

Heat Treatments Control Extension Growth and Enhance Microbial Disinfection of Minimally Processed Green Onions

M.I. Cantwell¹, G. Hong², and T.V. Suslow³

Department of Vegetable Crops, One Shields Avenue, University of California, Davis, CA 95616-8631

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Abstract. Extension growth of minimally processed (removal of roots and compressed stem) green onions (*Allium cepa* L. × *A. fistulosum* L.) was greatly reduced by storage in air at 0 °C, while growth of 10–20 mm occurred at 5 °C over 10 days. Heat treatments of 52.5 and 55 °C water for 4 and 2 min, respectively, were especially effective in reducing growth to less than 5 mm during 12–14 days at 5 °C. Growth was inhibited irrespective of whether the heat treatments were applied before or after cutting. Heat treatments resulted in higher average respiration rates during 12 days at 5 °C, but did not affect the overall visual quality or shelf life. Treatments with 52.5 °C water alone or in combination with different chlorine concentrations (50 to 400 mg·L⁻¹ NaOCl, pH 7.0) were more effective than use of water or chlorine solutions at 20 °C for initial microbial disinfection.

Minimal or fresh-cut processing of vegetables provides convenience to food service and retail customers, but may result in limited postcutting shelf life because of undesirable physiological changes (Cantwell, 1997; Ohlsson, 1994; Saltveit, 1997). A food service green onion product now available is one in which most of the roots are cut off, senescent and damaged outer leaves are removed, and leaves are trimmed to a length of 26–28 cm. A more desirable product for consumers is one in which both the roots and the compressed stem are removed, but this degree of preparation usually results in extension growth of the white inner leaf bases. This extension is also referred to as “telescoping” and can rapidly reduce marketability of the fresh-cut product.

Heat treatments have been demonstrated to be effective as a nonchemical means of improving postharvest quality of a range of horticultural products. Heat treatments are usually applied as hot water dips, vapor heat, or hot air treatments (Lurie, 1998). They may affect ripening and protect against physiological disorders (Klein and Lurie, 1992), and

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¹Extension Postharvest Specialist; to whom reprint requests should be addressed. E-mail address: micantwell@ucdavis.edu

²Visiting Scientist.

³Extension Postharvest Specialist.

have been used as an effective alternative treatment for decay control (Cantwell and Nie, 1996; Conway et al., 1994). Heat shock treatments prevented browning of minimally processed lettuce (*Lactuca sativa* L.) (Loaiza-Velarde et al., 1997) and reduced discoloration of avocado (*Persea americana* Mill.) pulp prepared from treated fruit (Trejo-González et al., 1992). Growth-related phenomena can also be affected by heat treatments. Short hot water dips controlled geotropic curvature in asparagus (*Asparagus officinalis* L.) (Paull and Chen, 1999), and reduced sprouting and spoilage of potatoes (*Solanum tuberosum* L.) with no loss in quality (Ranganna et al., 1998). Elongation of isolated stems of cucumber (*Cucumis sativus* L.) seedlings was blocked by heat treatments, and subsequently restored by the addition of expansin proteins (Cosgrove, 1996).

Preliminary work (Hong et al., 2000) showed that a hot water dip was more effective in reducing extension growth than modified atmosphere storage. Our objectives were to determine the effect of various handling and storage parameters on growth and to develop a treatment to significantly reduce undesirable extension growth in fresh-cut green onions. In this study we focus on effective, noninjurious heat treatments and also evaluate their effect on the microbial load of the minimally processed green onions.

Materials and Methods

Plant material. Green onions were produced in the Salinas Valley of California using standard production practices (Voss and Mayberry, 1999), harvested by pulling plants 2 h after undercutting the beds, and brought to the laboratory in coolers containing an ice-water slurry. At the laboratory the onions

were trimmed (leaf tips and roots removed) and washed with clean chlorinated water (50 mg·L⁻¹ NaOCl, pH 7.0). The tests were conducted on these onions unless specified that “commercial” onions were used. For the latter, onions were harvested into plastic crates in Mexicali, Baja California, Mexico, shipped overnight in a refrigerated truck to Salinas, trimmed, washed with cold chlorinated water, and packed in plastic film bags in 0.9-kg units. The bags were placed in polystyrene-lined boxes with gel ice and transported overnight to the laboratory at Davis, where they were placed at 0 °C until used.

Prestorage treatments. Intact trimmed onions (control) consisted of samples with roots and leaf tips cut off, but with the compressed stem intact. Minimally processed or fresh-cut onions were those in which roots and the compressed stem base were completely removed with a razor blade. Intact or fresh-cut onions were subjected to water dips at a range of temperatures and times. In most experiments, the onions were placed in the water bath so that 4 cm (from the cut end) was exposed to a large volume of water (15 L) in the circulating, temperature-controlled water bath. In other experiments, the entire onion was submerged in the heated water. Following treatment, onions were cooled in air to the indicated storage temperature. In preliminary testing, 4 cm of the cut ends of the fresh-cut onions were dipped in 10 °C solutions of acid (2% acetic acid), salt (1.5% NaCl) or an osmoticum (0.8 M mannitol) for 10 and 30 min.

Storage conditions. Treated and cooled onions were stored horizontally on moist paper towels in plastic trays enclosed in polyethylene bags (unsealed with ends overlapped) or placed horizontally in 18-L glass storage containers with a humidified air flow. These conditions simulated the high humidity (>95% RH) conditions found in the commercially packaged green onion products. Onions were routinely stored at 5 °C, but in some experiments they were stored at 0 or 10 °C.

Quality evaluations. Leaf extension during storage was measured with a vernier caliper to the nearest 0.1 mm as the length from the cut surface of the white base to the end of the most extended portion. Overall visual quality was scored on a 9 to 1 scale, where 9 = excellent, 7 = good, 5 = fair, 3 = poor, and 1 = unusable. A score of 6 was regarded as the limit of marketability. The visual quality assessment included discoloration defects but did not include curvature or “telescoping” defects. Leaf discoloration and heat injury were scored separately on 1 to 5 scales, where 1 = none, 2 = slight, 3 = moderate, 4 = moderately severe, and 5 = severe. The discoloration of the white portion of the leaves was referenced to objective color values measured with a color difference meter (Minolta Chroma Meter CR-200; Minolta, Ramsey, N.J.) with illuminant A and a 10° viewing angle and calibrated on a white tile. L*, a*, and b* values were recorded and chroma [C* = (a*² + b*²)^{-1/2}] and hue [h° = tan⁻¹(b*/a*)] were calculated. Green leaf color was measured with the same instrument.

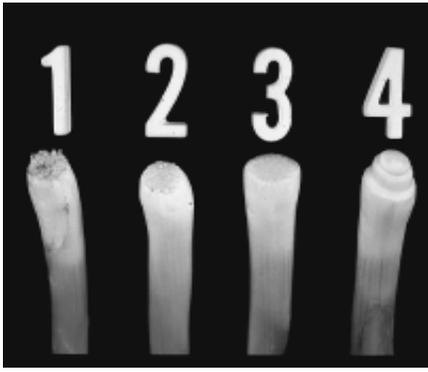


Fig 1. Green onions with (1) partially trimmed roots, with (2, 3) partial removal of the compressed stem, and (4) extension growth or “telescoping” after complete stem removal. All forms may occur in commercial product.

Respiration measurements. Onions were placed in plastic containers connected to a flow of humidified air ($\approx 95\%$ RH) at 5°C , controlled by capillaries at flow rates calculated to maintain CO_2 concentrations $<0.5\%$. The CO_2 concentrations were monitored daily by taking 1-mL gas samples and injecting into

an infrared gas analyzer (Horiba PIR-2000; Horiba, Irvine, Calif.). For calibration, a standard mixture of 0.5% CO_2 was used, and calculations of respiration rates were based on the difference between inlet and outlet CO_2 concentrations.

Microbiological testing. Microbiological examinations consisted of aerobic plate counts (APC) of samples taken immediately after treatment (4 min with 20 or 52.5°C water with 0 to $400 \mu\text{L}\cdot\text{L}^{-1}$ NaOCl) or after treatment + storage at 5 or 10°C . Replicate samples of 25 g of the white portion of the onions were homogenized in 225 mL sterile 0.1% peptone water. Total aerobic plate counts were determined by a dilution series with peptone water followed by spread plating on standard method agar (SMA) (Association of Official Analytical Chemists, 1984). A modified technique, involving incubation of SMA plates at 29°C , was used to determine the growth of important psychrophiles typically associated with produce spoilage. Duplicate plates were used for each 10 -fold dilution step. Data were expressed as colony forming units (CFU) on a tissue weight basis.

Sodium hypochlorite solutions were prepared from newly opened household bleach

solutions (5.25% a.i.) and were adjusted to pH 7.0 with HCl. The redox potential of each solution was determined using a temperature-compensating oxidation-reduction potential (ORP) meter (Orion Model 250A with Pulse Instrument Probe SOTA SP-3; Pulse Instruments, Torrance, Calif.).

Preliminary taxonomic characterization of bacterial isolates from nonheated and heated onions was conducted using Biolog GN and GP plates (Microlog™ System, release 4.0; Biolog Inc., Hayward, Calif.). Representative predominant colony-types from each treatment were purified by resuspension in sterile 0.1% peptone water and standard single colony-streak transfer to tryptic soy agar (TSA) with 5% lysed sheep blood (Media Room, Veterinary Medicine, Univ. of California, Davis), as recommended by the manufacturer.

Experimental design and statistical analysis. Experiments were conducted as completely randomized designs with three replicates of 10 – 12 green onions each per treatment unless otherwise specified. For growth and quality measurements, the highest and lowest values in each treatment were discarded and data were analyzed as averages \pm standard deviations, or by analysis of variance with calculation of least significant difference or mean separation by Student-Newman-Keuls

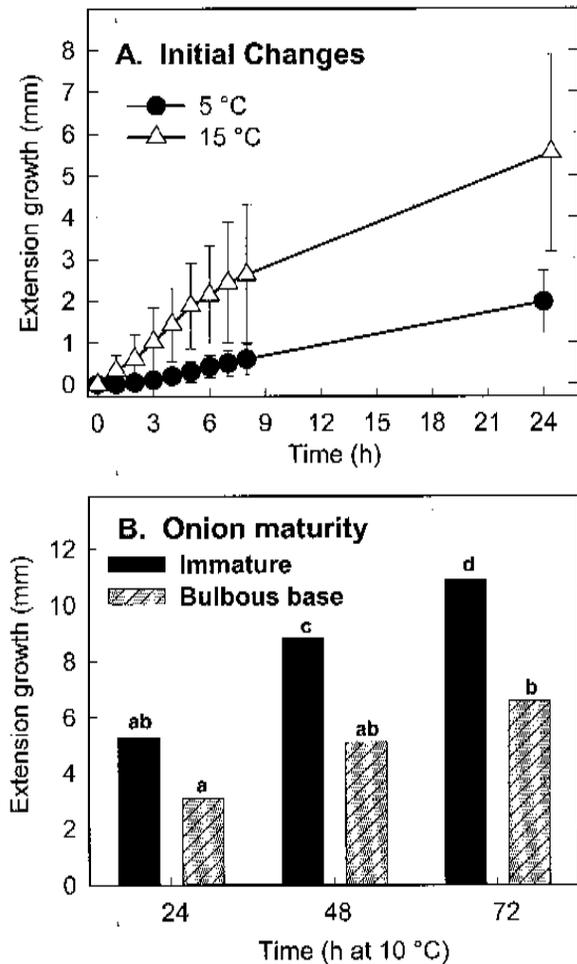


Fig 2. Effect of (A) temperature and (B) onion maturity on extension growth of cut commercial green onions. Data points are (A) averages for 10 onions \pm standard deviations or (B) averages for 15 onions with mean separation at $P \leq 0.05$. An immature onion is one with a uniform diameter along the length of the leaf sheath; the onions were more mature when the base was bulbous with a diameter >2 -fold that of the green leaf sheath.

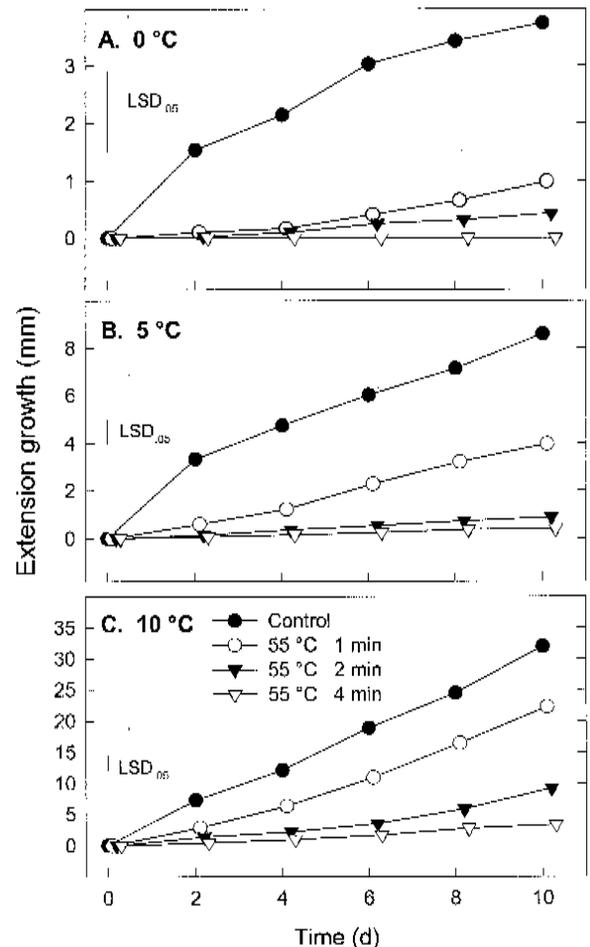


Fig 3. Extension growth of green onions subjected to water dips at 55°C for various periods and then cut and stored in air at (A) 0°C , (B) 5°C , or (C) 10°C . Note that the vertical scale is different for each graph. Data are averages for 10 onions.

method (SigmaStat 2.0; Jandel Scientific, San Rafael, Calif.). Summary data were analyzed as an inverse-order polynomial regression (SigmaPlot 4.0; Jandel Scientific).

Results

Extension growth. Green onions were trimmed to various degrees. If any cut roots remained after trimming, they grew several millimeters (Fig. 1) during storage. Further trimming, with complete removal of roots and compressed stem, provided a more desirable minimally processed product (Fig. 1). However, extension growth occurred during storage if the compressed stems were completely removed (Fig. 1). Extension growth was directional and occurred from the cut leaf end but not the cut stem. Cutting the leaf at different distances (0 to 27 mm) from the stem plate did not affect extension growth at 5 °C (data not shown).

The rate of extension growth was highly temperature-dependent and extension growth was measurable within 1 h of stem removal (Fig. 2A). Less mature, uniform-diameter onions exhibited a more rapid rate of extension growth than onions in which the basal leaves had become bulbous (Fig. 2B). All subsequent work was done with the immature, uniform-diameter green onions.

Based on these initial results, a supplementary treatment was needed to provide greater control of inner leaf extension growth. Dipping the cut ends for 10 or 30 min in different chemical solutions (2% acetic acid, 1.5% NaCl, or 0.8 M mannitol) had no effect on extension growth during 96 h at 10 °C (data not shown). However, a 2-min dip in water at 55 °C substantially reduced (>40%) extension growth, and subsequent work focused on improving the efficacy of the hot water treatment.

Storage at 0 °C in air effectively controlled extension growth, with <4 mm of growth occurring during 10 d (Fig. 3). Storage at 5 °C, however, resulted in >8 mm of growth on the cut end, and storage at 10 °C resulted in >30 mm of extension growth over the same 10-d period. A 1-min dip at 55 °C (prior to root and stem removal) was partially effective in controlling extension growth at the three storage temperatures. However, the 2- or 4-min treatments were much more effective (Fig. 3). Similar results were obtained if the onions were cut and then dipped in water at 55 °C (data not shown). None of the hot water dips at 55 °C resulted in any injury to the cut onions.

Longer treatment periods at slightly lower water temperatures were also effective in controlling extension growth (Fig. 4A–C). In this experiment the onions were cut and then treated with hot water dips. With 50 °C water, periods of 10 to 14 min were required to reduce extension growth to 5 mm or less during the 12-d storage period. With 52.5 °C water, treatment periods of 4 to 10 min were effective, with very little measurable growth after the 10-min dip. In other experiments, a 2-min treatment at 52.5 °C provided substantial control (data not shown, Fig. 3), although it did not in this experiment. At 55 °C, 2- to 6-min treatments

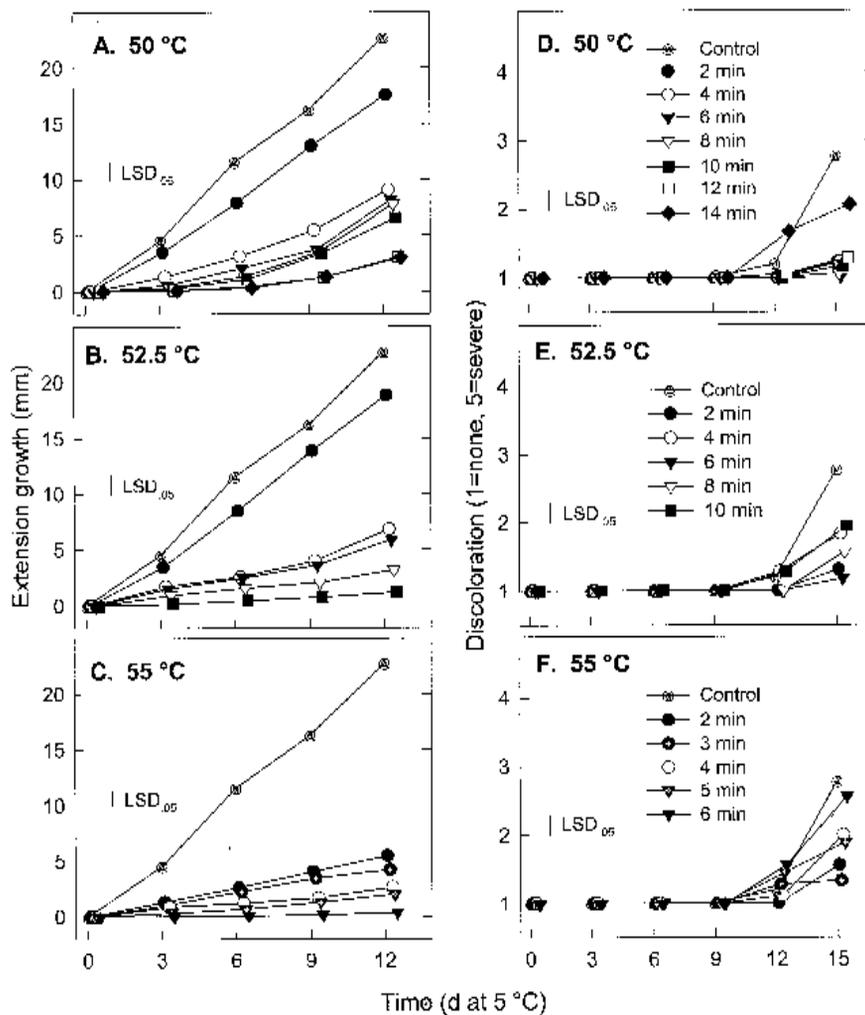


Fig. 4. (A–C) Extension growth and (D–F) discoloration of green onions cut (compressed stem removed) and then subjected to water dips at (A, D) 50 °C, (B, E) 52.5 °C, or (C, F) 55 °C for different periods. Data are averages for 10 onions.

Table 1. The relationship between subjective discoloration scores and the objective measurement of color on the white portions of onion leaves. Data are averages \pm standard deviations from three individual white stem bases of onions representative of each discoloration score.

| Discoloration score | Color values | | | | |
|-----------------------|----------------|----------------|----------------|-----------|--------|
| | L | a | b | Hue angle | Chroma |
| 1 (none) | 76.4 \pm 2.6 | -1.2 \pm 0.1 | 3.2 \pm 0.5 | 110.6 | 3.4 |
| 2 (slight) | 72.6 \pm 1.6 | -3.0 \pm 0.1 | 9.0 \pm 1.0 | 108.4 | 9.5 |
| 3 (moderate) | 71.6 \pm 4.0 | -3.5 \pm 0.3 | 9.1 \pm 1.6 | 111.0 | 9.7 |
| 4 (moderately severe) | 73.6 \pm 3.0 | -5.3 \pm 1.4 | 13.0 \pm 4.9 | 112.2 | 14.0 |
| 5 (severe) | 74.2 \pm 3.5 | -7.1 \pm 1.5 | 19.6 \pm 1.4 | 109.9 | 20.8 |

were effective with virtually no measurable growth after the 6-min treatment.

Hot water dips at temperatures up to 60 °C were tested for short periods. A 1- or 2-min treatment at 60 °C, and a 2-min treatment at 57.5 °C were effective but resulted in some product injury (data not shown). Common symptoms of injury were leaf discoloration and loss of texture. Water dips at 40 or 45 °C were not effective in controlling extension growth, regardless of the period of exposure (up to 30 min) (data not shown).

Discoloration of cut ends. The cut ends of fresh-cut green onions may show discoloration and deterioration, especially after \approx 12 d of storage. The appearance of these defects was variable but was reduced by some heat

treatments, such as 50 °C for 2 to 10 min (Fig. 4D–F). The discoloration usually appeared as a yellowing of the cut ends, and sometimes was associated with tissue softening. Discoloration resulted in decreased a and increased b and chroma color values (Table 1), but no consistent changes in L and hue values. The discoloration and deterioration associated with storage were distinct from heat injury defects. A water treatment such as 55 °C for 6 min or 60 °C for 2 min may cause visible injury to the cut end, and this injury was characterized as a reddish discoloration with or without loss of texture.

Curvature. Curvature was another common defect in the commercial green onion product. For most of the experiments, only the

lower 4 cm of the onions was heat-treated. In an experiment where the entire onion was dipped, a 2-min treatment at 55 °C was effective in reducing extension growth from 16 to 3 mm after 7 d at 5 °C, but was not effective in reducing geotropic curvature (data not shown). In cut, nontreated onions, curvature averaged 45 to 60° from the horizontal under these storage conditions.

Respiration. During the day following treatment, respiration rates of heat-treated onions were similar to those of the controls except for onions treated at 55 °C for 4 min (Fig. 5). Respiration rates of nontreated onions decreased during storage at 5 °C for 8 d, whereas the hot water dips resulted in rates averaging 20% to 40% higher than those of the nontreated onions (Fig. 5). Although the 4-min treatment at 55 °C resulted in a substantial and continuous increase in respiration rates, it did not reduce the visual quality or shelf life of the onions (data not shown). In a separate experiment, respiration rates of onions held at 55 °C for 4 min or 60 °C for 2 min were significantly higher than those of nontreated onions, while the rates of those treated at 55 °C for 2 min were not affected (data not shown).

Leaf color. For most of the experiments only the lower 4 cm of the onions was exposed to the hot water. However, commercial heat treatments could involve complete submergence. The effect of representative heat treatments on the color of the leaf portions is shown in Fig. 6. Two 55 °C treatments were selected, since in previous tests this temperature appeared not to affect overall visual quality but did affect respiration (Fig. 5). A 2-min treatment at 60 °C that usually resulted in some visible injury to the cut area (red-brown discoloration and texture loss) was also selected. The 60 °C treatment caused a rapid darkening of the green leaves, as indicated by the lower L* values (Fig. 6a). Color intensity of the 60 °C-treated leaves also decreased after 6 d (Fig. 6b), but L* and chroma values of the leaves were not affected by exposure for 2 or 4 min at 55 °C. During storage at 10 °C, hue values of the leaves of the control onions decreased due to yellowing, whereas the heat treatments retarded loss of green color (Fig. 6c).

Effective treatment combinations. A treatment that reduced “telescoping” to <5 mm during 12 d of storage at 5 °C without causing visible injury to the fresh-cut green onions was considered effective. A growth of 5 mm was not sufficiently noticeable to make the onions unmarketable unless they also had other defects. For effective treatments, a curvilinear relationship existed between water temperature and exposure time (Fig. 7).

Microbial disinfection. A 20 °C water wash reduced the microbial population of the fresh-cut green onions only slightly (Table 2). A 4-min water wash at 52.5 °C reduced the aerobic plate count by 1 to 2 logs in both experiments. Hot water treatment before cutting resulted in improved disinfection in one experiment but had no effect in the second experiment when compared with cutting followed by heat treatment (Table 2). After storage at 5 °C for 7 d, but not 14d, hot water-treated onions had lower

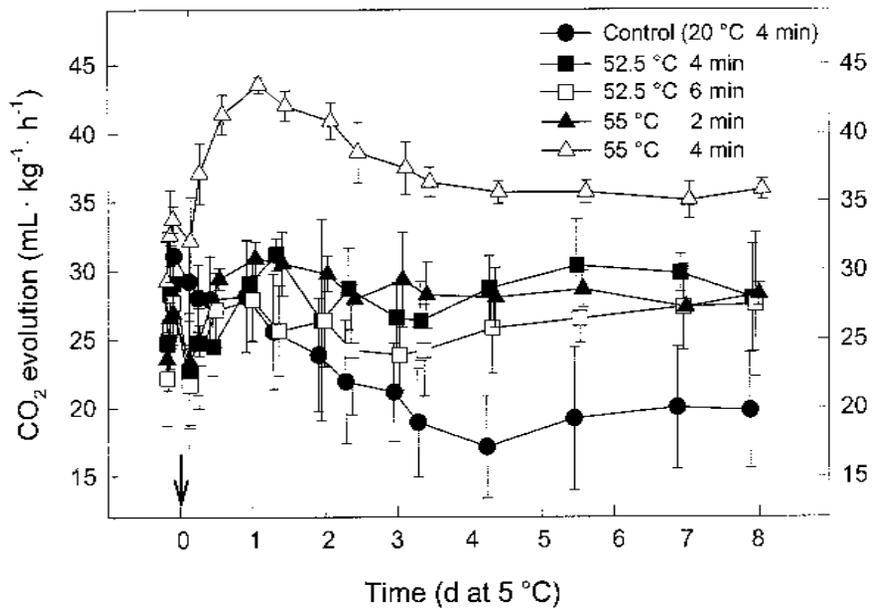


Fig 5. Respiration rates of cut green onions subjected to water dips at 20, 52.5, or 55 °C for different periods. The arrow indicates when treatments were applied. Data are averages of three replications of eight onions each \pm standard deviations.

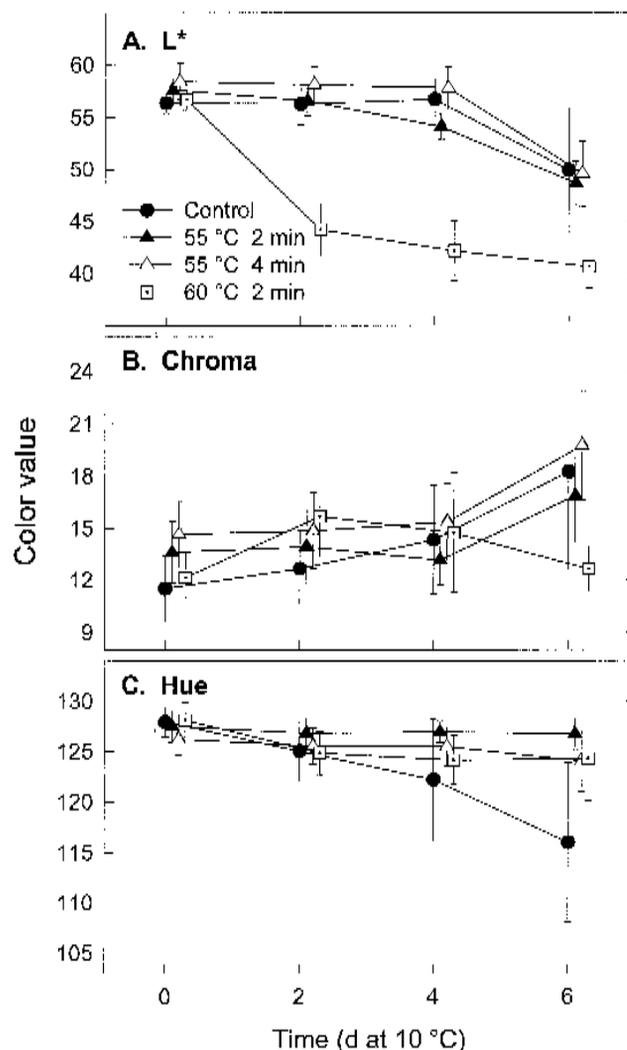


Fig 6. Effect of hot water dips on (A) L*, (B) chroma, and (C) hue color values of the outer green leaf of commercial onions. The entire onion was submerged in the water treatments. Data are averages for 10 onions \pm standard deviations.

microbial loads than did those treated with water at 20 °C. Hot water improved the efficacy of all sodium hypochlorite solutions (Table 3). Heating chlorinated water reduced the microbial populations ≈2 logs vs. only 1 log without heating. A 400 μL·L⁻¹ NaOCl solution may cause some injury to the onions, as indicated by lower visual quality scores after 3 d of storage at 10 °C compared with onions treated with lower chlorine concentrations (Table 3).

Discussion

Extension growth rates of cut, nontreated green onions varied among experiments from <1 to >3 mm per day at 5 °C. The most effective treatments were the 2-min dips in water at 55 °C and the 4-min dips at 52.5 °C. These treatments not only controlled extension growth of the cut ends, but also maintained the bright green color of the leaves during storage. Leaf color was also effectively maintained by CA storage of green onions, although CA was ineffective in controlling extension growth (Hong et al., 2000). The most effective CA treatment (0.2% O₂ + 7.5% CO₂) reduced growth to 6–7 mm during 10 d at 5 °C (Hong et al., 2000), while a 2-min dip in water at 55 °C reduced growth to 1 mm over the same period. Heat treatments may reduce the pungency of the onions slightly, but modified atmosphere storage following heat treatment mitigated this change so that thiosulfinate concentrations were similar to those of the nontreated product (Hong et al., 2000). The heat-treated, trimmed product stored in modified atmospheres had a shelf life of at least 2 weeks at 5 °C (Hong et al., 2000).

Discoloration of the cut ends of the green onions, often associated with loss of firm texture, could be related to decay. Very high microbial loads (APC >7 log CFU·g⁻¹) were associated with the discolored cut ends, and preliminary identification indicated that the predominant isolates were *Pseudomonas fluorescens* Biovar II, *Pantoea agglomerans*, and *Bacillus* sp. Discoloration occurred only on some onions and usually appeared after ≈12–14 d of storage at 5 °C in air. The discoloration was not caused by the heat treatments, and in some cases such treatments reduced the incidence of discoloration. In tests where the whole onions were dipped, some hot water dips (i.e., 60 °C for 2 min) increased deterioration of the oldest, most senescent leaf.

Curvature is a defect observed in green onions and asparagus stored horizontally under high humidity at temperatures above 0 °C (Hong et al., 2000; Lipton, 1990). Paull and Chen (1999) reported that a 50 °C water dip for 3 min completely controlled curvature of asparagus spears. Although a hot water treatment (55 °C for 2 min) of whole green onions had no effect on curvature during subsequent storage at 5 °C, other noninjurious hot water dip treatments may be effective.

Chlorination is considered a means to maintain sanitation of process waters rather than to disinfect the product (Suslow, 1997). Typically, chlorinated wash water reduces micro-

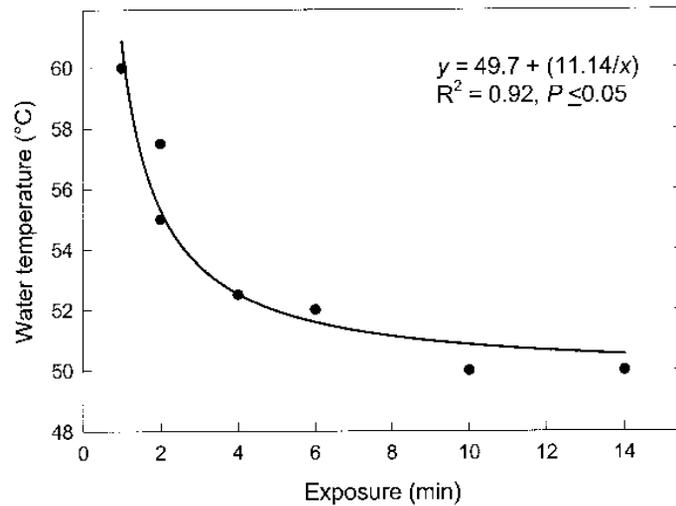


Fig 7. Average time–temperature water dip conditions required to control extension growth of cut green onions. An effective treatment combination was one in which extension growth was <5 mm after 12 d at 5 °C and in which there was no visible injury.

Table 2. Effect of 4-min water dips at 20 and 52.5 °C on the microbial population of commercial green onions. The compressed stem was cut before or after water dips. Aerobic plate counts (APC as log CFU·g⁻¹) were determined immediately after treatment (Expt. 1) or after treatment and storage at 5 °C for 0 to 14 d (Expt. 2), and are averages of duplicates for each sample period.

| Temp. (°C) of water wash | Cut | Expt. 1 | | Expt. 2 | |
|--------------------------|--------|---------------------|--------|---------|------|
| | | 0 d | 0 d | 7 d | 14 d |
| No wash | Before | 4.60 a ² | 5.12 a | 7.60 a | 7.93 |
| 20 | Before | 4.19 ab | 5.09 a | 7.59 a | 7.91 |
| 20 | After | 3.75 b | 4.79 a | 7.76 a | 8.04 |
| 52.5 | Before | 2.95 c | 3.43 b | 6.66 b | 8.04 |
| 52.5 | After | 2.18 c | 3.24 b | 6.89 b | 8.33 |

²Mean separation within columns by Student-Newman-Keuls method at $P \leq 0.05$.

bial populations of apples (*Malus × domestica* Borkh.), tomatoes (*Lycopersicon esculentum* Mill.), and lettuce (Beuchat et al., 1998) and watercress (*Nasturtium officinale* R. Br.) (Park and Dong, 1995) by <100-fold. In our study, a chlorinated water wash reduced microbial populations on green onions by <10-fold, the same result obtained with a clean water wash. A 52.5 °C chlorinated water dip resulted in a further average reduction of >200-fold. Heated, chlorinated water treatments prior to cutting were as effective as postcutting treatments. Although our original objective was to control extension growth, the proposed treatments also substantially improved initial disinfection of the fresh-cut onions. Park et al. (1998) showed a similar reduction in microbial populations of soybean [*Glycine max* (L.) Merrill] sprouts and watercress with a 30-s water dip at 60 °C. We found this treatment to be ineffective for control of growth, although the 1- and 2-min treatments at 60 °C were effective.

In conclusion, extension growth of minimally processed, cut green onions (removal of roots and compressed stem) was reduced by low temperature storage (0 °C). At the more typical storage and handling temperature of 5 °C, extension growth of 10 to 20 mm occurred over 10 d and reduced marketability. Several hot water treatments were effective in controlling or greatly retarding extension growth. Treatment combinations of 55 °C for 2 min and 52.5 °C for 4 min were the most effective and did not cause injury to the white or green

Table 3. Effects of washing for 4 min in chlorinated water (NaOCl) at two temperatures on total aerobic plate counts and visual quality of commercial green onions. Onions were cut after the disinfection treatment. Aerobic plate counts (APC as log CFU·g⁻¹) were determined immediately after treatment and are averages of two samples. Visual quality was evaluated after 3 d at 10 °C and values are averages for 10 onions.

| NaOCl (mg·L ⁻¹) | Temp (°C) | APC (log CFU·g ⁻¹) | Visual quality ² |
|-----------------------------|-----------|--------------------------------|-----------------------------|
| 0 | 20 | 6.19 a ³ | 7.4 ab |
| 0 | 52.5 | 5.11 ab | 7.3 ab |
| 50 | 20 | 5.43 ab | 8.4 a |
| 50 | 52.5 | 3.79 c | 7.7 ab |
| 100 | 20 | 5.49 ab | 8.4 a |
| 100 | 52.5 | 3.43 c | 8.1 a |
| 200 | 20 | 5.01 b | 8.0 ab |
| 200 | 52.5 | 2.85 c | 7.9 ab |
| 400 | 20 | 5.73 ab | 7.4 ab |
| 400 | 52.5 | 3.30 c | 6.8 b |

²Visual quality rated on a 9 to 1 scale, where 9 = excellent, 1 = unusable; a score of 6 is minimum for salability.

³Mean separation within columns by Student-Newman-Keuls method at $P \leq 0.05$.

portions of the onion leaves or reduce shelf life. Heating (52.5 °C) increased the disinfection potential of chlorinated water by 200-fold, and treatment before cutting was at least as effective as a postcutting treatment. Heat treatments were not effective in reducing curvature of onions stored horizontally.

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