based on the average specific gravity (SG; Smith, 1983), immature: $SG < 0.905$; mature: $0.920 < SG < 0.960$, these were about the same as commercial mature fruit; and over-mature: $SG > 1.000$. Immature fruit were approximately $6 > 8$ weeks from commercial maturity. All fruits were stored at 10 °C for 3 weeks, followed by 1 week at 25 °C. PPO activity was assayed concurrently with blackheart assessment.

2.3. Blackheart determination

Ten fruits from each treatment were halved longitudinally, and the incidence and severity of blackheart injury were visually scored from 0 (no blackheart) to 6 (maximum blackheart) according to the method of Paull and Rohrbach (1985).

2.4. Polyphenoloxidase (EC 1.10.3.2 or 1.14.18.1)

The extraction of PPO followed a modified method of Robinson, Loveys, Chacko, Trejo, and Soto (1993). A 7-g sample (pulp, core, skin or crown of fruit) was homogenized for 2 min in a Waring Blender (IKA-T25, Labortechnik) with 30 ml 0.1 M Na-Pi buffer, pH 6.0 at 4 °C. The homogenate was then centrifuged at 12,000 rpm for 10 min and filtered through Whatman No. 4 paper. The supernatant was used for PPO activity assay. PPO activity was assayed by the oxygen-uptake method, with a Clark-type oxygen electrode (Hansatech, UK), using 4-methycatechol as the substrate. The instrument was calibrated daily with sodium dithionite at 25 °C. To determine PPO activity, the reaction chamber contained 0.2 ml crude enzyme, and 2.3 ml 0.1 M Na-Pi buffer (pH 6.0) with 0.5% SDS (w/v). Following equilibration, the reaction was initiated by adding 0.5 ml 36 mM 4-methylcatechol, which was prepared in the same buffer. The reaction was carried out at 25 °C and the initial linear phase of the curve was used to calculate the rate of O_2 consumption. PPO activity was expressed as nmole O_2 min^{-1} mg^{-1} protein.

2.5. Peroxidase (EC. 1.11.1.7)

Pulp tissue (5 g) was homogenized in 20 ml of 100 mM Na-Pi buffer (pH 5). The solution was allowed to

stir at 4 °C for a minimum of 1 h. The homogenate was centrifuged at 12,000 rpm for 20 min, filtered through Whatman No. 4 paper and the supernatant used for POD assay. For the activity assay, 1 μl fresh 30% H_2O_2 was added to 100 ml Na-Pi buffer (pH 5) containing 0.4 mg/ml *o*-phenylenediamine dihydrochloride, added just before assay. This mixture was used as the substrate solution. A sample of supernatant (0.1 ml) and 2.9 ml substrate solution were mixed and immediately measured at 446 nm at 20-s intervals for 2 min at 25 °C. POD activity was expressed as A_{446} increase min^{-1} mg^{-1} protein.

2.6. Phenylalanine ammonia-lyase (EC 4.3.1.5)

The assay of PAL followed a modified method of Martinez, Chaves, and Anon (1996). Fruit pulp tissue (10 g) was homogenized in 15 ml 0.1 M borate buffer (pH 8.8) with 5 mM *b*-mercaptoethanol, 2 mM EDTA and 1% (w/v) PVPP. After stirring for 1 h at 4 °C, the solution was filtered through two layers of cheesecloth and centrifuged at 12,500 rpm for 15 min. The supernatant was used for PAL assay. For PAL activity assay, the reaction mixture contained 2.75 ml 60 mM L-phenylalanine in 0.1 M borate buffer (pH 8.8) and 0.25 ml supernatant extract. The substrate was pre-incubated at 40 °C for 15 min before mixing with the enzyme extract. The reaction was at 40 °C for 1 h and was stopped by adding 0.1 ml 6 N HCl. The change in OD_{290} was measured against a blank without substrate. PAL activity was expressed as A_{290} increase h^{-1} g^{-1} FW.

2.7. Catalase (EC 1.11.1.11), and ascorbate peroxidase (EC 1.11.1.6)

The extractions of catalase and ascorbate peroxidase were carried out according to the modified method of O'Kane et al. (O' Kane, Gill, Boyd, & Burdon, 1996). Fruit pulp tissue (5 g) was homogenized in 20 ml 0.1 M K-Pi buffer (pH 7.0) containing 0.1 mM EDTA, 1% PVPP and 1 mM ascorbate. The homogenate was stirred at 4 °C for 1 h. After centrifugation for 20 min at 12 500 rpm, the supernatant was filtered through Whatman No. 4 paper, and used immediately for the assays.

Catalase activity was determined by measuring the reduction of H_2O_2 at 240 nm (Schoener & Krause, 1990). The reaction contained 2.9 ml 0.34 M H_2O_2 in 50 mM K-Pi buffer pH 7.0 and 0.1 ml enzyme extract. The enzyme activity was determined using the linear portion of the rate curve and expressed as A_{240} decrease min^{-1} g^{-1} FW. Ascorbate peroxidase activity was determined by a modified method of Schoner and Krause (Schoener & Krause, 1990). The reaction mixture contained 2.9 ml ascorbic acid in 50 mM K-Pi buffer (pH 7.0) with 0.3 mM H_2O_2. The reaction was initiated by adding 0.1 ml enzyme extract and the reduction of ascorbate was measured at 290 nm during the first 20–30 seconds of reaction. Corrections were made for ascorbate disappearance due to non-enzymatic oxidation and H_2O_2-independent oxidation. Activity was expressed as A_{290} decrease min^{-1} g^{-1} FW.

2.8. Protein assay

Protein concentration was determined with a DC Bio-Rad assay kit and using bovine serum albumin as standard according to the manufacturer's instruction.

2.9. Statistical analysis

All data was analyzed using analysis of variance. For comparison of different tissues, the analysis of variance considered the effects of tissue, storage temperature and storage time and the interaction between factors. Within the stored fruit treatments, the effects of temperature, time and their interaction were assessed. All terms were tested against the variation between fruit within a treatment. After variance analysis, pairwise comparisons were made using the protected least significant difference procedure at $P < 0.05$.

3. Results

3.1. Comparison of PPO and POD activity during blackheart development

Blackheart was not observed in pineapple fruit stored at 25 °C for 4 weeks (Fig. 1). Fruit developed blackheart symptoms immediately upon removal from the 13 °C storage, with the severity of damage increasing with subsequent storage at 25 °C. Small watery, translucent

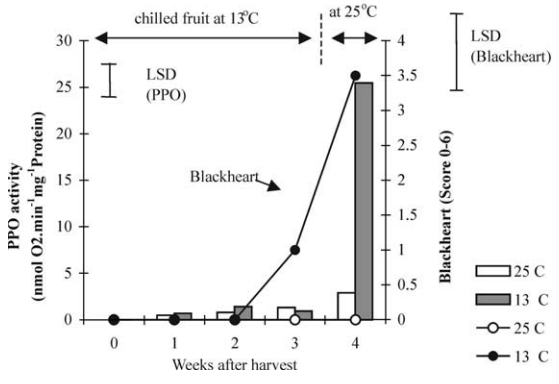

Fig. 1. Change of PPO activity in pineapple fruit accompanying blackheart development after chilling. Blackheart rating was based on a 0 (no visible injury) to 6 (maximum discoloration) scale and represented a visual assessment of 10 fruit per treatment, LSD = 1.35 ($P < 0.05$). PPO activity values are the means representing the average of triplicate experiments. LSD = 3.46 ($P < 0.05$).

spots, the first sign of blackheart symptoms (Paull & Rohrbach, 1985) were also observed in some fruits after sufficient periods of low temperature storage alone (3 weeks at 13 °C). While it is difficult to predict the temperature and exposure period under which the maximum level of blackheart is induced, many researchers use 10–15 °C to induce blackheart (Smith, 1983; Paull & Rohrbach, 1985). PPO activity increased significantly after 3 weeks at 13 °C, followed by 1 week at 25 °C, with activity being 4 times higher than those held for 4 weeks at 25 °C (Fig. 1). The PPO activity was assayed in crude homogenates of the full pulp tissue of whole fruit, thus representing the total PPO activity of the sample. As SDS was used in the PPO assay, the total PPO activity included soluble and membrane-bound forms (Mayer, 1987).

On the other hand, POD activity was present in pineapple fruit during the whole postharvest period (Fig. 2). The activity remained constant during 3 weeks at 13 °C, and the following week at 25 °C.

3.2. Spatial relationship between PPO activity and blackheart development

PPO activity varied between different parts of the fruit at harvest, with significantly higher levels in the skin and crown leaf, but there was negligible PPO activity (<0.1 nmol $O_2 \cdot min^{-1} mg^{-1}$ protein) in any parts of the fruit pulp (Fig. 3). No increase in PPO activity was observed in any part of pulp tissue during 4 weeks of storage at 25 °C. However, significant increase in PPO activity was found in the pulp of fruit after 3 weeks at 13 °C followed by 1 week at 25 °C (Table 1). The bottom pulp and the pulp near the core were found to have the highest increase compared to other sections of the fruit, with about 8 times and 20 times higher PPO, respectively, than the same tissues stored continuously at 25 °C (Table 1).

PPO activity at the skin and the crown decreased during the first 2 weeks of storage, then increased after 3 weeks and 4 weeks of storage. Fruit stored continuously at 13 °C and 25 °C showed the same trend. There was no difference in the skin PPO activity between chilled and un-chilled fruit (Table 1). After 3 weeks of storage, some fruit skin and crown leaves were brown and with rot, so the increase of PPO activity in the skin and crown leaves at this stage might be pathogen-related.

3.3. Effect of fruit maturity to blackheart development and PPO activity

No blackheart was present in fruit of any maturity at harvest. After low temperature storage, negligible blackheart was found in immature fruit or over-mature fruit, but mature fruit developed intense blackheart injury (Fig. 4 A). Low temperature at 10 °C was used in this experiment to avoid the rotting of fruit.

There was no significant difference between PPO activities of fruit at different maturity at harvest (Fig. 4 B). PPO activity was low and unaffected by storage in immature and over-mature fruit. However, after 3 weeks at 10 °C plus 1 week at 25 °C, mature fruit, where blackheart symptoms were observed, showed a significant increase in PPO activity (Fig. 4B). Furthermore, a chi square test showed that a low value of PPO activity was significantly correlated to low blackheart symptoms ($P<0.01$).

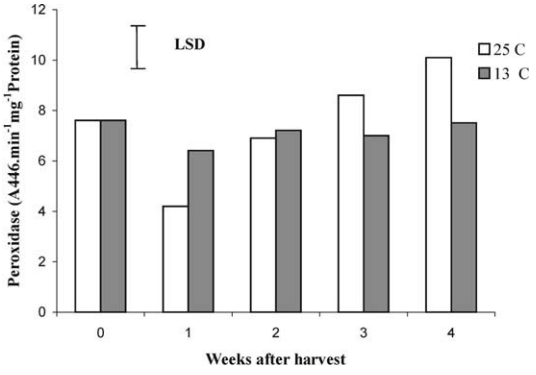

Fig. 2. Change of POD activity in pineapple fruit during storage at 13 and 25 °C. POD activity values are the means representing the average of triplicate experiments. LSD = 1.65 ($P<0.05$).

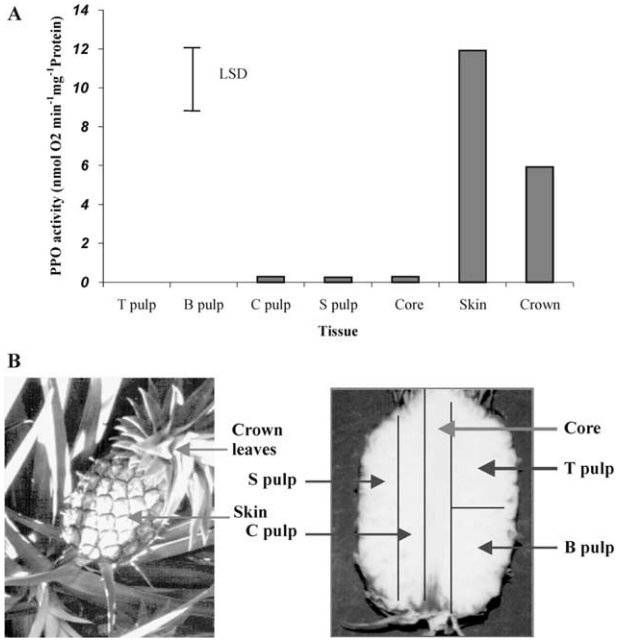

Fig. 3. Comparison of PPO activity in different parts of pineapple fruit at harvest. A. Distribution of PPO activity in the fruit at harvest. T pulp, top part of the fruit pulp tissue; B pulp, bottom part of the fruit pulp tissue; C pulp, pulp tissue near the core; S pulp, pulp tissue near the skin. LSD = 3.46 ($P<0.05$). B. Spatial location of pineapple fruit tissue.

Table 1
Effect of low temperature on the spatial PPO activity in pineapple fruit (as nmole O_2 mix^{-1} mg^{-1} protein)

Time after harvest (w)	Treatment	Blackheart (0–6)	T pulp	B pulp	C pulp	S pulp	Core	Skin	Crown
1	Non-chilled[a]	0	0.862	0.477	0.528	1.03	1.65	8.145	5.49
	Chilled[b]	0	0	0.697	0	0.524	0.7385	4.769	2.501
2	Non-chilled	0	0.817	0.807	0.780	0.983	0.666	3.59	0.632
	Chilled	0	0	1.42	0	1.127	0	0	0
3	Non-chilled	0	1.59	1.32	0	2.484	1.302	7.89	5.449
	Chilled	1.1	0	0.925	0.495	0	0	6.879	1.0168
4	Non-chilled	0	0	2.91	1.25	2.35	0	8.21	NA[c]
	Chilled	3.15	6.41*	25.5*	33.1*	7.96*	5.20*	8.21	7.42

PPO activity values are the means representing the average of triplicate experiments. B pulp, bottom part of the fruit pulp tissue; C pulp, pulp tissue near the core; S pulp, pulp tissue near the skin.

[a] Fruit continuously stored at 25 °C.

[b] Fruit stored at 13 °C for 3 weeks, followed by another week of storage at 25 °C.

[c] Data not available.

* Significantly different ($P < 0.05$). LSD = 3.46.

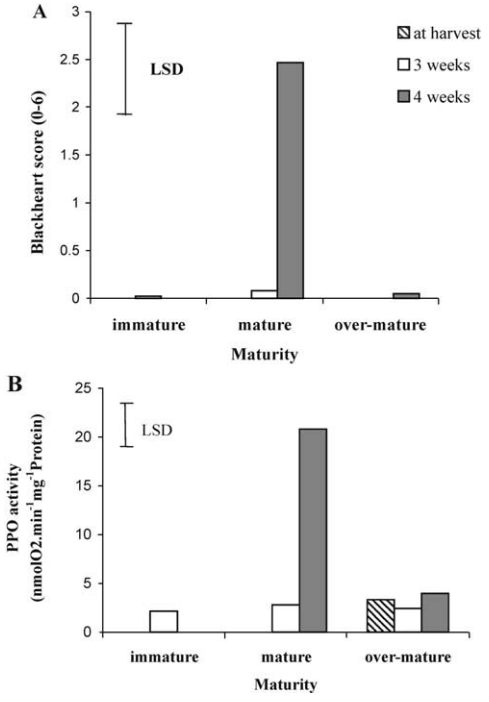

Fig. 4. Effect of maturity on PPO activity related to blackheart development after low temperature storage. A. Effect of maturity on blackheart incidence after low temperature storage (10 °C). LSD = 1.17 ($P < 0.05$). B. Effect of maturity on PPO activity after low temperature storage. The values of PPO activity are the means representing the average of five replicates. LSD = 4.92 ($P < 0.05$).

3.4. Changes of phenylalanine ammonia-lyase activity during blackheart development

While PAL activity did not increase during storage at 25 °C, a significant increase was observed under low temperature storage at 13 °C (Fig. 5). Significant increase of PAL activity started after 2 weeks at 13 °C

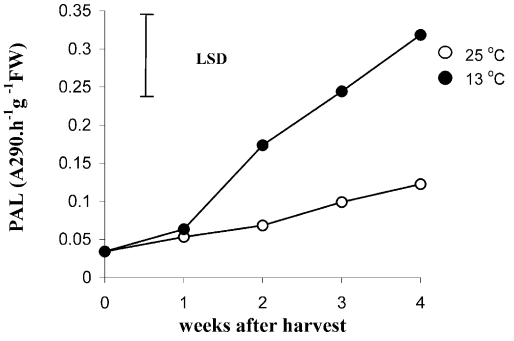

Fig. 5. Effect of low temperature storage on phenylalanine ammonia-lyase activity in pineapple fruit. PAL activity values are the means representing the average of triplicate experiments. LSD = 0.12 ($P < 0.05$).

and continued when the fruit was removed from chilling to 25 °C. PAL activity was also increased under chilling at 6 °C and 18 °C (see Fig. 7 B).

3.5. Changes of catalase and ascorbate peroxidase activity during blackheart development

The activity of AsPOD and CAT increased during continuous storage at 25 °C (Fig. 6). However, the activities of these enzymes showed no significant changes after chilling at 13 °C for 3 weeks, followed by 1 week at 25 °C. When compared with fruit stored continuously at 25 °C, a significantly lower level of AsPOD activity was observed at the 3rd week of chilling. Chilling at 6 and 18 °C also significantly inhibited the increase of AsPOD activity that was observed in fruit continuously stored at 25 °C (Fig. 7B). Lower CAT activity was observed after 3 weeks at 6 °C, but there was no significant difference between chilling at 18 °C or 13 °C and continuous storage at 25 °C (Fig. 7C).

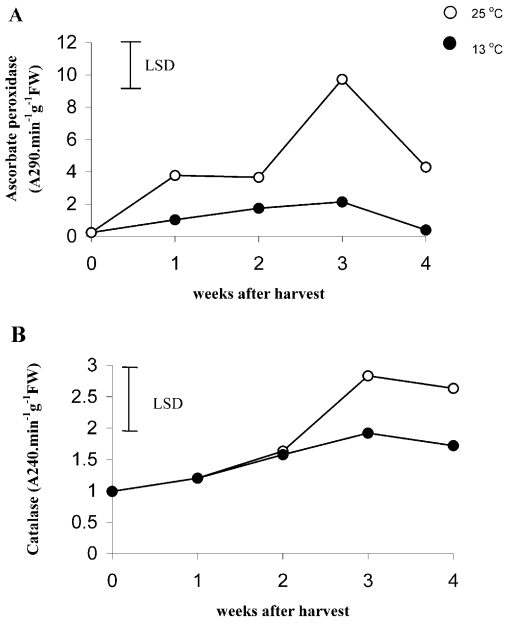

Fig. 6. Effect of low temperature storage on ascorbate peroxidase and catalase activity in pineapple fruit. A. Change of ascorbate peroxidase activity during storage at 13 and 25 °C. LSD = 2.90 ($P < 0.05$). B. Change of catalase activity during storage at 13 and 25 °C. LSD = 1.084 ($P < 0.05$). The activity values are the means representing the average of triplicate experiments.

4. Discussion

Blackheart in pineapple fruit is induced by low temperature and commonly observed following postharvest storage and transportation. Most blackheart symptoms were found to develop during another week of storage at 25 °C, following 3 weeks of storage at 13–0 °C, which is consistent with previous reports (Paull & Rorhbach, 1985; Smith, 1983; Teisson, 1972; Teisson, Combres, Martin, & Marchal, 1979). PPO is a key enzyme for enzymatic browning in many fruits (Mayer, 1987). Our results indicated that PPO is directly related to the development of blackheart symptoms, both temporally and spatially. PPO activity was very low in harvested fruit and remained low during postharvest storage at 25 °C for 4 weeks. However, PPO activity dramatically increased in the fruit following low temperature storage. PPO is present in a latent form in many plant species, and is tightly bound to the chloroplast membrane (Mayer, 1987). The latent form of PPO is often activated during ripening, senescence or stress condition when the membrane is damaged, which results in increase of PPO activity (Mayer, 1987). As detergents, such as SDS were found to be the activators of latent PPO, in the current experiment, SDS was added into all PPO assays. The results were consistent with the previous report that the increase of PPO activity, after low temperature treatment was not related to the conversion of membrane-bound PPO into soluble PPO, but involved a *de novo* PPO synthesis induced by low temperature (Stewart et al., 2001). The variable distribution

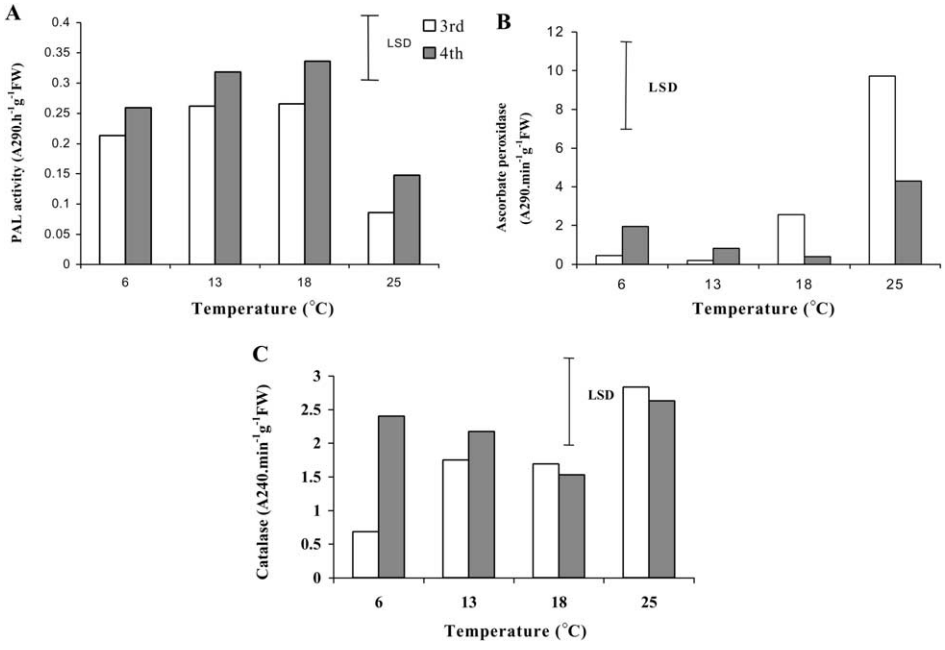

Fig. 7. Comparison of phenylalanine ammonia-lyase, ascorbate peroxidase and catalase activity in pineapple fruit after storage at 6, 13, 18 or 25 °C. A. PAL activity, LSD = 0.12 ($P < 0.05$). B. Ascorbate peroxidase activity, LSD = 4.862 ($P < 0.05$). C. Catalase activity, LSD = 1.385 ($P < 0.05$). All the activity values are the means representing the average of triplicate experiments.

of PPO activity within fruit has been observed in many other fruits, and has been correlated with the spatial occurrence of browning (Underhill & Critchley, 1995). Pineapple PPO activity was barely detectable in any pulp tissue at harvest or during postharvest ripening at 25 °C. In contrast, PPO activity in the skin and crown leaves was higher at harvest, with activity decreasing during the onset of senescence and was not significantly affected by chilling. The chilling-induced increase in PPO activity only occurred in the pulp tissue. Blackheart symptoms start as small brown translucent spots at the base of fruitlets near the core (Paull & Rorhbach, 1985; Smith, 1983), resulting in a more intense injury in the pulp near the core and the bottom part of the fruit. A comparison of the spatial distribution of PPO showed that these parts of the fruit had the highest PPO increases. This result indicated that the induction of PPO by chilling was tissue-specific, and consistent with the proposed role for PPO in blackheart development.

Our results showed that fruit maturity significantly affected blackheart incidence. Immature and overmature fruits were found to be less susceptible to blackheart injury. The results reflect the Queensland practice of harvesting immature fruit to reduce blackheart incidence. The mechanism of the maturity effect on blackheart susceptibility is unclear. Pineapple PPO activity was very low in fruit of all maturities at harvest, although maturity affected the later development of blackheart. It appears that factors, other than pre-existing PPO, are responsible for subsequent blackheart development during storage. PPO induction after chilling was found to be developmental-specific; an increased PPO found in mature fruit was highly correlated to only mature fruit displaying blackheart symptoms after chilling. The result supports the theory that *de novo* synthesis of PPO after chilling, rather than the pre-existing PPO, is important in blackheart development, but also suggests that other factors, including factors triggering PPO induction, are also involved. In fact, PPO activity did not increase until after the fruit was returned from chilling to 25 °C, indicating that PPO, despite being important for enzymatic browning in blackheart development, may act as a secondary factor induced by chilling.

Involvement of POD in enzymatic browning of fruit and vegetables has been widely reported (Nicolas, Richard, Goupy, Amiot, & Aubert, 1994; Williams, Lim, Chen, Pangborn, & Whitaker, 1985). PODs can oxidize phenols to quinones, then condense tannins to brown polymers in the presence of H_2O_2, which may then contribute to enzymatic browning (Robinson, 1991; Vaughn & Duke, 1984). Increased POD activity has been observed upon exposure to ozone, pollution, nutritional disorders, wounding and chilling injury (Campa, 1991). However, in contrast to PPO, our results showed POD activity was present in pineapple fruit throughout all the postharvest period; the activity showed no significant change after chilling. These results suggest that POD is not a key browning enzyme causing blackheart development in pineapple fruit after chilling.

PAL is a key enzyme for the biosynthesis of polyphenols (Ke et al. 1989), and is rapidly synthesized de novo with various stimuli (Hahlbrock et al., 1989). PAL activity increased in low temperature storage at 6, 13 and 18 °C; significant increase occurred during chilling and continued during storage at 25 °C. The availability of polyphenol compounds is the prerequisite for the PPO enzymatic browning. Increased polyphenol has been reported in blackheart affected fruit (van Lelyveld, 1977). The results suggest that chilling stimulates the biosynthesis of polyphenol compounds by enhancing PAL activity. Further characterization of the browning substrates involved would help to better understand the specific biosynthetic pathway associated with blackheart development.

A significant decrease in AsPOD activity was observed in pineapple fruit after 3 weeks at 6, 13 and 18 °C compared with that continuously stored at 25 °C. The significance of decreased AsPOD to blackheart development is not clear. A lower AsPOD activity was related to chilling injury in maize (Anderson, Prasad, Martin, & Stewart, 1994), mandarin (Sala, 1998), cucumber (Shen, Nada, & Tachibana, 1999b) and zucchini (Wang, 1996). Another antioxidant enzyme, CAT, showed no change in activity after chilling at 13 and 18 °C, indicating that the enzyme is not associated with blackheart development in pineapple fruit.

Taken together, the results indicate blackheart in pineapple fruit is a consequence of the disturbance of many metabolic pathways. The increase of PPO was directly related to the incidence of blackheart symptoms, but was most likely a secondary response to chilling. Chilling enhanced the activity of PAL, the key enzyme for phenol biosynthesis. Chilling also decreased the activity of AsPOD, an important antioxidant enzyme for H_2O_2 degradation, but further experimentation is required to identify the role of this enzyme in the blackheart development after chilling.

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