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Pre- and post-harvest salicylic acid treatments alleviate internal browning and maintain quality of winter pineapple fruit

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ABSTRACT

The post-harvest internal browning (IB) and quality in pineapple (*Ananas comosus* L. 'Comte de Paris') fruit were studied in relation to pre-harvest salicylic acid (SA) spray (PSS) or/and post-harvest salicylic acid immersion (PSI) treatments at 10 °C for up to 20 days plus 2 days at 20 °C (shelf-life). In addition, the activities of polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) were measured during cold storage. The results showed that all SA treatments significantly reduced IB incidence and intensity. Furthermore, SA did not affect soluble solids content (SSC), titratable acidity (TA) and total phenolics (TP) content, but delayed the decline of ascorbic acid (AsA) content. At the same time, SA significantly inhibited PPO and PAL activities. The study indicated the beneficial effect of SA by pre-harvest spray and/or post-harvest immersion on pineapple fruit quality and resistance to IB, and PSS + PSI treatment showed the best effect.

1. Introduction

Internal browning (IB, also referred to as endogenous brown spot or blackheart) is the most important physiological disorder of pineapple that is induced by exposure to low temperature, either pre-harvest or post-harvest, and it develops quickly within 2–3 days when fruit are moved to physiological temperatures of 15–30 °C (Akamine et al., 1975; Paull and Rohrbach, 1985; Smith, 1983). In China, pineapple harvested in winter show higher levels of IB compared to those harvested in other months of the year, which severely restricts the pineapple industry because of its negative impact on the consumer's acceptance and on sensorial evaluation of the fruit. This also has been reported in Australia (Smith, 1983). Therefore, finding methods of extending shelf-life and maintaining adequate fruit quality, especially reducing the occurrence of IB, are most important for pineapple cultivation.

Recently, it has been observed that salicylic acid (SA) treatment could be used to reduce deterioration and chilling injury symptoms in some fruit (Sayyari et al., 2009; Wang et al., 2006). Both pre- and post-harvest SA treatments have been reported as being effective in fruit quality maintenance and storage life extension of strawberry (Babalare et al., 2007). Pineapple is a good source of ascorbic acid (AsA), thus is very important to avoid the loss of this vitamin, which commonly occurs during cold storage and marketing conditions.

Polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) have been reported to play important roles in the browning process of many fruits and vegetables (Ke and Saltveit, 1989). PAL is the first key enzyme involved in the biosynthesis of phenols, and PPO oxidizes these phenols to quinines, which simultaneously polymerize into brown pigment (Vamos-Vigyazo, 1981). PPO and PAL have been proposed to be related to IB development in pineapple (Teisson, 1972; Zhou et al., 2003).

Previous research mainly focused on the effect of summer pineapple fruit by treatment with some strategies, such as heat treatment, 1-methylcyclopropene, potassium treatment (Weerahewa and Adikaram, 2005; Selvarajah et al., 2001; Gomes Soares et al., 2005). Lu et al. (2010) reported that fruit IB index decreased and some anti-oxidant enzymes increased in summer pineapple fruit with post-harvest SA (5.0 mM) treatment. However, the mode of SA action in reducing IB and avoiding losses of nutritive compounds has not been clearly explained. Therefore, we presented its pre- and post-harvest treatments effects to study the alleviation of IB and quality with interval, as well as to determine changes in activities of pertinent enzymes of winter pineapple fruit during cold storage.

2. Materials and methods

2.1. Plant materials and treatments

Pineapple (*Ananas comosus* L. 'Comte de Paris') fruit were harvested from a commercial orchard in China. All experimental plants were grown according to regular practices. They were induced to

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Table 1
The treatments of pineapple fruit by SA pre- and post-harvest were conducted in this experiment.

Treatment	Pre-harvest spray		Post-harvest immersion	
	Water	SA (2.0 mM)	Water	SA (5.0 mM)
CK	+		+	
PSI	+			+
PSS		+	+	
PSS + PSI		+		+

Note: CK, control; PSI, post-harvest SA immersion; PSS, pre-harvest SA spray; PSS + PSI, the combination of pre-harvest SA spray and post-harvest immersion.

flowering by ethephon (Bayer Crop Science, Germany) was provided with a commercial dose of 0.25 kg ethephon per hectare dissolved in 1000 l water on 25 August, 2009. Mature green fruit were harvested on 13 January, 2010, then immediately transported to the laboratory for experiments.

Based on the results of our preliminary experiment, 2.0 mM SA solution sprayed or 5.0 mM immersed was the suitable concentration and induced the greatest responses. Therefore, SA at 2.0 or 5.0 mM containing 1% (v/v) ethanol and 0.01% (v/v) Tween 20 was dissolved for experimental use and pre- and post-harvest treatments were conducted as follows in Table 1. Then the SA solution (2.0 mM) was sprayed on pineapple fruit by using a hand-sprayer until fruit were wet to runoff. Additional fruit were also sprayed with distilled water as the control. The sprays were applied four times at 15-day intervals before harvest. At harvest time, each of the groups of fruit with either SA or water before harvest were divided into further two groups: some fruit were immersed into the solution of 5.0 mM SA for 15 min, and the others in distilled water, accordingly. All the fruit were air-dried for approx. 30 min, then stored at 10 °C and 90% relative humidity (RH) for 20 days. Each treatment contained three replicates of 75 fruit for this experiment. At 5-day intervals, 5 fruit from each replicate were sampled for enzyme assays, while another 10 fruit were removed from cold storage and kept for 2 days at 20 °C (shelf-life), and then evaluated for the occurrence of IB and quality.

2.2. Evaluation of IB incidence and intensity

Upon removal from storage, fruit were cut longitudinally in half and the incidence of IB was determined. IB intensity was scored from 0 to 5 according to Teisson (1979) after modification: 0 = no browning, 1 = 1–25% browning, 2 = 26–50% browning, 3 = 51–75% browning, 4 = 76–100% browning. The average IB intensity was calculated for 10 fruit per replicate from each treatment.

2.3. Measurements of SSC, TA, AsA and TP

Fruit pulp (100 g) was cut into small pieces and homogenized separately in a blender. After being filtered, the filtrate was used to analysis the soluble solids content (SSC), titratable acid (TA) and AsA contents according to methods previously reported (Lu et al., 2010). Total phenolics (TP) content was measured according to Pirie and Mullins (1976) using the Folin–Ciocalteu reagent. A standard curve of gallic acid was used for quantifying the TP content, and the results expressed as mg in 100 g of fresh weight (FW).

2.4. Extraction and assay of PPO and PAL activities

Five fruit were sampled per replicate after 0, 5, 10, 15 or 20 days of storage, respectively. Enzyme activities were then determined. Samples (150 g of flesh per fruit) were used immediately or frozen in liquid nitrogen and stored at −80 °C until use. All enzyme extraction procedures were conducted at 0–4 °C. For PPO activity, 3 g of pineapple fruit flesh was homogenized in 8 ml 50 mM sodium phosphate buffer, pH 7.8, containing 50 g l^{-1} polyvinylpolypyrrolidine (PVP). For PAL, 3 g of flesh was homogenized in 5 ml pre-cooled 50 mM sodium borate buffer, pH 8.8, containing 5 mM β-mercaptoethanol, 2 mM ethylene diaminetetraacetic acid (EDTA). The homogenate was then centrifuged at 12,000 × *g* for 25 min at 4 °C, and the supernatants were used for enzyme assays.

PPO activity was determined based on the method of Zauberman et al. (1988). Each 3 ml reaction mix contained 0.1 M catechol, 0.1 M sodium phosphate buffer, pH 6.8, and 100 μl enzyme extract. The increase in absorbance at 420 nm was monitored. One unit of PPO activity was defined as the amount of enzyme that caused a change in A_{420} of 0.01 Units min^{-1} g^{-1} FW.

PAL activity was measured according to the method of Cheng and Breen (1991). The assay mixture consisted of 0.2 M sodium borate buffer, pH 8.8, 2 mM L-phenylalanine, and 100 μl enzyme extract and was incubated for 1 h at 30 °C. The increase of 0.01 units in absorbance at 290 nm, due to the formation of *trans*-cinnamate, was measured. PAL activity was expressed as A_{290} Units h^{-1} g^{-1} FW.

2.5. Statistical analysis

All data were analyzed by analysis of variance (ANOVA) using SPSS v 11.5(SPSS Inc., Chicago, USA), significant difference ($P < 0.05$) among means was determined by Duncan's multiple range test.

3. Results

3.1. IB incidence and intensity

As shown in Fig. 1A, 11.11% of the control pineapple fruit showed IB after 5 days of storage at 10 °C plus 2 days at 20 °C, and then the symptoms became serious (Fig. 1A). All treatments with SA showed IB after 10 days plus shelf-life, but markedly reduced the incidence. IB intensity associated with IB symptoms incidence and development was increased. From day 10 to day 20 storage plus 2 days, IB intensity in PSS + PSI treated fruit and the control ranged from 0.1 to 1.0 and 2.4 to 3.6, accompany IB incidence ranging from 7% to 27% and 92% to 100%, respectively. Treatments with SA completely eliminated IB initially, and effectively delayed the development of discoloration with a more marked effect on the intensity of IB afterwards (Fig. 1B). After 20 days plus shelf-life, PSS + PSI treatment showed lowest incidence (27%) and symptoms (25% of surface area), so it had the best effect on IB control.

3.2. The effect on SSC, TA, AsA and TP content

The SSC of the pineapple fruit showed higher and was not significantly affected by PSS treatment at harvest time (Fig. 2A). After 5 days of storage plus shelf-life, the SSC increased in the control and PSS treated fruit, but decreased in PSI and PSS + PSI treated fruit. Afterwards, the SSC of all treatments increased and reached a peak at 10 days plus shelf-life, then decreased and indicated no significant difference.

At harvest time, PSS treatment increased the level of TA, but did not differ significantly as compared to the control (Fig. 2B). During cold storage plus shelf-life, the TA content of the pineapple fruit initially increased significantly and reached a peak value at 10 days, then underwent a downward trend, following a similar pattern in all treatments. Pre- or/and post-harvest SA treatments had higher TA values than the control. However, no significant differences were detected among these treatments.

A significant transient increase of AsA content in all treatments was found at 5 days plus shelf-life (Fig. 2C), while the AsA content