Effects of Mild Heat Treatment on Microbial Growth and Product Quality of Packaged Fresh-Cut Table Grapes

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ABSTRACT: The changes in packaged fresh-cut grape quality and microbial growth as affected by mild heat treatments and the retention of grape cap stems during 5 °C storage were evaluated. Each individual grape was either manually pulled off (stemless) from the stems, or cut (cut stem) to allow for a 1- to 2-mm cap stem remaining on the berry. The samples were sanitized in 100 mg/L chlorine solution for 1 min, followed by a mild heat treatment in a water bath (45 °C, 8 min) or an oven (55 °C, 5 min). After cooling, the berries were packaged in rigid trays sealed with a gas permeable film and stored at 5 °C. Product quality and decay rate were evaluated periodically during storage. The results indicate that in the package headspace for hot water treatment of stemless grapes, partial pressures of O_2 declined significantly ($P < 0.05$) less and C_2H_4 increased significantly ($P < 0.001$) less than for the control and hot air treatment. Stem removal and heat treatment had significant ($P < 0.05$) effects on the decay rate of grapes during storage. Hot water treatment maintained a significantly lower decay rate than the control and hot air treatment throughout the entire storage. Color and texture were not significantly ($P > 0.05$) affected by either heat treatment or stem removal. Grapes that retained the cap stems and received hot water treatment had the lowest decay rate and lowest microbial growth with the absence of any negative impact on grape color, texture, and flavor.

Keywords: hot water treatment, microbial growth, packaging, quality, table grapes

Introduction

Packaged fresh-cut fruits and vegetables are becoming increasingly popular because they are convenient and nutritious snack alternatives. The fresh-cut produce industry has experienced a double-digit growth rate in response to increased demand by consumers. However, fresh-cut produce has limited shelf stability due to rapid quality deterioration (Watada and others 1996; Jacxsens and others 2002). The major technical issue associated with packaged fresh-cut grapes is decay. Few fresh-cut grapes are currently found in supermarkets and food service distribution chains. Limited studies have been published regarding the optimal conditions for maintaining quality of these products.

Mild heat treatment has recently emerged as a potential alternative to chemical treatment in maintaining quality of fresh and fresh-cut produce. Researchers found that a mild heat treatment was beneficial to maintain the quality of packaged fresh-cut pears (Abreu and others 2003), reduce chilling injury in tomatoes (Lurie and others 1993; Lurie and Sabehat 1997; Lurie 1998), and retain texture in fresh-cut cantaloupes (Lamikanra and others 2005). The mechanism by which a heat treatment maintained quality of fresh produce is believed to be associated with the synthesis of a heat shock protein (Saltveit 1997; Loaiza-Velarde and others 2003). Loaiza-Velarde and Saltveit (2001) found that a hot water treatment at 50 °C for 90 s, applied either after or before cutting, effectively inhibited lettuce and celery browning by diverting protein synthesis to heat shock protein and phenylalanine ammonia-lyase. Studies reported that a mild heat treatment significantly reduced the decline of peroxidase super oxide dismutase activities of grapes (Kou and others 2006a, 2006b) and inhibited ripening in various fruits (Paull and Chen 2000). Kou and others (2006a, 2006b) further compared the hot water and hot air treatment temperature of 40, 45, 50, 55, 60, and 65 °C and contact time of 2, 4, 6, 8, and 10 min on packaged cluster "Red Globe" and "Kyoho" grapes. They found that 45 °C for 8 min and 55 °C for 5 min provided the best hot water and hot air treatments, respectively.

Current commercial practice for preparation of fresh-cut grapes includes removal of cap stems. In our previous studies on fresh-cut grapes, we noticed that decay and quality loss were primarily caused by tissue injury sustained during stem removal. Physical injury often stimulates oxygen uptake, because of increased respiration rate and nonmitochondrial activities (for example, lipoxygenase, polyphenol oxidase, peroxidase) (Taiz and Zeiger 1991). In addition, tissue injury sustained during stem removal and the exposure of internal tissues resulting from stem removal make stemless grapes susceptible to microbial growth, product decay, and quality deterioration.

The main objectives of this study were to evaluate the effects of different preparation methods of fresh-cut grapes (pulling stems out entirely, or leaving 1- to 2-mm stems on the fruits) on quality maintenance and microbial growth, and the response to hot water and hot air treatments of grapes both with and without cap stems.

Materials and Methods

Sample preparation

Table grapes (*Vitis vinifera* L.) were obtained from W.D Class wholesale produce market in Jessup, Md., U.S.A. The commercially packaged grapes were transported (within 30 min) to the Agricultural Research Center in Beltsville, Md., U.S.A. and used immediately. The grapes were prepared according to the following

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2 methods: (1) the grape stems were manually removed so that the grapes were completely stemless; (2) the rachis of the grapes was removed and the cap stems (pedicels) were cut short with a pair of sanitized scissors so that the grapes retained 1 to 2 mm of cap stem. Grapes were sorted to eliminate undersize (diameter less than 15 mm) or damaged grapes. The grapes were then sanitized with 100 mg/L chlorine solution (NaOCl) adjusted to pH 6.5 with HCl for 1 min followed by draining and air-drying.

Heat treatment, storage, and sampling

Each 300-g sample of grapes was placed into a mesh bag (Linens N' Things, Clifton, N.J., U.S.A.) and was subjected to either hot water or hot air treatment. Hot water treatment was conducted by immersing the bag of grapes in a water bath at 45 °C for 8 min, followed by draining and air-cooling. Care was taken to ensure that all berries were completely submerged in the water during the hot water treatment. Hot air treatment was performed by placing the grapes contained in the mesh bag in an oven set at 55 °C for 5 min. Care was taken to ensure that the grapes did not touch the metal surface of the oven. A large pan of water was placed in the oven on the day prior to treatment to maintain the desired humidity and thermocouples were used to monitor the temperature during the entire heating process. The grapes were cooled before packaging.

Modified atmosphere packaging

Each 300-g sample of grapes, including those from both heat treatment groups and untreated controls, was packaged in a 13.5 × 17 × 3 cm^3 rigid polypropylene tray (Pactiv Corp., Lake Forest, Ill., U.S.A.) and sealed with a 29.2 pmol/s/m^2/Pa oxygen transmission rate film. The packages were stored at 5 ± 1 °C for 14 d with quality evaluation performed on days 0, 7, and 14.

Analysis of grape respiration rate and package atmospheres

Grapes, 300 g each, were placed in sealed containers at 5 °C with humidified air flowing through at a rate of 20 mL/min. The CO_2 content of the outlet streams from sample containers was monitored every 6 h using a gas chromatograph (GC; HP 5890a, Hewlett Packard Co., Rockville, Md., U.S.A.) fitted with a Hayesep Q column (2.4 m × 3 mm) and a thermal conductivity detector. Respiration rate is expressed in unit of mg CO_2/kg/h.

The partial pressures of O_2 and CO_2 in the headspace of grape packages were measured using O_2/CO_2 infrared gas analyzers (Model S-3A/I and Model CD-3A, respectively; Ametek Pittsburgh, Pa., U.S.A.). Package atmospheres are expressed in units of kPa.

Ethylene levels in the headspaces of the sealed packages were measured using a gas chromatography (HP 5890a) equipped with a GS-Q column (3.0 m × 0.53 mm; J & W Scientific, Folsom, Calif., U.S.A.) and a flame ionization detector according to Saftner (1999). Ethylene levels were expressed in units of μL/L.

Color, texture, and decay measurement

Both color and texture were determined using 30 grapes of each replicate. Grape color (L^*, a^*, b^*) was determined on 2 sample points on the opposite sides of the equatorial region of each grape using a Minolta chroma meter (model CR-300, Tokyo, Japan) calibrated with

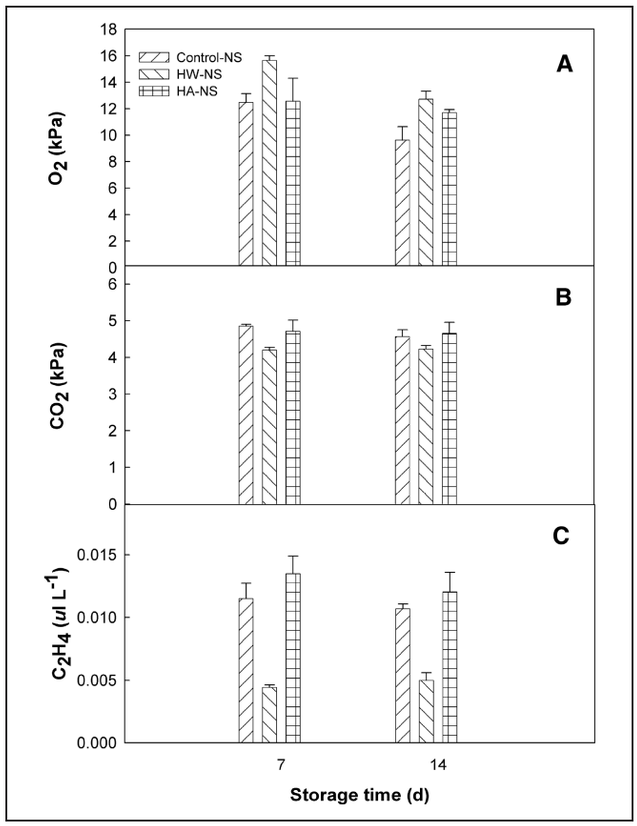

Figure 1 — Changes of partial pressures in (A) O_2, (B) CO_2, and (C) C_2H_4 in the headspace of packages containing stemless grapes (NS) treated with hot water (HW), hot air (HA), and the control. Vertical bars represent means of 3 replications ± SE.

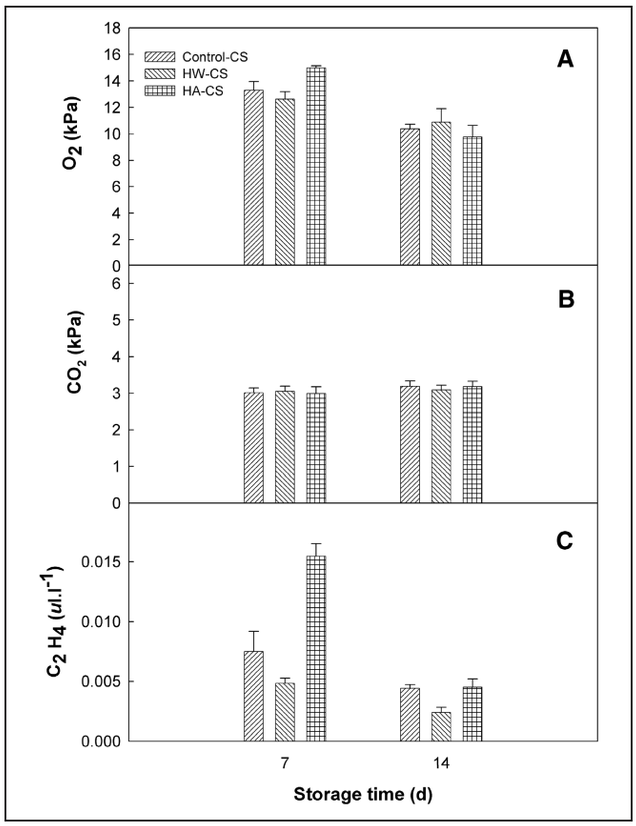

Figure 2 — Changes of partial pressures in (A) O_2, (B) CO_2, and (C) C_2H_4 in the headspace of packages containing grapes retaining 1- to 2-mm cap stems (CS) treated with hot water (HW), hot air (HA), and the control. Vertical bars represent means of 3 replications ± SE.

a white tile. The color values of a^* and b^* were further converted into hue angle [hue = $\tan^{-1}$ (b/a)] and chroma [chroma = $(a^2 + b^2)^{0.5}$] according to Nunes and Emond (1998).

The firmness of grapes was determined using a texture analyzer (Model TA-XT2, Texture Technologies Corp., Scarsdale, N.Y., U.S.A.). Compression firmness was measured with a 38-mm-dia cylindrical probe to a deformation of 10 mm at 2.0 mm/s. The peak force was recorded and used to indicate firmness of grapes.

Decay rate was calculated by dividing the number of grapes in each package showing any visible decay appearance by the total number of grapes in that package and multiplying the dividend by 100 (Loaiza and Cantwell 1997).

Table 1—Color changes of packaged stemless grapes stored at 5 °C.

Color parameter	Treatment	Storage time (d) 0	7	14
L^*	Control-NS[a]	32.9 ± 0.4	32.9 ± 0.2	33.0 ± 0.2
	HW-NS	33.0 ± 0.3	33.0 ± 0.2	32.7 ± 0.2
	HA-NS	32.8 ± 0.4	33.0 ± 0.2	32.3 ± 0.2
Hue	Control-NS	28.6 ± 1.8	30.8 ± 1.5	32.5 ± 1.5
	HW-NS	25.6 ± 1.8	30.8 ± 1.67	28.8 ± 1.23
	HA-NS	31.8 ± 2.6	33.5 ± 1.6	32.5 ± 1.7
Chroma	Control-NS	7.24 ± 0.3	7.64 ± 0.2	8.13 ± 0.2
	HW-NS	7.74 ± 0.2	7.73 ± 0.2	8.12 ± 0.2
	HA-NS	7.11 ± 0.2	7.09 ± 0.1	7.39 ± 0.1

[a]Control = no heat treatment; HW = hot water treatment; HA = hot air treatment; NS = stemless grapes.

Sensory evaluation

A trained sensory panel of 10 individuals was used to evaluate the quality attributes of grapes with cut stems, either treated with hot water, or without heat treatment, and stored at 5 °C for 28 d. The panelists had previously received intensive training on sensory evaluation techniques and were experienced at assessing the sensory profile of fresh fruits and vegetables. The grapes were first destemmed and then placed in the sampling trays (3 to 5 grapes per tray) labeled with random 3-digit numbers. Sample trays, including a tray with blind samples, were presented in a random order to the panelists in single cabins in the sensory evaluation laboratory. The panelists were required to cleanse their palates with a bite of low-salt saltine crackers, a sip of room-temperature water, and a 30-s time

Table 2—Color changes of packaged grapes retaining 1- to 2-mm cap stems stored at 5 °C.

Color parameter	Treatment	Storage time (d) 0	7	14
L^*	Control-CS[a]	32.9 ± 0.3	34.8 ± 0.3	35.1 ± 0.3
	HW-CS	34.6 ± 0.4	34.2 ± 0.4	34.9 ± 0.3
	HA-CS	33.3 ± 0.4	35.1 ± 0.4	35.1 ± 0.4
Hue	Control-CS	26.7 ± 1.50	33.5 ± 1.8	30.5 ± 1.8
	HW-CS	33.0 ± 1.9	31.5 ± 1.7	31.7 ± 1.5
	HA-CS	27.8 ± 2.0	35.1 ± 2.0	33.4 ± 2.1
Chroma	Control-CS	10.5 ± 0.3	10.9 ± 0.3	11.3 ± 0.3
	HW-CS	9.78 ± 0.3	10.6 ± 0.3	10.7 ± 0.3
	HA-CS	8.58 ± 0.3	11.0 ± 0.3	11.0 ± 0.3

[a]Control = no heat treatment; HW = hot water treatment; HA = hot air treatment; CS = grapes retaining 1- to 2-mm cap stems.

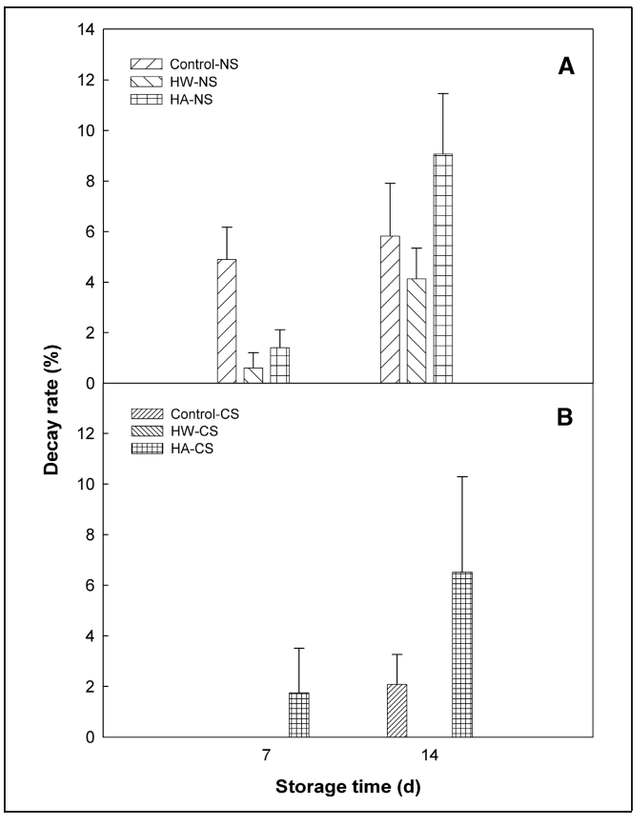

Figure 3—Decay rate of packaged fresh-cut table grapes stored at 5 °C; panel A—stemless grapes (NS) and panel B—grapes retaining 1- to 2-mm cap stems (CS). HW = hot water treatment; HA = hot air treatment; control = no heat treatment. Vertical bars represent means of 3 replications ± SE.

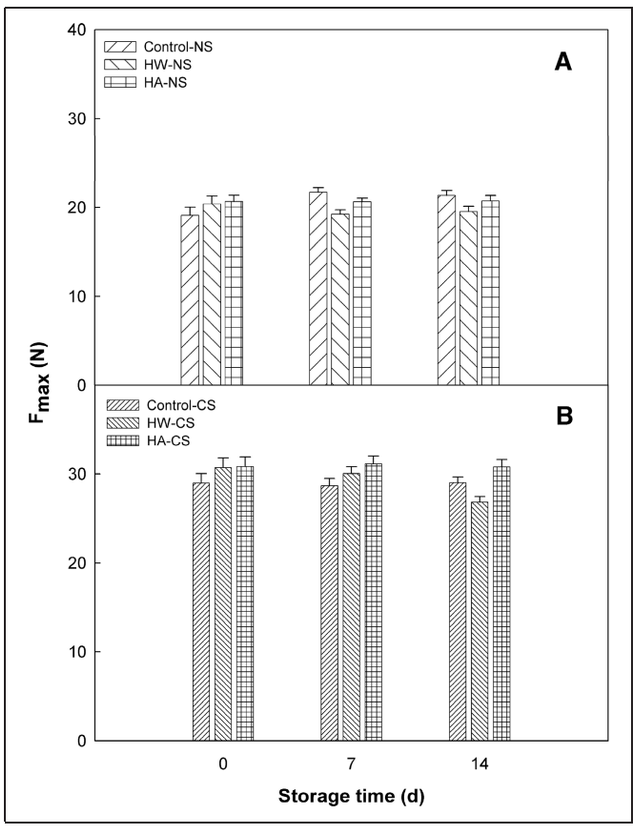

Figure 4—Changes in texture of stemless grapes (panel A; NS) or grapes retaining 1- to 2-mm cap stems (panel B; CS) that were treated with hot water (HW), hot air (HA), or no heat treatment (control). Vertical bars represent means of 3 replications ± SE.

lag before every sample. The sensory attributes of grapes in terms of skin toughness, grape flavor, sweetness, sourness, juiciness, and firmness were evaluated on unstructured 100-mm scales from none (zero) to very strong (100). Overall visual quality and eating quality were rated from bad (zero) to excellent (100), following a similar procedure by Saftner and others (2002). On-screen ballots were prepared and data were collected using Compusense Five (Compusense Inc., Guelph, Canada). All 3 replications were evaluated by the same sensory panel on 3 consecutive days.

Microbial enumeration

Samples, each containing 25 g of grape tissue, taken from 16 grapes per replicate were macerated in 225 mL PBS, with a model 400 Lab Stomacher (Seward Medical, London, U.K.) for 1 min at 260 rpm in filtration stomacher bags. A 50-μL sample of each filtrate or its appropriate dilution was logarithmically spread on agar plates with an automatic spiral plater (Autosprial^{TM}, Don Whitley Scientific Ltd., West Yorkshire, U.K.). Enumeration of the selected microorganisms was performed with the following culture media and conditions: (1) total aerobic mesophilic bacteria were plated on tryptic soy agar (TSA, Difco Lab, Sparks, Md., U.S.A.) and incubated at 30 °C for 24 to 48 h; (2) yeasts and molds were plated on potato dextrose agar (PDA, Difco Lab) supplemented with 200 μg/mL chloramphenicol and incubated at 30 °C for 48 h; (3) lactic acid bacteria (LAB) were plated on lactobacilli Man-Rogosa-Sharpe agar (MRS, Difco Lab) and incubated at 30 °C for 72 h under 20 kPa CO_2 and 5 kPa O_2 provided with a water-jacketed incubator with automatic gas control (Forma Scientific Inc., Marjetta, Ohio, U.S.A.). Microbial colonies were counted using a Protos Colony Counter (Model 50000; Synoptics, Cambridge, U.K.) and reported as log CFU/g of tissue.

Experimental design and statistical analysis

The experiment was conducted using a completely randomized design with 3 replications. A preliminary experiment on grapes was run prior to the experiment reported here. Data were analyzed as a 2-factor linear model using the PROC MIXED procedure (SAS 1999)

Figure 5—Sensory attributes of grapes retaining 1- to 2-mm cap stems (CS) that were treated with hot water (HW) or the control. The samples were stored at 5 °C for 28 d before evaluation by a panel of 10 trained judges. For each attribute, the score is given as the value at which the axis labeled with that attribute is intersected. Ordinates of axes are scores, shown in increments of 20 on a 100-point scale.

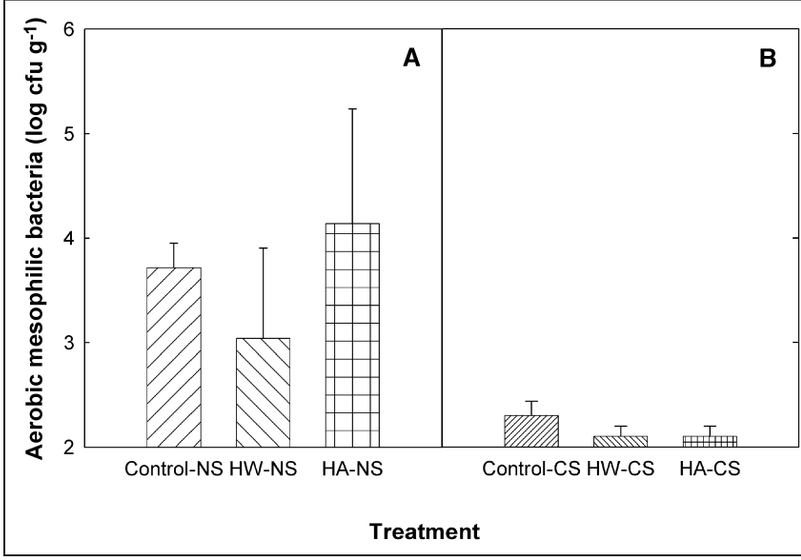

Figure 6—Aerobic mesophilic bacterial populations on fresh-cut grapes stored at 5 °C for 14 d; (A) stemless grapes and (B) grapes retaining 1- to 2-mm cap stems. HW = hot water treatment; HA = hot air; control = no heat treatment. Vertical bars represent means of 3 replications ± SE. Detection level = 100 CFU/g.

with treatment and storage time as the factors. Prior to the current experiment, a series of experiments were conducted to identify the optimum hot water and hot air treatment temperature and duration to be used in this experiment. Differences of least squared means were considered to be significant at $P < 0.05$.

Results and Discussion

Respiration rate and gas composition

The respiration rate as CO_2 evolution of grapes with and without stems ranged from 0.63 ± 0.03 to 0.77 ± 0.12 and 0.68 ± 0.06 to 0.86 ± 0.13 mg/kg/h, respectively, during 14-d storage period. The respiration rate of grapes with stem removed was higher than that of grapes with cap stems remaining intact. This result is a consequence of the damage sustained by the grape tissue when the cap stem is pulled out, because the damaged tissue stimulates high oxygen uptake (Taiz and Zeiger 1991). Respiration rates for hot water and hot air treated grapes with stems ranged from 0.50 ± 0.08 to 0.63 ± 0.11 and 0.55 ± 0.05 to 0.68 ± 0.10 mg/kg/h during the testing. This is similar to the findings from Kou and others (2006b) with hot water treated "Kyoho" grapes. Other researchers have also reported inhibition of respiration rate by heat treatment for tomatoes (Cheng and others 1988) and mangos (Mitcham and McDonald 1993).

Oxygen partial pressures in the headspace of the grape packages decreased significantly ($P < 0.05$) during storage (Figure 1). Among all packages containing stemless grapes, O_2 partial pressure in the control (no heat treatment) and hot air treated samples decreased rapidly, reaching average values of 12.5 and 12.6 kPa O_2, respectively, on day 7 and 9.6 and 11.7 kPa O_2 on day 14 (Figure 1A) while the O_2 partial pressure decreased at a significantly ($P < 0.05$) slower rate in the packages containing hot water treated samples. The changes in CO_2 partial pressure (Figure 1B) followed a reverse trend in comparison to O_2. Packages containing hot water treated grapes had significantly ($P < 0.05$) lower CO_2 partial pressure than the controls after 7 d. The same tendency was observed after 14 d, but the difference was not significant ($P > 0.05$). All of these findings suggest that hot water was beneficial in maintaining a lower tissue metabolic rate in the stemless grapes during storage. The benefit of hot water treatment in maintaining lower tissue metabolic rate is more pronounced in terms of ethylene production (Figure 1C). Although all samples accumulated ethylene over time, the ethylene partial pressure in the headspace of the packages containing hot water treated grapes was at least 50% less than the control and hot air treated samples.

The changes in O_2, CO_2, and C_2H_4 partial pressures in the packages containing grapes with stems followed similar trends as in those containing stemless grapes, except that the benefit of hot water treatment in maintaining a high O_2 and lower CO_2 in the headspace of the packages was less pronounced in this group of grapes than those with stemless grapes (Figure 2A and 2B). However, hot water treatment also maintained a lower ethylene production and accumulation than the control and those treated with hot air (Figure 2C). This finding is consistent with other studies, which indicate that effective heat treatments inhibit ethylene production (Lurie 1998). Although the exact mechanisms for the reduced O_2, and elevated CO_2 and ethylene levels measured in the headspace of the hot air treated grapes, compared to those obtained for hot water and control treatments are unknown, the hot air treatment conducted in this study (55 °C for 5 min) may have caused tissue injury that simulated increased metabolic rate.

Decay rate and product quality

Storage duration, stem removal, and heat treatment significantly affected the decay rate. For stemless grapes, decay percentages were 4.8%, 0.6%, and 1.4% for the control, hot water, and hot air treatments, respectively, on day 7, and increased to 5.8%, 4.1%, and 9.1%, respectively, on day 14 (Figure 3A). Considerable mold growth was observed on hot air treated grapes on day 14.

Among grapes with stems, no decay was found in either control or hot water treated samples while a few decayed berries (1.8%) were observed in hot air treatment on day 7 (Figure 3B). While the percent decay in the control and hot air treated samples increased from day 7 to day 14, no decay was found in the hot water treated samples, suggesting that hot water treatment was effective in reducing grape decay during storage. The beneficial effect of hot water in reducing decay was further confirmed by extending the storage of grapes with cap stems to 28 d; the control had a 5.7% decay rate whereas hot water treated samples had only a 1.5% decay rate. Similar results were found with packaged cluster "Red Globe" grapes in our previous study (Kou and others 2006a, 2006b).

Stem removal had an important effect on the percent decay of grapes with or without heat treatment (comparing Figure 3A to 3B). More decayed berries were observed in packages of stemless grapes than in those that retained stems in all of the corresponding

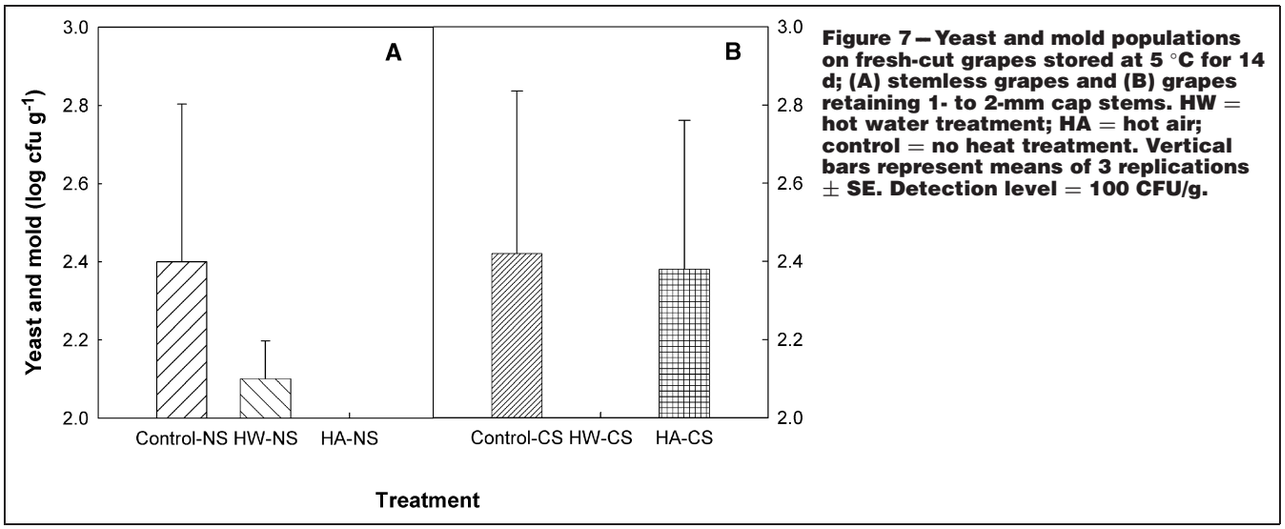

Figure 7 — Yeast and mold populations on fresh-cut grapes stored at 5 °C for 14 d; (A) stemless grapes and (B) grapes retaining 1- to 2-mm cap stems. HW = hot water treatment; HA = hot air; control = no heat treatment. Vertical bars represent means of 3 replications ± SE. Detection level = 100 CFU/g.

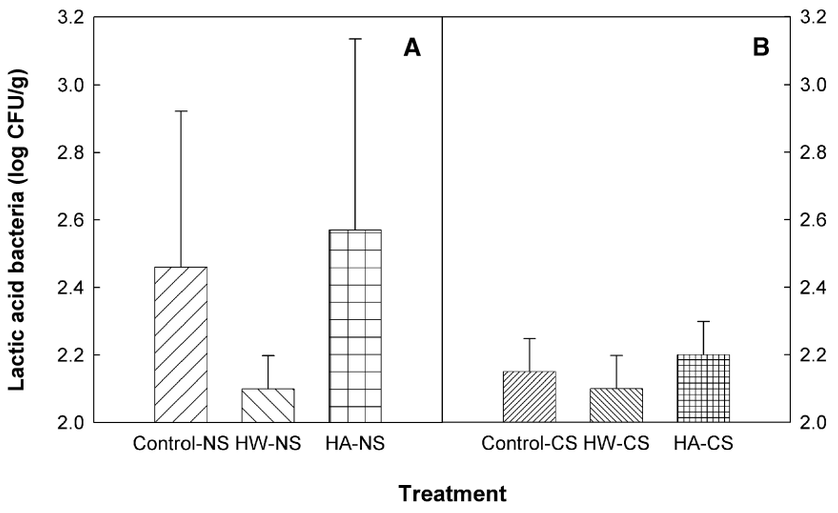

Figure 8—Lactic acid bacterial populations on fresh-cut grapes stored at 5 °C for 14 d; (A) stemless grapes and (B) grapes retaining 1- to 2-mm cap stems. HW = hot water treatment; HA = hot air; control = no heat treatment. Vertical bars represent means of 3 replications ± SE. Detection level = 100 CFU/g.

treatments. The damages sustained during grape removal (tearing out the stems) and the openings created after stem removal may have made the grape berries more susceptible to microbial growth and decay.

Grape color varies largely among each individual fruit and the different locations of the same fruit. Color of grapes both with and without stems was stable during 5 °C storage for 14 d and there were no significant ($P > 0.05$) differences in terms of lightness (L^*), hue angle, or chroma, over time and among treatments (Table 1 and 2).

Both hot water and hot air treatments displayed slightly higher firmness readings than the control on day 0, regardless of whether they retained stems. However, no significant difference ($P > 0.05$) was found among the treatments during storage (Figure 4A and 4B).

Because of the high quality and low decay rate on grapes with cut stems that were treated with hot water or the control at the end of 14 d, additional decay rate and sensory evaluation was performed on these samples after 28 d in storage at 5 °C. The results indicate that hot water treated grapes with cut stems again had significantly ($P < 0.01$) lower decay rate (1.5%) than the control (5.7%). The sensory evaluation results indicate that the grapes with cut stems that received a hot water treatment had similar grape flavor intensity and overall eating quality, slightly higher visual color acceptability, and was slightly more sweet, less tart, and firm; no significant difference was found between the control and hot water treated samples in any of the sensory attributes tested (Figure 5). This suggests that hot water was beneficial in reducing decay rate without altering the flavor and taste of grapes.

Microbial growth

Total aerobic mesophilic bacterial populations on the stemless grapes after 14-d storage were 0.7 and 1.1 log CFU/g lower in hot water treated samples than those found on control and hot air treatments, respectively (Figure 6A). A similar trend was also demonstrated on grapes with stems (Figure 6B). However, neither of these differences reached a statistically significant level.

There was a large variation in yeast and mold counts, because several plates exhibited no growth. Consequently, there was no significant difference found between treatments. However, there was little or no growth on hot water and hot air treatments on any plates, while more growth was observed on some control plates (Figure 7A). No yeast growth was observed for hot water treated grapes with stems on day 14 (Figure 7B), suggesting that hot water treatment was beneficial in inhibiting fungal growth.

Lactic acid bacterial counts of the hot water treatment were lower than in control and hot air treated fruit after 14-d storage (Figure 8A and 8B). There was no significant difference in lactic acid bacterial counts between grapes with or without stems.

Low microbial counts were observed for all treatments, frequently below detection limits. Consequently, there are few significant differences in spite of observed trends.

Conclusions

Grapes that retained 1 to 2 mm of cap stem maintained significantly better quality than those without stems. Hot water treatment at 45 °C for 8 min significantly reduced the decay rate for grapes both with and without stems. Hot water treated grapes retaining 1- to 2-mm cap stems maintained high quality for 14 d with no decay, and had the lowest population of yeast and mold, lactic acid bacteria, and total mesophilic aerobic bacteria in comparison to all other treatments. Hot water treatment of grapes combined with close cutting of their stems is an effective process to control decay and retain quality.

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