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Research Report

Effect of CaO Treatment on Quality Characteristics and Storage of Mulberry (*Morus alba* L.) Fruits in *Yecheon*

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Abstract: The effects of aqueous calcium oxide (CaO) treatment on the quality characteristics and shelf life of mulberry (*Morus alba* L.) were investigated. Mulberry fruits were immersed in 0, 0.5, 1, and 2 g·L^{-1} CaO solutions for 0, 1, 3, 6, and 12 min. Mulberries were then rinsed with potable tap water for 1 min and stored at -1°C for 14 days. CaO treatment was effective at promoting the retention of titratable acid, pH, and ascorbic acid as well as total flavonoid contents. CaO concentration and treatment time were significant factors affecting the sensory qualities of the fruits, including off-odor, flavor, and texture. For shelf life determinations, the total bacterial count was reduced by CaO treatment so that the samples treated with 1 g·L^{-1} CaO for 12 min had bacterial levels at 14 days comparable to those of the control at 4 day, and no coliform group was detected after CaO treatment. These results indicate that calcium oxide treatment is a promising approach for the preservation of mulberry fruit.

Additional key words: calcium oxide, *Morus alba* L., storage, quality characteristics, *Yecheon*, *Gwasang* No. 2

Introduction

The mulberry (*Morus alba* L.), a deep-colored berry of the family *Moraceae*, is a traditional Korean edible fruit that can be eaten fresh. Traditionally, mulberry fruit has also been used in Korean folk medicine to effectively treat fever, strengthen joints, protect the liver from damage, lower blood pressure, and facilitate discharge of urine (Kim, 1991). The mulberry has been reported to be rich in various polyphenolic compounds such as flavonoids, anthocyanins, and stilbenes. The major flavonoids present in mulberry fruits are the 3-O-glycosides of quercetin and kaempferol, while the major anthocyanins consist of the 3-O-glycosides of cyanidin and pelargonidin (Pawlowska et al., 2008). Numerous researchers have shown that mulberry fruits have various beneficial health effects such as antioxidant activity, anti-inflammatory and neuroprotective effects, as well as anticancer activity (Jeong et al., 2010; Lee et al., 2008; Liu et al., 2008). Thus, in recent years, mulberries have been widely converted to juice for use in the production of wine, fruit juice, jam, and canned food (Lee and Choi, 2011).

Mulberries must be harvested at full maturity to achieve maximum flavor, texture, and nutritional value. Mulberry fruits are perishable after harvest due to their high (> 70%) water content and thin flesh (Ercisli and Orhan, 2007). Because of their short harvesting season and sensitivity to storage, mulberry fruits are mainly processed and consumed locally, during which a large part of the fruit is damaged, causing a great economic loss. Conventional thermal processing, which is an effective method for preserving fruits and vegetables, is not suitable for thermo-sensitive products such as mulberries (Terefe et al., 2009), because it results in the loss of organoleptic and nutrient levels of the produce, despite the process having desirable effects such as microbial and enzyme inactivation. An alternative technology for the preservation of mulberry fruit is sun drying, which can reduce moisture content to inhibit the growth of microbes

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and hinder quality losses (Doymaz, 2004). However, the sensory quality of mulberries can be seriously damaged during exposure to sunlight. Therefore, the development and application of new preservation methods to improve the storage qualities and shelf life of mulberry fruit is necessary for enhancing levels of commercial sales.

Scallop shells have found use as food additives, and are also found in plastering and paving materials. However, most of the shell is considered as commercial waste in Korea and Japan (Bodur and Cagri-Mehmetoglu, 2012). According to the KFDA (2006), Calcium oxide (CaO) is currently used to adjust the pH of foods and is specified as a safe substance with an unrestricted amount of usage. CaO is generated by the loss of CO_2 following the heat treatment of $CaCO_3$—a main component of materials such as natural mollusk shells—at more than 825°C under conditions where the air is blocked. Research on CaO treatment related to food has shown that its excellent bactericidal effects for vegetables such as lettuce, sesame, cabbage, tomato, radish, and sprouts, and cherry tomatoes can be used in conjunction to remove residual pesticides and extend the shelf life of kimchi and tofu (Bae et al., 2006; Kim et al., 2012; Sawai et al., 2001a; Sawai, 2011)

To the best of our knowledge, there have been no studies on the effects of CaO treatment on the nutritional components and shelf life of mulberry fruit to date. The objective of this work was to evaluate the effects of aqueous CaO treatment on flavonoid, ascorbic acid, reducing sugar, and titratable acid contents as well as shelf life of mulberry fruit. This study is anticipated to provide valuable information for preserving mulberry fruit for its expanded use in the food industry.

Materials and Methods

Materials and Preparation

Mulberry (*Morus alba* L.) fruit was provided by Yecheon Red Pepper Powder Farm Corporation and harvested at full maturity at Yecheon (Kyungbuk, Korea) in June 2011. The ripe berries were randomly divided into 1 kg batches, packaged, and immediately stored at -20°C until further use. Calcium oxide (CaO) powder (B&F Korea Co., Ltd., Seoul, Korea), used as a food additive in this study, was dissolved in distilled water.

CaO Treatment of Mulberry Fruit

Mulberries were immersed in aqueous CaO solutions with different concentrations (0.5, 1, and 2 $g \cdot L^{-1}$) for different time periods (0, 3, 6, and 12 min); the suspensions were prepared in a ratio of 500 g:2.5 L (mulberry: CaO solution, w/v) at 22 ± 2°C. The CaO solution was used as a control sample instead of distilled water. Immersed samples were rinsed with potable tap water for 30 sec, and solution residues on the fruit surface were drained. Each group was packed into a bag and stored at -1°C for 14 days for subsequent analysis.

Quality Characteristics

Total flavonoid contents of the mulberry fruit were analyzed according to the method described by Liu et al. (2008). A 250 μL aliquot of mulberry fruit juice was mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNO_2$. After 6 min, 150 μL of 10% $AlCl_3$ was added. Finally, 500 μL of 1 M NaOH was added and the total volume was made up to 2.5 mL with distilled water. Absorbance was measured at 510 nm. The total flavonoid content was expressed as mg of catechin equivalents per 100 g of mulberry.

Total ascorbic acid, reducing sugar, and titratable acid contents of the mulberry fruit were analyzed according to the method described by Li et al. (2009). Ascorbic acid was titrated using 2,6-dichloroindophenol. Briefly, 50 g of fruit samples were homogenized in 50 mL of a 0.02 $g \cdot L^{-1}$ oxalic acid solution and centrifuged at 15,000 g and 4°C for 15 min. Afterwards, 10 mL of the supernatant was titrated until reaching a permanent pink color using 0.1% 2,6-dichlorophenolindophenol titration. Ascorbic acid content was expressed as mass (mg) per 100 g of mulberry fruit. The content of reducing sugar was analyzed by Fehling's method (Lane and Eynon, 1934). Fruit samples (50 g) were first homogenized; then, a 25 g quantity of the homogenate was transferred to a beaker containing 150 mL of distilled water. The mixture was heated in a water bath at 80°C for 30 min. Aliquots of 10 mL were titrated, and reducing sugar content was expressed as mass (g) of glucose per 100 g of mulberry fruit. The content of titratable acid was obtained by titration with 0.1 M NaOH to pH 8.2 and expressed as mass (g) of citric acid per 100 g of mulberry fruit.

Storage Study

After analyses of the effects of CaO treatment on flavonoid, ascorbic acid, reducing sugar, and titratable acid contents, the ideal CaO treatment condition for maintaining nutritional components of the mulberry fruit was assessed by conducting a shelf life study. Control and CaO-treated samples

were packaged and stored at -1℃ for 14 d. Mulberries were examined using a microbial growth assay and evaluated for sensory quality during storage. Samples prepared without washing using tap water or CaO (fresh fruit) were used to determine the inherent background microflora. The end of shelf life was defined as when the population of a microbial group reached an unacceptable level or the sensory quality evaluation panelists rejected the sample. A mulberry fruit sample (25 g) was homogenized for 2 min in 225 mL of sterile buffered peptone water using a Stomacher 400 circulator (Steward Ltd., London, UK). Ten-fold dilution series were made in peptone saline solutions for plating. The following media and conditions were used for micro-organism incubation: plate count agar (Becton, Dickinson & Co., Maryland, USA) for total aerobic mesophilic bacteria incubated at 30°C for 3 d and also for total aerobic psychrotrophic bacteria incubated at 22°C for 5 d; de Man-Rogosa-Sharpe (MRS) medium containing 0.14% sorbic acid (Becton, Dickinson & Co., Maryland, USA) for lactic acid bacteria incubated at 30°C for 3 d; Rose Bengal agar (Becton, Dickinson & Co., Maryland, USA) for yeasts and molds incubated at 30°C for 3 d. Colonies were counted and results expressed as log CFU (colony-forming units)/g. The following microbiological specifications were used to determine the end of shelf life (12): 8 log CFU/g for aerobic mesophilic bacteria and aerobic psychrotrophic bacteria, 7 log CFU/g (plus sensory analysis) for lactic acid bacteria, and 5 log CFU/g for yeasts and molds.

Sensory quality was evaluated by a semi-trained panel of ten food researchers. Overall visual quality (OVQ) was scored based on a modification of the nine-point hedonic scale reported by Wright and Kader (1997): 9 = excellent, extremely fresh; 7 = very good, marketable; 5 = good, limit of marketability; 3 = fair, limit of usability; 1 = poor, unusable. The following sensory quality attributes were also evaluated according to Gómez-López et al (2008): off-odor (1 = none, 3 = acceptable, 5 = severe); flavor (1 = fresh, 3 = acceptable, 5 = spoiled); texture (1 = fresh, 3 = acceptable, 5 = spoiled). Off-odor, flavor, and texture were scored under red light, while OVQ was evaluated under white light. The end of shelf life from the sensory quality point of view was reached when at least one of the mean scores was above the acceptability limit.

Statistical Analysis

All experiments were carried out in triplicate and data were expressed as mean ± standard deviation (SD) using SPSS version 17.0 (SPSS Inst., U.S.A.). One-way analysis of variance (ANOVA) and Duncan's multiple-range test were used to determine the significance of the difference among samples with a significance level of 0.05.

Results

Flavonoid, Ascorbic Acid, Reducing Sugar, and Titratable Acid Contents

The effects of CaO treatment on the flavonoid content of mulberry fruit are shown in Table 1. Flavonoid contents of the control and CaO-treated samples decreased over time. In the first 2 d, flavonoid contents of the control were higher than those of the CaO-treated samples (p > 0.05). The treatment with 0.5 g·L^{-1} CaO for 3 min was similar with the control during storage, which was not effective in maintaining acceptable flavonoid content. After 6 d, the treatments with 1 and 2 g·L^{-1} CaO were more effective and significantly different from the control and 0.5 g·L^{-1} CaO treatments (p > 0.05). The 1 and 2 g·L^{-1} CaO treatments for 3 min were very similar. For both 1 and 2 g·L^{-1} CaO treatments, flavonoid contents for the samples treated over 12 min were higher than those samples using 3 and 6 min treatments. From these results, CaO concentration and treatment time was established to have a significant effect on flavonoid contents for all the samples.

In consideration of mulberry as a dietary source of ascorbic acid, the ascorbic acid content of mulberry fruit during storage was next determined. As shown in Table 2, a remarkable decrease in ascorbic acid content was detected in all samples. Contents of the CaO-treated samples were similar to that of the control sample during storage up until the first 6 d, which was not effective in maintaining ascorbic acid. From 10 d onwards, the ascorbic acid contents of CaO-treated samples became higher in comparison with the control (p > 0.05).

CaO concentration and treatment time had significant effects on ascorbic acid content (p > 0.05). The 1 and 2 g·L^{-1} CaO treatments were more effective than the treatment using 0.5 g·L^{-1} CaO in retaining ascorbic acid (p > 0.05). For treatments using 1 and 2 g·L^{-1} CaO, ascorbic acid contents in samples after 12 min were higher than those treated over 3 and 6 min (p > 0.05). The 1 g·L^{-1} CaO treatment was similar with the 2 g·L^{-1} CaO treatment (p > 0.05).

The effects of CaO treatment on the content of reducing sugars in mulberry fruit are shown in Table 3. No significant differences in initial reducing sugar contents among all samples could be observed (p > 0.05). Reducing sugar contents of the control and CaO-treated samples decreased as storage time was prolonged. Although the correlations

between treatment time and analyte content were similar for all treatments, contents of the CaO-treated samples were higher than those of the control ($p > 0.05$). The reducing sugar content became higher with increasing CaO concentration and treatment time ($p > 0.05$). Compared to the treatments using 0.5 g·L^{-1} CaO, reducing sugar contents of the 1 and 2 g·L^{-1} CaO-treated samples were higher during storage ($p > 0.05$). The 1 g·L^{-1} CaO treatments were similar

Table 1. Flavonoid content according to CaO treatment of mulberry fruits at full maturity at Yecheon in June 2011.

Treatment time (min)	Treatment concentration (g·L^{-1})	Storage time (days)					
		0	2	6	10	12	14
3 min	0 (Control)	21.02 ± 0.33^{aA}	17.35 ± 0.21^{bcB}	15.40 ± 0.14^{fC}	14.22 ± 0.11^{fD}	11.77 ± 0.17^{fE}	10.57 ± 0.07^{iF}
	0.5	20.30 ± 0.68^{aA}	16.75 ± 0.21^{efB}	15.34 ± 0.10^{fC}	14.15 ± 0.17^{fD}	12.22 ± 0.16^{fE}	10.99 ± 0.10^{hcF}
	1	20.91 ± 0.11^{aA}	16.83 ± 0.05^{defB}	16.06 ± 0.06^{eC}	15.55 ± 0.06^{deD}	14.35 ± 0.05^{deE}	13.01 ± 0.03^{deF}
	2	20.52 ± 0.03^{fA}	16.49 ± 0.06^{fB}	15.89 ± 0.14^{eC}	15.21 ± 0.14^{eD}	14.09 ± 0.02^{eE}	12.54 ± 0.20^{fF}
6 min	0 (Control)	20.04 ± 1.59^{aA}	17.54 ± 0.38^{abB}	16.51 ± 0.50^{dBC}	15.45 ± 0.49^{deCD}	14.21 ± 1.00^{eD}	11.51 ± 0.61^{gE}
	0.5	19.72 ± 1.41^{aA}	17.01 ± 0.39^{cdeB}	16.55 ± 0.38^{cdB}	16.01 ± 0.36^{bcBC}	14.87 ± 0.23^{dC}	12.75 ± 0.22^{efD}
	1	19.85 ± 0.26^{aA}	17.12 ± 0.18^{cdeB}	17.05 ± 0.18^{abB}	16.65 ± 0.22^{aC}	16.01 ± 0.11^{bcD}	14.21 ± 0.25^{cE}
	2	20.01 ± 0.10^{aA}	17.21 ± 0.29^{bcdB}	17.11 ± 0.10^{aB}	16.99 ± 0.30^{aB}	16.23 ± 0.11^{abC}	14.58 ± 0.12^{bcD}
12 min	0 (Control)	21.02 ± 0.33^{aA}	17.89 ± 0.23^{aB}	16.57 ± 0.44^{cdC}	15.79 ± 0.30^{cdD}	14.58 ± 0.12^{deE}	11.06 ± 0.08^{hF}
	0.5	20.01 ± 0.10^{aA}	16.77 ± 0.07^{efB}	16.61 ± 0.18^{bcdB}	16.22 ± 0.22^{bC}	15.54 ± 0.29^{cD}	13.24 ± 0.11^{dE}
	1	20.33 ± 0.68^{aA}	16.87 ± 0.08^{defB}	16.78 ± 0.11^{abcdB}	16.73 ± 0.02^{aB}	16.69 ± 0.14^{bB}	14.89 ± 0.13^{abC}
	2	19.85 ± 0.26^{aA}	16.99 ± 0.21^{cdeB}	16.97 ± 0.02^{abcB}	16.88 ± 0.10^{aB}	16.78 ± 0.03^{aB}	15.01 ± 0.07^{aC}

$^{a-i}$Different lowercase letters within columns indicate significantly different values base on Duncan's multiple range test ($p < 0.05$).
$^{A-F}$Different lowercase letters within rows indicate significantly different values base on Duncan's multiple range test ($p < 0.05$).

Table 2. Ascorbic acid content according to CaO treatment of mulberry fruits at full maturity at Yecheon in June 2011.

Treatment time (min)	Treatment concentration (g·L^{-1})	Storage time (days)					
		0	2	6	10	12	14
3 min	0 (Control)	15.14 ± 0.53^{abA}	14.56 ± 0.22^{abcB}	13.61 ± 0.36^{abcdC}	11.32 ± 0.24^{fD}	10.13 ± 0.11^{gE}	8.18 ± 0.08^{hF}
	0.5	15.27 ± 0.24^{abA}	14.25 ± 0.15^{deB}	13.36 ± 0.10^{cdefC}	12.41 ± 0.16^{eD}	10.77 ± 0.17^{fE}	8.79 ± 0.03^{gF}
	1	15.40 ± 0.06^{abA}	14.45 ± 0.21^{bcdB}	13.63 ± 0.08^{abcC}	12.95 ± 0.05^{dD}	11.82 ± 0.18^{cdE}	9.96 ± 0.04^{eF}
	2	15.27 ± 0.13^{abA}	14.31 ± 0.19^{cdeB}	13.29 ± 0.25^{cdefC}	13.16 ± 0.09^{cdC}	11.95 ± 0.06^{bcD}	10.35 ± 0.20^{dE}
6 min	0 (Control)	14.87 ± 0.23^{bA}	14.45 ± 0.22^{bcdB}	13.77 ± 0.20^{abC}	11.32 ± 0.18^{fD}	10.16 ± 0.15^{gE}	8.59 ± 0.12^{gF}
	0.5	15.41 ± 0.27^{aA}	14.31 ± 0.02^{cdeB}	13.66 ± 0.22^{abcC}	12.27 ± 0.07^{eD}	11.59 ± 0.39^{deE}	9.21 ± 0.31^{fF}
	1	15.13 ± 0.08^{abA}	14.53 ± 0.17^{abcdB}	13.91 ± 0.36^{aC}	13.23 ± 0.10^{bcD}	12.01 ± 0.04^{bcE}	10.64 ± 0.06^{cF}
	2	15.00 ± 0.05^{abA}	14.12 ± 0.08^{eB}	13.51 ± 0.06^{bcdeC}	13.42 ± 0.03^{abC}	12.14 ± 0.05^{bD}	10.91 ± 0.01^{bE}
12 min	0 (Control)	15.02 ± 0.10^{abA}	14.79 ± 0.12^{aB}	13.11 ± 0.11^{fC}	11.18 ± 0.10^{fD}	10.15 ± 0.16^{gE}	8.59 ± 0.12^{gF}
	0.5	14.87 ± 0.17^{cA}	14.26 ± 0.13^{deB}	13.38 ± 0.14^{cdefC}	12.27 ± 0.05^{eD}	11.52 ± 0.12^{eE}	9.21 ± 0.08^{fF}
	1	15.14 ± 0.04^{abA}	14.72 ± 0.15^{abB}	13.16 ± 0.11^{efC}	13.30 ± 0.19^{bcC}	12.07 ± 0.05^{bcD}	11.62 ± 0.28^{aE}
	2	15.00 ± 0.05^{abA}	14.23 ± 0.11^{deB}	13.24 ± 0.04^{defC}	13.57 ± 0.05^{aD}	12.55 ± 0.08^{aE}	11.79 ± 0.09^{aF}

$^{a-h}$Different lowercase letters within columns indicate significantly different values base on Duncan's multiple range test ($p < 0.05$).
$^{A-F}$Different lowercase letters within rows indicate significantly different values base on Duncan's multiple range test ($p < 0.05$).

with the 2 g·L^{-1} CaO treatments (p > 0.05).

The effects of CaO treatment on titratable acid content are presented in Table 4. Initial titratable acid contents in all samples were similar (p > 0.05). Titratable acid contents of the control and CaO-treated samples decreased during storage. In particular, the contents of the control decreased markedly during storage, while contents of the CaO treated samples remained higher (p > 0.05). Both factors—CaO

Table 3. Reducing sugar content according to CaO treatment of mulberry fruits at full maturity at Yecheon in June 2011.

Treatment time (min)	Treatment concentration (g·L^{-1})	Storage time (days)					
		0	2	6	10	12	14
3 min	0 (Control)	6.36 ± 0.10^{bA}	5.62 ± 0.05^{dB}	5.27 ± 0.02^{fC}	5.07 ± 0.05^{fD}	4.92 ± 0.06^{hE}	4.77 ± 0.02^{jF}
	0.5	6.30 ± 0.03^{abA}	5.81 ± 0.09^{bcB}	5.36 ± 0.03^{eC}	5.32 ± 0.01^{dC}	5.16 ± 0.02^{eD}	5.07 ± 0.01^{gE}
	1	6.33 ± 0.00^{abA}	5.87 ± 0.06^{abB}	5.51 ± 0.01^{bC}	5.46 ± 0.02^{cC}	5.37 ± 0.03^{cD}	5.32 ± 0.01^{cE}
	2	6.35 ± 0.08^{aA}	5.89 ± 0.02^{abB}	5.51 ± 0.01^{bC}	5.50 ± 0.00^{bC}	5.40 ± 0.01^{bcD}	5.27 ± 0.03^{dE}
6 min	0 (Control)	6.35 ± 0.02^{aA}	5.66 ± 0.06^{dB}	5.29 ± 0.01^{fC}	5.12 ± 0.03^{eD}	5.07 ± 0.05^{fD}	4.82 ± 0.02^{iE}
	0.5	6.28 ± 0.01^{abA}	5.75 ± 0.02^{cB}	5.42 ± 0.01^{dC}	5.33 ± 0.00^{dD}	5.31 ± 0.01^{dD}	5.15 ± 0.00^{fE}
	1	6.29 ± 0.03^{abA}	5.86 ± 0.03^{abB}	5.61 ± 0.00^{aC}	5.51 ± 0.01^{bD}	5.51 ± 0.00^{aD}	5.32 ± 0.00^{cE}
	2	6.30 ± 0.02^{abA}	5.90 ± 0.01^{aB}	5.60 ± 0.01^{aC}	5.56 ± 0.02^{aD}	5.45 ± 0.00^{bE}	5.33 ± 0.01^{cF}
12 min	0 (Control)	6.25 ± 0.02^{bA}	5.68 ± 0.03^{dB}	5.27 ± 0.02^{fC}	5.12 ± 0.01^{eD}	4.97 ± 0.02^{gE}	4.92 ± 0.01^{hF}
	0.5	6.27 ± 0.01^{bA}	5.77 ± 0.01^{cB}	5.44 ± 0.02^{cC}	5.44 ± 0.03^{cC}	5.32 ± 0.03^{dD}	5.21 ± 0.01^{eE}
	1	6.28 ± 0.03^{abA}	5.92 ± 0.01^{aB}	5.60 ± 0.02^{aC}	5.53 ± 0.01^{abD}	5.51 ± 0.00^{aD}	5.44 ± 0.01^{bE}
	2	6.32 ± 0.00^{abA}	5.91 ± 0.02^{aB}	5.61 ± 0.01^{aC}	5.56 ± 0.01^{aD}	5.53 ± 0.01^{aE}	5.50 ± 0.01^{aF}

$^{a-j}$Different lowercase letters within columns indicate significantly different values base on Duncan's multiple range test (p < 0.05).
$^{A-F}$Different lowercase letters within rows indicate significantly different values base on Duncan's multiple range test (p < 0.05).

Table 4. Titratable acid content according to CaO treatment of mulberry fruits at full maturity at Yecheon in June 2011.

Treatment time (min)	Treatment concentration (g·L^{-1})	Storage time (days)					
		0	2	6	10	12	14
3 min	0 (Control)	0.64 ± 0.00^{cdA}	0.56 ± 0.00^{eB}	0.41 ± 0.00^{fC}	0.36 ± 0.00^{iD}	0.32 ± 0.01^{hE}	0.26 ± 0.01^{kF}
	0.5	0.64 ± 0.01^{dA}	0.61 ± 0.00^{cdB}	0.52 ± 0.01^{eC}	0.44 ± 0.01^{gD}	0.40 ± 0.00^{gE}	0.33 ± 0.00^{iF}
	1	0.65 ± 0.00^{bcA}	0.62 ± 0.01^{cB}	0.60 ± 0.01^{bC}	0.54 ± 0.01^{dD}	0.53 ± 0.00^{eE}	0.48 ± 0.00^{fF}
	2	0.66 ± 0.00^{aA}	0.63 ± 0.00^{bB}	0.61 ± 0.00^{aC}	0.57 ± 0.00^{cD}	0.54 ± 0.00^{dE}	0.50 ± 0.00^{eF}
6 min	0 (Control)	0.65 ± 0.01^{abcA}	0.56 ± 0.00^{eB}	0.41 ± 0.00^{fC}	0.39 ± 0.01^{hD}	0.31 ± 0.00^{iE}	0.29 ± 0.00^{jF}
	0.5	0.65 ± 0.00^{cdA}	0.60 ± 0.00^{dB}	0.54 ± 0.00^{dC}	0.46 ± 0.00^{fD}	0.40 ± 0.00^{gE}	0.36 ± 0.00^{hF}
	1	0.66 ± 0.01^{aA}	0.63 ± 0.00^{bB}	0.60 ± 0.01^{bC}	0.55 ± 0.01^{dD}	0.54 ± 0.00^{dD}	0.52 ± 0.00^{dE}
	2	0.66 ± 0.01^{abA}	0.64 ± 0.00^{aB}	0.62 ± 0.01^{aC}	0.58 ± 0.00^{bD}	0.56 ± 0.01^{cE}	0.53 ± 0.00^{cF}
12 min	0 (Control)	0.65 ± 0.00^{cdA}	0.57 ± 0.01^{eB}	0.40 ± 0.01^{gC}	0.36 ± 0.01^{iD}	0.33 ± 0.01^{hE}	0.25 ± 0.00^{lF}
	0.5	0.65 ± 0.00^{bcA}	0.63 ± 0.01^{bB}	0.56 ± 0.01^{cC}	0.51 ± 0.01^{eD}	0.46 ± 0.01^{fE}	0.43 ± 0.00^{gF}
	1	0.65 ± 0.00^{bcA}	0.64 ± 0.00^{aB}	0.62 ± 0.00^{aC}	0.62 ± 0.00^{aC}	0.59 ± 0.00^{bD}	0.56 ± 0.00^{bE}
	2	0.66 ± 0.00^{abA}	0.64 ± 0.00^{aB}	0.62 ± 0.00^{aC}	0.62 ± 0.00^{aC}	0.60 ± 0.00^{aD}	0.58 ± 0.01^{aE}

$^{a-l}$Different lowercase letters within columns indicate significantly different values base on Duncan's multiple range test (p < 0.05).
$^{A-F}$Different lowercase letters within rows indicate significantly different values base on Duncan's multiple range test (p < 0.05).

concentration and treatment time—had significant effects on titratable acid content ($p > 0.05$). From day 6, compared to the 0.5 g·L^{-1} CaO treatment, titratable acid contents of the samples treated using 1 and 2 g·L^{-1} CaO solutions were higher ($p > 0.05$). The titratable acid contents of the 3, 6, and 12 min CaO-treated samples were similar over the first 6 d ($p > 0.05$). From day 10, the contents of the 12 min CaO-treated samples increased in comparison with the 3 and 6 min CaO treatments ($p > 0.05$). No significant differences were noted between the 1 and 2 g·L^{-1} CaO treatments for 12 min ($p > 0.05$).

Storage

The ideal CaO treatment conditions for maintaining the nutritional components of mulberry fruit were identified as those using 1 and 2 g·L^{-1} CaO for 12 min. Therefore, these two conditions were used to further investigate the effects of CaO treatment on shelflife from microbiological and sensory quality perspectives.

Changes in the microflora of mulberry fruit during storage were evaluated with changes in microbial counts for aerobic mesophilic bacteria, aerobic psychrotrophic bacteria, lactic acid bacteria, and yeast and mold immediately after CaO treatments and during storage (Table 5). The presence of aerobic mesophilic bacteria (3.3 ± 0.2 log CFU/g) and aerobic psychrotrophic bacteria (3.5 ± 0.3 log CFU/g) of untreated fresh mulberry fruit was detected. Aditionally, lactic acid bacteria (1.8 ± 0.4 log CFU/g) as well as yeasts and molds (1.5 ± 0.3 log CFU/g) were present in relatively lower numbers.

Microbial populations decreased in the control and CaO-treated samples after washing, whereas the CaO treatments significantly reduced the microflora in mulberry fruit compared to the control (p > 0.05). Microbial populations increased in all samples during storage; the populations in the CaO-treated samples were maintained at a lower level than those in the control ($p > 0.05$). No significant differences were detected between the 1 and 2 g·L^{-1} CaO treatments up to 12 min ($p > 0.05$). The counts of aerobic mesophilic bacteria and aerobic psychrotrophic bacteria reached more than 8 log CFU/g in the control sample on d 14 while maintained acceptable in CaO treated samples throughout storage period ($p > 0.05$). Populations of lactic acid bacteria, yeasts, and molds increased during storage in the control and CaO-treated samples; however, their counts did not reach 7 and 5 log CFU/g, respectively, in the storage duration. Therefore, lactic acid, yeasts, and molds could not be used as a determinant in the storage study. When analyzed together with the populations of aerobic mesophilic bacteria and aerobic psychrotrophic bacteria, it can be concluded that, from the microbiological point of view, a storage extension of an additional 5 d could be achieved for mulberry fruits treated with 1 and 2 g·L^{-1} CaO for 12 min.

As shown in Table 6, immediately after washing, no significant differences were noted in the same sensory

Table 5. Effects of 1 and 2 g·L^{-1} CaO treatments for 12 min on microbial counts (log CFU/g) of mulberry fruit at full maturity at Yecheon in June 2011.

Microbial	Treatment (g·L^{-1})	Storage Time (days)				
		0	4	8	10	14
Aerobic Mesophilic Bacteria	0 (Control)	3.2 ± 0.3^{a}	5.8 ± 0.2^{a}	6.9 ± 0.4^{a}	7.5 ± 0.3^{a}	8.0 ± 0.4^{a}
	1	1.2 ± 0.3^{b}	2.7 ± 0.4^{b}	4.5 ± 0.3^{b}	5.2 ± 0.3^{bD}	5.8 ± 0.2^{b}
	2	0.6 ± 0.2^{b}	2.9 ± 0.1^{b}	4.4 ± 0.3^{b}	5.0 ± 0.5^{b}	5.2 ± 0.3^{b}
Aerobic Psychrotrophic Bacteria	0 (Control)	3.4 ± 0.3^{a}	5.4 ± 0.2^{a}	6.9 ± 0.5^{a}	7.4 ± 0.4^{a}	8.1 ± 0.3^{a}
	1	0.9 ± 0.2^{b}	3.0 ± 0.2^{b}	4.0 ± 0.4^{b}	4.8 ± 0.5^{b}	5.5 ± 0.5^{b}
	2	0.6 ± 0.1^{b}	2.1 ± 0.3^{b}	3.4 ± 0.2^{b}	4.5 ± 0.2^{b}	5.1 ± 0.4^{b}
Lactic Acid Bacteria	0 (Control)	1.6 ± 0.4^{a}	2.6 ± 0.2^{a}	3.7 ± 0.2^{a}	4.5 ± 0.3^{a}	5.3 ± 0.3^{a}
	1	0.4 ± 0.3^{b}	1.5 ± 0.1^{b}	2.6 ± 0.3^{b}	3.4 ± 0.3^{b}	4.1 ± 0.1^{b}
	2	0.2 ± 0.3^{b}	1.0 ± 0.4^{b}	2.4 ± 0.3^{b}	3.0 ± 0.2^{b}	3.7 ± 0.3^{b}
Yeasts and Molds	0 (Control)	1.7 ± 0.1^{a}	2.7 ± 0.3^{a}	3.5 ± 0.2^{a}	4.0 ± 0.3^{a}	4.7 ± 0.2^{a}
	1	1.1 ± 0.3^{b}	1.8 ± 0.2^{b}	2.5 ± 0.3^{b}	3.0 ± 0.2^{b}	3.9 ± 0.2^{b}
	2	0.4 ± 0.1^{b}	1.1 ± 0.2^{b}	1.6 ± 0.2^{b}	2.1 ± 0.3^{b}	3.1 ± 0.3^{b}

$^{a-b}$Different letters within columns indicate significantly different values base on Duncan's multiple range test ($p < 0.05$)

Table 6. Effects of 1 and 2 g·L^{-1} CaO treatments for 12 min on sensory qualities of mulberry fruit at full maturity at Yecheon in June 2011.

Sensory Quality Attributes	Treatment (g·L^{-1})	Storage Time (days)				
		0	4	8	10	14
Overall Visual Quality	0 (Control)	9.0 ± 0.0^{a}	6.3 ± 0.2^{a}	5.5 ± 0.2^{a}	4.3 ± 0.5^{a}	1.5 ± 0.6^{a}
	1	9.0 ± 0.0^{a}	8.7 ± 0.2^{b}	7.9 ± 0.5^{b}	7.2 ± 0.4^{b}	5.9 ± 0.3^{b}
	2	9.0 ± 0.0^{a}	8.5 ± 0.5^{b}	7.5 ± 0.5^{b}	6.9 ± 0.3^{b}	5.6 ± 0.3^{b}
Off-odor	0 (Control)	1.0 ± 0.1^{a}	2.0 ± 0.3^{a}	2.5 ± 0.2^{a}	3.0 ± 0.6^{a}	4.3 ± 0.4^{a}
	1	1.0 ± 0.0^{a}	1.3 ± 0.2^{b}	1.8 ± 0.3^{b}	2.1 ± 0.3^{b}	2.4 ± 0.3^{b}
	2	1.0 ± 0.0^{a}	1.2 ± 0.1^{b}	1.7 ± 0.2^{b}	2.0 ± 0.3^{b}	2.3 ± 0.6^{b}
Flavor	0 (Control)	1.1 ± 0.1^{a}	2.2 ± 0.1^{a}	2.7 ± 0.1^{a}	3.5 ± 0.3^{a}	4.4 ± 0.4^{a}
	1	1.1 ± 0.1^{a}	1.5 ± 0.3^{b}	1.9 ± 0.3^{b}	2.2 ± 0.4^{b}	2.5 ± 0.3^{b}
	2	1.0 ± 0.2^{a}	1.2 ± 0.3^{b}	1.5 ± 0.4^{b}	2.0 ± 0.3^{b}	2.3 ± 0.4^{b}
Texure	0 (Control)	1.2 ± 0.1^{a}	2.0 ± 0.2^{a}	2.3 ± 0.3^{a}	2.7 ± 0.1^{a}	3.3 ± 0.5^{a}
	1	1.2 ± 0.1^{a}	1.4 ± 0.2^{b}	1.8 ± 0.2^{b}	2.0 ± 0.2^{b}	2.2 ± 0.3^{b}
	2	1.2 ± 0.1^{a}	1.4 ± 0.3^{b}	1.6 ± 0.3^{b}	1.9 ± 0.4^{b}	2.1 ± 0.3^{b}

$^{a-b}$Different letters within columns indicate significantly different values base on Duncan's multiple range test ($p < 0.05$).

quality attributes between the control and CaO-treated samples ($p > 0.05$). Sensory quality declined in all samples as storage time was prolonged. Overall visual quality, off-odor, and flavor qualities of the control were above the acceptability limit after 10 d, while texture became unacceptable at the end of the examined storage duration. In contrast, sensory qualities of the CaO-treated samples remained acceptable throughout the storage period. The samples treated using 1 g·L^{-1} CaO for 12 min maintained better sensory qualities during storage compared to the control ($p > 0.05$). The treatment using 2 g·L^{-1} CaO for 12 min generated higher OVQ, off-odor, flavor, and texture scores than the control ($p > 0.05$). From the sensory quality point of view, a storage prolongation of 6 d was obtained by treatment of mulberry fruits with 1 and 2 g·L^{-1} CaO for 12 min, which was consistent with the microbial growth assay. Furthermore, minimal differences in OVQ, off-odor, flavor, and texture scores were observed for the 1 and 2 g·L^{-1} CaO-treated samples ($p > 0.05$). Therefore, the 1 g·L^{-1} CaO treatment for 12 min was established as more acceptable in maintaining the sensory qualities of mulberry fruit during storage.

Discussion

The efficacy of CaO treatment on the inhibition of antibacterial activity, extension of storage, and elimination of pesticide residues has been previously established. In terms of strong antibacterial activity, Sawai et al. (2001c) reported that CaO in the heated scallop-shell powder form effectively demonstrated bactericidal activity against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Bacillus subtilis*. Sawai et al. (2003, 2007) also reported that CaO (heated scallop-shell powder) exhibited substantial sporicidal activity against *B. subtilis* spores. Sawai (2003) also found that metallic oxide (ZnO, MgO, and CaO) powders effectively demonstrated antibacterial activity against *S. aureus* and *E. coli*; of the examined powders, CaO was the most effective against *E. coli*, followed by MgO and ZnO. CaO (heated scallop-shell powder) has also been demonstrated to have high disinfection efficacy against *E. coli*, *S. typhimurium*, *S. aureus*, and *B. subtilis*. The good disinfection efficacy of CaO (heated scallop-shell powder) against *Salmonella* biofilm (Nagasawa et al., 2011), *S. aureus* biofilm (Sawai et al., 2013) and *E. coli* ATCC 25922 biofilm (Kubo et al., 2013) has also been reported. With regards to storage, the efficacy of CaO treatment on the storage extension of both kimchi (Choi et al., 2006) and tofu (Sung et al., 2012) has been shown. Lee et al. (2005) reported that CaO is highly useful as a natural eliminator of pesticides in mini-tomatoes.

CaO treatment has been proven to be effective in preserving a variety of fruits and vegetables. Gandhi and Matthews (2003) reported that CaO was effective in the elimination

of *Salmonella* from alfalfa seeds and sprouts. Bari et al. (1999) reported that calcinated calcium was useful in controlling *E. coli* O157H7 contamination during the production of fresh radish sprouts. Sawai et al. (2001b) also reported that CaO treatment effectively reduced the aerobic bacterial count in shredded cabbage. CaO treatment also exhibited good bactericidal activity against total aerobic bacteria, *E. coli*, *Bacillus cereus*, *Listeria monocytogenes*, *S. aureus*, and *S. typhimurium* found in sesame leaf (Yeon et al., 2005) and lettuce (Kim et al., 2006).

At present, there is a lack of available scientific literature concerning the preservation of mulberry fruit. Lin et al. (2006) prolonged the storage of mulberry fruit for 5-6 d at 5-6°C by washing the fruits with solutions containing 5 $g \cdot L^{-1}$ potassium sorbate, 5 $g \cdot L^{-1}$ sodium benzoate, or 30 $g \cdot L^{-1}$ sodium propionate for 2 min. Chen et al. (2011) demonstrated that the storage of mulberry fruit could be prolonged for 14 d under storage at -1°C by immersion in solutions containing 20, 60, and 80 $mg \cdot L^{-1}$ ClO_2 solutions for 5, 10, and 15 min.

The results obtained in this study revealed the beneficial effects of CaO treatment to maintain the flavonoid, ascorbic acid, reducing sugar, and titratable acid contents of mulberry fruit. Treatment of the fruit with 1 $g \cdot L^{-1}$ CaO for 12 min could prolong the storage of mulberry fruit up to 14 d compared to 4 d for the control sample. From these results, CaO treatment is comparatively more efficient, convenient, and low-cost in practical storage applications.

With regard to the effects of CaO treatment on nutritional quality, different results have also been reported. Sawai et al. (2001b) have reported that ascorbic acid contents of CaO-treated shredded cabbage were higher than those of the control; this effect exhibited a concentration dependence. In our study, ascorbic acid contents of the CaO-treated samples were lower than those of the control in the early days of the storage duration; however, as storage time was prolonged, CaO treatment of the fruit showed the ability to slow down the loss of nutritional components.

Choi et al. (2006) have reported that the titratable acid content of CaO-treated kimchi increased as the fermentation time was increased. The addition of 0.05%, 0.1% and 0.5% CaO in powdered shell form yielded acid contents of 1.19%, 1.18%, and 0.59%, respectively, where total acidity was calculated as a percentage of lactic acid. In our study, the titratable acidities of the CaO-treated samples were lower than those of the control in the early days of storage; however, as storage time was prolonged, the CaO treatment was shown to slow down the loss of nutritional components. In the present study, total acidity was calculated as a percentage of citric acid.

Previously reported studies have described the remarkable antimicrobial efficacy of CaO treatment on the microbiota of fruits and vegetables. For example, Yeon et al. (2005) reported that CaO-treated sesame leaf showed a concentration-dependent reduction of total aerobic bacteria by 0.55-1.49 log 10 CFU/g. Kim et al. (2006) reported the effectiveness of CaO treatment of lettuce, showing a dramatic reduction rate of 5.9 × 103 for total aerobic bacteria. Choi et al. (2006) reported that CaO-treated kimchi showed a rapid increase in the total number of microbes up to the 5th day of fermentation; up to 10th day of fermentation, no significant difference ($p > 0.05$) was detected among the control and the test groups treated with 0.05% and 0.1% CaO in powdered oyster-shell form. However, the addition of 0.5% shell powder showed a significant ($p < 0.05$) inhibitory effect on microbial growth.

Sung et al. (2012) have reported that CaO-treated tofu showed an increase in the total number of aerobic bacteria (3.93-4.18 log CFU/g) up to the 3rd day of storage, with significant differences noted between treatment groups. After the 3rd day, however, aerobic bacteria counts in the control sample increased most rapidly with prolonged storage time; aerobic bacteria counts of CaO-treated tofu was inhibited the multiplication according treatment concentration. Moreover, yeast and mold counts for the control showed the fastest growth—almost 7 log CFU/g in the experimental group—by the 7th day of storage, while those counts for treated tofu increased gradually up to the 8th day of storage. CaO-treated tofu showed an increased yeast and mold count of almost 7 log CFU/g by the 9th day of storage. These results suggested that treatment of tofu with CaO delayed the growth rates of yeast and mold. In our study, the mulberry fruit samples treated with 1 and 2 $g \cdot L^{-1}$ CaO concentrations for 12 min exhibited significantly reduced microflora numbers ($p < 0.05$); the CaO-treated samples had microflora populations at a lower level during storage compared to the control ($p < 0.05$).

Studies have been performed to describe the sensory qualities of fruits and vegetables subjected to CaO treatment. For example, Bari et al. (1999) found that a 0.4% CaO treatment did not affect the quality parameters (taste, color, and appearance) of radish sprouts in comparison to untreated samples. Choi et al. (2006) reported that the sour taste of kimchi treated with 0.5% CaO in powdered oyster-shell form was lower than that of the control; however, significant differences were not seen ($p > 0.05$) among the control and 0.05%, and 0.1% shell powder treatments. The bitter taste of kimchi treated with 0.5% shell powder was lower

than that of the control without a significant difference ($p > 0.05$). The increased addition of shell powder resulted in an increase in crispness. In terms of overall taste evaluation, the control kimchi was rated lower than that treated with shell powder; a significant difference ($p < 0.05$) was observed between the control and kimchi treated with 0.5% shell powder.

In our study, sensory quality declined in all samples as storage time was prolonged. For the samples treated with 2 g·L^{-1} CaO for 15 min, higher OVQ, off-odor, flavor, and texture scores were generated in comparison to the control ($p > 0.05$). From these results, the treatment using 1 g·L^{-1} CaO for 12 min was established as more acceptable in maintaining the sensory qualities of mulberry fruit.

Presently, mulberry cultivation in mulberry-growing countries has focused on promoting foliage production to feed silkworms (*Bombyx mori* L.) rather than enhancing fruit sales, which leads to a shortage of marketable mulberry fruit. However, this situation is not for a lack of potential markets for mulberry fruit, but largely attributed to its perishability. From this perspective, application of the as-described novel CaO treatment for mulberry fruit preservation seems to be of far-reaching significance for expanding the marketability of this fruit. Our research provides some evidence for the feasibility of CaO for prolongation of mulberry fruit storage in practical food industries. However, the above conclusions related to CaO treatment of mulberry fruit were drawn under specific experimental conditions; thus, more work is needed to determine whether this protocol can be generalized to other produce.

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