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Use of Copper Cast Alloys To Control *Escherichia coli* O157 Cross-Contamination during Food Processing

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The most notable method of infection from *Escherichia coli* O157 (*E. coli* O157) is through contaminated food products, usually ground beef. The objective of this study was to evaluate seven cast copper alloys (61 to 95% Cu) for their ability to reduce the viability of *E. coli* O157, mixed with or without ground beef juice, and to compare these results to those for stainless steel. *E. coli* O157 (NCTC 12900) (2×10^7 CFU) mixed with extracted beef juice (25%) was inoculated onto coupons of each copper cast alloy or stainless steel and incubated at either 22°C or 4°C for up to 6 h. *E. coli* O157 viability was determined by plate counts in addition to staining in situ with the respiratory indicator fluorochrome 5-cyano-2,3-ditolyl tetrazolium. Without beef extract, three alloys completely killed the inoculum during the 6-h exposure at 22°C. At 4°C, only the high-copper alloys (>85%) significantly reduced the numbers of O157. With beef juice, only one alloy (95% Cu) completely killed the inoculum at 22°C. For stainless steel, no significant reduction in cell numbers occurred. At 4°C, only alloys C83300 (93% Cu) and C87300 (95% Cu) significantly reduced the numbers of *E. coli* O157, with 1.5- and 5-log kills, respectively. Reducing the inoculum to 10^3 CFU resulted in a complete kill for all seven cast copper alloys in 20 min or less at 22°C. These results clearly demonstrate the antimicrobial properties of cast copper alloys with regard to *E. coli* O157, and consequently these alloys have the potential to aid in food safety.

Escherichia coli O157:H7 has emerged as a serious foodborne pathogen, with outbreaks associated primarily with consumption of undercooked ground beef (17), although other transmission routes exist, including potable (19) and recreational water (1). The bacterium was first identified as a pathogen in 1982, and the numbers of cases reportedly caused by this strain have increased over the last decade in many countries (18). The physiological effects of *E. coli* O157:H7 infection range from diarrhea (2% of all cases in the western world) to serious and life-threatening conditions, including hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (6). A recent outbreak (September 2005) in southern Wales resulted in 157 cases over a period of 20 days, with 65% affecting school age children and one unfortunate fatality in a 5-year-old male. Evidence to date traced the source to a supplier of cooked meats to a school meals service (9).

The intestinal tract of cattle is considered the major reservoir of *E. coli* O157 (2), and the contamination with beef is attributed to contact with feces from the ruptured gut, hide, hair, or hooves of the animals during the slaughter process (10). Once contaminated, subsequent downstream processing can potentially lead to cross-contamination from the meat to any point of contact. The metal of choice for food preparation and handling is stainless steel (types 304 and 316) due to its mechanical strength, corrosion resistance, longevity, and ease of fabrication (11). However, it has been shown that even with cleaning and sanitation procedures consistent with good manufacturing practices, microorganisms can remain in a viable state on stainless steel equipment surfaces (14). In addition, this alloy has been shown to be ineffective at reducing microbial load once it is contaminated. A study conducted by Kusumaningrum et al. demonstrated that *Salmonella enteritidis*, *Staphylococcus aureus*, and *Campylobacter jejuni* remained viable on dry stainless steel surfaces for many hours after inoculation (13), which raises the issue of alternative materials for surfaces in food-processing environments. Pure copper and copper-containing alloys such as brass and bronze have the potential to control microbial populations due to the well-documented antimicrobial properties of copper itself (4, 7, 8, 15, 16). With this in mind, a selection of the most widely used cast copper alloys (including brasses, bronzes, and copper-nickel-zinc) were tested for their ability to reduce the viability of *E. coli* O157 cultured in a high-protein (50%) medium, tryptone soy broth (TSB), with or without the addition of beef liquid (to reflect the presence of meat residue during processing) extracted from minced beef (19% protein, 26% fat content), with results compared to those for food-grade stainless steel.

MATERIALS AND METHODS

Preparation of *E. coli* O157 cultures. *E. coli* O157 (NCTC 12900) was maintained on glycerol protect beads (Fisher Scientific, United Kingdom) at −80°C. For experimental tests, 15 ml of TSB was aseptically inoculated with a single bead and incubated at 37°C for 16 h. After this incubation period, the culture contained ~1.25×10^9 CFU per ml. Unless otherwise stated, media were obtained from Oxoid (Basingstoke, Hampshire, United Kingdom).

Preparation of liquid beef extract. Minced beef (500 g; 19% protein, 26% fat) was purchased from a leading United Kingdom supermarket and stored in bags (50-g amounts) at −20°C until required, and then they were defrosted and stored

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TABLE 1. Metal samples and their constituent components

Metal type	UNS[a] no.	% Composition								
		Cu	Al	Zn	Sn	Ni	Pb	Mn	Fe	Si
Silicon bronze	C87300	95						1		1
Red brass	C83300	93		4	1.5		1.5			
Brass	C83600	85		5	5		5			
Ni-Al bronze	C95800	81	9			5		1	4	
Al bronze	C95500	78	11			4		3	4	
Ni sliver	C97600	66		6	4	20	4			
Yellow brass	C85700	61		37	1		1			
Stainless steel 304[b]	S30400					10		2	65.45	1

[a] UNS, Unified Numbering System.
[b] Stainless steel 304 also contains 0.8% C, 20% Cr, 0.45% P, and 0.3% S.

at 4°C. For experimental procedures, a 20-ml sterile syringe housing was modified by the addition of a series of small holes to the top end of the tube using a syringe needle. The syringe tube was then filled approximately one third (9 ± 0.9 g) with beef mince, and the plunger was reinserted into the syringe housing. Pressure was then applied to the mince, and the liquid extract that appeared from the holes was removed with a sterile pipette tip and transferred to a 1.5-ml Eppendorf tube. The liquid extract was stored at 4°C and used on the day of production. Microbial contamination of the beef extract was determined by serially diluting the juice in sterile phosphate-buffered saline (PBS) and plating out on nutrient agar plates. The CFU count detected from all meat samples used was 0.

Preparation of alloy coupons. Table 1 lists the compositions of the alloys tested during this study. Sample ingot blocks (1 cm by 1 cm by 1 cm) of each metal type (provided by the Copper Development Association, New York, NY) were cut into sections (3 mm thickness) and then into small coupons (1 cm by 1 cm by 0.3 cm). Prior to testing, these coupons were degreased and cleaned by vortexing for 30 s in 10 ml acetone containing ~30 2-mm-diameter glass beads (Merck, United Kingdom). After cleaning, coupons were immersed in ethanol and flamed in a Bunsen burner before being transferred to a sterile plastic container with a lid to prevent contamination prior to inoculation. Coupons remained within the container during the experimental procedures.

Alloy testing. For experiments testing the effect of meat residue, liquid beef extract (100 μl) was added to 300 μl of *E. coli* culture and gently mixed by pipetting. Coupons were aseptically inoculated with either 20 μl of *E. coli*-beef extract suspension (2×10^7 CFU) or *E. coli* culture (2.7×10^7 CFU) alone. Droplets were spread evenly across the whole surface of the coupon using a separate sterile pipette tip. Following inoculation, the coupons were incubated at either room temperature (22 °C ± 2°C) or 4°C (to represent cold storage areas) for varying time periods, ranging from 15 min to 6 h. Control coupons were removed immediately after inoculation at time zero to determine the initial number of viable bacterial cells. Relative humidity in the laboratory was monitored and recorded (50% ± 10%). The effect of desiccation on the viability of *E. coli* O157 with or without beef extract over 6 h was investigated, and no effect was seen (data are from stainless steel coupons). Mean drying time at room temperature for the evenly spread 20-μl droplet was 65 min (±7 min) for all the cast alloys tested (with or without beef extract).

After incubation, cells were removed from the coupons by vortexing for 30 s in 10 ml sterile PBS containing ~20 2-mm glass beads. The effect of copper release into the PBS on the viability of recovered cells (measured in CFU) was investigated by the addition of 20 mM EDTA, which readily complexes free copper (20). No significant difference was seen in the number of colonies formed (data not shown) between samples recovered into PBS or PBS with EDTA. Thorough analysis of coupons by episcopic differential interference contrast (EDIC) microscopy revealed no attached cells after washing (12). To ascertain the number of viable cells removed from the coupons, 100 μl was removed and serially diluted to 10^{-4} in sterile PBS. Nutrient agar plates were then inoculated with 50 μl of each dilution, which was spread evenly over the surface of the agar with a sterile, glass spreader. This provided a detection limit of 200 CFU, although subsequent analysis using a viability stain (see the next section on reduced inoculum testing) confirmed zero counts when they occurred. Postinoculation, plates were incubated at 37°C for 18 h, and the number of CFU was counted and used to calculate the number of viable CFU per coupon. Three plates were completed for each dilution, and the means were calculated. Three replicates were completed for each alloy sample as well as for each time period and temperature regime.

Reduced inoculum testing. Contamination of a work surface of 10^7 CFU cm^{-2} would represent a significant breakdown in hygiene practices. Contamination by *E. coli* O157 of beef carcasses and boned head meat after slaughter was found at concentrations of 1.41 $\log_{10}$ CFU g^{-1} and 1.0 $\log_{10}$ CFU g^{-1}, respectively (5). Consequently, contamination of food-processing surfaces would be significantly less than 10^7 CFU cm^{-2}. To determine the effect of a reduced inoculum size on the time required for total kill on each of the cast alloys and stainless steel, the number of *E. coli* O157 cells inoculated onto sample coupons was reduced by serially diluting the original cell culture-beef extract solution. Four serial 1:10 dilutions were performed, and sample coupons were inoculated with 20 μl of the final dilution (10^3 CFU). Tests were conducted at room temperature (22 °C ± 2°C), and samples taken every 10 min up to a period of 30 min. After exposure, coupons were transferred to tubes containing 2 ml sterile PBS (detection limit of 40 CFU) with glass beads and then were treated as described above for alloy testing. Zero counts were additionally confirmed by viability staining in situ on the metal surfaces with 5-cyano-2,3-ditolyl tetrazolium (CTC), as described below.

Episcopic differential interference contrast (EDIC) and epifluorescent microscopy analysis. To confirm results obtained from the direct culturing of CFU recovered from sample coupons in addition to investigating the possibility of the presence of sublethally damaged or viable but nonculturable cells, images were taken of inoculated coupons by both EDIC and epifluorescent microscopy. For the epifluorescent analysis, *E. coli* cells on inoculated coupons were stained with 5-cyano-2,3-ditolyl tetrazolium (CTC), which detects actively respiring bacteria (3). Coupons were flamed first and then inoculated with 20 μl of beef extract-*E. coli* culture as described in the alloy testing protocol. Only metal samples which produced zero viable cell counts were tested. For stainless steel, sample coupons were analyzed after an exposure period of 6 h. After the exposure period, coupons were transferred to 55-mm petri dishes, and 50 μl of 10 mM CTC was added to the surface and incubated in the dark for 4 h. Postincubation, the coupons were thoroughly examined using an EDIC/epifluorescent microscope (Nikon Eclipse Model ME600; Best Scientific, Swindon, United Kingdom) equipped with a ×40 objective and epifluorescent filters appropriate for CTC. For each coupon tested, representative EDIC and epifluorescent pictures were taken using a digital camera (Model CoolSnap CF; Roper Industries, United Kingdom) connected to a personal computer with digital image analysis software (Image-Pro Plus, version 4.5.1.22; Media Cybernetics, United Kingdom).

Statistical analysis. Data are expressed as the means ± standard errors of the means (SEM). For group comparison, a Mann-Whitney U test was used. Statistical significance was defined as $P < 0.05$. Statistical procedures were performed using SigmaStat version 2.03, and graphical analyses were performed with SigmaPlot version 8.0.

RESULTS

***E. coli* viability on copper cast alloys and stainless steel.** The effect of *E. coli* O157 viability on exposure to stainless steel or copper cast alloys at either 22°C or 4°C without the presence of beef extract can be seen in Fig. 1 and 2, respectively. From Fig. 1 it is evident that exposure to stainless steel for 6 h at 22°C had no significant effect ($P > 0.05$) on the mean number of CFU per coupon. At time zero, the mean number of viable CFU per coupon was 2.7×10^7, with 1.7×10^7 CFU $coupon^{-1}$ remaining after 6 h. What is also clear from Fig. 1 is that the percentage of copper content of the cast alloys is not directly linked to their ability to reduce viability of *E. coli* O157. Of the seven cast alloys tested, three reduced the inoculum CFU to 0 (>7-log kill) within the exposure period of 6 h: C87300 (95% Cu), C83600 (85% Cu), and C83300 (93% Cu). For alloy C87300, a significant 4-log reduction was achieved in only 45 min, with no viable *E. coli* organisms remaining after 75 min. However, alloy C83600 completely killed the inoculum in 3 h, compared to 4.5 h for alloy C83300, which actually contained a higher copper content (8% higher). In addition, alloy C95500 (78% copper content) demonstrated no significant reduction ($P > 0.05$) in the numbers of cells recovered after 6 h compared to those of control coupons at time zero. Comparison between C97600

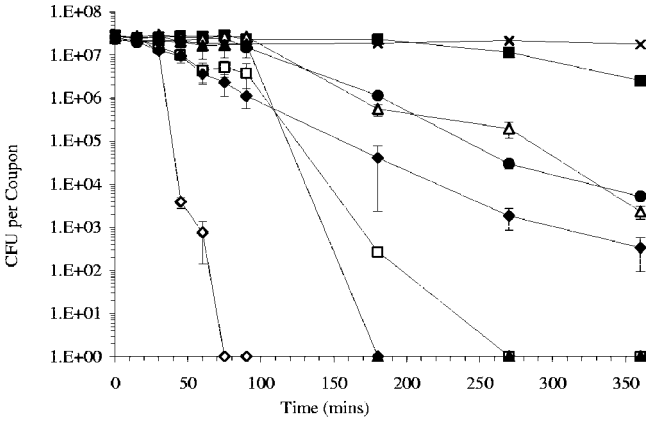

FIG. 1. Effect on *E. coli* O157 viability of a 6-h exposure to either stainless steel (×), C873000 (◇), C83600 (▲), C83300 (□), C97600 (◆), C95800 (●), C85700 (△), or C95500 (■) at 22°C. Coupons (1 cm by 1 cm) were inoculated with 20 μl of a 19-h *E. coli* O157 culture. Following the exposure period, coupons were transferred to tubes containing 10 ml sterile PBS with 2-mm-diameter glass beads. Cells were subsequently removed from the coupons into suspension by vortexing, and 100 μl was removed and serially diluted to 10^{-4} in sterile PBS. TSB plates were then inoculated (50 μl) for each dilution and subsequently incubated at 37°C for 18 h. Postincubation, the number of CFU on each plate was counted and used to calculate the number of viable CFU per coupon. Points represent the means ($n = 3$) ± SEM.

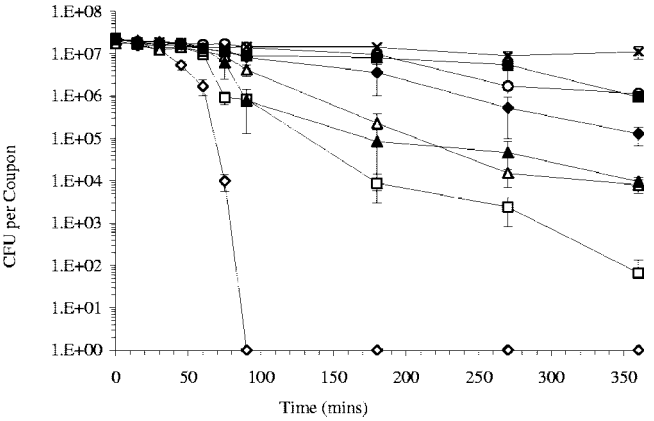

FIG. 3. Effect on *E. coli* O157 viability of a 6-h exposure to either stainless steel (×), C873000 (◇), C83600 (▲), C83300 (□), C97600 (◆), C95800 (●), C85700 (△), or C95500 (■) at 22°C in the presence of liquid beef extract. Coupons (1 cm by 1 cm) were inoculated with 20 μl of a 19-h *E. coli* O157 culture. Following the exposure period, coupons were transferred to tubes containing 10 ml sterile PBS with 2-mm-diameter glass beads. Cells were subsequently removed from the coupons into suspension by vortexing, and 100 μl was removed and serially diluted to 10^{-4} in sterile PBS. TSB plates were then inoculated (50 μl) for each dilution and subsequently incubated at 37°C for 18 h. Postincubation, the number of CFU on each plate was counted and used to calculate the number of viable CFU per coupon. Points represent the means ($n = 3$) ± SEM.

and alloys C95800 and C85700 at 6 h revealed a significant difference ($P < 0.05$) in the numbers of *E. coli* killed. At 6 h, the mean number of viable *E. coli* cells remaining on alloy C97600 was 333 CFU, compared to 2,567 and 2,300 CFU for alloys C95800 and C85700, respectively.

The effect on *E. coli* O157 viability at 4°C is shown in Fig. 2. Of the alloys tested, C87300 (95% Cu), C83600 (85% Cu), and C83300 (93% Cu) were the only ones which demonstrated an effect on *E. coli* viability, with all three completely killing the 10^7-CFU inoculum in 3 h. However, it must be noted that for alloy C83300, the complete kill of the inoculum was faster at 4°C than at 22°C, a pattern which is the reverse for all the other alloys. After 3 h at 22°C, a mean number of 267 viable cells remain on alloy C83300, with this diminishing to zero at 4.5 h. This reverse trend can be attributed to a single isolated replicate where viable cells were recovered. All other replicates for this alloy after 3 h at room temperature resulted in no recovery of viable cells. Additional replicates ($n = 3$) (data not shown) have also resulted in total kill of the inoculum for this time point and temperature regimen, which indicates that the kill rate at room temperature is in fact faster than that at 4°C for alloy C83300. Viability for the four remaining copper alloys and stainless steel remained unaffected for *E. coli* O157, with no significant difference in cell numbers at 6 h compared to that at time zero.

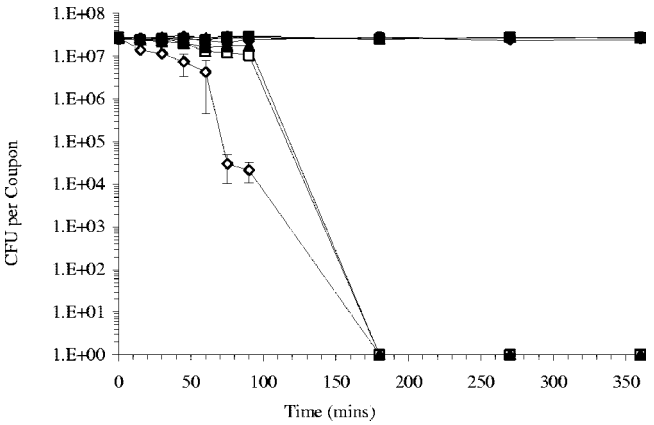

FIG. 2. Effect on *E. coli* O157 viability of a 6-h exposure to either stainless steel (×), C873000 (◇), C83600 (▲), C83300 (□), C97600 (◆), C95800 (●), C85700 (△), or C95500 (■) at 4°C. Coupons (1 cm by 1 cm) were inoculated with 20 μl of a 19-h *E. coli* O157 culture. Following the exposure period, coupons were transferred to tubes containing 10 ml sterile PBS with 2-mm-diameter glass beads. Cells were subsequently removed from the coupons into suspension by vortexing, and 100 μl was removed and serially diluted to 10^{-4} in sterile PBS. TSB plates were then inoculated (50 μl) for each dilution and subsequently incubated at 37°C for 18 h. Postincubation, the number of CFU on each plate was counted and used to calculate the number of viable CFU per coupon. Points represent the means ($n = 3$) ± SEM.

The effect of the addition of the liquid beef extract on exposure to stainless steel or copper cast alloys on *E. coli* O157 viability at either 22°C or 4°C can be seen in Fig. 3 and 4, respectively. From Fig. 3, it can be seen that once again exposure to stainless steel for 6 h at 22°C had no significant effect ($P > 0.05$) on the mean number of CFU per coupon. What is also immediately clear is the reduced antimicrobial activity for all the alloys to which beef juice had been added. Of the seven cast alloys tested, only one reduced the inoculum CFU to zero, C87300 (95% Cu), with complete kill achieved after 90 min, a result previously accomplished after 75 min. For alloy C83300 (93% Cu) with beef extract, a significant ($P < 0.05$) 5-log kill was achieved after 6 h, compared to complete kill in under 6 h with no beef extract. Both alloys C85700 (61% Cu) and C83600

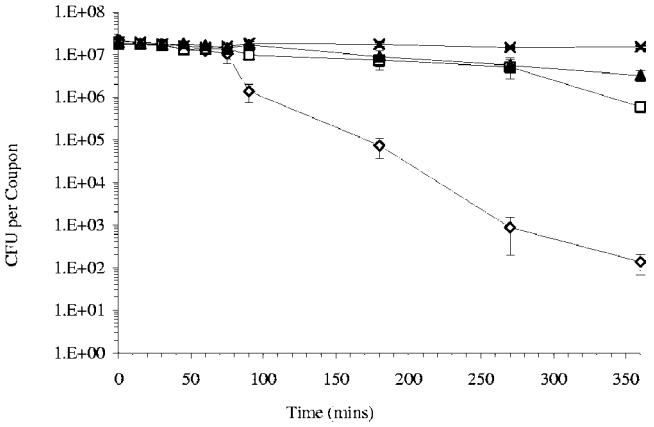

FIG. 4. Effect on *E. coli* O157 viability of a 6-h exposure to either stainless steel (×), C873000 (◇), C83600 (▲), or C83300 (□) at 4°C in the presence of liquid beef extract. Coupons (1 cm by 1 cm) were inoculated with 20 μl of a 19-h *E. coli* O157 culture mixed with liquid beef extract (25%). Following the exposure period, coupons were transferred to tubes containing 10 ml sterile PBS with 2-mm-diameter glass beads. Cells were subsequently removed from the coupons into suspension by vortexing, and 100 μl was removed and serially diluted to 10^{-4} in sterile PBS. TSB plates were then inoculated (50 μl) for each dilution and subsequently incubated at 37°C for 18 h. Postincubation, the number of CFU on each plate was counted and used to calculate the number of viable CFU per coupon. Points represent the means ($n = 3$) ± SEM.

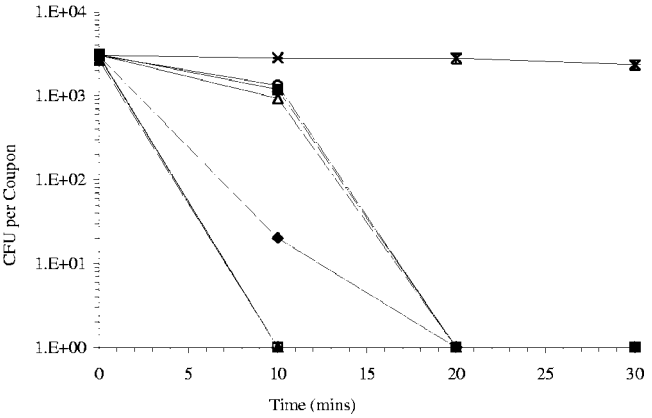

FIG. 5. Effect of reduced inoculum size on time for total kill when exposed to copper cast alloys C873000 (◇), C83600 (▲), C83300 (□), C97600 (◆), C95800 (●), C85700 (△), or C95500 (■) or stainless steel (×) at 22°C. Coupons (1 cm by 1 cm) were inoculated with 20 μl of a serially diluted *E. coli* culture-liquid beef extract solution (10^3 CFU). Following the exposure period, coupons were transferred to tubes containing 10 ml sterile PBS with 2-mm-diameter glass beads and vortexed for 30 s, and 50 μl was removed and TSB plates inoculated, followed by incubation at 37°C for 18 h. Postincubation, the number of CFU on each plate was counted and used to calculate the number of viable CFU per coupon. Points represent the means ($n = 3$) ± SEM.

(85% Cu) achieved 3-log kills, with mean CFU of 7,867 and 10,000, respectively, remaining viable at 6 h. Nearly identical kill rates were observed, even with a difference in total copper content of the alloys of 24%. Both C95800 (81% Cu) and C95500 (78%) at 6 h produced 1-log reductions in the viability of *E. coli* O157, although alloy C97600 with a lower copper content of 66% produced a significant ($P < 0.05$) 2-log kill.

The effect of added beef juice on *E. coli* O157 viability at 4°C is shown in Fig. 4. For clarity, in the figure only plots for alloys C87300, C83300, C83600, and stainless steel have been shown. As previously shown at room temperature, antibacterial activity is reduced. From the alloys tested, only C87300 (95% Cu) and C83300 (93% Cu) demonstrated significant ($P < 0.05$) antimicrobial ability on *E. coli* at chill temperatures, with 5 and 1.5-log kills, respectively. Viability for the five remaining copper alloys and stainless steel remained unaffected for *E. coli* O157, with no significant difference ($P > 0.05$) in cell numbers at 6 h compared to that at time zero.

The effect on total kill time of reducing the inoculum size of *E. coli* O157 when exposed to the seven cast copper alloys can be seen in Fig. 5. Reducing the number of CFU to 10^3 resulted in complete kill for all the alloys tested in 20 min or less. Once again, viability on stainless steel remained unaffected at the 30-min time point. For three of the copper alloys, C87300, C83300, and C83600, complete kill was achieved in 10 min.

Epifluorescent microscopy and digital image analysis. To confirm that the low numbers of cells recovered on the copper alloys was indeed due to cell death, epifluorescent images were taken of sample coupons stained with CTC before cells were due to be removed for culture. The images of cells at time zero on either copper alloys or stainless steel indicated active respiration, shown by the numerous points of red emission within the images due to the intracellular reduction of CTC to the water-insoluble fluorescent product 3-cyan-1,5-di-tolyl-formazan (data not shown). Subsequently, by contrast, there was no fluorescent labeling of cells incubated on the copper surfaces where no subsequent culture could be obtained. The EDIC microscopy images showed that the cells were still present but not respiring. In contrast, images of inoculated stainless steel after 6 h of incubation at 22°C clearly show the presence of respiring cells whose numbers matched the culturable numbers of cells recovered on the agar medium.

DISCUSSION

Infections from *E. coli* O157 are serious and life threatening, with contamination of ground beef representing a significant source. Preparation of meat products requires surfaces that are resilient and easily cleaned to reduce the risk of contamination. Stainless steel, although hard wearing and easily cleaned, is not intrinsically effective at reducing numbers of viable bacteria, which suggests that food-processing environments would benefit from the installation of materials that are inherently biocidal.

The data from this study demonstrate that the viability of the pathogen *E. coli* O157 can be significantly affected by three factors: the composition of the substrate alloy on which it is placed, the ambient temperature, and the presence of beef juice. The addition of the liquid beef extract in these tests was used to represent soiling of preparation surfaces, although regular cleaning as part of any normal hygiene policy should normally prevent contact areas from becoming this dirty, i.e., meat residue allowed to remain on a surface for up to 6 h. With regards to the metal of choice, *E. coli* O157 was able to persist in a viable state in dried deposits on stainless steel at room temperature for periods of 6 h regardless of whether beef juice

was present. In contrast, survival on the high-copper alloy C87300, for example, was significantly reduced, with complete kill of 10^7 cells achieved after 75 min without beef extract and in 90 min even with the beef juice.

Also apparent is the effect of temperature on antimicrobial activity. Reducing the exposure temperature to 4°C increased the time required to totally kill the inoculum on the cast copper alloys which had previously achieved this at 22°C. Further reductions in antimicrobial activity were found with the addition of the liquid beef extract. For example, only two alloys, C87300 and C83300, with mean CFU counts at 6 h of 133 and 4.7×10^5, respectively, showed any effect on cell viability. For the remaining five cast copper alloys, antimicrobial activity was effectively removed at chill temperatures, which in turn suggests that alloys with >90% copper should be utilized under these conditions to provide significant disinfection ability.

What is clear, though, is the significantly faster and greater kill rates on the high-copper cast alloys (>80%) without the addition of liquid beef extract, which suggests that the extract itself provides a protective matrix for the bacterial cells to "hide in" from the detrimental effects of copper exposure. This may be due to the fat content, since the raw minced beef contained 26% fat before the juice was extracted. What these findings also suggest is that copper-based work surfaces that are free from meat residue would be even more effective at reducing microbial load if contamination occurs. However, as shown from the results presented here, significant reductions in viability are still achieved with the presence of a "meat residue." In addition, results from the reduced inoculum tests, representing possible levels of processing contamination, show rapid disinfection for all the copper alloys sampled, with four achieving this in 20 min and the remaining three (with the highest copper content) in only 10 min.

In addition, reducing the copper content in the alloys tested in general reduced the numbers of *E. coli* O157 cells killed, although this was not the case for all alloys. In particular, the aluminum-bronze alloy C95500 (11% Al, 78% Cu) and the nickel-aluminum-bronze alloy C95800 (9% Al, 81% Cu) both demonstrated poor antimicrobial ability regardless of the presence of beef residue. This lack of antimicrobial property from these high-copper alloys could be attributed to the formation of a protective aluminum oxide layer during the cutting of the sample ingot. This possibility was investigated by cleaning the surface of the C95500 coupons with a coarse grit paper and repeating the tests for 6 h. This cleaning procedure resulted in a 3-log-greater reduction in *E. coli* viability after 6 h (data not shown) when meat juice was not present. However, with the beef juice added, the cleaning procedure resulted in no significant difference in the reduction in *E. coli* viability. Findings suggest a "protective layer" was present, but even after removal the copper itself is "locked" into the alloy by some unknown action of the beef residue. Further investigation into these findings is required. Tests on pure aluminum showed no detrimental effect (data not shown) on the viability of *E. coli* O157.

To conclude, the inhibitory effects observed in these commonly used cast copper alloys are an intrinsic property of these materials. Although stainless steel surfaces may appear to be clean, this study has shown that bacteria can survive on these surfaces for considerable periods of time. In comparison, survival on many copper alloys is limited to just a few hours or even minutes. Due to the intrinsic characteristics of copper alloys, i.e., homogeneous and solid, superior lifetime antimicrobial efficacy, wear resistance, and durability, they could be utilized in facilities where bacterial contamination cannot be tolerated. As such, copper-based work surfaces could provide an important additional protective barrier to complement what should always be existing good cleaning practices in food production and retail facilities. Considering the low infectious dose of a dangerous pathogen such as *E. coli* O157 and its ability to survive for long periods in the environment, all possible protective barriers to prevent transmission through the food chain should be utilized.

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