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SHORT COMMUNICATION

Efficacy of washing treatments in the reduction of post-harvest decay of chestnuts (*Castanea crenata* 'Tsukuba') during storage

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Abstract: This research evaluated the influence of different washing treatments (i.e., tap water, ozone, microbubbles, and ozone combined with microbubbles) on post-harvest decay of chestnuts (*Castanea crenata* 'Tsukuba') during storage. Overall, treatments with ozone and microbubbles significantly reduced the decay frequency and the associated microbial populations (aerobic bacteria, mold/filamentous fungi, and yeasts) during post-harvest storage compared with the traditional practice (tap water washing). Enhancing the efficacy of chestnut washing treatments will contribute to the improved storage of high-quality chestnuts.

Key words: *Castanea*, disease, microbial population, post-harvest decay.

Résumé : Par ces recherches, les auteurs voulaient évaluer l'influence de diverses méthodes de lavage (à savoir, à l'eau courante, par l'ozone, au moyen de microbulles et par l'ozone avec microbulles) sur la détérioration des châtaignes (*Castanea crenata* 'Tsukuba') après leur récolte, durant l'entreposage. Dans l'ensemble, comparativement à la pratique usuelle (lavage à l'eau courante), les traitements à l'ozone et aux microbulles diminuent sensiblement la fréquence des dommages et la population microbienne qui en est responsable (bactéries aérobies, moisissures/champignons filamenteux, levures) pendant l'entreposage qui suit la cueillette. Un lavage plus efficace concourra à un meilleur stockage des châtaignes de qualité. [Traduit par la Rédaction]

Mots-clés : *Castanea*, maladie, population microbienne, détérioration après récolte.

Introduction

Globally, chestnuts (*Castanea* spp.) are cultivated in 26 countries on more than 536 000 ha. According to the FAO (2014), the estimated worldwide production of chestnuts in 2012 was more than 1 998 000 t and geographically distributed as follows: China (82.8%), Republic of Korea (3.5%), Turkey (3.0%), Bolivia (2.9%), Italy (2.6%), Greece (1.4%), and Japan (1.1%), with other countries contributing less than 1% each.

Most harvested chestnuts are stored to increase the sweetness and harden the pericarp. In addition, improved post-harvest storage of chestnuts can provide potential economic benefits by reducing spoilage and extending the marketing period. Nonetheless, storage-associated spoilage of chestnuts represents a major post-harvest problem for chestnut marketing, and fungi have been identified as the major cause of chestnut rots (Overy et al. 2003; Jermini et al. 2006; Donis-González 2008). Infection of the fruits by spoilage agents usually occurs after the chestnuts fall to the ground and are exposed to soil, plant matter, and (or) dirty water during the harvest (Mencarelli 2001; Kader 2003). Unlike many other fruits and vegetables, little information is available on the efficacy of different washing and sanitizing treatments for chestnuts prior to post-harvest storage.

One traditional pre-storage treatment still widely used in Europe is water curing, where chestnuts are soaked in water for 7–9 days to reduce fungal inoculum during storage and kill insect larvae inside the chestnut (Mencarelli 2001; Botondi et al. 2009). Unfortunately, 2 days of water curing induces the development of a water-associated odor in the chestnut kernel (Kim et al. 2007). Therefore, in many countries, especially Korea, where raw chestnuts are preferentially consumed, either short-time, water-washing treatments are applied or the chestnuts are left untreated prior to post-harvest storage. Although chemical sanitizers containing diverse acids offer an alternative method to reduce fungal development, traditional chemical treatments are deemed unsuitable for consumers due to regulations and potential health-

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related concerns problems (Wells and Payne 1975; Kwon et al. 2004; Fernandes et al. 2011; Carocho et al. 2012).

Our study objective was to evaluate the effectiveness of different non-chemical washing treatments against decay and microbial (aerobic bacteria, mold/filamentous fungi, and yeasts) growth on the pericarp of chestnuts (*Castanea crenata* 'Tsukuba') during post-harvest storage.

Materials and Methods

Chestnuts were collected from the ground less than 24 h after dropping from the trees in a commercial orchard in the Cheongyang region in South Korea (36°23′N, 126°50′E) in 2012. Collected chestnuts were inspected, and decayed, damaged, and otherwise defective nuts were discarded. Our experimental design comprised five washing treatments × nine storage durations × three replications of randomized complete design with each experimental unit containing 15 chestnuts (2025 chestnuts total). The five different washing treatments were ozone, microbubble, ozone + microbubble, tap-water soak, and untreated (control). All treatments except the control were conducted in a 300 L water tank and chestnuts simultaneously exposed to the respective treatment for 10 min. An ozone generator (WHOZ-10, Cheonglimtech, Seoul, Korea) equipped with an oxygen generator (Integra 7, SeQual Technologies Inc., Ball Ground, GA, USA) supplied 0.4 ppm ozone confirmed with a portable dissolved ozone meter (DO 3, Himax Tech. Co., Ltd., Seoul, Korea). Microbubbles were produced using a microbubble generator (O.N.B MIN/100, O.K. Nano Tech, Bucheon, Korea) until the water tank was filled with microbubbles. The ozone + microbubble treatment combined the previous two treatments. For the tap-water treatment (traditional method), chestnuts were simply soaked. After treatment, chestnuts were air-dried for 40 min prior to sealing them in low-density polyethylene film (0.05 mm). A total of 135 polyethylene film packing units (five washing treatment × nine storage durations × three replications) was stored at 4 ± 1°C with 90 ± 3% of relative humidity. Each treatment contained ca. 20 kg of chestnuts. In addition, chestnuts were stored for nine time periods: 0, 2, 4, 6, 8, 10, 12, 14, and 16 weeks. Visual observations were made to calculate the frequency of decayed pericarp (% = decayed chestnuts/total chestnuts) for each storage duration. At five of those durations (0, 4, 8, 12, and 16 weeks), microbial population counts were made by sampling 10 g pericarp samples excised from 3 to 5 chestnuts per treatment (randomly selected from three replicates/packing units within treatment). Samples were placed in sterile water (90 mL) and agitated at 120 rev min^{-1} in a shaker (SK-760A, Jeio Tech Co., Ltd., Daejeon, Korea) for 30 min at room temperature. Ten-fold serial dilutions of the homogenized solution were prepared and 1 mL of each solution was inoculated onto 3M™ Petrifilm™ Aerobic Count Plates (3M™ Microbiology Products, St. Paul, MN, USA) or 3M™ Petrifilm™ Yeast and Mold Count Plates. The populations of aerobic bacteria were determined after growing for 48 h at 35 °C in an incubator (SH-77BL, Human Corporation, Seoul, Korea). Molds (filamentous fungi) and yeasts were counted after 96 h incubation at 25 °C. The data were analyzed using ANOVA (analysis of variance) and the mean difference was assessed by Duncan's multiple range test (P = 0.05) using the SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The population counts of aerobic bacteria were highest in control-treatment chestnuts, while significant differences were revealed among the other treatments, which follow in an order of decreasing effectiveness: ozone + microbubble, ozone, microbubbles, and tap water (Table 1A). In general, the mean count of aerobic bacteria tended to increase during storage regardless of treatment. Prior to storage, the highest mean counts of aerobic bacteria were detected on the control treatment, compared with the other treatments. At 4-weeks storage, the ozone treatment showed the lowest mean counts of aerobic bacteria compared with the other treatments, and this effect was maintained after 8-weeks storage; however, the bacterial counts of ozone-treated chestnuts were not statistically different from the ozone + microbubble treatments. At 12-weeks storage, all washing treatments exhibited lower mean counts of aerobic bacteria than the control, although the tap-water-treated chestnuts exhibited the lowest mean counts of aerobic bacteria. At 16-weeks storage, both the ozone + microbubble and ozone treatments featured low mean counts of aerobic bacteria, whereas these bacterial counts were the highest in the control. Unfortunately, it remains largely unknown whether different bacterial species can contribute to increased, decreased, or no effect on chestnut decay. Thus, focused studies are needed to better determine the role of bacteria in chestnut decay.

Overall, the mean counts of molds (filamentous fungi) tended to increase with increasing storage period regardless of the treatments (Table 1B). Prior to storage, the highest mean mold counts were detected on the control treatment, compared with the other treatments. The tap-water treatment and control showed high mean counts of molds at 4-weeks storage. In contrast, the ozone + microbubble treatment showed the lowest mean counts of molds at 8-weeks storage and 12-weeks storage, which was significantly different from the other treatments. The ozone treatment resulted in the lowest mean mold counts at 16-weeks storage, but this treatment did not significantly differ from the ozone + microbubble and microbubble treatments. With increasing post-harvest storage periods, it appears that the ozone and ozone + microbubble treatments are more effective in reducing mold populations on stored chestnuts compared with the traditional practice.

Prior to storage, the highest mean counts of yeasts were detected after tap-water treatment, followed

Table 1. The effect of different washing treatments on mean counts (log CFU g^{-1}) of (A) aerobic bacteria, (B) molds (filamentous fungi), and (C) yeasts of chestnuts (*Castanea crenata* 'Tsukuba') during storage.

	Week				
	0	4	8	12	16
(A) Aerobic bacteria					
Ozone	4.77*b*	4.85*b*	5.57*b*	5.82*b*	5.30*c*
Microbubble	4.85*b*	5.38*a*	6.02*a*	5.70*bc*	6.18*b*
Ozone + microbubble	4.65*b*	5.40*a*	5.60*b*	5.58*bc*	5.25*c*
Water	4.93*b*	5.30*a*	5.88*a*	5.43*c*	6.02*b*
Control	5.52*a*	5.43*a*	6.13*a*	6.43*a*	7.75*a*
(B) Molds					
Ozone	2.85*b*	3.30*b*	4.07*b*	4.50*b*	4.13*c*
Microbubble	3.03*b*	3.30*b*	4.05*b*	4.57*b*	4.67*bc*
Ozone + microbubble	2.80*b*	3.47*b*	3.35*c*	3.55*c*	4.20*c*
Water	3.07*b*	4.03*a*	4.70*a*	5.17*a*	5.23*b*
Control	4.07*a*	4.32*a*	5.17*a*	5.20*a*	6.02*a*
(C) Yeast					
Ozone	5.18*a*	4.93*a*	4.98*b*	5.23*b*	5.07*c*
Microbubble	5.08*a*	5.00*a*	5.15*b*	4.97*b*	5.10*c*
Ozone + microbubble	5.20*a*	4.88*a*	5.23*b*	4.83*b*	4.80*c*
Water	5.50*a*	5.15*a*	5.33*b*	5.13*b*	6.15*b*
Control	5.28*a*	5.33*a*	6.37*a*	6.18*a*	6.73*a*

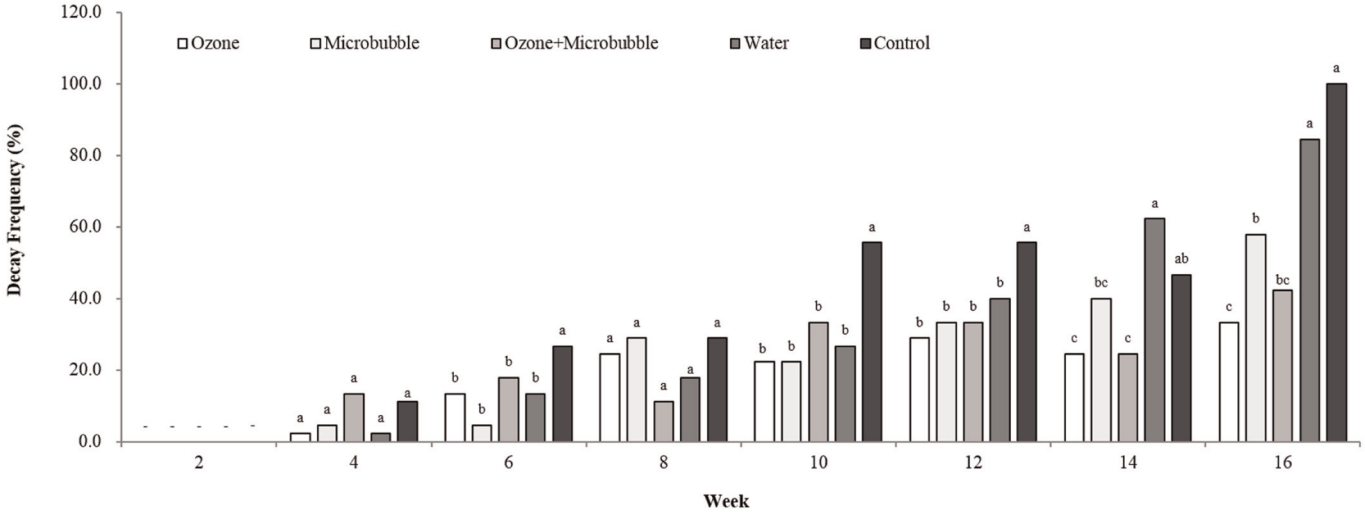
Note: Different letters in italic type indicate significant differences among the washing treatments at $P = 0.05$ (Duncan's multiple range test).

by the control, ozone + microbubble, ozone, and microbubble treatments; however, these observations were not statistically significant (Table 1C). During storage, the highest mean yeast counts were recovered from control chestnuts at 8- and 12-weeks storage, which significantly differed from the other treatments. At 16-weeks storage, ozone + microbubble, ozone, and microbubble treatments were significantly different from the traditional practice. Compared with the traditional practice, both microbubble and ozone treatments were very effective in reducing the yeast populations in chestnut after post-harvest storage.

A similar study on chestnut (*Castanea* spp.) decay in North America previously showed that fungi, bacteria, and yeast significantly increased during storage (Donis-González et al. 2010b). That study and another study (Washington et al. 1997) also showed that microbial populations were much higher on the shells (pericarps) compared with the kernels. Molds are reported as the most prominent post-harvest, decay agents for chestnut (both shells and kernels) (Jermini et al. 2006; Donis-González 2008). However, it is possible that some microbes associated with shells and kernels of chestnut might be antagonistic to decay-causing microbes. More studies are needed to survey the microbial community associated with chestnut and determine the interactive roles of diverse microbes during decay processes. In this study, only shell decay was evaluated after different washing treatments during storage. However, in our parallel study, we tested seven major chestnuts cultivars (including 'Tsukuba') grown in Korea to compare differences in chestnut quality characteristics (shell color, kernel hardness, kernel soluble solids content, shell decay frequency, etc.) according to storage temperatures and storage duration (S. Joo, M.J. Kim, M.-S. Kim, and U. Lee, unpublished data). In that study, we observed that a generally high correlation between uninfected shells and sound kernels among tested chestnut cultivars (data not shown).

We observed that post-harvest, chestnut decay was more prevalent after traditional practice (tap-water washing) and the control treatment. In contrast, ozone treatment diminished chestnut decay (Fig. 1). No decayed chestnuts were observed after the initial 2-weeks storage; however, chestnut decay frequency tended to increase during storage, regardless of treatment. Chestnut decay was initially detected after a 4-week storage period, but no major differences in the decay frequency were observed among treatments until 8-weeks storage. Decay frequencies among treatments appeared to rapidly change after 10-weeks storage ($P < 0.05$). In the control treatment, decay frequency increased from 55.6% after 10-weeks storage to 100% after 16-weeks storage, which was not statistically different than the tap-water treatment (84.4%). Chestnut-decaying microbes, which can originate from diverse sources (air, dust, animal feces, soil, plant organic matter, etc.), can contact the chestnut surface before and after harvest and can subsequently contribute to post-harvest decay during storage. The tap-water treatment was apparently ineffective at removing/reducing microbes on the chestnut surface, and therefore this treatment failed to reduce chestnut decay during post-harvest storage. After 16-weeks storage, the ozone treatment showed the lowest decay frequency (33.3%) followed by the ozone + microbubble (42.2%) and microbubble (57.8%) treatments ($P < 0.05$). Our results revealed that the ozone and microbubble washing treatments are very effective in reducing the frequency of decayed chestnuts compared with traditional washing practice. In particular, the ozone treatment of the chestnuts caused a disinfestation rate of 70% with regards to fungal contamination, while this treatment was previously shown to be less effective for other fresh fruits, such as peaches (20% disinfestation rate) (Cho et al. 2003). Donis-González et al. (2010a) also applied the ozone (0.92 ppm) and other post-harvest treatments to reduce molds and decay of fresh Michigan chestnuts. They demonstrated that ozone treatment was less

Fig. 1. The effect of different washing treatments on the decay frequency (%) of chestnuts (*Castanea crenata* 'Tsukuba') during storage. Different letters indicate significant differences among the washing treatments at $P = 0.05$ (Duncan's multiple range test).



effective for reducing mold frequency on the chestnut shells than other products, such as the StorOx™ (hydrogen dioxide plus peracetic acid). However, in that previous study, the ozone treatment was as effective as the StorOx™ for reducing decay incidence in the kernels. Because our study was focused on the mold on shells, we cannot draw inferences about treatment effect on kernel decay. Washington et al. (1997) showed that chestnut storage under an initial 6% carbon monoxide atmosphere followed by controlled atmosphere (2.5% carbon dioxide, 2.5% oxygen, and 95% nitrogen) combined with fungicide (e.g., benomyl, iprodione) treatment resulted in significantly reduced incidence of chestnut rot from 15.3% to 6.1% over 172 days. However, because of health and environmental concerns, many consumers prefer chestnuts with limited or no exposure to synthetic chemicals, including fungicides, before or during storage. Thus, treatments, such as ozone or microbubbles, that leave no chemical residue on chestnuts are more compatible with customer demand. Currently, harvested chestnuts are typically only subjected to tap-water treatment prior to storage in Korea. The results from this study showed that non-chemical washing treatments can significantly reduce pericarp decay, and these results are useful for small-scale commercial operations, especially those that have relied on tap-water washes prior to chestnut storage. This study also represents an essential first step toward developing methods for large-scale commercial applications. For example, additional studies are needed to (1) develop efficient methods for selecting damaged nuts after harvesting; (2) optimize storage conditions (temperature, modified atmosphere, etc.); (3) select optimal packing materials or methods (density of polyethylene film or other packaging materials, methods of package sealing); and (4) estimate consumer demands and market fluctuations.

In conclusion, our results provide useful baseline information for developing appropriate post-harvest management systems for the chestnut production industry, and further contribute to increased product value and associated economic benefits for chestnut producers.

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