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Inhibitory effect of sucrose laurate ester on degreening in *Citrus nagato-yuzukichi* fruit during storage

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Abstract

An inhibitory effect of sucrose laurate ester (SLE) on the degreening of Nagato-yuzukichi (*Citrus nagato-yuzukichi* hort. ex Y. Tanaka) fruit was determined. SLE treatment suppressed the degreening of the fruit during storage at 20 °C more efficiently than the treatment with any other sucrose fatty acid ester, such as myristate, palmitate or stearate. SLE itself did not have an inhibitory effect on chlorophyllase and chlorophyll (Chl)-degrading peroxidase activities, but laurate, which was de-esterified from SLE, had a significant effect. Laurate inhibited both enzyme activities more effectively than any other fatty acid, such as caprylate, caprate, myristate, palmitate or stearate. The fruit flavedo extract had an activity to decompose SLE to laurate and sucrose, and treatment of the fruit with laurate significantly suppressed degreening during storage at 20 °C as well. These results indicate that the suppression of degreening in SLE-treated Nagato-yuzukichi fruit could be in part due to the formation of laurate from SLE by an esterase, such as a lipase, which is present in the flavedo, and the laurate formed might be involved effectively in the inhibition of Chl-degrading enzyme activities. Moreover, the suppression of degreening by SLE treatment could be due to the inhibition of degreening by laurate in addition to the coating effect of SLE.

Keywords: Chlorophyll degradation; *Citrus nagato-yuzukichi*; Degreening; Lauric acid; Sucrose laurate ester

1. Introduction

Nagato-yuzukichi (*Citrus nagato-yuzukichi* hort. ex Y. Tanaka) fruit, which belong to the Yuzu (*Citrus junos*) group, are harvested in late summer in the Yamaguchi Prefecture, Japan, when the rind is still green. The quality characteristics of the fruit are highly flavored and acidic. For the maintenance of postharvest quality, it is necessary to retain the green peel as long as possible.

Coating treatments are known to maintain the quality of stored horticultural crops by suppressing water loss, improving the strength of peel tissue and retaining volatile components, and by controlling ripening by modifying CO_2 and O_2 concentrations inside the fruit (Baldwin, 1994, 2003). Waxes such as carnauba and paraffin, oils, gums, polysaccharides, etc., have been used as coating reagents. Sucrose fatty acid esters are also used as edible coating reagents for maintaining quality in horticultural produce and have been shown to effectively delay degreening of Kabosu (*Citrus sphaerocarpa*) and banana fruit. Murata (1989) demonstrated that a treatment of sugar fatty acid esters delayed the degreening of the flavedo tissue in green Kabosu fruit. Momen et al. (1997) found that treatments with sucrose laurate ester (SLE), sucrose palmitate ester and sucrose stearate ester delayed the degreening of ethylene-treated banana fruit. We also reported in a previous paper (Yamauchi et al., 2003) that *Citrus nagato-yuzukichi* fruit treated with SLE at 50 °C for 3 min effectively reduced the degreening of flavedo tissue during storage at 20 °C and that the degreening control by SLE treatment at 50 °C could be due to the formation of modified atmosphere conditions by the coating. In addition, SLE treatment reduced the enhancement of the activities of chlorophyll (Chl)-degrading enzymes such as chlorophyllase and Chl-degrading peroxidase, especially the latter, which could imply that SLE has control over Chl degradation of fruit flavedo in addition to having an effect on the concentration of internal gases.

This study deals with the inhibitory role of SLE on degreening in Nagato-yuzukichi fruit during storage.

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2. Materials and methods

2.1. Plant materials

Green Nagato-yuzukichi (*Citrus nagato-yuzukichi* hort. ex. Y. Tanaka) fruit were harvested in summer at the Hagi Citrus Research Station. The fruit were kept for 4 days at room temperature as a pretreatment (4–5% weight loss from the peel) and then transported to the laboratory of Horticultural Science, Yamaguchi University.

2.2. Surface color and chlorophyll assays

The surface color of the fruit was measured using a color difference meter (Nippon-denshoku NF777). For Chl analysis, 1 g of flavedo segments obtained from five fruit was immersed in *N*,*N*-dimethylformamide for 1 day at 5 °C in the dark, and Chl was measured by reading the absorbance at 664 nm and 647 nm according to the method of Moran (1982).

2.3. Sucrose fatty acid ester treatment and storage

The fruit were treated with 2% sucrose fatty acid esters (SLE, sucrose laurate ester; SME, sucrose myristate ester; SPE, sucrose palmitate ester; SSE, sucrose stearate ester) or 0.1% sodium laurate at ambient temperature for 3 min and then dried for 1 day at ambient temperature. After drying, five fruit per perforated polyethylene-film bag (20 cm × 14 cm, 0.04 mm thick, with two 6 mm holes) were stored at 20 °C for 20 days. All analyses were conducted using triplicate samples, and the data are presented as means and S.E. ($n = 3$).

2.4. Chlorophyll-degrading enzyme assay

For the enzyme assay, an acetone powder (300 mg) was suspended in 8 mL of a 5 mM phosphate buffer (pH 7.0) containing 1% CHAPS for chlorophyllase activity or without it for peroxidase activity. The suspension was stirred for 1 h at 0 °C and then filtered through Miracloth (Calbiochem). Afterwards, the filtrate was centrifuged at 16,000 × *g* for 15 min at 4 °C. The supernatant was used as a crude enzyme extract.

Chlorophyllase activity was determined by a modification of the method of Amira-Shapira et al. (1987). The reaction mixture contained 0.3 mL enzyme solution, 0.2 mL Chl *a* (Tama Chemical) acetone solution (500 μg mL^{-1}) and 0.5 mL 100 mM phosphate buffer (pH 7.0). The mixture was incubated in a water bath at 25 °C for 1 h, and the enzyme reaction was stopped by the addition of 4 mL acetone. The remaining Chl *a* was extracted with 4 mL hexane and assayed by reading the absorbance by Chl *a* at 663 nm.

Chl-degrading peroxidase was determined as described by Yamauchi et al. (1997). The reaction mixture contained 0.1 mL enzyme solution, 0.1 mL 1% Triton X-100, 0.2 mL Chl *a* acetone solution (500 μg mL^{-1}), 0.1 mL 5 mM naringin, a flavonoid of the enzyme substrate included in the Nagato-yuzukichi fruit flavedo (Yamauchi and Eguchi, 2002), 0.1 mL 0.3% hydrogen peroxide, 0.4 mL distilled water and 1.5 mL 100 mM phosphate–citrate buffer (pH 4.5). The activity was determined spectrophotometrically by measuring the decrease of Chl *a* at 668 nm at 25 °C.

One unit of those enzymes was defined as a change of 1 μg Chl *a* degradation per min. The enzyme protein content was assayed by the method of Bradford (1976).

2.5. Laurate formation from sucrose laurate ester by flavedo tissue or lipase

The flavedo tissue (2.5 g) was homogenized in 20 mL of a 100 mM phosphate buffer (pH 7.5) containing 5 mM dithiothreitol and 250 mg Polyclar AT. The homogenate was filtered through Miracloth and centrifuged at 16,000 × *g* for 20 min at 4 °C. The supernatant was passed through a PD-10 desalting column (GE Healthcare Bioscience) to remove low-molecular-weight substances. The elute was used as a flavedo extract. Five milliliters of a 100 mM phosphate buffer (pH 7.0) containing 2% SLE was added to the flavedo extract (2.5 mL), the flavedo tissue segments (2.5 g, 5 mm × 5 mm) or 2.5 mL of a lipase solution (Sigma–Aldrich, 150 Units mL^{-1}) and the mixture was incubated at 25 °C. After the incubation, 3 mL of a hexane solution was added to the reaction mixture (2 mL) and stirred vigorously. The hexane layer and the aqueous layer which were separated into two phases, were used for lauric acid and sucrose analyses, respectively.

2.6. Lauric acid and sucrose assays

The hexane solution, which was dehydrated by the addition of anhydrous sodium sulfate, was concentrated using a rotary evaporator, and the dried residue was then dissolved with 0.25 mL of benzene. HCl–methanol (5% (v/v), 0.5 mL) was added to the aliquot in a vial and heated to methylate the lauric acid at 100 °C for 2 h after the vial was sealed. Afterwards, both 1.5 mL of hexane and 4 mL of 2% $KHCO_3$ were added to the aliquot and the mixture was kept at 5 °C for 1 day. The hexane layer or the $KHCO_3$ layer was used for the analysis of lauric acid or sucrose.

Lauric acid methyl ester in the separated hexane layer was identified and measured by GC–MS (Hewlett Packard, GC 5890 and a mass selective detector 5972) and GC (Hitachi G-3900) with FID, respectively, by a modified procedure of Takano and Koike (2000). A capillary column (GC–MS: HP-5MS, 0.25 mm i.d. × 30 m, GC: DB-1, 0.25 mm i.d. × 30 m) was used in a temperature-gradient mode (0–3 min at 120 °C; 3–23 min linear gradient from 120 to 220 °C, followed by 2 min at 220 °C). The temperature at the injection port or detector was 250 °C and the injection volume was 5 μL. Margaric acid was used as an internal standard for GC analysis.

For sucrose analysis, ethanol was added to the $KHCO_3$ solution to adjust to 70% at the final concentration, and the ethanol solution was filtered with DISMIC filter (0.45 μm, Advantec). The filtrate was analyzed by HPLC according to a slightly modified procedure of Yamashita et al. (1993). A Hitachi Model L-7100 pump and L-7490 RI detector were used for HPLC analysis. Sucrose was separated on a LiChrosphere NH_2 column