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Postharvest application of wax controls pineapple fruit ripening and improves fruit quality

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ABSTRACT

Pineapple (*Ananas comosus* (L.) Merr.) fruit ripen and soften rapidly after harvest. This study focused on examining the effect of wax application on the ripening and storage quality of the fruit. An optimized waxing treatment (65 g L^{-1} for 1 min) could effectively control fruit ripening by delaying fruit color change, decreasing the respiration rate and ethylene production, decreasing content of organic acids and relieving the symptoms of internal browning of pineapple fruit. Waxing enhanced the relative level of pentose phosphate pathway of respiration and affected the enzymes involved in organic acid metabolism. Waxing also reinforced the antioxidant system and enhanced the expression levels of genes related to defense, such as *PGIP*. These results indicated that wax treatment could effectively improve the fruit quality, mainly through the reduction of organic acid content.

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is an economically important fruit in many tropical and subtropical regions due to its unique flavor and great health benefits (Avallone et al., 2003; Hossain, 2016). Pineapple is a non-climacteric fruit and needs to be harvested at higher maturity to ensure optimal edible quality (Hardenburg et al., 1968; Lobo and Yahia, 2017). Pineapple fruit usually tend to be perishable and susceptible to physiological disorders and pathogen infection, resulting in a short shelf life (Hardenburg et al., 1968).

Various technologies have been investigated for the preservation of pineapple fruit, including modified atmosphere packaging (MAP) (Martínez-Ferrer et al., 2002), wax coating treatment (Hu et al., 2012; Wilson Wijeratnam et al., 2006), heat treatment (Weerahewa and Adikaram, 2005), $CaCl_2$ treatment (Youryon and Wongsaree, 2015), ABA treatment (Pusittigul et al., 2012; Zhang et al., 2015), and 1-methylcyclopropene (1-MCP) treatment (Selvarajah et al., 2001). However, most technologies mainly focuse on the inhibition of fruit internal browning (IB), a physiological disorder of pineapple fruit often occurs at low temperature storage (Zhang et al., 2015). Few studies have paid attention to the preservation of the exterior quality and taste quality of pineapple fruit.

Coating treatments have been applied to maintain the quality of stored horticultural crops, such as citrus (Fan et al., 2014), mango (Baldwin et al., 1999), pineapple (Hu et al., 2012), apple (Bai et al., 2003), and mamey sapote (Ergun et al., 2005). Coating act by reducing water loss, maintaining the strength of peel tissue, retaining fruit volatile components, and modifying CO_2 and O_2 concentrations inside the fruit (Baldwin, 2003; Lobo and Paull, 2017). However, coating treatment effects may depend on the materials and methods (Bai et al., 2003; Baldwin et al., 1999). Waxing has been applied to reduce postharvest diseases and stress damage in various horticultural crops (Hu et al., 2012; Petracek et al., 1998). As waxing creates modified atmospheres, which can influence fruit metabolism, especially respiration, and affect fruit interior quality. Wax treatment could reduce IB of pineapple under chilling stresses via maintenance of cell integrity (Hu et al., 2012). Pineapple fruit maturity and quality are mainly judged by sugar to acid ratios, skin color and aroma compounds (Lobo and Paull, 2017). Our previous work showed that wax treatment can delay pineapple fruit ripening and maintain fruit quality (Lin et al., 2013), but the mechanisms are not clear. In this work, we explored the effect of wax treatment on fruit quality, including sugar, organic acid and volatile production, and the effect on controlling IB and several related genes in pineapples was studied. This work aimed to study the overall effects of wax application, as well as mechanism of action, on the ripening and storage quality of pineapple fruit.

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2. Materials and methods

2.1. Plant materials and treatments

Pineapple fruit (*A.comosus* (L.) Merr. cv. 'Comte de Paris') were harvested from a commercial plantation in Chaozhou, Guangdong province in China. Fruit at commercial maturity (about 80% of full maturity), with uniform color and size but without any physical injuries and/or disease symptoms were selected for the experiment. The crown of fruit were half-cut, cleaned, dipped in 0.1% hypochloride solution for 8 min, and then soaked in 0.05% (w/v) iprodione solution (Kuaida, Jiangsu, China) and 0.05% (w/v) prochloraz solution (Huifeng, Jiangsu, China) for 2 min to eliminate potential microbes, and air dried at ambient temperature. Fruit were dipped in Sta-Fresh 2952 (FMC, USA, the main ingredients are water, food-grade sorbitan fatty acids, food-grade vegetable derived fatty acid salts, food-grade vegetable derived mono- and diglycerides, propylene glycol) wax solution at 40 g L^{-1}, 65 g L^{-1}, and 90 g L^{-1} for 1 min, respectively. Another group of fruit were dipped in water for 1 min and used as the control. Three replication of 60 fruit were used for each treatment. Fruit were then allowed to dry for 2 h at 25 °C, stored at 25 ± 1 °C, and covered with polyethylene film (0.02 mm thick) to maintain a high (~90%) relative humidity (RH).

Fruit were monitored by periodic measurements of respiration, ethylene, firmness and peel color for a minimum of fifteen fruit each time. Samples of fruit pulp and different parts of fruit (peel tissue, pulp tissue closed to peel, pulp tissue closed to core and core tissue) were collected at 0, 1, 3, 5, 7, 9, 11, 13, and 15 d. All the samples were frozen in liquid nitrogen immediately after sampling, and stored at −80 °C freezer for further use.

2.2. Fruit ripening index assessment

Fruit color scales were evaluated as described by Selvarajah et al. (2001), and fruit pulp color and firmness were measured as describe by Hu et al. (2012). For fruit respiration and ethylene production, two fruit were placed in a 10.5 L airtight container equipped with a rubber stopper for 2 h at 25 °C. Measurement was conducted as described by Zhu et al. (2015).

For the respiration measurement of samples treated with different inhibitors involved in different respiration pathway, fruit pulp were cut into 1 × 1 cm pieces with 3 mm thick, dipped in water or solutions containing respiratory inhibitors (0.015 mol L^{-1} Na_3PO_4, 0.025 mol L^{-1} NaF or 0.025 mol L^{-1} malonic acid, respectively) and incubated in a container after being vacuumed for 3 min. Then the gas from the container was used for measuring the CO_2 concentration, which represented the residual respiration rate, excluding the proportion suppressed by certain inhibitors.

2.3. Analysis of volatile compounds

Pulp samples were mixed using a food processor. The pulp juice samples were used for the analysis of volatile compounds, which were extracted by solid-phase microextraction: 5 mL of homogenated juice samples was introduced into a glass vial and the SPME syringe was then manually inserted into the headspace of the vial with a fiber coating of 50 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) purchased from Supelco. Aroma compounds were extracted at 35 °C for 30 min. After extraction, the SPME fiber was placed into the injector of the GC/MS instrument. Volatile compounds were analyzed by the methods described by Zhu et al. (2010).

2.4. Determination of total soluble solids, titratable acids, total sugar and organic acids

The total soluble solids (TSS) and titratable acids (TA) were determined as described by Zhang et al. (2015). An ion chromatography (Dionex ICS3000, USA) was applied to determine the contents of sugar and organic acid in pineapple fruit during storage as described as Wang et al. (Lin et al., 2016; Wang et al., 2010).

2.5. Assay of enzymatic activities related to organic acid metabolism

Crude enzymes were extracted from the fruit flesh with a modified method of Hirai and Ueno (1977). All procedures were carried out at 4 °C. Two gram of fruit tissues were ground with a mortar and pestle with 4 mL of grinding buffer (200 mM Tris–HCl, pH 8.2), 600 mM sucrose, and 10 mM isoascorbic acid on an ice-bath. The mixture was centrifuged at 4000*g* for 20 min. The supernatant was then collected and re-centrifuged. Both supernatant and pellet were used to assay enzyme activity. The supernatant was diluted to 5 mL with an extracting buffer (200 mM Tris–HCl (pH 8.2, 10 mM isoascorbic acid and 0.1% Triton X-100). Then 2 mL of the diluted supernatant was centrifuged at 15,000*g* for 15 min at 4 °C. The resulting pellet was diluted to 2 mL with an extracting buffer for mitochondral-aconitase (Mit-ACO, EC 4.2.1.3) and isocitrate dehydrogenase (IDH, EC 4.1.1.41) assays. The resulting supernatant was diluted to 4 mL with the extracting buffer, which was used as Cytochrome-Aconitase (Cyt-ACO, EC 4.2.1.3). The remaining 3 mL of the diluted supernatant was further diluted to 6 mL with the extracting buffer, after which, 2 mL was used for measuring the activities of malate dehydrogenase (MDH, EC 1.1.1.37) and malic enzyme (ME, EC 1.1.1.40) assays. The remaining 4 mL was dialyzed (Spectra/Por 7 dialysis tubing, 2 K MWCO (Spectrumlabs, USA)) with the extracting buffer for 10 h and then used for measuring the activities of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) and citrate synthetase (CS, EC 4.1.3.7) assays.

The enzyme activities were assayed according to Hirai and Ueno (1977) and Tang et. al (2010) with slight modifications. The assay mixture for IDH contained 1.2 mL of supernatant, 0.3 mL of 40 mM Hepes buffer (pH 8.2), 0.2 mL of 2 mM sodium isocitrate, 0.15 mL of 16 mM NAD, 0.15 mL of 4 mM $MnSO_4$ and with the final volume of 3 mL. The mixture was mixed rapidly and the increase in OD_{340} nm was recorded. IDH activity was expressed in units per gram fresh weight (U g^{-1}), where one unit was defined as the amount of enzyme causing an increase in OD_{340} of 0.01 min^{-1}.

To assay ACO activity, the enzyme was preliminarily incubated with equivalent volume of 2 mM GSH for 1 h. The reaction mixture contained 1.2 mL of the crude enzyme solution, 0.3 mL of 800 mM Tris–HCl buffer (pH 7.5), 0.15 mL of 2 mM NaCl, 0.45 mL of 1.2 M *cis*-aconitate and 0.9 mL of ddH_2O in a final volume of 3 mL. The mixture was mixed rapidly and the increase in OD_{340} was recorded. ACO activity was expressed in units per gram fresh weight (U g^{-1}).

For CS activity assay, the reaction mixture contained 0.9 mL of supernatant, 0.3 mL of 800 mM Tris-HCl buffer (pH 9.0), 0.15 mL of 800 uM 5,5′-dithiobis-(2-nitrobenzoic acid), and 0.15 mL of 800 uM acetyl CoA in a final volume of 3 mL. The mixture was mixed rapidly and the increase in OD_{412} was recorded. CS activity was expressed in units per gram fresh weight (U g^{-1}), where one unit was defined as the amount of enzyme causing an increase in OD_{412} of 0.01 min^{-1}.

PEPC was assayed in a reaction mixture containing 0.6 mL of supernatant, 0.15 mL of 800 mM Tris-HCl buffer (pH 8.5), 0.15 mL of 40 mM $KHCO_3$, 0.15 mL of 40 mM $MgCl_2$, 0.15 mL of 4 mM PEP, 0.15 mL of 10 mM GSH, 0.15 mL of 3 mM NADH and 0.15 mL of ddH_2O in a final volume of 3 mL. The mixture was mixed rapidly and the increase in OD 340 nm was recorded. PEPC activity was expressed in units per gram fresh weight (U g^{-1}).

MDH activity was determined in a 3 mL of mixture composed of 0.3 mL of supernatant, 0.3 mL of 800 mM Tris-HCl (pH 8.2), 0.15 mL of 2 mM $MgCl_2$, 0.15 mL of 200 mM $KHCO_3$, 0.15 mL of 10 mM GSH, 0.15 mL of 3 mM NADH, 1.5 mL of 4 mM oxaloacetate (OAA) and 0.3 mL of ddH_2O. The mixture was mixed rapidly and the increase in OD_{340} was recorded. MDH activity was expressed in units per gram