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Hot water treatments delay cold-induced banana peel blackening

Surassawadee Promyou [a], Saichol Ketsa [a,*], Wouter G. van Doorn [b]

[a] *Faculty of Agriculture, Department of Horticulture, Kasetsart University, Bangkok 10900, Thailand*
[b] *Wageningen University and Research Centre, PO Box 17, AA 6700 Wageningen, The Netherlands*

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Abstract

Banana fruit of cv. Gros Michel (*Musa acuminata*, AAA Group, locally called cv. Hom Thong) and cv. Namwa (*Musa* × *paradisiaca*, ABB Group) were immersed for 5, 10 and 15 min in water at 42 °C, or in water at 25 °C (control), and were then stored at 4 °C. Hot water treatment for 15 min delayed peel blackening during cold storage by about 4 days in cv. Gros Michel and by 2 days in cv. Namwa. In both cultivars the delay of blackening was correlated with an increase in the ratio of unsaturated to saturated fatty acids. Hot water treatment in cv. Gros Michel but not cv. Namwa was correlated with lower lipoxygenase (LOX) activity and lower levels of thiobarbituric acid-reactive compounds. The results suggest that the rapid peel blackening of cv. Gros Michel is related to detectable membrane degradation, whereas the membrane-associated changes might be below the detection limit in the slower blackening cv. Namwa. The delay of peel blackening in cv. Gros Michel was associated with reduced expression of a catechol oxidase gene, which might partially explain the lower catechol oxidase activity after hot water treatment. The hot water treatment also increased the abundance of a *Hsp70* transcript. The changes in gene expression found in cv. Gros Michel were not observed in cv. Namwa. Taken together the delay of blackening by hot water treatment in cv. Namwa was only correlated with a change in the ratio of unsaturated to saturated fatty acids, whereas that in cv. Gros Michel was additionally correlated with lower LOX activity, lower mRNA abundance of a gene encoding a catechol oxidase and lower catechol oxidase activity.

Keywords: Banana; Blackening; Chilling injury; Hot water treatment; Lipid composition; TBA-reactive compounds; Phenolics; Lipoxygenase; Catechol oxidase; Gene expression

1. Introduction

Banana fruit cannot be stored for prolonged periods at temperatures below 12–14 °C because of rapid peel blackening and other chilling injury (CI) symptoms, such as failure to ripen, hardening of the central placenta and loss of flavour (Grierson et al., 1967).

One of the primary physical events leading to chilling injury, apparently, is a phase transition (from liquid to gel) in membrane polar lipids. This transition is related to loss of activity of membrane-bound enzymes and loss of membrane semi-permeability. A higher proportion of unsaturated fatty acids in the polar lipids generally result in lowering of the transition temperature and in lowering the temperature at which chilling injury occurs (Ariizumi et al., 2002).

Membranes can also become disrupted as a result of lipid peroxidation. This peroxidation can be mediated by enzymes such as lipoxygenase (LOX; Berger et al., 2001). Malondialdehyde (MDA), one of the main products of lipid peroxidation, is often measured using the thiobarbituric acid (TBA) method. However, since the TBA method also detects other degradation products of lipid peroxidation, the measured parameter is more properly designated "TBA-reactive compounds". The level of TBA-reactive compounds is considered to be a useful index of lipid peroxidation (Queiroz et al., 1998). Campos et al. (2003) observed lower levels of TBA-reactive compounds in leaves of coffee cultivars exhibiting higher cold tolerance.

Blackening as a result of chilling injury seems mainly the result of oxidation and polymerisation of free phenolics, starting with the activity of catechol oxidase (also called tyrosinase or polyphenol oxidase [PPO]). The enzyme and its substrate are believed to be located, normally, in separate cellular compartments but can come in contact with each other after membranes have become disrupted (Robinson, 1991; Hind et al., 1995).

A previous report has indicated that blackening of the banana peel during cold storage was related to the concerted activities of phenylalanine ammonia lyase (PAL) and catechol oxidase (Nguyen et al., 2003). In banana, and in many other fruit, a

* Corresponding author. Tel.: +66 2579 0308; fax: +66 2579 1951x112.
E-mail address: agrsck@ku.ac.th (S. Ketsa).

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relationship between low temperature-induced blackening and membrane-related properties such as fatty acid composition, the activity of lipid degrading enzymes such as LOX and the level of lipid peroxidation products, has apparently not been reported.

A relatively short exposure (min up to 2 h) to water at 40–60 °C, called hot water treatment, has been shown to delay chilling injury in a number of tropical and subtropical fruit (Fallik, 2004; Lurie, 2006). It is not known how this treatment increases resistance to low temperature. The beneficial effect of the treatment was found to be correlated with an increase in heat-shock protein (Hsp) levels (Woolf et al., 2004), but the function of these chaperone proteins in protection against low temperature, if any, is unknown.

'Gros Michel' and 'Namwa' bananas are commercially important cultivars in Thailand. Preliminary experiments showed that fruit of cv. Namwa was less CI sensitive than cv. Gros Michel. Here, we report the activities of catechol oxidase and LOX, the total free phenolics content, the TBA content and the composition of membrane fatty acids in the banana peel. We also used treatments in hot water (42 °C for 5–15 min) to modulate the onset of peel blackening. It was hypothesized that peel blackening would be correlated with each of these physiological parameters.

2. Materials and methods

2.1. Plant material

Fruit at 80% maturity (i.e. commercial maturity) of cv. Gros Michel (*Musa acuminata*, AAA Group; locally called cv. Hom Thong) and cv. Namwa (*Musa* × *paradisiaca*, ABB Group) were harvested from a plantation in the Petchaburi province (Western Thailand). Dehanded bananas were placed in corrugated cardboard boxes and transported by temperature-controlled truck (25 °C) to the laboratory within 2 h of harvest. In the laboratory, the hands were selected for uniformity of finger size and washed with water containing 100 mg/L sodium hypochlorite, then treated with 500 mg/L thiabendazole solution to control fruit rot and allowed to air-dry at ambient temperature (29–30 °C, 85% RH). In both cultivars, the hands were dipped in distilled water at 42 °C for 5, 10 or 15 min. Hands immersed in distilled water at 25 °C served as controls. Hands were randomly placed in corrugated cardboard boxes and stored at 4 °C, 85% RH. Fruit were regularly inspected for peel blackening. Peel was also regularly collected for chemical analysis, using three replications per treatment. Each replication consisted of the whole peel from 10 to 15 randomly sampled fruit. The peels were pooled, frozen in liquid nitrogen and stored at −80 °C for later analysis. In order to prevent oxidative damage, each individual peel was placed in liquid nitrogen, immediately after removal from the fruit. The peel material was thoroughly homogenized to ensure that the 5 g samples taken for analysis were representative.

2.2. Peel blackening

CI was evaluated every 2 days, by determining the brown area on the peel of 30 individual fruit using a scale from 1 to 5; 1: no chilling injury; 2: mild injury (1–20% of fruit affected); 3: moderate injury (21–50% of fruit affected); 4: severe injury (51–80% of fruit affected); 5: very severe injury (81–100% of fruit affected). A CI index was calculated as $\sum$ (CI level × number of fruit at that level)/total number of fruit in each group (30 in the present experiments).

2.3. Lipid extraction and analysis

Methyl esters of fatty acids were separated and quantified by the AOAC (1995) method, using a gas chromatograph (Varian, model CP 3800, Palo Alto, CA, USA) with a 60 m × 0.25 mm DB-23 capillary column and a film thickness of 0.25 mm (Agilent Technologies, Wilmington, DE, USA), coupled to a flame ionization detector. The injector and detector were maintained at 230 and 250 °C, respectively. Samples were injected on the column at a split rate of 1:50 with a helium carrier gas flow rate of 1 mL/min. All solutions were injected using three replications (each from another biological sample) and standards were also injected three times. Total saturated fatty acids is the sum of palmitic (16:0), stearic (18:0) and beheric (22:0) and total unsaturated fatty acids is the sum of elaidic (18:1), linoleic (18:2) and linolenic (18:3).

2.4. TBA-reactive compounds

Five grams of banana peel were homogenized with 25 mL of 5% (w/v) trichloroacetic acid (TCA). The mixture was centrifuged for 10 min at 4000 × *g*. Thiobarbituric acid (TBA) reactivity was determined by adding 2.5 mL of 0.5% TBA in 15% TCA to 1.5 mL of the supernatant. The reaction solution was held for 20 min in a boiling water bath, then cooled quickly and finally centrifuged at 12,000 × *g* for 10 min to clarify the solution. Absorbance was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm, calculated with an extinction coefficient of 1.55 nmol L^{-1} m^{-1} and expressed as nmol g^{-1} FW.

2.5. Lipoxygenase (LOX) activity

LOX was extracted and assayed by the method described by Lara et al. (2003). Five grams of banana peel were homogenized in 1 mL of extraction solution containing 0.1 M phosphate, pH 7.5, 2 mM DTT, 1 mM EDTA, 0.1% (v/v) Triton X-100 and 1% (w/v) PVPP. The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C and the supernatant held at 0 °C. LOX activity assayed by mixing 100 μL of the supernatant with 2.5 mL 0.1 M phosphate, pH 8, 400 μL substrate solution (8.6 mM linoleic acid, 0.25% (v/v) Tween-20, 10 mM NaOH, in 0.1 M phosphate, pH 8). Activity was measured by following the increase in absorbance at 234 nm over time, due to formation of hydroperoxides from linoleic acid. One unit of enzyme activity was defined as the increase in absorbance per min and per ml enzyme solution.