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Quality of Iceberg (*Lactuca sativa L.*) and Romaine (*L. sativa L. var. longifolial*) lettuce treated by combinations of sanitizer, surfactant, and ultrasound

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A B S T R A C T

The effects of sonication, sanitizers and sodium dodecyl sulfate (SDS) on the quality of fresh-cut Iceberg and Romaine lettuce were examined. Lettuce samples were treated for 1 min with and without ultrasound with one of the following solutions: tap water, chlorine, Tsunami, and a combination of Tsunami with 1 g/L SDS. Washed samples were packed under modified atmosphere conditions and stored at 4 °C for up to 14 days. Changes in headspace gases, texture, color, tissue damage, visual quality, and natural flora were determined. The O_2 concentrations and CO_2 accumulation in Romaine lettuce were not significantly different among the treatments. In Iceberg lettuce, a lower O_2 and high CO_2 content in the headspace of samples treated with Tsunami and Tsunami + SDS were recorded. After 14-day storage, the tissue damage expressed by electrolyte leakage, total color difference, firmness, and total aerobic plate counts were not significantly different among treatments in two types of lettuce samples. Treatment of Iceberg lettuce with sonication in combination with Tsunami or Tsunami + SDS did not degrade quality compared to samples treated with chlorine alone, whereas for Romaine lettuce, chlorine-treated samples had a significantly higher overall quality score than that from the other treatments.

1. Introduction

Consumption of lettuce in the U.S. has increased over the last decade due to new trends in diet that emphasize the importance and popularity of vegetable salads, the convenience offered by fresh-cut products, and increases in salad bar patronage and meals eaten outside the home (Buck, Walcott, & Beuchat, 2003; USDA, 2002). This increase in lettuce consumption has led to annual U.S. production of nearly 3950 Gg of lettuce in 2010, while in the same year 3270 Gg were imported from Mexico and Canada to meet demand (Boriss & Brunke, 2011). Increased production and consumption of lettuce has drawn significant public interest to the potential for foodborne illness associated with lettuce and other leafy green vegetables. During the period 2010–2012, three multi-state outbreaks of Shiga toxin-producing *Escherichia coli* O157:H7 and *E. coli* O145 associated with consumption of lettuce were reported (CDC-Centers for Disease Control and Prevention, 2012). These high-profile foodborne illness outbreaks highlight the importance of further improving the microbial safety of fresh produce.

Currently, the produce industry processes lettuce by cutting it into bite-size pieces, washing the cut lettuce with chlorinated water, followed by rinsing, dewatering or drying, and packaging. However, washing produce with chorine in industrial-scale operations, for instance at a throughout of 45 kg/min, has been reported to reduce the survival count of *E. coli* O157:H7 by no more than one log cycle (Luo et al., 2012). In addition, chlorine is consumed when organic matter is present, leading to an increase in turbidity of the wash water (Luo et al., 2012; O'Beirne & Zagory, 2009). The presence of organic matter in wash water can also enhance formation of chloroform ($CHCl_3$), haloacetic acids or other trihalomethanes (THM), all of which are known to be harmful to human health (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009). Efforts have thus been made to find alternative and/or more effective sanitization agents/methods to enhance reduction of microbial populations.

Treatments that create an acidified environment in a washing system through the use of organic acids such as lactic, citric, peroxyacetic, and levulinic acids, or their salts, have been reported as an alternative to the traditional chlorine wash (Oms-Oliu et al.,

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2010). In tests performed in a beaker, 1.74 log CFU/g reduction of *E. coli* O157:H7 on lettuce washed with 20 g/L lactic acid for 5 min was achieved (Sagong et al., 2011). Another study reported more than a 6 log CFU/g reduction of *E. coli* O157:H7 population on lettuce when treated with 30 g/L levulinic acid in combination with the surfactant sodium dodecyl sulfate (1 g/L SDS) for 1 min (Zhao, Zhao, & Doyle, 2009). The use of a surfactant aims to allow the (dissolved) sanitizer to penetrate small cracks and crevices on the complex topography of lettuce. The combination of a chemical wash with a physical process, such as sonication, has also been tested for enhancing the efficacy of a sanitizer wash (Zhou, Feng, & Luo, 2009; Zhou, Feng, & Pearlstein, 2012).

Lettuce, unlike other fresh produce, lacks an external protective tissue, and processes like cutting expose its tissues to air, leading to a series of chemical reactions that cause damage and make the plant material vulnerable to dehydration. Several studies have shown that many sanitizing agents, such as chlorine, organic acids, ozone and some surfactants are excellent antimicrobials, especially for planktonic microorganisms. However, many of these compounds have a detrimental effect on the quality of leafy produce when used beyond certain critical concentrations, leading to quality degradation through browning, tissue damage, color changes, water segregation, and overall poor appearance (Garcia, Mount, & Davidson, 2003). For instance, Guan, Huang, and Fan (2010) reported that treatment with 5 g/L to 30 g/L levulinic acid plus 0.5 g/L SDS rendered fresh-cut Iceberg lettuce sensorially unacceptable beyond seven days due to development of sogginess and tissue damage. In general, for the development of any sanitizer or sanitization method, the effect of the treatment on produce quality is a primary consideration. The only meaningful microbial count reductions are those that are achieved for treatment times and sanitizer concentrations below the threshold for unacceptable quality changes during storage long enough to be consistent with retail sale. For this reason, this study was undertaken to examine the effects of sonication in combination with two sanitizers (chlorine and Tsunami 100®) and a surfactant (sodium dodecyl sulfate) on the quality of fresh-cut Iceberg and Romaine lettuce during 14-day refrigerated storage.

2. Materials and methods

2.1. Ultrasound-wash system

This study was carried out in a custom-made ultrasonic washing tank. The tank was made of welded aluminum sheet, with a capacity of 115 L. Two ultrasound (US) transducer blocks (each operating at 25 kHz, and with 2 kW nominal power), with sound emitting planes facing each other, were vertically placed in the tank against two walls. Prior to the start of each test the wash tank was filled with chilled tap water (10 °C) to which was added chlorine (active ingredient sodium hypochlorite), Tsunami 100® (active ingredient peroxyacetic acid), or Tsunami 100®+ sodium dodecyl sulfate (SDS). To minimize "blockage" (Zhou et al., 2012) and allow ultrasonic waves to reach each piece of the cut lettuce, a plastic holder (Fig. 1) measuring 30.48 cm × 15.25 cm × 12.70 cm ($L \times W \times H$) with mesh size of 1.21 cm × 1.21 cm was used to hold lettuce samples. The walls of the holder were made of stretchable molded polyethylene mesh (McMaster-Carr, Elmhurst, IL, USA) and the holder can hold up to 450 g of cut lettuce. The holder was submerged in the tank during treatment.

2.2. Preparation of lettuce samples

Iceberg (*Lactuca sativa L.*) and Romaine (*L. sativa L. var. longifolial*) lettuce were purchased at a local supermarket and immediately transported to the laboratory, where they were stored at 6 ± 1 °C and used within 24 h of purchase. The three outermost leaves of each head of lettuce were removed. A kitchen knife was used to cut lettuce into pieces of 6.45 cm^2. The lettuce pieces were randomized at the beginning of the experiment and divided into batches of 300 g each for treatment.

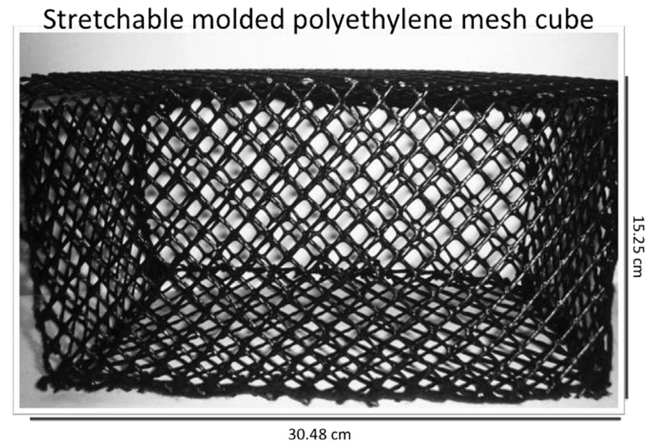

Fig. 1. Ultrasound wash system.

2.3. Treatment procedure

Three hundred grams of fresh-cut lettuce were submerged in the water tank containing one of the following solutions: tap water (control), sodium hypochlorite (final free chlorine concentration 100 mg/L), Tsunami 100® (peroxyacetic acid as active ingredient, final acid concentration 80 mg/L), and Tsunami100® in combination with 1 g/L SDS. For each washing solution, samples were treated for 1 min with and without ultrasound, except for the tap-water control. After the one-minute treatment the samples were rinsed with tap water for 1 min and de-watered with a manual salad spinner (OXO, New York, NY, USA). One hundred grams of each de-watered sample were placed in polypropylene plastic film bags (OTR 7000 cc/m^2/day and CO_2 21,000 cc/m^2/day) (PD-961 EZ, Cryovac, Duncan, SC). The lettuce bags were vacuumed, flushed with N_2 using an Audionvac 101/151 packaging machine (Audion Elektro, Hogeweyselaan, Netherlands), sealed, and stored at 4 ± 1 °C until further analysis. Nine bagged samples were set aside for sampling, with three bags taken at days 0, 7 and 14 to perform triplicate quality analyses, including electrolyte leakage rate, texture, color, sensory evaluation, headspace O_2 and CO_2 content, total aerobic plate count, and yeasts and molds.

2.4. Analysis of headspace O_2 and CO_2 in package

Headspace gas in the packages was analyzed at days 0, 7 and 14 of storage. To measure the content of O_2 and CO_2 inside the packages, gas from the headspace was withdrawn through a needle using a built-in pump into a portable dual headspace analyzer (model 650, Mocon Inc. Minneapolis, MN, U.S.A.)

2.5. Visual quality

Visual quality was assessed immediately after headspace analysis of packages by a 5-member trained panelists using the same parameters as Guan et al. (2010). Overall visual quality was rated on a 9 to 1 scale: 9 = excellent, essentially free from defects; 7 = good, minor defects, not objectionable; 5 = fair, slightly to moderately objectionable defects, lower limit of sales appeal; 3 = poor, excessive defects, limit of salability; 1 = extremely poor, not usable. Cut edge tissue browning, surface browning, and sogginess/watery were rated on a scale of 5 to 1: 5 = severe; 4 = moderately severe; 3 = moderate; 2 = slight; 1 = none.

2.6. Texture measurement

The firmness of fresh-cut lettuce leaves was measured using a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, U.S.A.) and a Kramer Shear press with five blades (TA-91). Twenty-five grams of sample were positioned in the press holder and the five-blade plunger was moved down at a velocity of 2 mm/s to 1 cm below the bottom of the holder. The maximum cut force (MCF) was recorded using the Texture Expert Software (version 1.22, Texture Technology Corp., Scarsdale, NY, USA). Two aliquots were taken for measurements from each bag, resulting in a total of six measurements for each combination of treatment and sampling day.

2.7. Electrolyte leakage analysis

Electrolyte leakage from fresh-cut lettuce was measured immediately after a treatment and during storage to determine the rate of tissue deterioration. Five grams of lettuce leaves were submerged in 100 mL of deionized water in a beaker and incubated for 1 min at 23 °C. During incubation the samples were agitated using a New Brunswick incubator with built-in shaker (Model I-24, New Brunswick Scientific, Enfield, CT, USA) at a speed of 100 rpm. Electrical conductivity (μS/cm) of the bathing solution was measured at 1 min (C_1) and 60 min (C_{60}) using a conductivity meter (Accumet Basic AB30, Fisher Scientific Co., Pittsburgh, PA, USA). The samples were then autoclaved (121 °C) for 25 min, and total conductivity (CT) was measured after cooling. Electrolyte leakage rate was calculated as (Zhou, 2010)

$$E = \left[\frac{C_{60} - C_1}{\mathrm{CT}}\right] \times 100 \tag{1}$$

2.8. Color measurement

For color measurement, five pieces of cut lettuce were withdrawn from each packed bag and analyzed using a Minolta Chroma Meter CR-300 (Minolta Corp., Osaka, Japan). Hunter's color values (L, a, b) were measured at 3 locations of each piece of lettuce and averaged for a total of 15 readings for each treatment/sampling day. Total Color Difference (TCD) was determined using the following equation (Heimdal, Kühn, Poll, & Larsen, 1995)

$$\Delta E^*_{ab} = \sqrt{\left(L^*_1 - L^*_0\right)^2 + \left(a^*_1 - a^*_0\right)^2 + \left(b^*_1 - b^*_0\right)^2} \tag{2}$$

where L^*_0, a^*_0, and b^*_0 are Hunter's color values from a reference and, L^*_1, a^*_1, and b^*_1 are Hunter's color values from the treated samples.

2.9. Microbiological analysis

Ten grams of treated lettuce were homogenized in 90 mL of 1 g/L sterile peptone water (pH 7.4) in a lab stomacher (model 400, Seward Medical, London, UK) and agitated for 2 min at 260 rpm. Homogenates were serially diluted in peptone water, and logarithmically plated (100 μL in duplicate). The total aerobic plate count (TPC) was determined by plating the samples on Tryptic Soy Agar (TSA, Difco Lab, Detroit, MI, USA) and incubated at 37 °C for 48 h. Yeasts and molds were determined by plating the samples in acidified Potato Dextrose Agar, pH adjusted with tartaric acid (PDA, Difco Lab, Detroit, MI, USA) and incubated at 25 °C for 5 days.

2.10. Statistical analysis

In this completely randomized experimental design (CRD), all treatments were replicated three times and analyzed at each sampling time. Data were analyzed with Statistical Analysis System version 9.1 (SAS Institute, Raleigh, NC, U.S.A) using a general linear model. Mean separation was determined using Tukey's test with $\alpha = 0.05$.

3. Results and discussion

3.1. Changes in headspace composition

Table 1 shows changes of the O_2 and CO_2 concentrations in the packages during storage at 4 °C. An increase in O_2 concentration can be observed in the control and three treated samples for both Iceberg and Romaine lettuce. At day 0, both lettuce types exhibited low oxygen levels, between 3.07 and 3.57% because of the N_2 flush and vacuum packing. From day 0 to day 7, the oxygen concentration increased rapidly, reaching high levels of 11.20–12.52% in Romaine lettuce and 8.86–9.73% in Iceberg lettuce. Afterwards, the O_2 concentration continued to increase but at a lower rate, and at day 14, the final oxygen content was between 12.55 and 14.48% for Romaine and 11.75–13.40% for Iceberg lettuce. It is known that fresh-cut produce has a relatively high respiration rate and therefore packaging films with high oxygen transmission rate (OTR) are normally used for this type of product (Toivonen, Brandenburg, & Luo, 2009). The polypropylene film used in this study had a high OTR of 7000 cc/m^2/day. This allowed rapid transport of O_2 by diffusion from the surroundings into the packages at the beginning of the storage period. The O_2 inflow was driven by the partial pressure difference of O_2 across the packaging film, as shown by the concentration differences, i.e., 20.95% in the air and 3.07–3.57% at day 0 in the packages. The O_2 consumption by lettuce in the bags was much less than the O_2 diffusion rate into the bags, and as a result, a rapid increase in O_2 concentration in the first 7 days of storage was observed. From day 7 to day 14, the partial pressure difference of O_2 across the film was much less than on day 0. However, since the amount of lettuce in each bag was small (100 g) and the size of the package was relatively large (12.25 × 8.5 inch, $L \times W$), the amount of O_2 that diffused in was still greater than that consumed by the lettuce, leading to a continued increase in O_2 concentration in the bags. This result was in agreement with the tests by Guan et al. (2010) with PDF961 films having an OTR of 7000 cc/m^2/day. Guan et al. washed Iceberg lettuce with combinations of levulinic acid and SDS and reported a rapid increase of O_2 concentration in the bags during the first 7 days of storage. From day 7 to day 14, they observed a decrease in O_2 concentration. This latter result differed from our observations, and could be due to differences in sanitization and lettuce species used in this study and that used by Guan et al.

Accumulation of CO_2 increased in the first 7 days storage. For instance, in the Romaine lettuce packages, the CO_2 levels reached 1.65–2.02% on the seventh day. This might be attributed to the respiration activity of cut and treated lettuce. After the seventh day, the CO_2 content in the Iceberg lettuce samples remained nearly unchanged while that for the Romaine lettuce was decreased slightly except for the Tsunami + ultrasound treatment. The differences in CO_2 concentration between lettuce samples treated with sonication in combination with chlorine, Tsunami, or Tsunami + SDS were not significantly different for

Table 1
Changes in headspace content of Romaine and Iceberg lettuce during storage.

Lettuce type	Treatment	O_2 content (%)			CO_2 content (%)		
		Day 0 Mean ± SE	Day 7 Mean ± SE	Day 14 Mean ± SE	Day 0 Mean ± SE	Day 7 Mean ± SE	Day 14 Mean ± SE
Romaine	Water	$3.50 \pm 0.20^{a\,(x)}$	$11.20 \pm 0.34^{a\,(y)}$	$14.48 \pm 2.32^{a\,(z)}$	$0.60 \pm 0.06^{bc\,(x)}$	$1.85 \pm 0.12^{a\,(y)}$	$1.70 \pm 0.23^{a\,(z)}$
	Chlorine + ultrasound	$3.34 \pm 0.20^{a\,(x)}$	$12.00 \pm 0.13^{a\,(y)}$	$13.42 \pm 0.98^{a\,(z)}$	$0.70 \pm 0.01^{a\,(x)}$	$1.65 \pm 0.05^{a\,(y)}$	$1.63 \pm 0.16^{a\,(z)}$
	Tsunami + ultrasound	$3.07 \pm 0.82^{a\,(x)}$	$11.48 \pm 0.51^{a\,(y)}$	$12.55 \pm 0.63^{a\,(z)}$	$0.53 \pm 0.05^{c\,(x)}$	$2.02 \pm 0.28^{a\,(y)}$	$2.23 \pm 0.14^{a\,(z)}$
	Tsunami + SDS + ultrasound	$3.15 \pm 1.30^{a\,(x)}$	$12.52 \pm 2.20^{a\,(y)}$	$13.97 \pm 0.67^{a\,(z)}$	$0.62 \pm 0.04^{b\,(x)}$	$1.95 \pm 0.45^{a\,(y)}$	$2.00 \pm 0.28^{a\,(z)}$
Iceberg	Water	$3.57 \pm 0.20^{a\,(x)}$	$8.86 \pm 0.43^{b\,(y)}$	$12.88 \pm 0.27^{ab\,(z)}$	$0.60 \pm 0.06^{bc\,(x)}$	$1.70 \pm 0.17^{a\,(y)}$	$1.63 \pm 0.16^{bc\,(y)}$
	Chlorine + ultrasound	$3.34 \pm 0.20^{a\,(x)}$	$9.73 \pm 0.51^{a\,(y)}$	$13.40 \pm 0.74^{a\,(z)}$	$0.70 \pm 0.01^{a\,(x)}$	$1.58 \pm 0.08^{a\,(y)}$	$1.60 \pm 0.13^{c\,(y)}$
	Tsunami + ultrasound	$3.08 \pm 0.81^{a\,(x)}$	$9.20 \pm 0.25^{ab\,(y)}$	$12.10 \pm 0.69^{bc\,(z)}$	$0.53 \pm 0.04^{c\,(x)}$	$1.65 \pm 0.12^{a\,(y)}$	$1.90 \pm 0.06^{a\,(y)}$
	Tsunami + SDS + ultrasound	$3.50 \pm 0.10^{a\,(x)}$	$8.75 \pm 0.35^{b\,(y)}$	$11.75 \pm 0.79^{c\,(z)}$	$0.65 \pm 0.12^{b\,(x)}$	$1.67 \pm 0.18^{a\,(y)}$	$1.92 \pm 0.19^{ab\,(y)}$

a–c: Treatment means within treatments (columns) with different letters are different at α 0.05.
x, y: Treatment means within days (rows) with different letters are different at α 0.05.
*SE: Standard error.

Romaine lettuce at day 14 ($P > 0.05$). For Iceberg lettuce samples, the lowest accumulation of CO_2 was observed when treated with Chlorine + ultrasound. Kim, Luo, Saftner, and Gross (2005) observed similar respiratory behavior after packaging romaine lettuce, where the CO_2 production rate increased at the beginning of storage and later decreased gradually towards the end of the storage period. It is expected that a relatively high level of O_2, accompanied by relatively low levels of CO_2 between days 7 and 14, will create an environment unfavorable for maintaining the quality of cut lettuce.

3.2. Electrolyte leakage rate

Changes in electrolyte leakage rate (ECR) in Romaine and Iceberg lettuce as a function of storage time are presented in Fig. 2. Romaine and Iceberg lettuce treated with Tsunami 100® + sonication had the highest ECR leakage rates on day 0, at 1.70 and 1.79, respectively ($P < 0.05$). A decrease in electrolyte leakage rate was observed by the end of day 7 of storage. On day 0, Romaine and Iceberg lettuce samples treated with chlorine + sonication had significantly lower ($P < 0.05$) electrolyte leakage rates than that of other treatments, an indication of less tissue damage. The decreased ECR observed on day 7 can be attributed to tissue recovery and electrolyte reabsorption by the plant material as a defense mechanism (Fan, Sokorai, Niemira, Mills, & l Zhen, 2012). Samples taken on day 14 showed an increased ECR, without significant differences among the treatments of Romaine and Iceberg lettuce. This increase in ECR can be attributed to permanent tissue damage and accumulation of CO_2 from respiration (Wang, 2004). Similar trends were reported by Luo, McEvoy, and Wachtel (2004), and Kim et al. (2005) who packed minimally processed cilantro and lettuce and reported a decrease in electrolyte leakage rate during first few days of storage followed by an increase in packages sampled towards the end of 14 days of storage.

3.3. Firmness

The effects of processing conditions (cutting, treatment, and modified atmosphere packaging) on the changes of Romaine and Iceberg lettuce texture during storage are shown in Table 2. At day 0, all samples were compared against an untreated raw sample. For Iceberg lettuce, the MCF values for the treated samples were

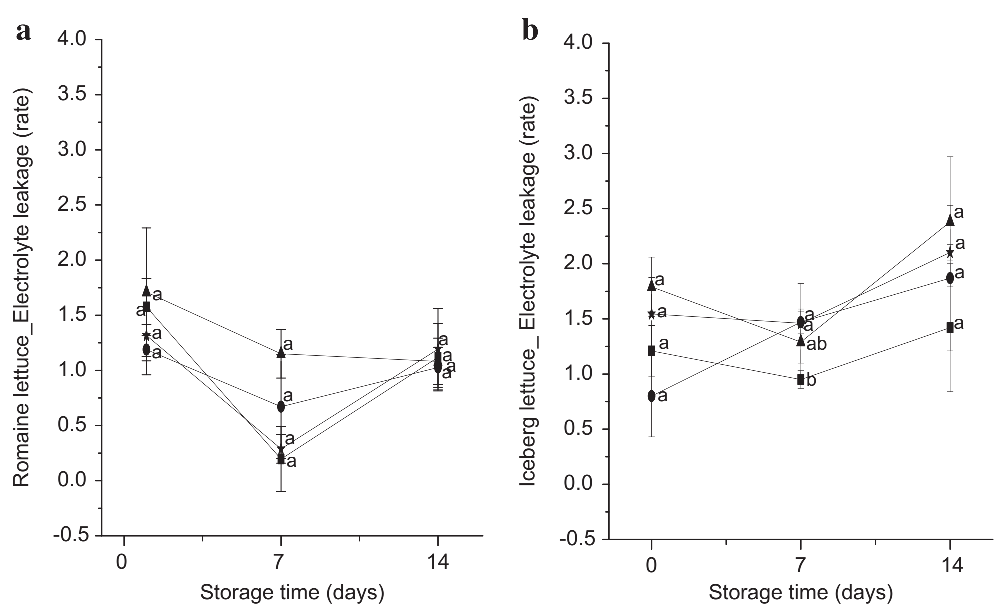

Fig. 2. Electrolyte leakage rate of Romaine and Iceberg lettuce during storage. (a) Electrolyte leakage rate (ECR) of Romaine lettuce during storage. (b) Electrolyte leakage rate (ECR) of Iceberg lettuce during storage –■–. Water, –●–, Chlorine + US, –▲– Tsunami + US, –★–Tsunami + US + SDS. **a–c** Treatment means within days of storage with different letters are different at α 0.05.

Table 2
Firmness of Romaine and Iceberg lettuce during storage.

Lettuce type	Treatments	Maximum cut force Day 1 Mean ± SE*	Maximum cut force Day 7 Mean ± SE	Maximum cut force Day 14 Mean ± SE
Romaine	Raw lettuce	376.50 ± 36.07^{b}	ND˜	ND
	Water	$449.42 \pm 26.28^{a(x)}$	$462.99 \pm 22.06^{b(x)}$	$497.61 \pm 79.05^{a(x)}$
	Chlorine + ultrasound	$464.10 \pm 40.39^{a(z)}$	$569.40 \pm 40.69^{a(y)}$	$629.04 \pm 33.07^{a(x)}$
	Tsunami + ultrasound	$446.19 \pm 36.45^{a(y)}$	$451.41 \pm 16.07^{b(y)}$	$584.25 \pm 103.62^{a(x)}$
	Tsunami + ultrasound + SDS	$343.42 \pm 51.60^{b(y)}$	$421.11 \pm 48.69^{b(x)}$	$584.35 \pm 103.51^{(x)}$
Iceberg	Raw lettuce	429.42 ± 29.92^{a}	ND	ND
	Water	$223.60 \pm 33.30^{b(y)}$	$331.14 \pm 29.92^{a(x)}$	$307.45 \pm 13.62^{b(x)}$
	Chlorine + ultrasound	$219.17 \pm 32.93^{b(y)}$	$293.98 \pm 35.14^{a(x)}$	$337.44 \pm 42.09^{a(x)}$
	Tsunami + ultrasound	$220.00 \pm 22.94^{b(y)}$	$310.97 \pm 36.18^{a(x)}$	$289.47 \pm 21.57^{bc(x)}$
	Tsunami + ultrasound + SDS	$182.21 \pm 35.46^{b(y)}$	$313.52 \pm 20.59^{a(x)}$	$275.84 \pm 24.25^{c(x)}$

a–c: Treatment means within treatments (columns) with different letters are different at α 0.05.
x, y: Treatment means within days (rows) with different letters are different at α 0.05.
˜: ND No determined.
*SE Standard error.

smaller and significantly different from the untreated sample ($P < 0.05$), indicating a loss of turgor in the treated samples. After day 7, an increase in MCF values was observed, which might be caused by self-repair and production of phenolic compounds as a defense mechanism (Qi, Hu, Jiang, & Tian, 2011; Wang, 2004). Nonetheless, on days 7 and 14, the MCF values for all four treatments were still lower than that of the untreated sample. On the other hand, the response of Romaine lettuce was quite different. Except for the Tsunami 100® + SDS + US treatment, the day 0 MCF values for treated Romaine were significantly higher than for the untreated sample, and the MCF values were higher on days 7 and 14 than on day 0. A similar trend was reported by Manolopoulou, Lambrinos, Chatzis, Xanthopoulos, and Aravantinos (2010), who after cutting and washing lettuce with chlorinated water and, reported a (statistically insignificant, $P > 0.05$) increase in textural properties during a 15-day storage at 5 °C.

3.4. Sensory evaluation

The mean assessments of visual quality parameters such as overall quality (OQ), surface browning (SB), and sogginess of Romaine and Iceberg lettuce stored for 0, 7 and 14 days are shown in Fig. 3. Progressive quality degradation as shown by decreasing OQ values was observed during storage for all treatments. On day 0, there were no significant differences in the hedonic rating of OQ, SB and sogginess in both lettuce types for all the treatments ($P > 0.05$).

On day 7, the highest hedonic rating for overall quality received by Romaine lettuce was 5.93 (treated with chlorine + US) while the corresponding value for Iceberg lettuce was 5.27 (treated with Tsunami 100® + SDS + US). The corresponding low values of the surface browning hedonic rating were 1.93 and 1.60 for Romaine and Iceberg, respectively. The samples were still appealing to panelists ($P < 0.05$). After 14 days of storage, the Romaine lettuce treated with chlorine received the highest OQ rating (4.68 ± 1.70) and the lowest surface browning (2.33 ± 0.72) hedonic rating ($P < 0.05$). On day 14, there were no significant differences in Iceberg lettuce hedonic rating for overall quality and surface browning ($P > 0.05$). However, none of the treatments were rated as appealing to panelists by the end of 14 days of storage, regardless of the relatively high rating in overall quality of samples treated with chlorine. These results are comparable with the work of Rodgers, Cash, Siddiq, and Ryser (2004), who stated that chlorinated products helped to preserve the overall quality of fresh-cut lettuce. The results are consistent with those of McWaters, Hashim, Walker, Doyle, and Rimal (2002), who in a combined wash of hydrogen peroxide and an organic acid also reported adverse effects of treatment on the sensory quality of lettuce, as well as decreasing sensory ratings during storage. We found no significant differences in sogginess during storage for Iceberg or Romaine lettuce ($P > 0.05$). Allende, Aguayo, and Artes (2004) observed that modified atmosphere packaging (MAP) of lettuce did not improve the quality of the product, but did delay decay during storage.

3.5. Total color difference

The total color changes during storage for Romaine lettuce subjected to different treatments were not very different (Table 3) except for the sample treated with water, which had a significantly lower color change on day 14 compared to that on day 7. The color changes observed for Iceberg lettuce were similar, except for the chlorine + US treatment at day 7. From this we infer that different chemical and ultrasound treatments had little effect on color change during 14 days of storage at 4 °C for lettuce that was cut and then washed. We note that the color readings all have relatively large standard errors, which we attribute, in part, to the heterogamous composition of different tissues in cut-lettuce samples, as discussed by Baur, Klaiber, Hammes, and Carle (2004).

3.6. Aerobic plate count

The total aerobic plate counts for Romaine and Iceberg lettuce during storage are presented in Table 4. At day 0, the samples treated with chlorine or Tsunami 100®, either in combination with ultrasound, had the lowest mean survival counts of aerobic bacteria on Romaine lettuce; however, there are no significant differences among the treatments ($P > 0.05$). The survival count of aerobic bacteria on Iceberg lettuce only washed with water was higher than samples treated with combinations of sonication and sanitizers. Treatment with Tsunami 100® + SDS + sonication achieved achieved a higher number in reduction of aerobic microorganisms but was no significantly different from other treatments ($P > 0.05$). At day 7, a sharp increase in total aerobic plate count for both Romaine and Iceberg is observed; and might be due to tissue damage, availability of O_2 inside the packages, or the presence of moisture and nutrients on produce surfaces that support microbial growth. Notably, the Romaine lettuce treated with chlorine + ultrasound had the lowest aerobic plate count at day 7, significantly different from other treatments ($P < 0.05$). Additionally on day 7, the Romaine lettuce treated with chlorine received the highest overall quality rating, showing a good correlation between the low natural micro-flora count and produce

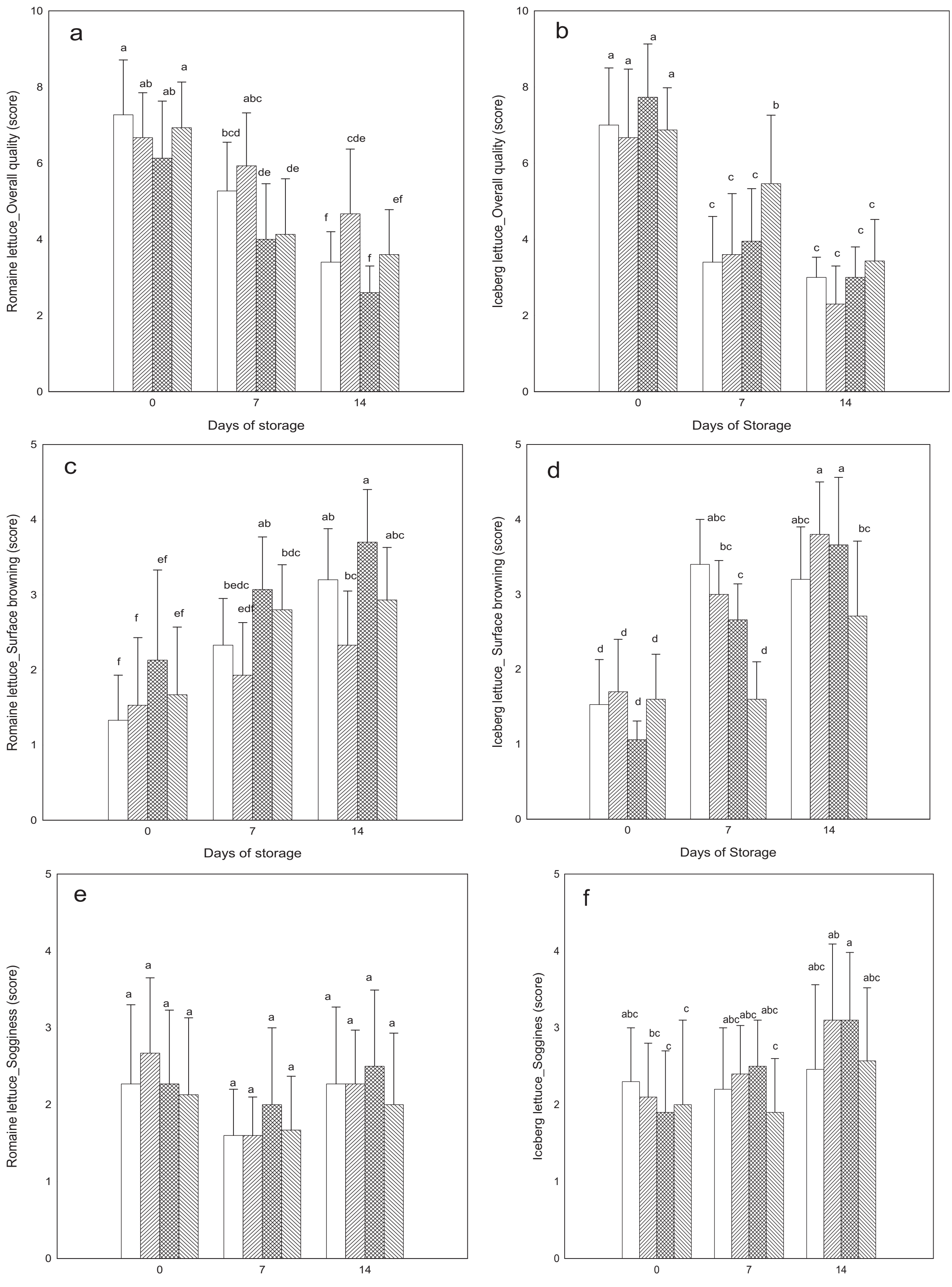

Fig. 3. Sensory evaluation parameters of Romaine and Iceberg lettuce during 14 days of storage. □ Water, ▨ Chlorine + US, ▩ Tsunami + US, ▧ Tsunami + US + SDS. (a) Overall quality of Romaine lettuce during storage. (b) Overall quality of Iceberg lettuce during storage. (c) Surface browning of Romaine lettuce during storage. (d) Surface browning of Iceberg lettuce during storage. (e) Sogginess of Romaine lettuce during storage. (f) Sogginess of Iceberg lettuce during storage. a–c Treatment means within sanitizer treatment with different letters are different at α 0.05.

Table 3
Total color difference (TCD) of Romaine and Iceberg lettuce during storage.

Lettuce type	Treatments	Day		
		TCD day 0 Mean ± SE*	TCD day 7 Mean ± SE	TCD day 14 Mean ± SE
Romaine	Water	NA	13.88 ± 4.60$^{a(x)}$	7.84 ± 5.87$^{a(y)}$
	Chlorine + ultrasound	13.48 ± 5.28$^{a(x)}$	12.47 ± 4.40$^{a(x)}$	12.04 ± 6.25$^{a(x)}$
	Tsunami + ultrasound	14.00 ± 5.88$^{a(x)}$	14.49 ± 5.57$^{a(x)}$	9.42 ± 6.30$^{a(x)}$
	Tsunami + SDS + ultrasound	9.81 ± 3.90$^{a(x)}$	9.78 ± 6.81$^{a(x)}$	10.80 ± 8.20$^{a(x)}$
Iceberg	Water	NA	10.78 ± 5.50$^{a(x)}$	11.90 ± 5.33$^{a(x)}$
	Chlorine + ultrasound	12.35 ± 6.20$^{a(x)}$	6.76 ± 3.68$^{a(y)}$	8.50 ± 4.36$^{a(xy)}$
	Tsunami + ultrasound	7.81 ± 4.40$^{a(x)}$	9.64 ± 3.60$^{a(x)}$	8.93 ± 3.19$^{a(x)}$
	Tsunami + SDS + ultrasound	11.33 ± 6.00$^{a(x)}$	11.31 ± 6.28$^{a(x)}$	11.60 ± 8.60$^{a(x)}$

a–c: Treatment means within treatments (columns) with different letters are different at α 0.05.
x, y: Treatment means within days (rows) with different letters are different at α 0.05.
*SE Standard error.

Table 4
Aerobic plate count (APC) of Romaine and Iceberg lettuce during storage.

Lettuce type	Treatments	Day of storage		
		APC day 0 Log_{10} CFU/g Mean ± SE*	APC day 7 Log_{10} CFU/g Mean ± SE	APC day 14 Log_{10} CFU/g Mean ± SE
Romaine	Water	4.42 ± 0.39$^{a(y)}$	6.97 ± 0.38$^{a(x)}$	7.14 ± 0.21$^{b(x)}$
	Chlorine + ultrasound	3.34 ± 0.26$^{a(z)}$	6.15 ± 0.17$^{b(y)}$	6.88 ± 0.12$^{b(x)}$
	Tsunami + ultrasound	2.70 ± 1.40$^{a(y)}$	6.84 ± 0.21$^{a(x)}$	7.24 ± 0.17$^{ab(x)}$
	Tsunami + SDS + ultrasound	4.03 ± 0.17$^{a(z)}$	7.06 ± 0.18$^{a(y)}$	7.52 ± 0.05$^{a(x)}$
Iceberg	Water	5.94 ± 0.32$^{a(y)}$	7.34 ± 0.25$^{a(x)}$	7.56 ± 0.23$^{a(x)}$
	Chlorine + ultrasound	5.53 ± 0.73$^{a(y)}$	7.14 ± 0.90$^{a(x)}$	7.37 ± 0.03$^{a(x)}$
	Tsunami + ultrasound	5.22 ± 0.78$^{a(y)}$	7.05 ± 0.35$^{a(x)}$	7.35 ± 0.23$^{a(x)}$
	Tsunami + SDS + ultrasound	5.19 ± 0.80$^{a(y)}$	7.12 ± 0.41$^{a(x)}$	7.20 ± 0.14$^{a(x)}$

a–c: Treatment means within treatments (columns) with different letters are different at α 0.05.
x, y: Treatment means within days (rows) with different letters are different at α 0.05.
*SE Standard Error.

quality. At the end of storage, the total aerobic plate count remained unchanged with no significant differences observed in both lettuce samples. This can be interpreted as stabilization in microbial growth during storage; the microorganisms reached a stationary phase of growth, with consumption of nutrients leading to decay in produce quality (Jacxsens, Devlieghere, & Debevere, 2002). A similar trend was reported by Akbas and Olmez (2007) who treated lettuce samples with organic acid and stored them at 4 °C for 12 days. Their counts of aerobic and psychrotrophic bacteria sharply increased from day 0, but remained constant after the mid-point of storage. Growth of yeasts and molds for the two lettuce types and among the three sanitization treatments were all below 0.7 Log 10 CFU/g, indicating the effectiveness of sanitization (Table 5). On the contrary, the Romaine lettuce only washed in water recorded 1.85 ± 0.99 and 1.57 ± 0.75 Log 10 CFU/g growth of yeasts and molds at days 7 and 14, respectively.

4. Conclusions

In this study, we compared the effect of washing Romaine and Iceberg lettuce in chlorine, Tsunami 100®, and in Tsunami 100® + 1 g/L SDS, with and without ultrasound, on the quality of lettuce samples. For Romaine lettuce after 14 days of storage, the overall quality when washed in chlorine was better than the other treatments as shown by OQ scores whereas no significant

Table 5
Yeasts and molds of Romaine and Iceberg lettuce during storage.

Lettuce type	Treatments	Day		
		Yeasts & molds day 0 Log_{10} CFU/g Mean ± SE*	Yeasts & molds day 7 Log_{10} CFU/g Mean ± SE	Yeasts & molds day 14 Log_{10} CFU/g Mean ± SE
Romaine	Water	<0.70^{a} $^{(x)1}$	1.85 ± 0.99^{a} $^{(x)}$	1.57 ± 0.75^{a} $^{(x)}$
	Chlorine + ultrasound	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$
	Tsunami + ultrasound	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$
	Tsunami + SDS + ultrasound	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$
Iceberg	Water	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$
	Chlorine + ultrasound	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$
	Tsunami + ultrasound	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$
	Tsunami + SDS + ultrasound	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$	<0.70^{a} $^{(x)}$

[1]Detection limit 0.70 Log_{10} CFU/g.
a–c: Treatment means within treatments (columns) with different letters are different at α 0.05.
x, y: Treatment means within days (rows) with different letters are different at α 0.05.
*SE Standard error.

differences among treatments were found for Iceberg lettuce samples. None of the washing treatments had a detrimental effect on the color of packaged lettuce, and no significant differences were observed in color changes for Iceberg and Romaine lettuces compared to the values on day 0. No significant differences among treatments were observed in plant tissue damage, as measured by either ECR or the firmness of fresh-cut lettuce. Treatments with sanitizers effectively reduced the initial count of natural flora compared to the water wash. During storage, regrowth of bacteria as shown by total aerobic plate counts was observed for all treatments. The use of SDS at low concentration did not cause additional quality changes.

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