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# Whole-head washing, prior to cutting, provides sanitization advantages for fresh-cut Iceberg lettuce (*Latuca sativa L.*)

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ABSTRACT

The efficacy of two leafy produce wash methods, the traditional *cutting-before-washing* process and a new *washing-before-cutting* method, on reduction of *Escherichia coli* O157:H7 inoculated on Iceberg lettuce was compared. The washing tests were conducted in a pilot-scale washer using combinations of water, chlorine, peroxyacetic acid, and ultrasound. The *washing-before-cutting* process recorded an *E. coli* O157:H7 count reduction 0.79–0.80 $\log_{10}$ CFU/g higher than that achieved with the *cutting-before-washing* process in treatments involving only a sanitizer. When ultrasound was applied to the *washing-before-cutting* process, a further improvement of 0.37–0.68 $\log_{10}$ CFU/g in microbial count reduction was obtained, reaching total reductions of 2.43 and 2.24 $\log_{10}$ CFU/g for chlorine and peroxyacetic acid washes, respectively.



## 1. Introduction

Washing is an important step in fresh produce processing. It reduces microbial populations, and is the only step that removes soil and debris. Cutting or shredding is a physical process to reduce the size of produce, providing convenience in packaging, transportation, and consumption. Currently, the produce industry normally applies a "triple-wash" procedure, where cut produce is prewashed in a primary flume/tank, followed by a sanitization wash (typically with chlorinated water) in a second flume/tank, and finally by a clean water rinse to remove residual sanitizer from produce surfaces (Li et al., 2008). In this process, the washing and rinsing are performed after cutting or shredding.

There are several potential problems associated with this process. First, cutting, and especially shredding, wounds the produce tissue. It allows latex and other produce exudates to leak into the washing solution, where reaction with sanitizer (particularly with chlorine) can lead to accelerated consumption of sanitizer (Pirovani et al., 2004; Nou and Luo, 2010). This can, in turn, lead to the sanitizer concentration falling below a critical level needed to kill microorganisms in the washing solution, which can allow cross contamination from one contaminated leaf to the washing solution and on to otherwise clean leaves (Luo et al., 2011). Reaction with chlorine can also lead to formation of potentially harmful disinfection by-products, including chloroform (López-Gálvez et al., 2010a; Van Haute et al., 2013). Beyond these effects on sanitizer concentration, cutting also provides sites for preferential attachment, sheltering, and internalization of pathogens (Singh et al., 2002; Gleeson and O'Beirne, 2005; Brandl, 2008; López-Gálvez et al., 2010b; Esseili et al., 2012), rendering them much less vulnerable to removal or inactivation by sanitization treatments.

It is thus clear that the interaction between produce cutting and sanitization needs to be carefully examined. In this work, we describe and test a new *washing-before-cutting* approach (Nou and Luo, 2010) to produce processing, whose aim is to minimize the aforementioned problems. Whole-head Iceberg lettuce (*Lactuca sativa* L.), inoculated with *Escherichia coli* O157:H7, was used as the model system, and was processed using both traditional *cutting-before-washing*, as well as *washing-before-cutting*, with washing in water, in water with sanitizer (20 mg/L free chlorine or 80 mg/L of peroxyacetic acid), and with and without simultaneous ultrasound treatment. The washing tests were performed in a previously developed pilot-scale continuous-flow ultrasonic washer (Zhou et al., 2012), and the acoustic power level was selected such that no unacceptable produce quality degradation would be observed during two-week storage (Salgado et al., 2014). In addition, we examined the use of peroxyacetic acid as an alternative sanitizer to reduce *E. coli* O157:H7 population on lettuce.

The present work differs in several key respects from the earlier work of Nou and Luo (2010). First, Nou and Luo used Romaine lettuce and compared washing of uncut individual leaves, followed by cutting, to washing of cut lettuce. We have used Iceberg lettuce, and compared washing of whole-head lettuce, followed by cutting, to washing of cut lettuce. The practicality of whole-head washing is considerably greater than that of individual leaf washing, especially for a closed-head

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produce type such as Iceberg. Second, Nou and Luo washed in a batch process in a bench-top container where each leaf has exactly the same exposure time, whereas we have washed in a pilot-scale continuous flow system, in which there is a dispersion of exposure times associated with the residence-time distribution of the flow.

## 2. Materials and methods

### 2.1. Specific gravity of, and water absorption by, whole-head Iceberg lettuce

Washing whole-head Iceberg lettuce before cutting requires that two issues be addressed. First, whole-head lettuce floats on water, in part due to inclusion of air between leaves and inside "ribs." Second, over a period of time, water infiltrates the head, which not only affects buoyancy, but can also transport sanitizer and bacteria into the interior of the head. To that end, we conducted a simple investigation of the buoyancy and water absorption of whole-head Iceberg lettuce as affected by wash water temperature and initial produce temperature.

The specific gravity (SG) of Iceberg lettuce submerged in water was determined using the method described by Mohsenin (1996) with the following equation

$$SG_{lettuce,submerged} = \frac{W_{lettuce\ \ in\ \ air} \times SG_{water}}{W_{displaced\ \ water}} \quad (1)$$

where $W_{lettuce\ in\ air}$ and $W_{displaced\ water}$ are the weight of the lettuce in air and the weight of the water displaced by the fully-submerged lettuce. The Iceberg lettuce heads were weighed and aseptically stored overnight at either 5 °C or 23 °C. We used two water temperatures, which were obtained by filling a tank with tap water at either 4 °C or 23 °C. For each of the four combinations of storage and immersion temperatures, individual heads were completely submerged for 1 min in a tank filled to capacity, and the weight of the displaced water was recorded. Each head was transferred to a second container and drained by gravity (without re-orientation[1]) for 40 min, as determined by a preliminary test,[2] to collect water trapped inside. After draining, each head was weighed a second time in order to determine the change in weight following the 1-min immersion and 40-min draining.

### 2.2. Pilot-plant wash system

Iceberg lettuce washing was carried out in a pilot-scale continuous-flow, recirculating, ultrasonic washing system described by Zhou et al. (2012). The washer consisted of a water tank with an approximate capacity of 1.51 $m^3$, equipped with submerged water jets that promote agitation of the samples, and with three pairs of ultrasound transducer boxes (25, 40, and 75 kHz), each pair of which is driven by an ultrasound generator (Quality Sonic Products, EZ, SOEST, Netherlands) with a rated power of 2 kW.

Prior to the start of each test, the wash tank was filled with chilled tap water (10 °C) and degassed for 10 min to remove dissolved gases and improve ultrasound efficacy. Chlorine solutions were prepared by dilution of Clorox® (active ingredient: sodium hypochlorite, 6.15%) to 20 mg/mL of free chlorine (60 mg/mL in §2.4). Peroxyacetic acid solutions were prepared by dilution of Tsunami-100® (active ingredient: peroxyacetic acid) to 80 mg/mL of acid concentration (60 mg/mL in §2.4). When a sanitizer was used, it was added to the degassed water to achieve the final desired concentration, and allowed to circulate in the washer in order to uniformize the concentration. Submerged water jets were used to ensure mixing of lettuce in the tank. Horizontal aluminum bars having a relatively smooth surface were placed just below the water surface to ensure that each whole head was fully immersed in the washing solution, and that every portion of its surface would be exposed to the same liquid environment.

### 2.3. Sample and chemical preparation

#### 2.3.1. Bacterial strain preparation

A nalidixic acid resistant mutant of nonpathogenic *E. coli* O157:H7 strain ATCC 87-23 obtained from the former Produce Quality and Safety Lab (USDA-ARS, Beltsville, MD, USA) was used in the experiments. The bacterial strain was prepared by repeated sub-culturing on a nutrient plate containing 50 mg/L of nalidixic acid (Sigma Aldrich, St. Louis, MO). Cultures of *E. coli* O157:H7 were grown in tryptic soy broth (TSB) (Sigma Aldrich, St. Louis, MO) overnight at 37 °C. Cells were harvested by centrifugation at 4 °C and 2,455 *g* for 10 min, and washed twice in sterile 0.1% peptone water. The recovered *E. coli* precipitate was diluted in 6 mL of 0.1 % peptone water; the final inoculation level was $2.5 \times 10^7$ CFU/mL.

#### 2.3.2. Sample preparation

Iceberg lettuce (*L. sativa* L.) heads were purchased from a local supermarket and immediately transported to a processing laboratory where they were stored at 5 ± 1 °C and used within 24 h of purchase. The three outermost leaves of each head were removed and discarded. A sterile kitchen knife was used to slice each head of lettuce into pieces of approximately 1 $in^2$ (6.45 $cm^2$).

#### 2.3.3. Sample inoculation

Each head of lettuce was inoculated at 10 different spots on the upper half surface with 200 μL of *E. coli* O157:H7 ATCC 87-23 inoculum and dried at room temperature for 2 h in a laminar-flow purifier PCR enclosure with a vertical airflow of 60–80 fpm (Labconco®, Kansas City, MO, USA) to allow bacterial attachment. The drying time was selected because it allows for good attachment but relatively little cell growth, and simulates produce pre-harvest scenarios (Han et al., 2001; Critzer et al., 2007). After drying, the head lettuce was cut using a sterile kitchen knife into 1 $in^2$ pieces prior to or after washing for 2 min in the continuous-flow ultrasound tank in a chlorine (final free chlorine concentration 20 mg/L) or peroxyacetic acid (final acid concentration 80 mg/L) solution.

### 2.4. Evaluation of degradation of chemicals

The decay of free chlorine and peroxyacetic acid during washing of Iceberg lettuce using a sample-to-solution ratio of about 1:27 (by mass) was investigated. The chlorine solution (free chlorine concentration 60 mg/L) and peroxyacetic acid solution (final acid concentration 60 mg/L) were prepared using distilled water. Four hundred and fifty grams of lettuce was submerged in 12 L of washing solution and washed for 1 min, during which time the samples were manually agitated. In each case, the concentration of sanitizer in the washing solutions was measured prior to addition of lettuce and after 1 min of treatment. The free chlorine concentration was measured using a free chlorine standard kit (Hach Company, Loveland, CO, USA). The concentration of peroxyacetic acid was measured by titration using a Peracid/Peroxide #311 test kit provided by Ecolab (St Paul, MN, USA).

### 2.5. *E. coli* O157:H7 inactivation with chlorine or peroxyacetic acid wash in combination with ultrasound

#### 2.5.1. Washing of lettuce

Lettuce was washed before and after cutting. In the traditional *cutting-before-washing* treatment, inoculated heads were cut across

[1] While it is certainly possible that the rate at which water will drain from the lettuce during the 40-min period (and indeed the total amount that will drain during that time) might depend on the orientation of the lettuce (e.g., stump up, stump down, etc.) no evidence of such an effect was evident in our data.

[2] This time is long enough for drainage from the head to have nearly ceased, but not so long that any significant dehydration of tissue has occurred.

the inoculation spots, in order to transport bacteria to the wound, and to simulate a cross-contamination scenario. The cut samples were then washed for 2 min in the continuous-flow ultrasonic tank (Fig. 1) with one of the following treatments: water, chlorinated water, peroxyacetic acid in water, chlorinated water + ultrasound, and peroxyacetic acid in water + ultrasound. In the *washing-before-cutting* tests, inoculated whole-head lettuce was washed for 2 min with the sanitizing treatments mentioned above. After washing, the head was cut into 1 $in^2$ pieces. Washed samples were drained for 1 min to remove excess water before subsequent microbial analysis.

#### 2.5.2. Enumeration of E. coli O157:H7

Washed lettuce samples were aseptically transferred to a sterile kitchen blender containing 0.1% peptone water, supplemented with 10% sodium thiosulfate to neutralize chlorine and stop chlorine-associated reaction. For treatment with peroxyacetic acid, phosphate buffered saline was added to stop reaction. Samples were macerated for 2 min, followed by a 2-min resting period to allow foams to dissolve. The filtrate was 10-fold serially diluted with 0.1% peptone water; 100 μL of the serially diluted samples was spread plated in duplicate over tryptic soy agar plates supplemented with 50 mg/L nalidixic acid. The plates were incubated at 37 °C for 24 h and colonies were counted manually.

### 2.6. Statistical analysis

The experiments were performed with a complete randomized design (CRD) with each treatment conducted three times. The *E. coli* O157:H7 population counts were subjected to log transformation before statistical analysis. Data were analyzed using a general linear model available in SAS version 9.1 (SAS Institute, Raleigh, NC, USA). Mean separation was determined using Tukey's test with $\alpha = 0.05$.

## 3. Results and discussion

### 3.1. Specific gravity of, and water absorption by, Iceberg lettuce

All heads absorbed water from the tank after 1-min soaking, as shown by weight increase (Table 1), with heads stored at 23 °C and immersed in 4 °C water having the greatest percentage increase. When the lettuce was soaked in water at 4 °C, samples that had been stored at 23 °C absorbed significantly more water than those stored at 5 °C ($P < 0.05$). All SG values were less than unity, and no significant differences in the SG value among the different storage temperatures and washing temperatures were observed ($P > 0.05$).

Since the SG of water is 1.0, the fact that lettuce's SG < 1 for all cases indicated that lettuce will float on the surface of the washing solution. As a result, a portion of the head will not be submerged in the washing solution, and will thus not be exposed to sanitizer. To address this problem, a special holder was designed and installed in the pilot-scale washer, as shown in Fig. 1.

Since there are air gaps between lettuce leaves (Cosgrove, 1993), it is not surprising that each head absorbed water and increased in weight (Table 1). This absorption will carry sanitizing solution (and possibly small suspended particles, such as dirt and microorganisms) into the interior of the head. When the sanitizer concentration is sufficiently high, this inflow of wash liquid might help to sanitize those interior leaves with which it comes into contact. However, if the sanitizer concentration is below a certain threshold for preventing cross contamination, the net result of this influx of washing liquid might be to carry microorganisms into the interior of the head, causing cross contamination (Luo et al., 2011).

Since all industrial lettuce washing is done at low temperatures, Table 1 makes clear that prewashed lettuce should be kept at similar temperatures, in order to minimize water absorption during washing. This is consistent with the work of Buchanan et al. (1999), who

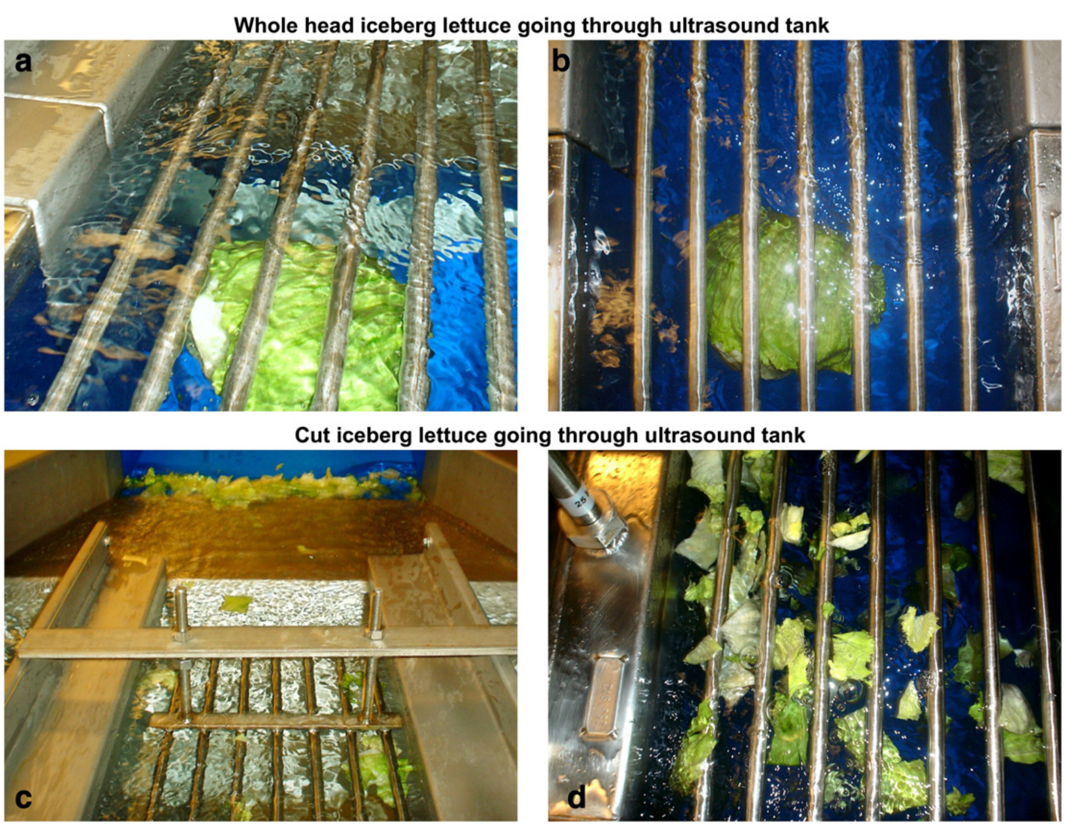


**Fig. 1.** Sanitization of Iceberg lettuce in ultrasound tank. (a) Whole head lateral view. (b) Whole head aerial view. (c) Cut lateral view. (d) Cut aerial view.

**Table 1**
Specific gravity and water absorption of lettuce at two storing (5°C, 23 °C) and two wash water (4°C, 23 °C) temperatures.

| Water temperature (°C) | Storage temperature (°C) | Specific gravity (SG) Mean ± SE* | Increase in weight (%) Mean ± SE |
|---|---|---|---|
| 4 | 5 | 0.86 ± 0.03 [x] | 7.59 ± 0.87 [b] |
| | 23 | 0.76 ± 0.13 [x] | 9.26 ± 0.45 [a] |
| 23 | 5 | 0.65 ± 0.20 [x] | 7.09 ± 1.74 [A] |
| | 23 | 0.69 ± 0.09 [x] | 7.60 ± 1.17 [A] |

**a–c** Treatment means for increase in weight within water temperature (4 °C) with different letters are significantly different (α 0.05).
**A–C** Treatment means for increase in weight within water temperature (23 °C) with different letters are significantly different (α 0.05).
**x–y** Treatment means within specific gravity (SG) with different letters are significantly different (α 0.05).
* **SE** Standard error

examined the effect of temperature differences on infiltration of *E. coli* O157:H7 for intact apples. After being immersed in cold (2 °C) 1% peptone water containing a high population of *E. coli* O157:H7 for 1 min, apples initially at 2 °C showed less internalization of *E. coli* O157:H7 in the outer core region than was observed for initially warmer (22 °C) apples. Moreover, studies by Kim and Harrison (2009) and Buchanan et al. (1999) provide substantial evidence that bacteria can passively enter produce through the movement of contaminated water. In addition, a drop in temperature will lead to contraction of interleaf air, which can give rise to a reduction in the volume of the head, or ingress of wash liquid (and suspended bacteria). Therefore, it is imperative to minimize the temperature difference between washing liquid and produce in order to minimize absorption of water during disinfection (Deering et al., 2012). Note that we attribute the observed increase in weight to water infiltration, and that while such infiltration is consistent with concomitant microbial transport and internalization, we have no direct evidence for such an effect.

### 3.2. Chlorine and peroxyacetic acid degradation

Degradation of free chlorine was monitored after 1-min contact with lettuce, a time mimicking the wash time used in the produce industry. The free chlorine concentration decreased by 35% and 65%, when whole-head and shredded iceberg lettuce, respectively, were submerged in the chlorine solution for one minute ($P < 0.05$) (Fig. 2). No changes in peroxyacetic acid concentration were observed after submerging whole-head and shredded iceberg lettuce for 1 min ($P > 0.05$), indicating the stability of peroxyacetic acid in the presence of vegetable and cut-vegetable tissues. This is in agreement with the report of González-Aguilar et al. (2012). It appears that free chlorine depletion occurs not only in the presence of cut lettuce (where the organic matter is expected in wash liquid), but also in the presence of whole-head lettuce. It is postulated that chlorine undergoes a number of chemical reactions in solution with organic matter, the result of which is to decrease the amount of active free chlorine and to generate chlorination by-products (Suslow, 1997; Gonzalez et al., 2004). According to Luo et al. (2011), it is imperative to maintain a certain concentration of free chlorine in washing water to inactivate *E. coli* O157:H7 present in the wash water. Therefore, regularly monitoring the free chlorine concentration and timely replenishment of chlorine during sanitization of fresh-cut produce are essential to ensure good anti-microbial activity of the wash liquid.

### 3.3. Reduction of *E. coli* O157:H7 on Iceberg lettuce washed before or after cutting

The efficacy of selected sanitizers in reducing *E. coli* O157:H7 populations on the surface of Iceberg lettuce washed with the traditional *cutting-before-washing* method and the new *washing-before-cutting* method was examined. Fig. 3 shows that when whole-head lettuce was first cut and then washed with chlorine or peroxyacetic acid, the reduction in *E. coli* O157:H7 count was 0.96 and 1.07 $\log_{10}$ CFU/g, respectively. However, by simply changing the order of these two processes, i.e., first washing the whole head followed by cutting, the reduction of *E. coli* O157:H7 counts was increased by 0.79 and 0.80 $\log_{10}$ CFU/g, reaching 1.75 and 1.87 $\log_{10}$ CFU/g in chlorine and peroxyacetic acid, respectively. Furthermore, when ultrasound was introduced, additional reductions of 0.68 and 0.37 $\log_{10}$ CFU/g were realized for washing liquids containing chlorine and peroxyacetic acid, respectively. Thus, washing before cutting, supplemented by ultrasound, provided a total reduction of 2.43 and 2.24 $\log_{10}$ CFU/g for the chlorine and peroxyacetic acid washes, respectively. Since the inoculated Iceberg lettuce was cut directly on inoculation sites (before or after washing), bacteria might have been carried by the knife into the interior of the produce, found

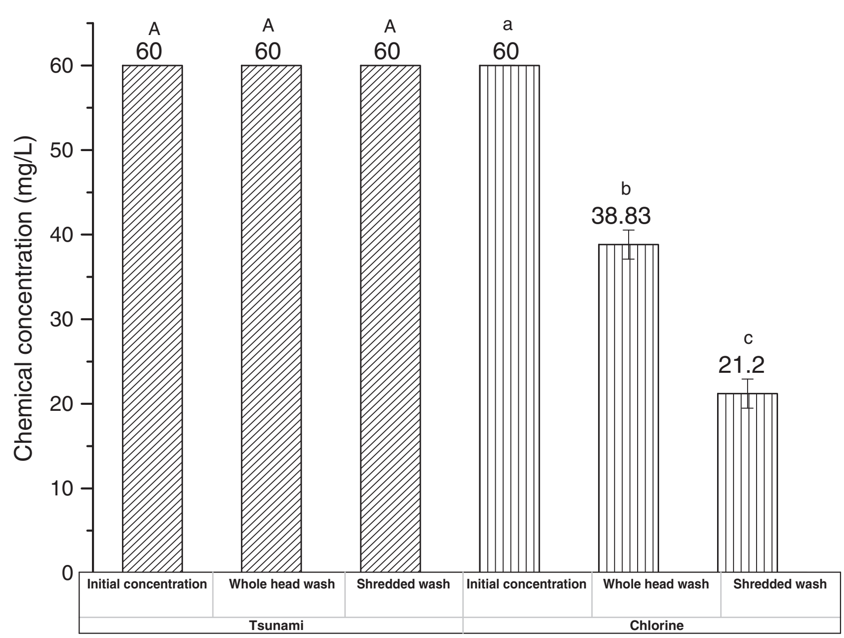


**Fig. 2.** Changes in chlorine and peroxyacetic acid concentration in the presence of organic matter. **A–C** Treatment means within Tsunami with different letters are significantly different (α 0.05). **a–c** Treatment means within chlorine with different letters are significantly different (α 0.05).

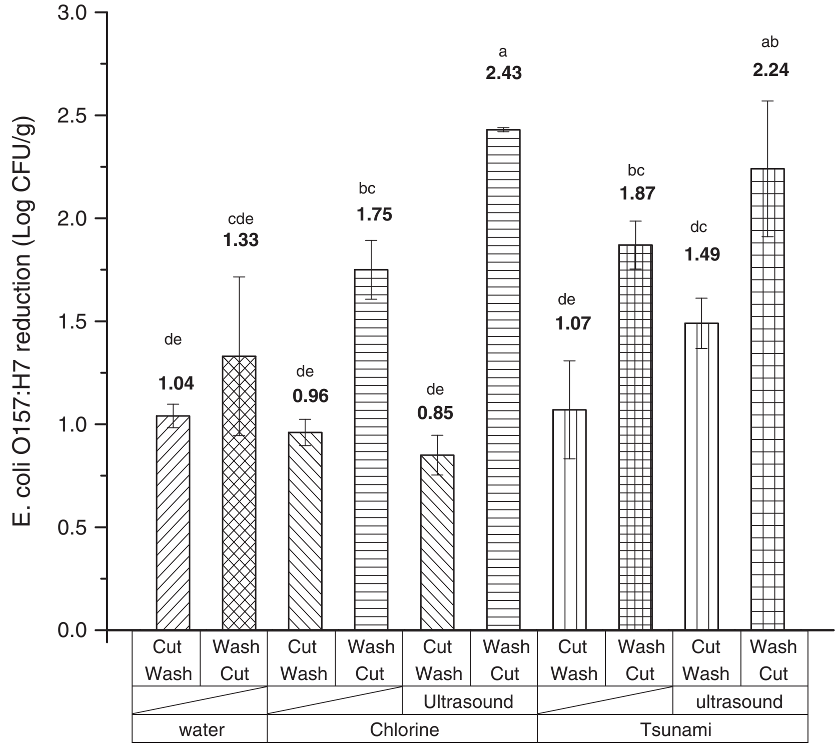


**Fig. 3.** *E. coli* O157:H7 reduction from Iceberg lettuce that was cut prior or post sanitization. **a–e** Treatment means within type of cut and chemical treatment with different letters are significantly different ($\alpha = 0.05$).

shelter on wounded sites, and attached to the cut surfaces, thus decreasing the efficacy of sanitization treatments. As a result, for samples washed *after* cutting and without ultrasound, the differences in microbial reduction observed between samples washed with water, chlorine, and peroxyacetic acid were not statistically significant. For samples washed *before* cutting, ultrasound increased the microbial reduction for both chlorine and peroxyacetic acid sanitization, with the increase for chlorine being statistically significant. For washing with water alone, the increased microbial reduction observed between washing before or after cutting was not statistically significant. This might result from the small sample size (three trials), or from batch-to-batch variation in the produce, inoculation, or cutting. Without ultrasound, the additional 0.79 and 0.80 log reduction of *E. coli* for the *washing-before-cutting* treatments, compared to the traditional *cutting-before-washing* treatment (for chlorine and peroxyacetic acid, respectively), might be the result of any of several factors: a) less exuded organic matter, which consumes less sanitizer in the wash liquid; b) no cut or wounded tissue surfaces, and c) better hydrodynamic flow conditions. In connection with the latter, we note that the whole heads are larger than cut leaves, so that the characteristic length $L$ in the Reynolds number $Re = VL/\nu$ (where $\nu$ is the kinematic viscosity) is larger, and hence the flow conditions at produce surfaces will be more turbulent, thus promoting a thinner concentration boundary layer for the sanitizer and stronger shear forces to dislodge bacteria (Bird et al., 1960).

Beyond the leakage of organic matter, cut and wounded lettuce surfaces also provide attachment sites for bacterial cells, and shelter them against sanitizer. According to Takeuchi and Frank (2000), the effectiveness of a sanitizer on inactivation of microorganisms depends on the accessibility of the cells. Therefore, produce-processing approaches that avoid cutting/wounding of unsanitized produce are advantageous (Allwood et al., 2004). The *E. coli* O157:H7 count reductions in lettuce washed with the two methods clearly demonstrate the advantage of washing whole-head lettuce before cutting. This simple change in the washing-cutting sequence may provide a practical means to significantly reduce food safety risk for both the produce industry and consumers. The work also confirms previous reports of enhanced microbial inactivation by application of ultrasonication to produce sanitation treatment (Zhou et al., 2009; Zhou et al., 2012).

While the "closedness" of the Iceberg head is not characteristic of all leafy produce, production volumes for fresh-cut and shredded Iceberg and for cabbage (which has a very "closed" head) are so high that the approach seems to be worthy of consideration. We also note that, even for less closed leafy produce (e.g., Romaine and green-leaf lettuces), there are still significant advantages to *washing-before-cutting*, because this approach provides major reductions of bacterial populations before creating wounds where bacteria can attach, shelter, and internalize, as shown by Nou and Luo (2010).

## 4. Conclusions

The temperature difference between produce and washing solution played an important role determining the water absorption of produce during a sanitation wash. The positive temperature difference between produce (23 °C) and washing solution (4 °C) resulted in the highest water absorption in this study, showing the need to minimize the difference between the initial temperature of the lettuce and the temperature of the wash water.

This work clearly shows the benefits of washing whole-head Iceberg lettuce before cutting. Significantly enhanced reductions in *E. coli* O157:H7 populations were obtained when whole-head Iceberg was washed with chlorine or peroxyacetic acid before cutting, compared to the traditional *cutting-before-washing* sequence. Additional increases in microbial reduction were achieved by applying ultrasound to the *washing-before-cutting* sequence, for both chlorine and peroxyacetic acid, with the increase for chlorine being statistically significant. When washed with sanitizer-free water (without ultrasound), the increase in bacterial reduction found for *washing-before-cutting*, compared to *cutting-before-washing*, was not statistically significant for the three-trial tests conducted. Similarly, the increased reductions found when ultrasound was used in cutting-before-washing treatments were also not statistically significant. The depletion of free chlorine in wash liquid was significantly higher for cut Iceberg lettuce compared to that for whole-head, and less reduction of *E. coli* O157:H7 from the surfaces of cut lettuce was recorded. Adoption of the new *washing-before-cutting* process will help the produce industry enhance the sanitization efficacy and reduce microbial hazards.

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