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Control of degreening in postharvest green sour citrus fruit by electrostatic atomized water particles

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ABSTRACT

The effect of electrostatic atomized water particles (EAWP) on degreening of green sour citrus fruit during storage was determined. Superoxide anion and hydroxyl radicals included in EAWP were present on the surface of the fruit peel after the treatment. Hydrogen peroxide was formed from EAWP in an aqueous solution, which could indicate that a hydroxyl radical of EAWP turns to hydrogen peroxide in the fruit flavedo as well as in the aqueous solution. EAWP treatment effectively suppressed the degreening of green yuzu and Nagato-yuzukichi fruits during storage at 20 °C. The enhancement in K+ ion leakage of both EAWP-treated fruits reduced in comparison with the control. In spite of EAWP treatment, total peroxide level in both fruits showed almost no changes during storage, suggesting that hydrogen peroxide formed by EAWP treatment could stimulate the activation of hydrogen peroxide scavenging system and control degreening of these fruits during storage.

1. Introduction

Green yuzu (*Citrus junos* Siebold ex. Tanaka) and Nagato-yuzukichi (*Citrus nagato-yuzukichi* hort. ex. Y. Tanaka), which are in the yuzu group, are a highly flavored, acidic, green citrus fruit like a lime (*Citrus latifolia* Tan.). These fruits are harvested in summertime in Japan when the rind shows a green color and the fruits are immature. Peel degreening, in which chlorophyll (Chl) is degraded in the flavedo tissue, is one of main factors of quality deterioration in these fruits when stored. It is necessary to retain the green peel as long as possible to preserve the quality.

Stress treatments, heat and UV treatments, after harvest have been reported to maintain fruit quality during storage. Heat treatments such as hot air, hot water, and vapor heat indicated physiological effects on the control of ripening and senescence and the tolerance of chilling injury in postharvest horticultural produce. We found that hot-air treatment for 2 h at 50 °C effectively suppressed Chl degradation during storage in broccoli florets (Funamoto, Yamauchi, Shigenaga, & Shigyo, 2002; Kaewsuksaeng et al., 2007). UV treatment after harvest is also known to maintain quality during storage. UV-C treatment seems to control the postharvest yellowing of broccoli florets (Büchert, Civello, & Martínez, 2010; Costa, Vicente, Civello, Chaves, & Martínez, 2006). Similarly, we reported that UV-B treatment efficiently inhibited Chl degradation in stored broccoli florets (Aiamla-or, Yamauchi, Takino, & Shigyo, 2009). However, UV-A was not effective to suppress floret yellowing. From these observations, we infer that active oxygen species, especially hydrogen peroxide, which is produced by the treatments, could induce activation of the ascorbate–glutathione (AsA–GSH) cycle, and enhancement of the cycle might be involved in the suppression of floret yellowing.

Electrostatic atomized water particles (EAWP) were found to be produced by electrostatic atomization from condensed moisture by applying high voltage to the discharge electrode, and the EAWP included active oxygen species such as the superoxide anion and hydroxyl radical (Yamauchi, Suda, & Matsui, 2007). Ma et al. (2012) noted that treatment with EAWP delayed yellowing and suppressed the reduction of ascorbic acid in broccoli florets during storage. Thus, it is likely that EAWP treatment could be used to maintain the quality of horticultural produce.

In this paper, we deal with the effect of EAWP on the degreening of green sour citrus fruit during storage.

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2. Materials and methods

2.1. Plant materials and treatment of electrostatic atomized water particles

Green yuzu fruit grown in a plastic-film house were harvested in early summer in Kochi Prefecture, and green Nagato-yuzukichi fruit were grown in open culture in late summer in Yamaguchi Prefecture. Lime (*C. latifolia* Tan.) fruit imported from Mexico were purchased from Tokio Fukuoka Co., Ltd., Japan. Green yuzu and Nagato-yuzukichi fruits were stored at 20 °C in a covered container (30 L) under a stream of humidified air (200 mL min^{-1}). A device (Panasonic, Japan) that generated EAWP was fitted to the downward direction on the lid of the container, and the EAWP formed was applied continuously to yuzu fruit and hourly to Nagato-yuzukichi fruit, respectively, as treated by an effective EAWP level to each fruit. Fruit were removed at scheduled intervals during storage and the flavedo tissues were used for the analyses.

2.2. ESR analyses of active oxygen species in electrostatic atomized water particles

For analysis of the active oxygen species included in EAWP, a small Petri dish contained 100 μL 100 mM CYPMPO and 100 μL distilled water put at a distance of 5 cm from the device and held for 30 min in a covered container (30 L). Active oxygen species, superoxide anions and hydroxyl radicals, included in EAWP were measured using an ESR spectrometer (E500, Bruker, Germany). Measurement was carried out at room temperature under the following experimental conditions: microwave frequency, 9.8 GHZ; microwave power, 10 mW; field modulation amplitude, 4 gauss; averaged scans, 100. CYPMPO (Radical Research, Japan), which shows selectivity for both superoxide anions and hydroxyl radicals, was used as a spin trapping reagent. All the experiments were conducted at room temperature.

2.3. Electrostatic atomized water particles on the surface of a fruit peel

Flavedo segments (3 × 20 mm) were prepared from lime fruit peel and held for 1 h in a desiccator. Ten μL aliquot of 500 mM CYPMPO in 0.1% Triton X-100 was dropped to the surface of a flavedo segment and then held for 20 min to be perfused. Afterward, the flavedo segments were exposed to EAWP for 1 h, and the active oxygen species on the flavedo tissue was determined directly using ESR.

2.4. Formation of hydrogen peroxide from electrostatic atomized water particles

For the assay of hydrogen peroxide formed from EAWP, 6 Petri dishes containing 30 mL distilled water were put down into the container and held at 20 °C for 24 h in a covered container (30 L) under a stream of humidified air (200 mL min^{-1}). Hydrogen peroxide was measured using a Pack Test (WAK-H2O2, Kyoritsu Chemical-Check Lab., Corp.) based on the peroxidase reaction with 4-aminoantipyrine.

2.5. Surface color and chlorophyll assay

The surface color of the fruit was determined by measuring the hue angle with a color difference meter (Nihon Denshoku NF 777). The Chl content was measured using *N,N*-dimethylformamide (Moran, 1982).

2.6. Total peroxide and potassium ion leakage assays

Total peroxide was measured according to the method of Sagisaka (1976). Flavedo tissue (1.0 g) was homogenized in 10 mL of cold 5% trichloroacetic acid with a mortar and pestle on ice. The homogenate was filtered through Miracloth (Calbiochem, USA) and the filtrate centrifuged at 15,000*g* for 10 min at 4 °C. The reaction mixture contained 2 mL supernatant solution, 0.5 mL 50% trichloroacetic acid, 0.5 mL 10 mM ferrous ammonia sulfate and 0.25 mL 2.5 M potassium thiocyanate. With the addition of potassium thiocyanate to the mixture, the color was developed and measured spectrophotometrically at 480 nm. Using hydrogen peroxide, the standard curve was prepared.

For the assay of K+ ion leakage, 2 g of flavedo segments were incubated at 25 °C for 2 h in a conical flask containing 50 mL deionized water after rinsing the flavedo segments. The samples were then incubated at 80 °C for one day to measure the total K+ ions. The K+ ion was analyzed using an atomic absorption spectrophotometer (Hitachi Z8200, Japan). The K+ ion leakage showed a ratio of leakage from flavedo segments during 2-h incubation for a total K+ ion level of the flavedo segments.

3. Results and discussion

3.1. Active oxygen species in electrostatic atomized water particles and their effects on the fruit surface

Fig. 1A shows ESR spectrum of active oxygen species within EAWP. In the ESR spectrum of CYPMPO spin adducts, the split in some signal such as lowest or highest field signal was observed. It is thought that both superoxide anion and hydroxyl radicals

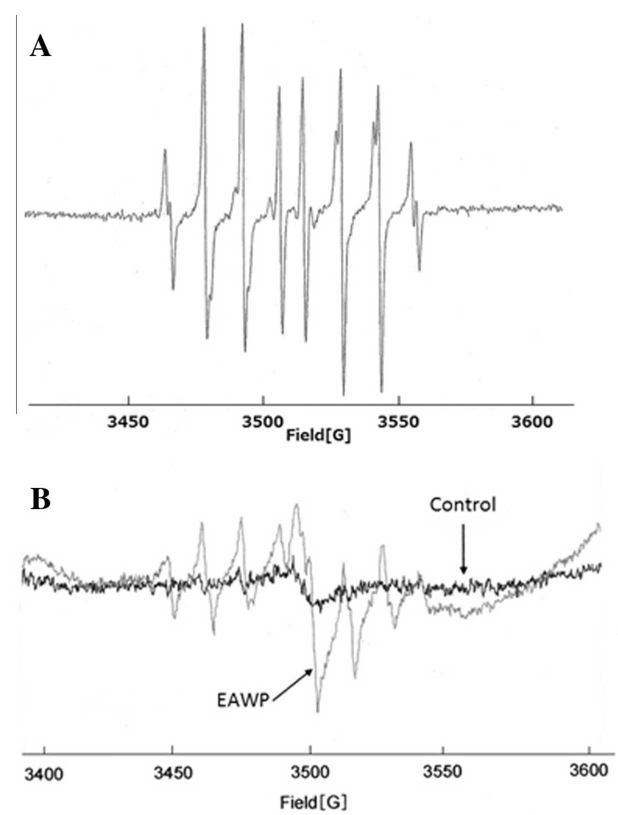

Fig. 1. ESR spectra of active oxygen species. Fig. 1A shows ESR spectrum of active oxygen species included in electrostatic atomized water particles (EAWP). Fig. 1B shows ESR spectrum of active oxygen species on the surface of fruit peel with the treatment of EAWP.