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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 2.5×10^7 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.