Journal of Food Protection, Vol. 75, No. 3, 2012, Pages 563–566
doi:10.4315/0362-028X.JFP-11-218

Research Note

Sanitation and Design of Lettuce Coring Knives for Minimizing *Escherichia coli* O157:H7 Contamination

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MS 11-218: Received 4 May 2011/Accepted 18 October 2011

ABSTRACT

This study was undertaken to examine the effect of ultrasound in combination with chlorine on the reduction of *Escherichia coli* O157:H7 populations on lettuce coring knives. Two new coring devices designed to mitigate pathogen attachment were fabricated and evaluated. The coring rings of the knives were dip inoculated with soil slurry containing 10^6 *E. coli* cells and treated with chlorinated water with and without ultrasonication for 30, 60, and 120 s. The rough welding joints on currently used in-field lettuce coring knives provided a site conducive to bacterial attachment and resistant to cell removal during sanitation treatment. The two modified coring knives harbored significantly fewer *E. coli* cells than did the currently used commercial model, and the efficacy of the disinfection treatment was high ($P < 0.05$). Ultrasound treatment reduced the *E. coli* O157:H7 counts to below the detection limit of 1.10 log CFU/cm^2 at both the coring ring blade and welding joint within 30 s in 1 ppm of chlorinated water. The redesigned coring knives and an ultrasound plus chlorine combination treatment may provide practical options for minimizing the microbial safety hazards of lettuce processed by core-in-field operations.

Before packaging, storage, and distribution, iceberg lettuce grown for fresh-cut processing is trimmed and cored in the field (CIF). Initiated in the late 1990s, CIF operations increase yields in processing plants, decrease nonmarketable tissues handled in the process *(1, 2),* and reduce shipping costs *(4, 5)*. Because the outer leaves are removed and only relatively clean inner leaves are harvested, soil contamination and total microbial populations are reduced. However, significant tissue damage occurs during core removal, and the additional human handling also increases the vulnerability of the lettuce to direct contamination, infiltration of microbes through the cut edges, and subsequent microbial growth *(7)*. Coring knives can become contaminated through direct contact with soil, plants, or workers' gloves and can transfer contaminating microbes to the edible portions of the lettuce heads *(11)*. McEvoy et al. *(8)* and Taormina et al. *(12)* found that a single contaminated coring knife can transfer *Escherichia coli* O157:H7 to 10 to 19 lettuce heads, depending on the initial contamination level. The commonly used coring knife consists of two parts, a cutting blade and a coring ring. The coring ring is attached to the metal tang by a rough-surface welding joint, a site conducive to the attachment of microbes. Strategies to minimize such contamination and maximize the removal of pathogens from the coring knives are therefore needed.

Ultrasound treatment can effectively remove biofilms on food processing surfaces *(3, 9, 10)*. In an ultrasonic cleaning operation, acoustic bubbles oscillate near the surface being cleaned. The flow resulting from the collapse of the bubbles creates drag and shear forces on the surface, causing the cleaning. Cavitation-related phenomena, such as those produced by water jets and micro-streaming, are also important in detaching particles and microbes from a surface *(6)*. This study was conducted to examine the effects of ultrasound in combination with chlorine on the reduction of *E. coli* O157:H7 populations on coring knives. An effort was made to design and fabricate two new prototype coring devices which can mitigate pathogen attachment.

MATERIALS AND METHODS

Inoculum preparation. *E. coli* O157:H7 strain 87-23 (nonpathogenic), which is resistant to 50 μg/ml nalidixic acid, was used in this study. The cells from a tryptic soy agar (TSA; Difco, BD, Sparks, MD) slant were transferred three times to tryptic soy broth (pH 7.3; Difco, BD) by loop inoculation at 24-h intervals and incubated at 37°C. Bacterial cells were harvested at 24 h by centrifugation (6,000 × *g*) at 4°C for 10 min, washed twice in sterile peptone water (0.85% NaCl, 0.1% Bacto Peptone), and resuspended in 10 ml of peptone water. The final level of *E. coli* O157:H7 in the inoculum was approximately 10^9 CFU/ml. Iceberg lettuce (867 ± 2 g) was purchased from a local wholesale store. Two heads were trimmed and cored, homogenized for 2 min with a blender (Waring Commercial Blender, Waring Commercial, Torrington, CT), and filtered through four layers of cheesecloth, producing 600 ml of lettuce juice. Sandy-loam soil samples were

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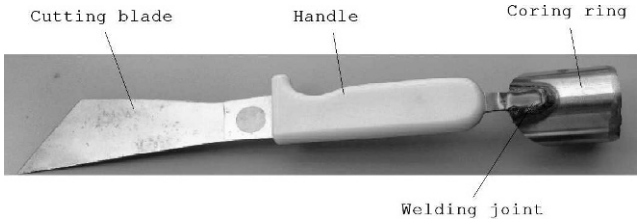

FIGURE 1. *Commercial lettuce coring knife.*

collected from a local field (Urbana, IL), dried at 55°C for 24 h, ground with a mortar and pestle, and autoclaved at 121°C for 40 min. Aliquots (125 g) of sterile soil powder were mixed with 250 ml of lettuce juice and agitated well with a stir bar. A 0.25-ml aliquot of *E. coli* O157:H7 inoculum (approximately 10^9 CFU/ml) was mixed thoroughly with 250 ml of the soil–lettuce juice slurry to obtain the desired initial inoculum level.

Design retrofit and fabrication of coring knives. Three types of lettuce coring knives were used in the study: a commonly used commercial CIF harvest knife (Agricultural Products, Inc., Salinas, CA) (Fig. 1) and two new redesigned knives with improved food safety features. The first new prototype (Fig. 2B1 and 2B2) was developed with no joint between the cutting blade and the coring ring. The cutting blade on prototype 1 was the same size and shape as that on the commercial knife. The new coring ring and a tang were crafted from a 316 stainless steel tube fabricated by wire electrical discharge machining (Fig. 2). The new coring knife handle was made of a Vero material (Objet Geometries, Inc., Billerica, MA) in two halves by the rapid prototyping technique. The coring ring was welded to the tang of the cutting blade with a tungsten inert gas weld, and the tang was embedded in the two halves of the Vero handle, which were subsequently bonded with liquid steel-epoxy resin (J-B Weld, Sulphur Springs, TX) and clamped for 24 h. Prototype 2 was fabricated by smoothing the rough welding joint of a commercial coring knife using a mechanical polishing method (Fig. 2C1 and 2C2).

Inoculation of coring knives. Because harvest crews in the field may use coring knives for about 2 h before disinfecting them, the knives may accumulate a significant amount of soil and lettuce extract before receiving a sanitizer dip-wash treatment. This scenario was simulated in the preparation of the slurry containing the *E. coli* O157:H7 inoculum, lettuce extract, and soil. The coring rings of the knives with and without the welding joint were dip inoculated for 1 min in the *E. coli*–spiked soil–lettuce juice slurry containing 10^6 CFU/ml *E. coli*. Knives were then dried in a biohood (Labconco Purifier 2-ft PCR enclosure, Labconco Corporation, Kansas City, MO) at 22°C for 2 h, which allowed complete drying of the inoculated knife surfaces before sanitization treatments.

Preparation of sanitizer. Chlorine solutions (1, 10, 50, 100, and 200 ppm) were prepared from concentrated food-grade sodium hypochlorite (6% Clorox bleach, Clorox Co., Oakland, CA), and the pH was adjusted to 6.5 with 1.0 M HCl (Fisher Scientific, Pittsburg, PA). Free chlorine concentration was determined with a chlorine test kit (no. 2231-01, HACH, Loveland, CO).

Treatment procedure. Four sets of experiments were conducted. The first test was performed to determine the differential attachment of microbial cells to different locations on the commercial coring knife (see Fig. 1). After 1 min of inoculation and 2 h of drying, the *E. coli* cells attached to the coring knife were sampled and analyzed. In the second test, the purpose was to determine the effectiveness of ultrasonication to remove microbial cells from the commercial coring knife (Fig. 1) when immersed in a chlorine solution. The inoculated coring ring was dipped in a washing tank containing five different concentrations of chlorine (1, 10, 50, 100, and 200 ppm of free chlorine) with and without ultrasonication at 25 kHz for 1 min at room temperature after a 10-min degassing (Quality Sonic Products, EZ Soest, The Netherlands). The third experiment was conducted to examine the effect of ultrasound treatment time and different chlorine concentrations (1, 10, 50, 100, and 200 ppm of free chlorine) on the *E. coli* cells inoculated on the commercial coring knife. The sonication (25 kHz) was performed for 0.5, 1, and 2 min at room temperature after a 10-min degassing. The residue *E. coli* cells on the coring knife were sampled and analyzed. The last experiment was conducted to determine whether the disinfection of *E. coli* cells attached to the surface of coring knives would be improved with the new coring knife designs. The coring rings of the three types of knives were dipped in a wash tank containing 10 ppm of chlorine solution without sonication for 1 min. The residual *E. coli* cells on the coring knives were sampled and analyzed. An inoculated coring knife without treatment was the control.

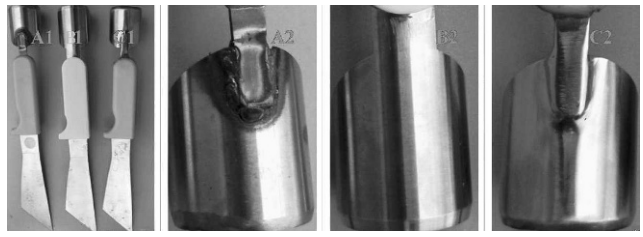

FIGURE 2. *Development of coring knife with improved food safety feature. (A1) Coring knife currently used in the industry; (A2) enlarged image showing the rough welding joint of the coring ring. (B1) Prototype 1, one piece design; (B2) enlarged image showing no welding point. (C1) Prototype 2, commercial coring knife with rough welding point smoothed out; (C2) enlarged image showing the smooth welding joint.*

Microbial analysis. The coring ring blade (smooth surface) and welding joint (rough surface) of a knife before and after polishing and before and after a treatment were sampled by swabbing a marked surface (20 by 20 mm) with three sterile cotton swabs moistened with 0.01 M peptone water *(13).* The cotton swabs were placed in stomacher bags containing 10 ml of 0.01 M peptone water and stomached for 1 min. The stomaching solution was serially diluted, and 200-μl aliquots were spread onto TSA containing 50 μg/ml nalidixic acid and incubated at 37°C for 24 h. Colonies were enumerated and recorded per square centimeter of the sampled area, and the detection limit was 1.10 log CFU/cm^2.

Statistical analysis. Each replicate involved one knife of each type. Three replications with three analytical replicates and three independent trials for each treatment were performed. For the different experiments, statistical analysis was performed using the general linear model of SAS (SAS Institute, Cary, NC). The Fisher's least significant difference test was used to determine differences between means at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Attachment of *E. coli* O157:H7 to commercial coring knife. The commercial CIF knife was composed

TABLE 1. *Recovery of* E. coli *O157:H7 on the smooth coring ring blade and rough welding joint of a commercial coring knife treated with chlorine alone or in combination with ultrasound for 1 min*[a]

Part of knife	Treatment	*E. coli* recovered (log CFU/cm^2) after chlorine treatment at[b]: 1 ppm	10 ppm	50 ppm	100 ppm	200 ppm
Coring ring blade	Chlorine only	2.11 ± 0.13 A	1.25 ± 0.25 B	ND	ND	ND
	Chlorine with ultrasound	ND	ND	ND	ND	ND
Welding joint	Chlorine only	3.15 ± 0.05 A	2.85 ± 0.15 B	ND	ND	ND
	Chlorine with ultrasound	ND	ND	ND	ND	ND

[a] Initial counts on the coring ring blade and welding joint were 2.5×10^2 and 2.1×10^3 CFU/cm^2, respectively.
[b] Within each knife part, means with different letters are significantly different. ND, not detectable (below the detection limit of 1.10 log CFU/cm^2).

of a cylindrical coring ring welded to the metal tang of the cutting blade (Fig. 1), which was set in a plastic handle. The rough welding joint (Fig. 2A2) provided a preferred attachment site for microorganisms, rendering the knife susceptible to direct contamination and cross-contamination and contributing to resistance to disinfection *(11)*. The results indicated that the number of *E. coli* O157:H7 cells (3.55 ± 0.06 CFU/cm^2) attached to the rough welding joint was significantly higher than the number of cells (2.68 ± 0.12 CFU/cm^2) attached to the smooth coring ring blade ($P < 0.05$). For enumeration, only the *E. coli* O157:H7 cells on the outer surface of an inoculated coring ring were counted because the outer surface would be in direct contact with the lettuce during a coring operation *(8, 11)*.

Disinfection of commercial coring knife using ultrasound plus chlorine wash. The current recommendation for in-field sanitation of a harvesting knife involves dipping it in a 200-ppm (total chlorine) chlorine solution for 10 s *(8)*. However, because chlorinated water is typically reused, reaction with organic matter such as lettuce extract and soil present on the knives may cause significant degradation of the chlorine. The results (Table 1) indicated that a wash of 50 ppm of free chlorine without ultrasound was sufficient to reduce the *E. coli* O157:H7 on both the smooth cutting blade and rough welding joint to below the detection limit (1.10 log CFU/cm^2) after 1 min. The bacterial count at the welding joint after a 1-min treatment with the 10 ppm of free chlorine alone was 2.85 log CFU/cm^2, and that after treatment with chlorine plus sonication was below the detection limit (1.10 log CFU/cm^2). At a concentration of only 1 ppm of free chlorine, 1 min of sonication reduced the *E. coli* O157:H7 counts to below the detection limit ($P < 0.05$). In the experiment to examine the effect of sonication time on the removal of *E. coli* O157:H7 from the commercial coring knife, all ultrasound treatments in chlorinated water with concentrations of 1 to 200 ppm of free chlorine reduced the bacterial count to below the detection limit (Table 2).

In the present study, a 25-kHz ultrasound tank with an acoustic power density of 80 W/liter was used in combination with chlorinated water for disinfection of the coring knives. The results (Table 2) indicate that the ultrasound-assisted washes effectively reduced the *E. coli* O157:H7 populations to below the detection limit (1.10 log CFU/cm^2) on both the blade and rough welding joints after a 30-s wash with 1 ppm of chlorinated water. The ultrasonication treatment dramatically decreased the chlorine concentration needed (from 50 to 1 ppm) to reduce the *E. coli* O157:H7 level to below the detection limit. The enhanced inactivation of *E. coli* may be attributed to the effect of acoustic cavitation, which is the formation, growth, and violent implosion of tiny bubbles in a liquid. In another study, the combination of ultrasound and ozonation (0.5 ppm) achieved a complete removal of *Listeria monocytogenes* biofilms on stainless steel chips after 60 s *(3)*. During in-field operations, organic matter from soil and produce juice can cause a rapid depletion of the chlorine concentration in the

TABLE 2. *Recovery of* E. coli *O157:H7 on the commercial coring knife treated with chlorine in combination with ultrasound*[a]

Part of knife	Treatment time (min)	*E. coli* recovered (log CFU/cm^2) after chlorine treatment at: 1 ppm	10 ppm	50 ppm	100 ppm	200 ppm
Coring ring blade	Control[b]	2.42 ± 0.18	2.27 ± 0.11	2.34 ± 0.15	2.47 ± 0.20	2.44 ± 0.11
	0.5	ND[c]	ND	ND	ND	ND
	1	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND
Welding joint	Control	3.26 ± 0.10	3.31 ± 0.07	3.20 ± 0.04	3.39 ± 0.07	3.35 ± 0.12
	0.5	ND	ND	ND	ND	ND
	1	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND

[a] Inoculum was prepared in lettuce juice.
[b] Control inoculated knife parts received no treatment.
[c] ND, not detectable (below the detection limit of 1.10 log CFU/cm^2).

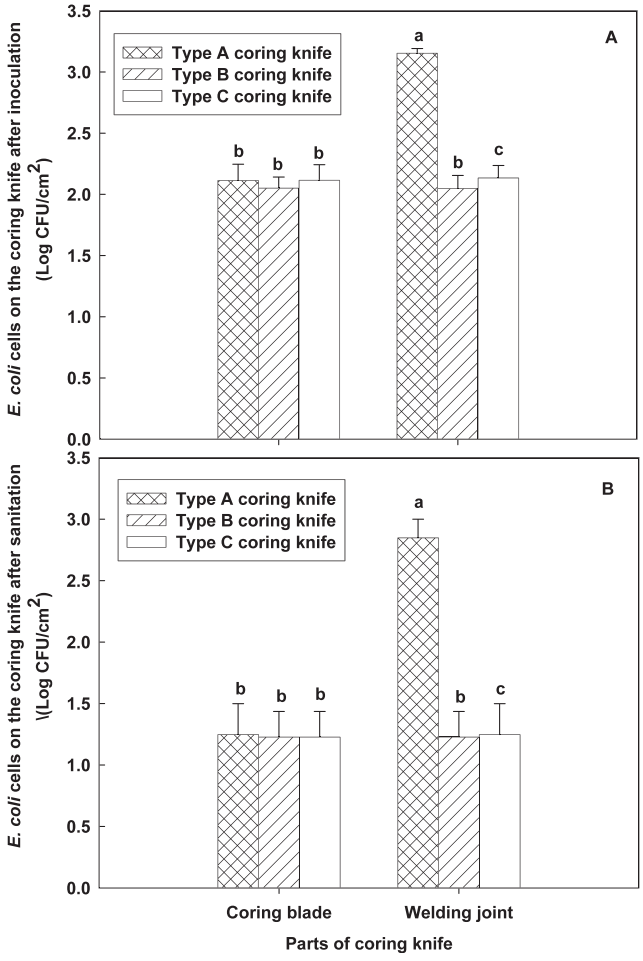

FIGURE 3. *Attachment (A) and removal and/or inactivation (B) of* E. coli *O157:H7 cells on the commercial coring knife and the two prototype knives with the rough welding joint removed or smoothed. Knives were treated with 10 ppm of chlorine without sonication. Different letters indicate significant differences between treatments.*

sanitation washes. Therefore, the ultrasound and sanitizer combination treatment may be useful for coring knife sanitation when an electric power source is available.

Attachment and removal of *E. coli* O157:H7 from two types of coring knife. Two new coring knives were designed and fabricated in this study (Fig. 2). Prototype 1 was designed and fabricated to removing the welding joint (Fig. 2B1 and 2B2). Prototype 2 was modified from the commercial coring knife used in current CIF operations by using a mechanical polishing method to smooth the rough welding joint (Fig. 2C1 and 2C2). The results of the experiments conducted to examine changes in microbial attachment and reduction with redesigned knives are shown in Figure 3. The prototypes (types B and C) retained significantly fewer *E. coli* cells on the welding joint than did the currently used knife (Fig. 3A) ($P < 0.05$). The *E. coli* cells attached to the welding joint of the two newly designed knives were reduced to below the detection limit (1.1 log CFU/cm^2), whereas 2.8 log CFU/cm^2 remained on the welding joint of the commercial coring knife (Fig. 3B) after treatment for 1 min in a 10-ppm chlorine solution.

The rough welding joint on the coring ring of commercial lettuce harvesting knives provides a site conducive to bacterial cell attachment and resistant to bacterial cell removal during sanitation treatments. The new coring knife designs proposed and tested in this study eliminated the rough surfaces and can thus potentially improve the microbial safety of in-field coring operations. The cleaning of coring knives with ultrasound plus 1 ppm of chlorine for 30 s reduced inoculated *E. coli* O157:H7 cells to levels that could no longer be detected by the swabbing and enumeration method. The redesigned coring knives and the ultrasonic sanitation method may provide a practical option for minimizing the microbial safety hazard associated with lettuce processed by CIF methods.

ACKNOWLEDGMENTS

We thank R. Keith Parrish (MechSE Machine Shop, University of Illinois, Urbana-Champaign) for his assistance with the design and fabrication of the two prototype lettuce coring knives. This project was sponsored by the California Leafy Green Research Board and the Center for Produce Safety (project 2009-48).

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