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Blackheart Disorder in Fresh Pineapple

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ABSTRACT

There are two different types of chilling-related physiological disorder in pineapple, common chilling injury (CI) and blackheart (BH). Unlike CI, the symptoms of BH are not discernible externally as it affects only the fruit flesh and core. Both disorders occur in two phases namely an induction phase at low temperatures with symptom expression at higher temperatures. While CI may be induced at temperatures below the optimal recommendation for storage i.e., 0-8°C, BH induction may take place at a wider temperature range of up to 21°C. BH can also occur at a preharvest stage and is associated with low field temperatures during growth. After harvest, BH occurs in fruit subjected to exposure to low temperature during storage, handling and transportation. Factors influencing BH development include temperature, length of exposure, varieties, maturity stage and growing climatic conditions. BH has been associated with reduction in ascorbic acid and an increase in polyphenol oxidase enzyme (PPO) activity. However, the initial ascorbic acid concentration and PPO activity do not indicate fruit susceptibility to BH. Partial control of BH was reported to be achieved by preharvest applications of chemicals such as parachlorophenoxyacetic acid (PCPA), α-naphthaleneacetic acid (ANA), potassium and calcium. Several postharvest methods have also been reported to provide some control of BH, including heat treatment, controlled and modified atmosphere, surface coatings and 1-methylcyclopropene (1-MCP) treatment. Plant breeding to develop BH-resistant cultivars provides an attractive alternative to postharvest treatments to control the disorder. New hybrids resistant to BH have been produced from conventional breeding while a genetic engineering approach has also shown promising development.

Keywords: biochemical changes, chilling injury, postharvest handling, storage
Abbreviations: BH, blackheart; **CI**, chilling injury; **PPO**, polyphenol oxidase; **POD**, peroxidase

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INTRODUCTION

Blackheart (BH), a temperature-related physiological disorder of pineapple (*Ananas comosus* L. Merr.) is a worldwide problem. The disorder is also known as “endogenous brown spots” and “internal browning”. The characteristic symptom of the disorder is the development of dark spots in the flesh area close to the core. In its early stage of development, the spots appear watery but they then enlarge and turn brown as the severity of the disorder increases. In severe cases the entire flesh and core tissue of a fruit may be visibly affected (**Fig. 1**). Fruits affected by BH appear normal externally and its presence is only known after the fruit has been cut open for processing or consumption (Akamine *et al.* 1975; Akamine 1976). The lack of detectable external symptoms creates substantial problems in the marketing of fresh fruit.

BH may occur before harvest in the field or after harvest following exposure to low temperature. The occurrence of BH in the field has been described by several workers and is usually associated with low field temperature. In Queensland, Australia, the BH problem is more common in winter than in summer months (Leverington 1968, 1971) and a higher incidence of BH was reported in Hawaii and Taiwan during the cooler months of the year (Akamine *et al.* 1975; Akamine 1976). In Malaysia, a tropical country where the normal field temperature is generally high throughout the year, BH can develop in pineapples subject to storage under refrigeration and was first reported in 1983 on pineapple cv. ‘Mauritius’ (Abdullah and Rohaya 1983). The

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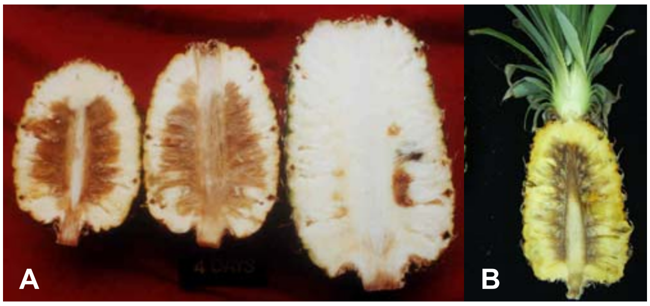

Fig. 1 Blackheart in (A) 'Babagon' and (B) 'Mauritius' pineapples after storage for 3 weeks at 8°C followed by holding for 1 week at 25°C.

occurrence of BH has also been reported on pineapples grown in other countries including Thailand (Haruenkit and Thompson 1993, 1996), Sri Lanka (Wijeratnam *et al.* 1996), Philippines (Akamine *et al.* 1975), South Africa (Van Lelyveld and de Bruyn 1976, 1977), Taiwan (Chang and Wu 1961) and Ivory Coast (Teisson 1979). The problem of BH is so critical in the global pineapple industry that its occurrence can lead to huge losses at the cannery or after shipment in refrigerated marine containers. The losses to the Australian pineapple industry alone due to BH amount up to US$1.3 million annually out of a total production value about US$30 million (Ko *et al.* 2006).

LOW TEMPERATURE AND PHYSIOLOGICAL DISORDERS

Physiological disorder refers to breakdown of tissue in response to an adverse environmental condition especially temperature, or nutritional deficiency during growth and development, which causes abnormal metabolism (Wills *et al.* 1981). BH in pineapple has been long considered a physiological disorder and it is different from black rot, a disease caused by *Thielaviopsis paradoxa* (Lim 1972, 1985) or any other diseases caused by fungi or bacteria. There are two different types of chilling-related physiological disorder in pineapple. Firstly, the common chilling injury (CI) for which symptoms are obvious externally and secondly, BH where the symptoms are not discernible externally. Both disorders occur in two phases namely an induction phase and symptoms expression phase. During the induction phase, there is a minimum exposure time at low temperature required before either disorder can take place although symptoms expression may occur at temperatures higher than the induction temperature (Teisson *et al.* 1979a).

CI in pineapple can be characterized by failure of the green shell to turn yellow, yellow-shelled fruit turning a brown or dull colour, wilting, drying and discolouration of crown leaves and a breakdown of internal tissue, giving a pale watery appearance (Dull 1971; Abdullah *et al.* 2008). In severe cases the fruit might be infected by severe fungal diseases (Dull 1971). Fruit are affected by CI when they are exposed to temperatures below the optimum level for storage for sufficient time to cause injury where the lower the temperature where the fruit is exposed to, the more severe is the disorder. The optimum storage temperature for pineapple has been recommended as between 7-13°C (Hardenburgh *et al.* 1986), whereas Paull (1997) recommended temperatures in the range of 7.5-12.5°C. For Malaysian pineapples, Abd Shukor *et al.* (1998) recommended temperatures of 8-10°C for optimal storage. Visual symptoms of CI develop faster when the fruits are transferred to higher temperatures following exposure to low temperature. Paull and Rohrbach (1985) reported faster development of CI symptoms in pineapple at 20-25°C while exposure to 25°C has been used as a normal procedure to evaluate changes in fruit quality after low temperature storage (Abdullah *et al.* 1996, 2002, 2008). Different parts of the fruit might have different levels of sensitivity towards CI. Abdullah *et al.* (2002) reported physiological responses of the fruit and

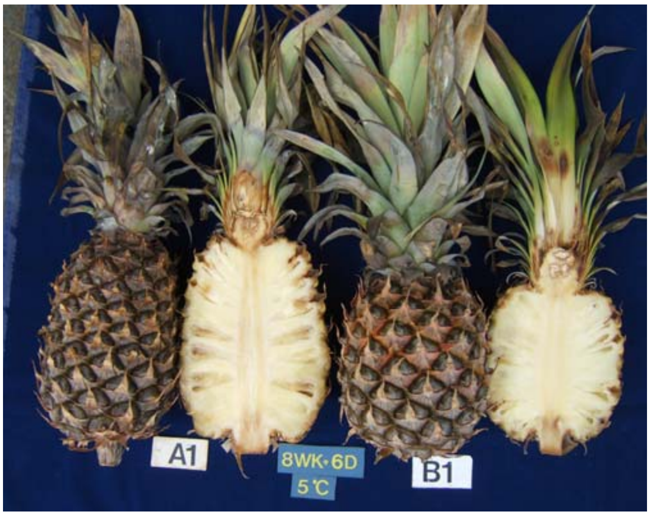

Fig. 2 Chilling injury in 'N36' pineapple, a blackheart resistant cultivar after being subjected to storage for 8 weeks at 5°C followed by holding for 6 days at 25°C.

crown of 'N36' pineapple subjected to storage at low temperature. **Fig 2** illustrates CI in 'N36' pineapples after being subjected to storage at 5°C for 8 weeks followed by holding for 6 days at 25°C.

Unlike CI, the BH disorder affects pineapple fruit without showing detectable external symptoms since the affected area is only the inner flesh and core of the fruit (Akamine *et al.* 1975; Akamine 1976). Microscopic examination by Kruger *et al.* (2000) revealed that the browning of BH affected tissues is associated with the vascular structure of the fruit. Vascular bundles enter the fruit ventrally from the pedicel and exit dorsally to the crown. In the fruit, the bundles are concentrated on the outside of the central cylinder from which they branch-off laterally to service the individual fruitlets. Only a few bundles are found in the middle of the cylinder. The browning is almost exclusively associated with the outer region of the central cylinder where there is a high concentration of vascular bundles.

BH has been reported in fruits stored at temperatures as low as 4°C and as high as 21°C (Teisson *et al*. 1979a; Wills *et al*. 1985; Smith 1987). Thus, in addition to the chilling at temperatures below the optimum, BH induction may also take place at temperatures higher than the normal range that causes CI. According to Teisson *et al.* (1979b), BH is a typical anomaly induced by cold, but involving other temperature sensitive factors, one sensitising the fruit and another allowing a manifestation of symptoms. They suggested 20°C as the threshold temperature for Ivory Coast pineapple where it is considered to be sufficiently low to cause injury and also sufficiently high for symptom expression to occur. Paull and Rohrbach (1985) divided the development of BH into two phases. The first phase occurred during storage at chilling temperatures (<12°C) and had no obvious symptoms. The second phase was after fruit were removed from chilling temperatures to temperatures in the range of 18 to 30°C, where symptoms developed. Zhou *et al*. (2003a) suggested that the development of BH symptoms in pineapple fruit results from the disturbance of a number of metabolic processes that occur at sub-ambient temperatures.

BLACKHEART BEFORE HARVEST

The occurrence of BH before harvest is usually related to low field temperatures during winter months in sub-tropical region, such as in Australia, South Africa and Taiwan. In Australia, the problem of BH is more serious in winter than summer months (Leverington 1968, 1971). It was found that the sharp peak in BH losses occurred a fortnight after three cold and cloudy days (Groszmann 1971, 1972). The worst affected fruits were those which had just commenced to colour when the cold, cloudy snap occurred. In addition, the fruits that had reached more than a quarter skin colour were more susceptible. Leverington (1968, 1971) also asserted that BH results from physiological disturbances brought about by unfavourable weather changes particularly sudden drops in temperature during the final stages of maturation. Kruger *et al.* (2000) reported the occurrence of BH as a cold damage in the field in South African 'Cayenne' pineapple where the damage did not seem to worsen during subsequent postharvest storage. Chang and Wu (1961) showed that when temperatures fall to 12-13°C or less for several days, harvested fruits would show signs of injury.

The susceptibility of fruits to BH is closely related to weather conditions especially the maximum temperature that the fruit experienced before harvest. With Ivory Coast pineapples, the disorder is favoured by a high diurnal temperature variation, a higher water deficit, and nocturnal temperature around 20°C (Teisson *et al.* 1978, 1979a, 1979b). The workers in Queensland, Australia believed that field conditions especially the lack of sunshine is the major factor in inducing the BH (Groszmann 1971, 1972). During winter months the northern part of Queensland receives more light than the southern part, and at this time of the year, the incidence of BH is higher in the area with less sunshine. The effects of environmental temperature, duration of temperature exposure and shading on the incidence and severity of BH development in growing pineapples were studied by Smith and Glennie (1987) using potted fruit-bearing plants in naturally lit controlled environment glasshouses during the final 4-6 weeks of maturation. Smith and Glennie (1987) observed that a day/night temperature of 25/15°C resulted in much less injury than did the average constant temperature of the same regime (20°C). Shaded fruit had more injury than fruit not shaded. Physiological breakdown of pineapples was reported earlier by Miller (1951) in Florida where the disorder was in fruits that were induced during winter months by forcing the bloom with acetylene gas during "off season". The stage of maturity determined the susceptibility of pineapple to the disorder where the greener fruits were more subject to the disorder. The importance of the stage of maturity in influencing the development of BH was also described by Van Lelyveld and de Bruyn (1976) who believed that BH would affect only the mature and ripe fruits while they were still on the plant. Groszmann (1971, 1972) recommended pineapples to be harvested earlier in order to avoid or reduce losses due to BH.

BLACKHEART AFTER HARVEST

BH development after harvest may take place by two methods. The first involves fruits that have been subjected to exposure to low temperature before harvest in the field and the BH symptoms are expressed postharvest after being exposed to higher ambient temperature. In the second method, both induction and symptoms expression stages take place after harvest where the fruits are initially subjected to exposure to low temperature during storage or handling and the symptoms may develop either during storage or after removal to higher ambient temperature.

Most pineapple varieties grown commercially including 'Smooth Cayenne' are highly susceptible to BH (Abd Shukor *et al.* 1998). In Malaysia, BH occurrence was reported on several pineapple varieties including 'Mauritius', 'Sarawak', 'Gandol', 'Babagon' and 'Maspine'. Pineapple cv. 'Mauritius' previously stored at 8°C and 12°C for up to 4 weeks followed by a week holding period at ambient (28°C) were affected by the disorder (Abdullah and Rohaya 1983). In fruits stored at 5°C, BH could only develop at ambient if the storage period was 2 weeks or less. BH was not observed in fruits stored for 3 weeks and longer at 5°C which indicated that prolonged storage at very low temperature can suppress induction and development of BH. Abdullah *et al.* (1986) observed that BH intensity in pineapple cv. 'Sarawak' subjected to storage at 5°C for 3 weeks was significantly lower than those stored at 10 and 15°C. 'Sarawak' pineapple stored for 1, 2 and 3 weeks at 10°C develop symptoms of BH after being exposed for 3 days at ambient whereas the one stored for 4 weeks at the same low temperature had already developed symptoms of BH when they were removed from the cold room. The reduction or absence in symptom development in fruits after prolonged storage can probably ascribed to damage to the metabolic pathway leading to browning (Paull and Rohrbach 1985).

Paull and Rohrbach (1985) reported browning symptoms in fresh 'Smooth Cayenne' pineapple began to appear within 2 days at 22°C after a period of storage at temperatures less than 12°C. Fruit not subjected to chilling temperatures during storage also developed similar symptoms when held for 0 and 10 days at between 18 and 30°C. Fruit stored for longer than 3 weeks at temperatures of 8 and 3°C showed fewer browning symptoms than similar fruit held at 12°C. Investigations carried out by Smith (1983) in Queensland, Australia showed that BH manifested itself in summer fruit following storage for 3 days at temperatures up to 21˚C, but the disorder was not initiated after 15 days at 25°C. He concluded that BH is a disorder produced by chilling at temperatures equal to or less than 21°C. According to Kruger *et al.* (2000), BH in 'Queen Victoria' pineapples produced in Kwazulu Natal, South Africa rarely developed in the field but can develop during storage.

Maturity stage at harvest may have some influence on BH development after harvest. Abdullah and Rohaya (1997) reported the effect of maturity stage on quality of stored pineapple cv. 'Mauritius'. In the study, pineapples were harvested at maturity stages of 120, 125 and 130 days from flower induction. BH development at ambient following low temperature storage was delayed in less mature fruits. However, the BH levels were the same for all maturity stages after holding for 6 days at ambient. Dahler *et al.* (2002) investigated the influence of fruit maturity on BH development by storing 'Smooth Cayenne' fruits of six maturity ranges for 3 weeks at 10°C, or for 3 weeks at 10°C followed by 7 days at 25°C. Immature fruits developed negligible BH, while fruit of mid-range maturity were susceptible. Over mature fruit developed a translucent breakdown of the pulp rather than BH.

SOME BIOCHEMICAL CHANGES ASSOCIATED WITH BLACKHEART

Ascorbic acid

The relationship of ascorbic acid content and BH development in pineapple has been reported by many workers including Miller (1951), Miller and Heilman (1952), Miller and Marsteller (1953), Abdullah and Rohaya (1983), Abdullah *et al.* (1986), Teisson *et al.* (1979), Van Lelyveld and De Bruyn (1977) and Dahler *et al.* (2002). The studies showed that pineapple fruits or fruitlets affected by BH are lower in ascorbic acid than the unaffected ones. The concentration of ascorbic acid is the highest in the top portion and the lowest in the bottom portion of pineapples which accords with the observations that symptoms of the disorder are usually more pronounced in the lower half of the fruit. It is possible that ascorbic acid acts as an anti-oxidant, which prevents the browning process occurring (Miller and Heilman 1952).

Singleton and Gortner (1965) found that the ascorbic acid content in pineapple seemed to fluctuate markedly and consistently according to the weather variation before harvest. There was a two week lag before the effects of the weather factors showed in the ascorbic acid content of the fruit. The two week lag period augurs well to the observation made by Groszmann (1971, 1972) who reported a sharp increase in BH losses which occurred two weeks after three cold and cloudy days in Australian pineapples.

Abdullah and Rohaya (1997) reported higher concentrations of ascorbic acid in less mature 'Mauritius' pineapple harvested between 120 to 130 days after flower induction.

Ascorbic acid content for the 120-day fruit stored for 3 weeks at 8°C was almost double the concentration in the 130-day fruit. Fruit of lesser maturity showed symptoms of BH a few days later than more mature fruit but reached the same scores after prolonged holding at ambient for 6 days. It was observed that ascorbic acid content in fruits of lesser maturity dropped sharply within 2 days to about the same level as the more mature ones while holding at ambient temperature. Higher initial concentration of ascorbic acid in lesser mature fruit could be one of the reasons for the delay in the expression of BH symptoms. A contrasting result was reported by Dahler *et al.* (2002) who did not find significant difference in ascorbic levels at harvest between fruit of different maturity. Dahler *et al.* (2002) also reported that ascorbic acid levels at harvest do not indicate fruit susceptibility to BH. Pineapple varieties higher in ascorbic acid may not necessarily to be more resistant towards BH since the 'N36' and 'Josapine' cultivars which are both BH resistant but have lower ascorbic acid contents than other varieties known to be BH susceptible (Abdullah *et al.* 1996).

Phenolic compounds and polyphenol oxidase (PPO)

BH in pineapple is associated with phenolic substances and polyphenol oxidase (PPO) (Van Lelyveld and De Bruyn 1977; Teisson *et al.* 1979a; Selvarajah *et al.* 2000; Stewart *et al*. 2002; Zhou *et al.* 2003). Van Lelyveld and De Bruyn (1977) identified the phenolic compounds in pineapple fruits as *p*-coumaric, caffeic, ferulic and sinapic acids. BH fruitlets showed an increased concentration of each phenol except sinapic acid, compared with healthy fruitlets. Exposure of the fruits to low temperature is responsible for cell disturbance, resulting in the accumulation of phenolic substances. However, PPO has also been implicated in black spots infected fruitlets of pineapple caused by *Penicillium funiculosum* and *Fusarium monoliforme* (Avallone *et al.* 2003).

PPO is a copper-containing enzyme which catalyses the conversion of *o*-dihydroxyphenols to *o*-quinones in higher plants. The *o*-quinones produced by this reaction can undergo polymerization and bind covalently to nucleophilic amino acids to form black or brown pigments (Mayer and Harel 1979; Walker and Ferrar 1998). PPO activity varies between different parts of the fruit at harvest, with significantly higher levels in the skin and crown leaves but negligible in any parts of the fruit pulp (Zhou *et al.* 2003a). Undetectable or very low PPO activity in BH unaffected pineapple was reported by Pauziah *et al.* (2005) on three pineapple cultivars. Stewart *et al.* (2002) reported similar PPO activity in 'Hawaiian Gold' (53-116) pineapple, a BH resistant cultivar and 'Smooth Cayenne', a BH susceptible cultivar. An increase in PPO activity is related to the development of BH symptoms in the flesh following exposure to low temperatures. In an earlier study, Stewart *et al.* (2001) found PPO activity was low in the leaves, roots, inflorescence tissues and developing and mature pineapple fruits. In fruit affected by the chill-induced BH, PPO activity was 10-fold higher than in unaffected fruit and there was a direct correlation between PPO activity and severity of BH symptoms. It was suggested that PPO is synthesised *de novo* in response to chilling of pineapple fruit and implicated a role for PPO in the development of BH.

Other compounds

Although PPO has been strongly associated with BH in pineapples, other enzymes have also been studied in relation to the development of the disorder. The association of peroxidase (POD) have been reported by several workers including Pauziah *et al.* (2005), Selvarajah *et al.* (2000), Zhou *et al.* (2003), Botrel and de Carvalho (1995) and van Lelyveld *et al.* (1991). POD has been implicated in discolouration of numerous fruits and vegetables, however, its association with BH is still questioned as both lower and higher POD activities have been reported. Being a key enzyme in phenolic biosynthesis, phenylalanine ammonia-lyase (PAL) has also been considered to be associated with browning (Ke and Saltveit 1989). Zhou *et al.* (2003a) observed that chilling stimulated the biosynthesis of polyphenol compounds by enhancing PAL activity in pineapple. Selvarajah *et al.* (2000) reported that PAL activity was not directly related to BH development. However, PPO, POD and laccase activities have also been determined in extracts from black rot-infected fruitlets of pineapples (Avallone *et al.* 2003).

CONTROL METHODS

Preharvest chemical treatments

Most of the chemical treatments to control BH in pineapples were applied during the growth stage of the fruits. Miller and Marsteller (1953) reported the effect of parachlorophenoxyacetic acid (PCPA) to control BH in pineapples grown in Florida. BH was found in varying degrees in all of the untreated controls but none in the treated fruits. Spraying the plants with PCPA 10 days before harvest caused a reduction in both amount and severity of BH. Groszmann (1971, 1972) reported that PCPA sprays had no effect on the severity of BH in Australian pineapple, but another spray α-naphthaleneacetic acid (ANA), apparently reduced the disorder. However, it was later discovered that the ANA treated fruits were comparatively backward in flesh maturity and in the fruits which developed BH, the disorder became more severe with time as the fruits advanced in ripeness.

Teisson *et al*. (1979b) reported the effect of preharvest application of potassium and calcium. Potassium was applied in the form of K_2O, K_2SO_4 and KCl whereas calcium was applied by dusting, soluble and assimilable form of acetate and lime after floral induction. The use of K_2O had only a very slight effect on browning but the controlling effect was enhanced when calcium was applied as well. The presence of boron which had been shown to increase browning destroyed the beneficial effect of K_2O and calcium. The use of K_2SO_4 and KCl especially before flora induction reduced browning greatly. The effect of calcium alone, and zinc in the form of sulphate, were also favourable. Teisson *et al.* (1979b) also studied the effect of iron, copper, manganese and boron but were unable to show any effects.

Soares *et al.* (2005) reported on reduction of BH of pineapple by preharvest soil application of potassium applied as a soil dressing at 4-20 g of K_2O per plant in three split applications at 8, 24 and 40 weeks after planting. Fruit were harvested 19 months after planting at two maturity stages, classified as colour break (CB) and half ripe (HR). BH was more severe in HR than in CB fruit after 15 days storage at 7°C followed by 5 days at 25°C. Application of potassium reduced BH in fruit of both maturity where maximum response was achieved with 16 g of K_2O per plant. There are some indications that adequate K in the soil may also contribute to quality improvement including levels of ascorbic acid which may prevent some degree of enzymatic browning by inhibition of PPO activity (Tisseau 1972; Teisson *et al.* 1979b). Hewajulige *et al.* (2006) reported pre-harvest application of calcium in the forms of CaO and $CaCl_2$ as spray in combination with basal dressing was effective in reducing BH in waxed fruits of 'Mauritius' pineapple after 17 days storage at 10°C. Pre-harvest calcium application in controlling BH of 'Mauritius' pineapple was also reported by Herath *et al.* (2003).

Postharvest treatments

1. Heat treatment

Akamine *et al.* (1975) reported the effectiveness of heat treatment between 32.3 to 37.8°C for 24 hours to reduce the incidence of BH in "Smooth Cayenne" pineapples held under refrigeration at 7.2°C. In simulated shipping experiments, heat treatment was effective when applied in transit in shipping containers before, during or after refrigeration, the last being most effective. All heat treatments were effective in terms of reduced number of spots and their degree of darkening. Similar methods controlled BH in fresh Australian pineapples where the quality of fruits after storage was also improved (Akamine 1976). A higher temperature range of 38 to 42°C for heat treatment was used for 'Mauritius' pineapple (Abdullah *et al.* 1983). Heat treatment at 42°C after refrigerated storage was the most effective where incidence of BH and browning scores were reduced from 80% to 13.3% and 2.63 to 0.27, respectively. Teisson *et al.* (1979a) found that BH in Ivory Coast pineapples could be reduced by heating at 43°C for 24 hours after cool storage but heating at 37°C increased the symptoms. Mizuno *et al.* (1982) reported heat treatment at 42°C one day before storage reduced BH. However, heat treatment can cause excessive weight loss, resulting in wilted or dried crown leaves of the fruit although Akamine *et al*. (1975) suggested that it was more than offset by the beneficial effect of the reduction in BH incidence, and improvement in appearance and flavour of the pulp. It would seem that pineapples grown in different areas respond differently to heat treatment as they are subjected to different climatic conditions and cultural practices before harvest. Kruger *et al*. (2000) reported that effective heat treatments for 'Smooth Cayenne' were proven to be ineffective for "Queen Victoria" pineapples. Effective reduction of BH was nevertheless attained by incubating the fruits at relatively high temperatures (28-32°C) for relatively long periods (5-7 days) before storage at 10°C. The treatment, however, leads to hardening of the fruit, loss in taste and deterioration of external appearance. A much shorter heating time for 60 minutes at 38°C was reported by Weerahewa and Adikaram (2005) as being effective enough in reducing BH in 'Mauritius' pineapple.

2. Modified and controlled atmosphere

Abdullah *et al.* (1985) reported on the effect of modified atmosphere in controlling BH in 'Mauritius' pineapple. Fruits sealed in polyethylene bags and stored for 1, 2, 3 and 4 weeks at 10°C followed by 1 week at ambient temperature were significantly lower in BH intensity than controls fruit. The presence of BH in fruits sealed and stored for less than 2 weeks at 10°C was almost zero. Fruits in which the polyethylene bags were opened immediately after removal from low temperature were found to be affected by BH but the intensity was half that of controls fruit. Mizuno *et al.* (1982) reported slow skin and flesh colour development in polyethylene packed pineapple where BH development was also inhibited. Nimitkeatkai *et al.* (2006b) found reduction in BH of stored pineapple which were packaged in sealed PE bags flushed with different combination of O_2 and CO_2 concentrations. Limited control of BH by modified atmosphere was also reported by Selvarajah *et al.* (1997). Modified atmosphere of pineapple may have its limitation due to off-flavour and off-odour development as has been reported on 'Mauritius' pineapple (Abdullah *et al.* 1985).

Paull and Rohrbach (1985) reported that low O_2 (3%), with or without high CO_2 (5%) during chilling did not reduce BH symptom expression. However, storage of fruit under low O_2 following chilling significantly reduced CI symptoms expression. Haruenkit and Thompson (1993) did not detect the presence of internal browning when pineapples were stored at 8°C for 3 weeks under controlled atmosphere containing a mixture of 2% O_2 and 10% CO_2, or 1% O_2 and 0% CO_2. However, BH symptoms were detected when the fruits were transferred to 22°C. They concluded that storage under controlled atmosphere could only delay BH symptoms development when fruits were exposed to normal air following CA storage. In another study, Haruenkit and Thompson (1996) reported that the development of BH was less in fruits following storage under CA with O_2 levels at 1.3 to 2.2% than at levels of 5.4% and the control (in air), and elevated CO_2 level of 11.2% did not influence BH development. Low incidence of BH symptoms development in Sri Lankan pineapple stored under CA was also reported by Wijeratnam *et al.* (1995). Lack of oxygen probably has not allowed the browning process to occur under MA or CA storage but the browning reaction started once the stored fruits are exposed to normal air. The enzymes responsible for browning possibly have a requirement for oxygen for symptom development with less than 5% oxygen leading to no symptoms (Paull and Rohrbach 1985).

3. Other postharvest methods

Rohrbach and Paull (1982) reported that waxing fruit either before or immediately after exposure to chilling temperatures was equally effective in reducing BH. Waxing was also found to reduce BH development in 'Mauritius' pineapples following low temperature storage (Abdullah *et al.* 1983). A cost-effective wax formulation was developed in Sri Lanka and reported to be effective in controlling BH in 'Mauritius' and 'Kew' pineapples (Wijeratnam *et al.* 2006). Suhaila and Safiah (1993) reported on the effectiveness of surface coating with liquid paraffin in reducing BH in 'Mauritius' pineapples where the effectiveness of the treatment was further enhanced by shrink-wrapping. The effectiveness of surface coating is greatly influenced by the type of coating materials and the pineapple cultivars as reported by Zaulia *et al.* (2007) and Nimitkeatkai *et al.* (2006a). Effect of edible coating on stored pineapple was also reported by Selvarajah and Herath (1997). Waxing or surface coating restrict access of oxygen to fruit tissues and perhaps explaining their effectiveness in reducing browning in stored pineapple.

Selvarajah *et al.* (2001) reported that treatment with 1-methylcyclopropene (1-MCP) at 0.1 ppm for 18 hours at 20°C effectively controlled BH in pineapples stored at 10°C for four weeks. 1-MCP is an inhibitor of ethylene synthesis as it binds irreversibly to the ethylene-binding protein (Blankenship and Dole 2003). The treatment with 1-MCP also delayed ascorbic acid decline, and arrested the decline in both total soluble solids and ethylene synthesis.

Rapid marketing strategies designed to deliver the fruit to consumers before BH symptoms are evident have been suggested by Swete-Kelly and Bagshaw (1993) as another approach. It takes a few days after refrigerated storage before the pineapple develops BH symptoms at higher ambient temperature (Abdullah and Rohaya 1983; Abdullah *et al.* 1986). Harvesting fruit at the immature, green stage tends to limit the development of BH and diseases including marbling and leathery pocket (Lim 1985). However, this fruit is generally of poorer eating quality than those harvested at a more advanced maturity.

Breeding of BH-resistant varieties

The use of plant breeding to develop BH-resistant cultivars of pineapple provides an attractive alternative to postharvest treatments used to control the disorder (Stewart *et al.* 2002). Attempts to control BH at the pre- or post-harvest stage through selective breeding have been unsuccessful with 'Smooth Cayenne' due to sterility factors (Sanewski and Giles 1997). The Pineapple Research Institute of Hawaii has successfully developed two hybrid cultivars from 'Smooth Cayenne' that show significant levels of resistance to BH. The two intergroup hybrids, '73-50' and '53-116', show different levels of BH resistance when harvested over winter in south-eastern Queensland, Australia. '73-50' shows some field resistance to BH, while '53-116' appears to be completely resistant to the disorder both in the field and following postharvest refrigeration at 10°C for 14 days (Sanewski and Giles 1997). The Malaysian N36 pineapple is resistant to BH disorder (Abdullah *et al.* 1996) but its other postharvest quality characteristics such as flesh colour and sweetness are lacking. Another pineapple hybrid developed by the Malaysian Agricultural Research and Development Institute (MARDI) called 'Josapine' that was released in 1996, as a result of hybridization between 'Johor and 'Sarawak' parents is resistant to BH disorder (Chan 2008). Both 'N36' and 'Josapine' have been used as the common varieties exported by sea shipment from Malaysia (Abdullah *et al.* 2000).

Research on BH control is also being made through molecular breeding approaches in Australia (Graham *et al.* 2000; Zhou *et al.* 2002) and Malaysia (Abu Bakar *et al.* 2008) by PPO gene silencing. Genetic modification permits the insertion of specific genes into the genome of target hosts. Ko *et al.* (2006) reported on the introduction of transgenes to control BH in 'Smooth Cayenne' pineapple by microprojectile bombardment. Leaf callus cultures capable of high frequency organogenesis with a short regeneration time were used as explants for transformation via biolistics. Basal leaf explants were used for transformation via *Agrobacterium tumefaciens*. Their results showed that biolistic gene delivery in pineapple can be used to efficiently introduce transgenes and stable incorporation of the genes was achieved in mature plants. The PPO transformed plants are currently being grown in field trials and fruit are being assessed for BH resistance.

CONCLUSIONS

BH disorder continues to be a major problem in the pineapple industry throughout the world. Some research activities have been conducted to provide greater understanding of the disorder at production and postharvest to finally coming out with practical solutions. Some pre- and postharvest treatments have been reported to give limited control of the disorder and offer some relief to the pineapple industry. Selective breeding programmes in some countries have come out with new BH-resistant cultivars which have been commercialised. Pineapple quality improvement by genetic engineering can be another alternative to overcome the problem in the future, as the present development is quite encouraging.

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