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Chilling injury in pineapple fruit: Fatty acid composition and antioxidant metabolism

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ABSTRACT

Chilling injury (CI) was investigated in fruit of pineapple (*Ananas comosus*) cv. Pattavia (Smooth Cayenne-type) and cv. Trad-See-Thong (Queen-type) stored at 10 °C for 21 days. We hypothesized that CI would be due to inadequate antioxidant response and/or membrane lipid composition. CI symptoms were only found in cv. Trad-See-Thong. Symptoms were correlated with an increase in ion leakage, with lower pulp total antioxidant capacity, determined by the FRAP method, and with lower ascorbate peroxidase (APX) activity. No correlation was found between CI symptoms and the concentrations of hydrogen peroxide or ascorbic acid. Additionally, no correlation was found between symptoms and the activities of superoxide dismutase (SOD) or catalase (CAT). Thiobarbituric acid-reactive substances (TBARS; indicative of fatty acid peroxidation), the membrane fatty acid composition, and the ratio of membrane saturated to unsaturated fatty acid also showed no correlation. It is concluded that the development of CI symptoms was correlated with reactive oxygen species metabolism, as reflected in total antioxidant capacity and APX activity, while no evidence was found for a correlation with membrane fatty acid composition or fatty acid oxidation.

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1. Introduction

Pineapple fruit show chilling injury (CI) symptoms during storage at low temperature. The fruit contains a central core surrounded by the pulp and peel. During storage at low temperature, water-soaked patches are initially found in fruit flesh close to the core, and these patches subsequently become brown. The final stage of internal browning (IB) comprises both the core and the fruit flesh. The symptoms develop faster after the fruit is transferred from cold storage to warmer temperatures (Akamine et al., 1975; Teisson, 1977; Abdullah, 1997).

During the development of CI symptoms ion leakage is often found to increase and concentrations of lipid peroxidation products, such as malondialdehyde (MDA) to increase, indicating that the membrane composition is compromised. Additionally, polyphenol oxidase (PPO) activity is often found to increase (Zhou et al., 2003a; Raimbault et al., 2011). Pineapple *PPO1* and *PPO2* genes are cold-inducible, indicating a role in the induction of chilling injury (Stewart et al., 2001; Zhou et al., 2003b). PPO is believed to be associated with symptom development rather than to be the cause of chilling injury (Zhou et al., 2003b).

Fruit from cultivars of the Queen-type were more susceptible to CI than those of the Smooth Cayenne-type (Hewajulige et al., 2003; Wilson Wijeratnam et al., 2006). The cause of this difference is not known. The causes of CI can be manifold, and according to two main current theories regarding the onset of chilling injury, it might be due to an effect on the lipid composition of membranes (Lyons, 1973; Lyons et al., 1979) or on the antioxidant system (Shewfelt and Rosario, 2000).

If the first theory (here called the fatty acid theory) were true, Smooth Cayenne-type pineapples would be expected to have a higher unsaturated fatty acid/saturated fatty acid ratio than fruit of Queen-type pineapple.

According to the latter theory, the symptoms are due to the detrimental action of reactive oxygen species (ROS). ROS attack cellular macromolecules such as DNA, proteins, lipids, and membranes. This leads to membrane degradation. ROS such as hydrogen peroxide peroxidize membrane lipids, resulting in degradation

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products such as malondialdehyde, which can be assessed using thiobarbituric acid. Chemicals detected by this method are called thiobarbituric acid-reactive substances (TBARS).

Intracellular antioxidant defense mechanisms can protect cells from ROS damage. According to this theory (the ROS theory) plant tissues with better antioxidant systems would be more resistant to low temperatures (Blokhina et al., 2003). If the ROS theory were true, Smooth Cayenne-type pineapples might have a better adapted antioxidant system than Queen-type fruit. Antioxidants include chemicals such as ascorbic acid, and the activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX).

We hypothesized that CI would be due to inadequate antioxidant response and/or membrane lipid composition. We therefore studied the relationship between chilling injury, electrolyte leakage, fatty acid composition, lipid peroxidation, hydrogen peroxide and ascorbic acid concentrations, total antioxidant capacity, as well as the activities of SOD, CAT, and APX, in fruit of two pineapple cultivars with different susceptibility to CI. The cultivars tested were cv. Pattavia (Smooth Cayenne-type) and cv. Trad-See-Thong (Queen-type).

2. Materials and methods

2.1. Plant materials

Cv. Pattavia (Smooth Cayenne-type) and cv. Trad-See-Thong (Queen-type) pineapple fruit [*Ananas comosus* (L.) Merr.] were harvested at the mature-green stage at a plantation in Trad province (Thailand). At harvest, all eyes on the fruit were still green but the pulp had turned pale yellow. Fruit were transported at 25 °C to the laboratory, where they arrived within 6 h of harvest. Cv. Trad-See-Thong and cv. Pattavia fruit that weighed about 1.0 and 1.5 kg, respectively, with no physical damage and no visible disease symptoms, were selected for the experiment.

2.2. Low temperature treatment; internal browning

The experiment was conducted in completely randomized design, whereby 60 fruit of each cultivar were packed into cardboard boxes and stored at 10 ± 2 °C, 85 ± 5% RH. Fruit were randomly sampled and evaluated 0, 7, 14, and 21 days after storage (3 replications; 5 fruits per replication). CI symptoms were assessed by cutting the fruit in half longitudinally, through the middle, 24 h after it had been transferred to room temperature. CI was estimating visually as the percentage of the cut area that exhibited water-soaking or browning symptoms.

Fresh pulp tissue within 2.5 cm around the core was used for the assessment of ion leakage, and the concentrations of TBARS and ascorbic acid. Other pulp tissue within 2.5 cm around the core was frozen with liquid nitrogen and kept at −70 °C for the estimation of fatty acid composition, hydrogen peroxide concentrations, antioxidant capacity (FRAP) and the activities of SOD, CAT, and APX.

2.3. Ion leakage

Ion leakage (EL) of the pulp around the core was determined using a method based on Hakim et al. (1999), 3.0 g of fresh pulp (2.5 cm around the core), cut into pieces of about 0.5 cm × 0.5 cm × 0.5 cm. The dices were washed with deionized water 3 times. After which 50 mL of a 0.4 M mannitol solution was added. The material was shaken at 100 rev/min on a rotary shaker for 1 h. The electrical conductivity was measured using a conductivity meter (Consort model C381, Turnhort, Belgium). The samples were placed in an autoclave at 121 °C for 30 min, left to cool to 25 °C, after which the electrical conductivity was measured again. Actual EL was expressed as a percentage of this maximum EL.

2.4. Hydrogen peroxide

H_2O_2 concentrations were determined according to Velikova et al. (2000). Three g of the frozen pulp (around the core) were homogenized in an ice bath with 5 mL of 0.1% (W/V) trichloroacetic acid (TCA). The homogenate was centrifuged at 14,000 × *g* for 20 min at 4 °C. One milliliter of the supernatant was added to 3.0 mL of 5 mM potassium phosphate buffer (pH 7.0) and 0.1 mL of 1 M potassium iodide. The absorbance of the supernatant was measured at 390 nm using a GENESYS 10 spectrophotometer, Madison, USA. The hydrogen peroxide concentration was expressed as mmol g^{-1} fw.

2.5. Ascorbic acid

Ascorbic acid concentrations were determined using the AOAC (1995) procedure. Two milliliter of juice squeezed from the pulp was mixed with 5 mL of 1% oxalic acid and were titrated with 1 mM 2,6-dichloroindophenol solution until a light rose pink persisted for 5 s. The amount of 2,6-dichloroindophenol solution used in the titration was determined and used in the calculation for ascorbic acid content.

2.6. Total antioxidant capacity (FRAP assay)

The method followed the one described by Benzie and Strain (1996). Frozen samples were prepared as described under 2.2. Five milliliter of 0.1% trichloroacetic acid (TCA) was added to 1.0 g of the frozen pulp and homogenized, then centrifuged at 12,000 × *g* at 4 °C for 20 min.

Scavenging capacity was assessed by mixing 1 mL of the supernatant with 1.5 mL of FRAP working solution (25 mL of 0.3 M acetate buffer pH 3.6 and 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid), and 2.5 mL of 20 mM ferric chloride solution ($FeCl_3$. 6 H_2O) dissolved in distilled water. The absorbance at 593 nm was recorded after 10 min using the GENESYS 10 spectrophotometer. A standard curve was prepared using 100–1000 μmol L^{-1} of $FeSO_4 \cdot 7H_2O$. The results were expressed in mmol Fe^{2+} L^{-1}.

2.7. Thiobarbituric acid-reactive substances (TBARS)

We followed the method of Health and Packer (1968). Five milliliter of 0.1% trichloroacetic acid (TCA) was added to 1.0 g of the fresh pulp and homogenized, then centrifuged at 12,000 × *g* at 4 °C for 20 min. One milliliter of the supernatant was mixed with 2.5 mL of 0.5% thiobarbituric in a 20% trichloroacetic acid, and boiled at 100 °C for 30 min. The reaction was stopped by placement in ice. The sample was centrifuged at 12,000 × *g* for 10 min. The absorbance of the supernatant was measured at 532 and 600 nm using the GENESYS 10 spectrophotometer. The TBARS were calculated as described by Health and Packer (1968).

2.8. Fatty acid composition

The method was based on AOAC (1995). Five g of the frozen pulp (around the core) was homogenized with chloroform/methanol (2/1) solution to a final volume of 30 mL. The chloroform layer was collected. The extraction was repeated twice, then the solution was filtered, and evaporated with EYELA rotary evaporator (Tokyo, Japan) at 40 °C until it was dry. It was weighed (total fat). Saponification occurred by adding 5 mL of 0.5 M KOH in methanol and 1 mL of 1 mg g^{-1} internal standard (tricosanoic acid, C23:0), refluxed at

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