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Antimicrobial activity of different copper alloy surfaces against copper resistant and sensitive *Salmonella enterica*

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ABSTRACT

Copper has shown antibacterial effects against foodborne pathogens. The objective of this study was to evaluate the antibacterial activity of copper surfaces on copper resistant and sensitive strains of *Salmonella enterica*. Six different copper alloy coupons (60–99.9% copper) were tested along with stainless steel as the control. The coupons were surface inoculated with either *S.* Enteritidis or one of the 3 copper resistant strains, *S.* Typhimurium S9, S19 and S20; stored under various incubation conditions at room temperature; and sampled at various times up to 2 h. The results showed that under dry incubation conditions, *Salmonella* only survived 10–15 min on high copper content alloys. *Salmonella* on low copper content alloys showed 3–4 log reductions. Under moist incubation conditions, no survivors were detected after 30 min–2 h on high copper content alloys, while the cell counts decreased 2–4 logs on low copper content coupons. Although the copper resistant strains survived better than *S.* Enteritidis, they were either completely inactivated or survival was decreased. Copper coupons showed better antimicrobial efficacy in the absence of organic compounds. These results clearly show the antibacterial effects of copper and its potential as an alternative to stainless steel for selected food contact surfaces.

1. Introduction

Foodborne disease outbreaks and recalls due to the presence of pathogens in foods continue to be a problem for the food industry. The Centers for Diseases Control (CDC) has reported that incidence of infection from pathogens commonly transmitted through food was 37.7 per 100,000 population in 2009, and the case fatality rate was 0.42% (CDC, 2010). Studies have shown that *Salmonella enterica* is amongst the more prevalent bacterial pathogens that cause foodborne infections (Butzby and Roberts, 1996; CDC, 2001). *Salmonella* is the second leading cause of foodborne illness cases (11%), and leading cause of foodborne illness hospitalizations (35%) as well as fatalities (28%) (Scallan et al., 2011). Significant sources of contamination include raw ingredients and cross contamination of food contact surfaces in a food processing environment. Continued use of antibiotics in poultry and animal feed has led to the emergence of antibiotic resistant bacterial strains. Hence, there is an urgent need for better control measures (other than antibiotics) to reduce the presence of *Salmonella* in the food production environment.

Copper is an essential micronutrient required in very small amounts for survival of most aerobic organisms. At higher concentrations it can become toxic and inhibit microbial growth; hence, it is essential for cells to maintain appropriate intracellular copper concentrations. The mechanisms of copper-mediated inhibition of cell growth may include substitution of essential ions and blocking of protein functional groups, inactivation of enzymes, production of hydroperoxide free radicals by membrane bound copper, and disruption of membrane integrity (Grass et al., 2011; Macomber and Imlay, 2009; Nies, 1999; Rodriguez-Montelongo et al., 1993; Ohsumi et al., 1988). The copper resistance determinants in the various *Salmonella* strains are quite similar to those of wild-type *Escherichia coli* (Rensing and Grass, 2003). Both contain the Cu(I)-translocating P-type ATPase CopA and the multicopper oxidase CueO (CuiD). In contrast to *E. coli*, most strains of *Salmonella* do not contain the CusCBA system responsible for transporting Cu(I) from the periplasm across the outer membrane. Instead, these strains often contain the periplasmic copper-binding protein CueP (Dupont et al., 2011). Some *E. coli* and *Salmonella* strains, such as the strains tested here, harbor the *pco* determinant responsible for an increased resistance to copper (Rensing and Grass, 2003). Copper alloys are effective in rapidly killing bacteria on contact and this inactivation is enhanced by low moisture conditions, minimal media, and high copper corrosion rates (Elguindi et al., 2011). Contact-inactivation of bacteria on copper alloys is a strategy which can be applied in food contact surfaces to prevent foodborne bacterial contamination.

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Copper is well known for its antimicrobial properties and has shown antibacterial effects against foodborne pathogens such as *Salmonella* Typhimurium as an ingredient in animal feed (Beal et al., 2003), and against *Listeria monocytogenes* as a food contact surface (Abushelaibi, 2005). Previous studies have shown that metallic copper and copper alloys have antibacterial activity against *S. enterica* and *Campylobacter jejuni* (Faundez et al., 2004; Grass et al., 2011). Copper alloys also inhibited the adhesion of bacteria during biofilm development (Kielemoes and Verstraete, 2001). Copper alloys reduced the viability of *E. coli* O157:H7, *L. monocytogenes* and methicillin-resistant *Staphylococcus aureus* (Michels et al., 2005), and these authors suggested that copper alloys be used in surfaces exposed to human touch or food contact. Noyce et al. (2006) showed that copper cast alloys significantly reduced the population of *E. coli* O157:H7 and concluded that these alloys have the potential to aid in food safety.

The objectives of this study were 1) to investigate the antimicrobial effects of different copper alloy surfaces against copper resistant and sensitive *S. enterica* strains suspended in rich medium or 0.8% sodium chloride solution and 2) compare survival after three different recovery methods.

2. Materials and methods

2.1. Bacterial culture preparation and media

The following *Salmonella* isolates were tested; *Salmonella* Enteritidis, which is a copper susceptible strain, and copper resistant strains *S.* Typhimurium DT193 S9, DT120 S19 and DT66 S20, obtained from Dr. Henrik Hasman at National Food Institute, Denmark. All three *S.* Typhimurium strains were isolated from healthy pigs in 2005. All strains contain the *pco* determinant responsible for additional copper resistance.

Each *Salmonella* culture was prepared by inoculating cryopreserved cells in tryptic soy broth (TSB; Difco-Becton Dickinson, Sparks, MD) and incubating overnight (18–20 h) at 37 °C. Two transfers were done before a working culture was prepared. *Salmonella* cells on the surface of coupons were recovered using phosphate buffered saline (PBS, Difco-Becton Dickinson). Buffered peptone water (BPW; Difco-Becton Dickinson) was used as the diluent in all experiments. Tryptic Soy Agar (TSA; Difco-Becton Dickinson) was used for enumeration of survivors.

2.2. Test surfaces preparation

The 6 different copper alloys with varying copper content were obtained from the International Copper Association, New York, NY (Table 1). Stainless steel coupons were used as control. Prior to use, the 2.5 × 2.5 cm copper alloy and stainless steel coupons were cleaned as follows: immersion for 30 s in a 3% sodium hydroxide (NaOH; Spectrum, Gardena, CA) solution, heating to 70 °C, rinsing in deionized water, followed by 5 s immersion in 10% sulfuric acid (H_2SO_4; Acros, NJ), and finally rinsing with deionized water. The coupons were then rinsed in 95% ethanol (Fisher Scientific, Fair Lawn, NJ), air-dried and kept in a sterile container until use.

Table 1
Copper alloys and their compositions (%).

Alloy	Copper	Zinc	Nickel	Iron	Chromium	Phosphorus	Tin
C11000	99.9						
C51000	94.8					0.2	5.0
C70600	88.6		10.0	1.4			
C26000	70.0	30.0					
C75200	65.0	17.0	18.0				
C28000	60.0	40.0					
Stainless steel			8.0	74.0	18.0		

2.3. Determining the minimum inhibitory concentration

In order to determine differences in copper sensitivity among the individual strains, TSA plates containing cupric chloride ($CuCl_2$; SIGMA Aldrich, St. Louis, MO) at concentrations ranging from 0 to 15 mmol l^{-1} were prepared by adding $CuCl_2$ from a stock solution to sterilized liquid TSA. The liquid TSA with $CuCl_2$ was mixed well and poured into petri dishes. Overnight cultures of *Salmonella* strains Enteritidis, S9, S19 and S20 were re-inoculated in TSB, grown to mid-log phase, and then streaked out on TSA-$CuCl_2$ plates. Growth was evaluated after 24 h incubation at 37 °C.

2.4. Determination of survival of Salmonella on copper alloy coupons

The testing methods were adapted from the Environmental Protection Agency (EPA) approved protocols. These protocols were prepared by ATS Laboratories (Eagan, MN) for the International Copper Association (Nada, 2005) and were initially described by Wilks et al. (2005). The sterile alloy coupons were inoculated with 25 μl of the overnight culture (suspended in TSB) of one of the test organisms. Selected coupons (99.9% and 70%) were also tested using cells suspended in 0.8% sodium chloride (NaCl; SIGMA Aldrich, St. Louis, MO) solution in order to compare the antimicrobial efficacy in the absence of organic compounds. Coupons were stored in sterile petri dishes and incubated at room temperature. Three methods involving varying incubation conditions were tested: in one condition, the inoculum was spread over the coupon surface and the coupon was placed in a petri dish that was kept slightly open; in the second condition, the inoculum was spread over the coupon surface and the coupon was placed in a petri dish that was kept closed; in the third condition, the inoculum was left as a droplet on the coupon surface and the coupon was placed in a petri dish that was kept closed. Samples were taken at various time intervals up to 2 h for enumeration of surviving *Salmonella*. At each sampling, the coupons were transferred into 50 ml centrifuge tubes containing 10 ml sterile phosphate buffered saline (PBS) and ten to twenty sterile 2 mm diameter glass beads. This was vortexed thoroughly for 30 s to 1 min. Then serial dilutions in BPW were done and samples plated on TSA. Enumerations were done after the plates were incubated at 37 °C for 24–48 h.

2.5. Statistical analysis

The experiments were repeated three times, and the mean and standard deviation were calculated. Data were analyzed by one-way analysis of variance (ANOVA) to determine differences at the 5% significance level (Minitab 16, Minitab Inc., State College, PA).

3. Results and discussion

3.1. Determining the minimum inhibitory concentration

The most copper susceptible serotype was *S.* Enteritidis which had sparse, small colonies on TSA plates containing 10 mM $CuCl_2$, while *S.* Typhimurium strains S19 and S20 showed normal growth at 10 mM $CuCl_2$, but no growth at 12 mM $CuCl_2$. *S.* Typhimurium S9, however, showed normal growth at 12 mM $CuCl_2$ and no growth at 14 mM $CuCl_2$ (Table 2). S9 was the most resistant among the 3 resistant strains.

Table 2
Minimum inhibitory concentration for *Salmonella* strains in Tryptic Soy Agar with $CuCl_2$.

$CuCl_2$ Conc.(mmol l^{-1})	*S.* Enteritidis SE	*S.* Typhimurium S 9	*S.* Typhimurium S 19	*S.* Typhimurium S 20
0	+[a]	+	+	+
5	+	+	+	+
7	+	+	+	+
10	+/−[b]	+	+	+
12	−[c]	+	−	−
14	−	−	−	−
15	−	−	−	−

[a] Normal colonies (+).
[b] Small colonies (+/−).
[c] No colonies (−).

3.2. Method 1: inoculum suspended in TSB spread over coupon surface and placed in petri dishes kept slightly open

Under this incubation condition, cell suspensions dried within 10–15 min on the copper alloy surfaces. The copper coupons in this experiment can be used to mimic the bacterial contamination of copper contact surfaces such as doorknobs which are usually dry. Fig. 1A shows that *S. enterica* serovar Enteritidis cell counts dropped rapidly upon exposure to copper alloy surfaces. On the copper alloys with the high copper concentrations (C11000, 99.9% Cu and C51000, 94.8% Cu), no survivors were detected at 10 min. On the alloy coupons with medium copper concentrations (C70600, 88.6% Cu and C26000, 70.0% Cu), no survivors were detected at 15 min. There were about 3 log reductions on coupons C75200 with 65% Cu and no survivors detected on coupons C28000 with 60% Cu at 15 min. No reduction was detected on control stainless steel coupons at 15 min. There were significant differences between copper coupons and control stainless steel coupons ($p < 0.05$).

Copper resistant strains S9, S19 and S20 (Fig. 1B, C, and D) showed better survival on some copper coupon surfaces than *S.* Enteritidis. This result is consistent with that obtained from the minimum inhibitory concentration (MIC) test, in which S9 showed the highest MIC, and *S.* Enteritidis showed the lowest (Table 2). For strain S9, on the coupons C11000 (99.9% Cu) and C51000 (94.8% Cu), the organism survived until 15 min as opposed to *S.* Enteritidis that survived only 10 min. On coupon C75200 (65.0% Cu) there were only 2 log reductions at 15 min for S9, while 3 log reductions were observed in the case of *S.* Enteritidis. Similar to S9, copper resistant strain S19 showed better survival on some copper coupons. On coupon C11000 (99.9% Cu), cells survived until 15 min as well. There were only 2 log reductions on C75200 (65.0% Cu) at 15 min, and 4 log reductions on C26000 (70% Cu). For strain S20, only 1 log reduction was seen on coupon C75200 (65.0% Cu) at 15 min.

On lower copper concentration alloys C70600 (88.6% Cu), C26000 (70.0% Cu) and C28000 (60.0% Cu), copper resistant strain S9 was completely inactivated at 15 min, and didn't show stronger resistance than did *S.* Enteritidis. The possible reason might be that for strain S9 copper ion-resistance is dependent on a genetically-based ability to withstand higher copper ion concentrations and is activated when there is a high rate of copper ion influx into the cells. Moreover, copper corrosion rates are variable on copper alloys and can affect the influx of copper ions into cells over time.

The results showed that under dry incubation conditions (method 1), *Salmonella* cells were extremely vulnerable to copper. In contrast, there was no decrease in viability for the cells on stainless steel coupons under the same conditions, and all strains survived more than 7 days on stainless steel (data not shown), this rules out desiccation as the main underlying bactericidal mechanism. The possible reason may be that the *Salmonella* cells become more sensitive to copper under desiccation stress. Copper and desiccation stress may interact to rapidly inactivate *Salmonella*. Beal et al. (2003) reported that acid stress decreased the tolerance of

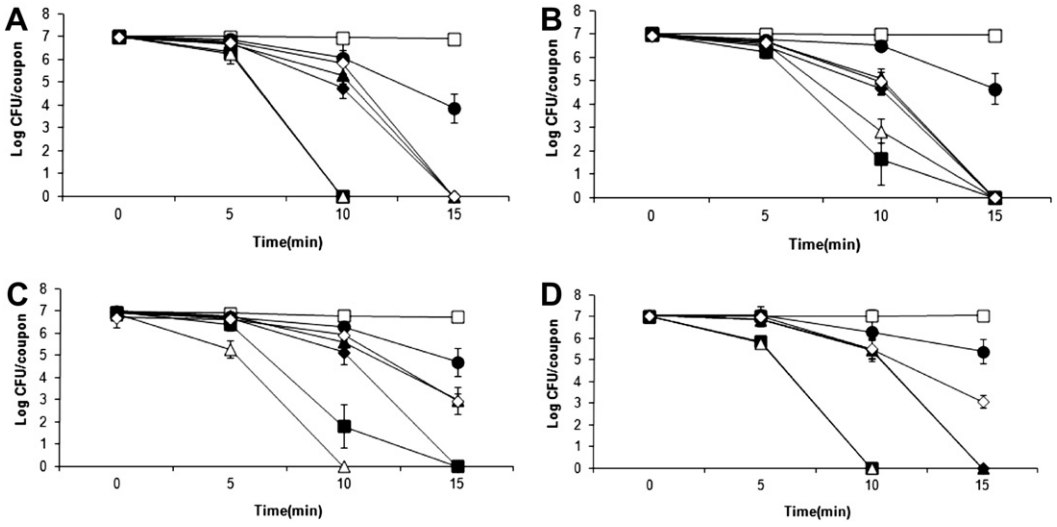

Fig. 1. Survival of *Salmonella* on copper coupon surfaces. The inoculum suspended in TSB was spread over the coupon surface, and placed in petri dishes kept slightly open. Shown are results for *S.* Enteritidis (A), S. Typhimurium S9 (B), S. Typhimurium S19 (C), and S. Typhimurium S20 (D). Stainless steel (□), C11000, 99.9% Cu (■), C51000, 94.8% Cu (△), C70600, 88.6% Cu (◆), C26000, 70.0% Cu (▲), C75200, 65.0% Cu (●) and C28000, 60.0% Cu (◇). Values plotted at each sampling point are an average of 3 replicates. Error bars represent standard deviation from the mean.

S. Typhimurium DT104:30 to copper, since the cells appeared to be extremely sensitive to very low (2.5 ppm) concentration of free Cu^{2+} at low pH values. A previous study (Espirito Santo et al., 2008) also showed similar results for *E. coli* cells, in which the culture was applied to a cotton swab and spread evenly across the copper alloy coupon, resulting in rapid drying of the culture. Their results showed that on coupon C11000 (99.9% Cu), *E. coli* wild-type strain was completely inactivated after 1 min at 23 °C. A recent study (Espírito Santo et al., 2011) reported that copper uptake by *E. coli* cells was faster from dry copper than from moist copper surfaces. Extensive cell membrane damage in *E. coli* cells was observed within minutes of exposure to dry copper.

3.3. Method 2: inoculum suspended in TSB spread over coupon surface and placed in closed petri dishes

When cell suspensions were spread over the copper coupon surfaces and stored in closed containers, *Salmonella* survived longer than that under dry conditions. This method was tested to simulate the conditions on wet surfaces in a food processing environment, in which little drops would be spread out on the surface. Fig. 2A shows the result for *S.* Enteritidis. On copper alloys C11000 (99.9% Cu) and C51000 (94.8% Cu), no survivors were detected at 30 min. On C70600 (88.6% Cu) and C26000 (70% Cu), no survivors were detected at 60 min and 2 h, respectively. There were 2 and 4 log reductions at 2 h on coupon C28000 (60.0% Cu) and C75200 (65.0% Cu), respectively. On control stainless steel coupons, bacterial population dropped by only 1 log.

Salmonella S9, S19 and S20 showed better survival than *S.* Enteritidis on all copper coupons except C11000 (99.9% Cu) and C51000 (94.8% Cu) (Fig. 2B, C, and D). All 3 strains survived until 2 h on coupon C70600 (88.6% Cu), and there was a significant difference when compared with *S.* Enteritidis ($p < 0.05$). There were only about 2 log reductions at 2 h for these strains on coupon C26000 (70% Cu), in contrast to no survivors being detected for *S.* Enteritidis ($p < 0.05$). Similarly, 1–3 logs more survivors of these resistant strains compared to *S.* Enteritidis ($p < 0.05$) were observed at 2 h on coupon C75200 (65.0% Cu) and C28000 (60.0% Cu).

A recent study (Elguindi et al., 2009) on *Pseudomonas aeruginosa* using the same inoculation method showed similar bacterial surviving patterns. When *P. aeruginosa* culture was spread on the copper coupons and stored in a closed container, the cells survived for 120 min on C11000 (99.9% Cu) and C70600 (88.6% Cu). Noyce et al. (2006) studied the survival of *E. coli* O157 on copper coupons using a similar inoculation method. The cells were not detectable on 95% Cu coupon after 75 min of exposure. A previous study (Mehtar et al., 2008) on nosocomial pathogens showed that when *Candida albicans* culture was spread on coupons containing 65–99.9% Cu, and stored in a sterile petri dish, the cells were inactivated after 60–90 min.

3.4. Method 3: inoculum suspended in TSB kept as droplet on coupon surface and placed in closed petri dishes

Because the 25 μl inoculum was kept as a droplet without spreading, and the coupons were stored in closed containers, the cell suspensions remained wet on the copper coupon surfaces for more than 2 h. This condition could simulate the heavy wet food contact surfaces in a food processing environment, which are usually wet for a long period of time. On copper coupons C11000 (99.9% Cu) and C51000 (94.8% Cu), cells survived longer under this condition (Fig. 3) than in method 2, where the inoculum was spread and stored in closed container. For non-resistant strain *S.* Enteritidis, the complete kill time on C11000 (99.9% Cu) and C51000 (94.8% Cu) was 1 h (Fig. 3A). For copper resistant strains S9, S19 and S20, organisms survived until 2 h on C11000 (99.9% Cu) and C51000 (94.8% Cu) (Fig. 3B, C, D). On coupon C70600 (88.6% Cu) and C26000 (70% Cu), S9 was the only resistant strain that survived longer than 2 h, while S19 and S20 died off at 2 h on both coupons. For *S.* Enteritidis, no viable cells were detected at 1 h and 2 h on coupons C70600 (88.6% Cu) and C26000 (70% Cu), respectively. Complete inactivation of *S.* Enteritidis on coupon C28000 (60.0% Cu) was achieved at 2 h, while there were about 3 log reductions

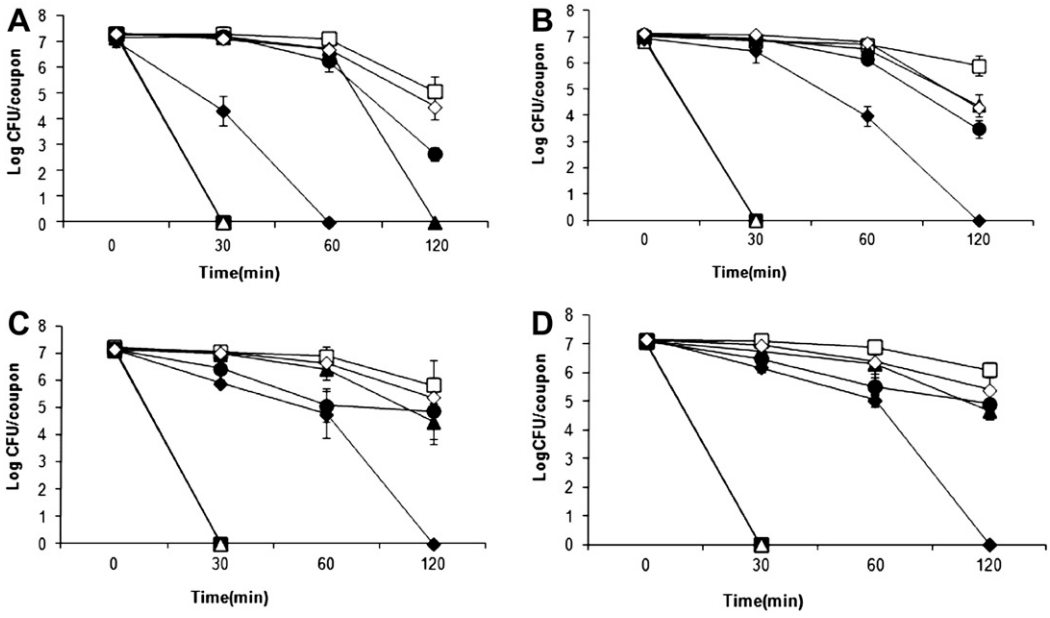

Fig. 2. Survival of *Salmonella* on copper coupon surfaces. The inoculum suspended in TSB was spread over the coupon surface, and placed in petri dishes that were kept closed. Shown are results for *S.* Enteritidis (A), S. Typhimurium S9 (B), S. Typhimurium S19 (C), and S. Typhimurium S20 (D). Stainless steel (□), C11000, 99.9% Cu (■), C51000, 94.8% Cu (△), C70600, 88.6% Cu (◆), C26000, 70.0% Cu (▲), C75200, 65.0%Cu (●) and C28000, 60.0% Cu (◇). Values plotted at each sampling point are an average of 3 replicates. Error bars represent standard deviation from the mean.

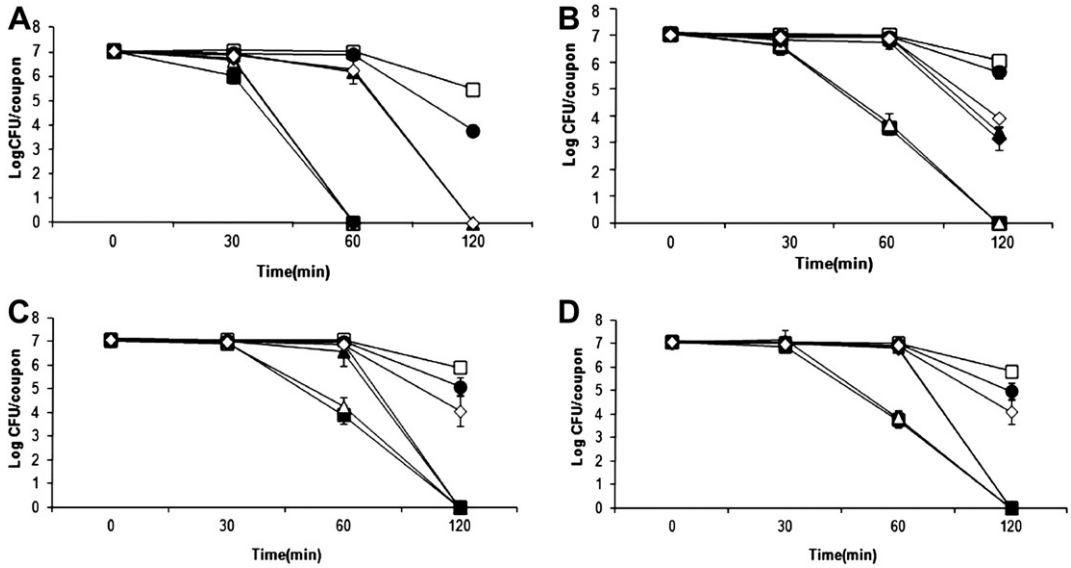

Fig. 3. Survival of *Salmonella* on copper coupon surfaces. The inoculum suspended in TSB was kept as droplet without spreading, and placed in petri dishes that were kept closed. Shown are results for *S.* Enteritidis (A), S. Typhimurium S9 (B), S. Typhimurium S19 (C), and S. Typhimurium S20 (D). Stainless steel (□), C11000, 99.9% Cu (■), C51000, 94.8% Cu (△), C70600, 88.6% Cu (◆), C26000, 70.0% Cu (▲), C75200, 65.0% Cu (●) and C28000, 60.0% Cu (◇). Values plotted at each sampling point are an average of 3 replicates. Error bars represent standard deviation from the mean.

each for strains S9, S19 and S20, respectively. Significant differences ($p < 0.05$) were observed between *S.* Enteritidis and these 3 resistant strains on coupon C28000 at 2 h. Coupon C75200 (65.0% Cu) had the least inactivation among all the copper alloys. There were 3 log reductions at 2 h for *S.* Enteritidis, and 1–2 log reductions for the resistant strains.

Elguindi et al. (2009) compared the droplet inoculation and spreading inoculation methods for their efficacy against *P. aeruginosa* on copper coupons. Their results showed that when the culture was left as a droplet on C11000 (99.9% Cu), the bacteria were completely inactivated at 4 h, while they survived only for 2 h when the culture was spread. On C70600 (88.6% Cu), cells survived until 8 h when left as a droplet, but only until 4 h when spread. Wilks et al. (2006) studied the survival of *L. monocytogenes* on copper coupons using the droplet inoculation method. The bacteria survived up to 90 min on copper coupon C75200 (65.0%).

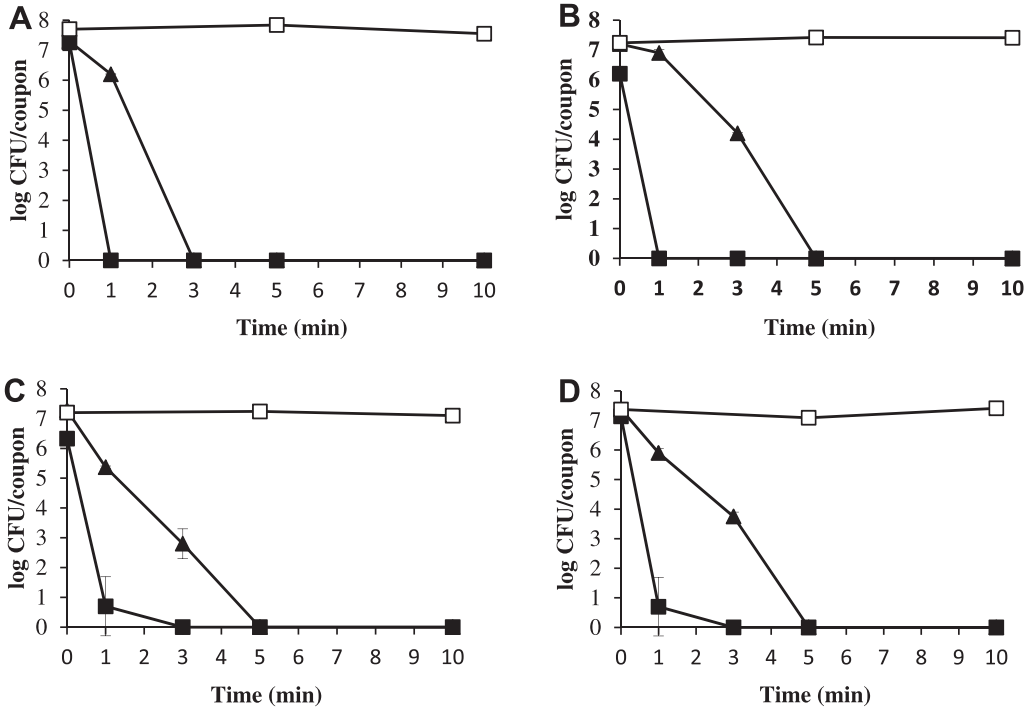

Fig. 4. Survival of *Salmonella* on copper coupon surfaces. The inoculum was suspended in 0.8% NaCl and spread over the entire surface in dry conditions (open container). Shown are results for *S.* Enteritidis (A), *S.* Typhimurium S9 (B), *S.* Typhimurium S19 (C), *S.* Typhimurium S20 (D) on C11000, 99.9% Cu (■), C26000, 70% Cu (▲), and stainless steel control (□).Values plotted at each sampling point are an average of 3 replicates. Error bars represent standard deviation from the mean.

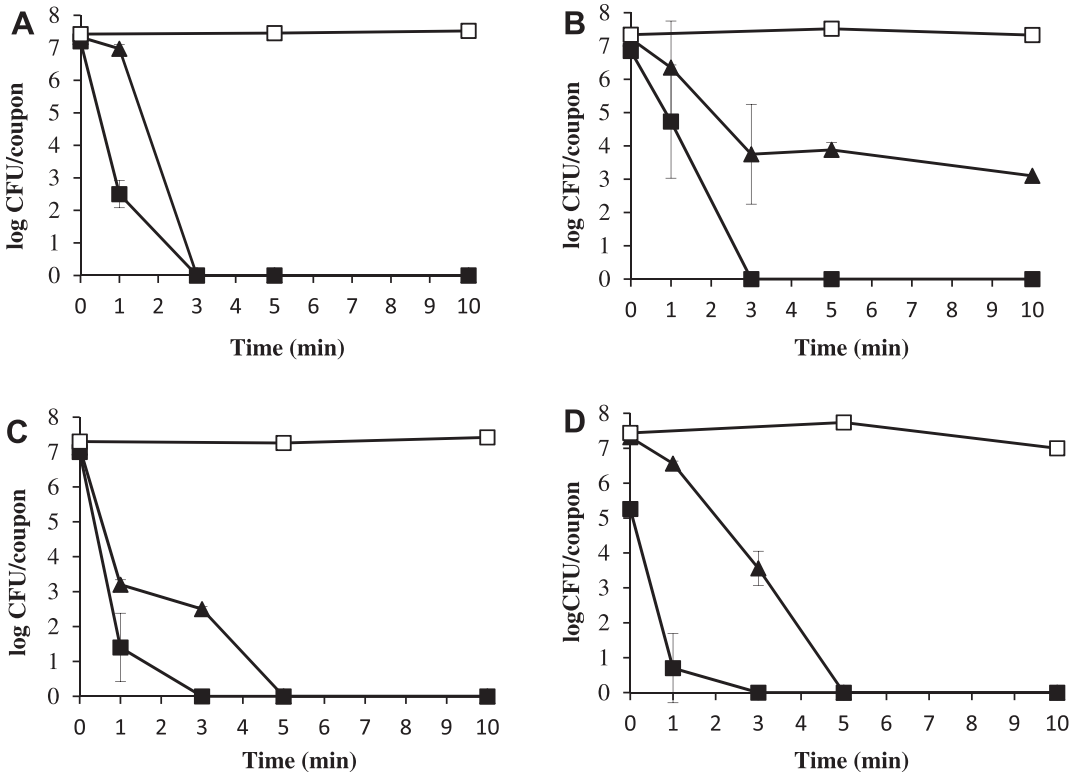

Fig. 5. Survival of *Salmonella* on copper coupon surfaces. The inoculum was suspended in 0.8% NaCl and spread over the entire surface under moist conditions (closed container). Results for *S.* Enteritidis (A), *S.* Typhimurium S9 (B), *S.* Typhimurium S19 (C), *S.* Typhimurium S20 (D) spread on C11000, 99.9% Cu (■), C26000, 70% Cu (▲), and stainless steel control (□).Values plotted at each sampling point are an average of 3 replicates. Error bars represent standard deviation from the mean.

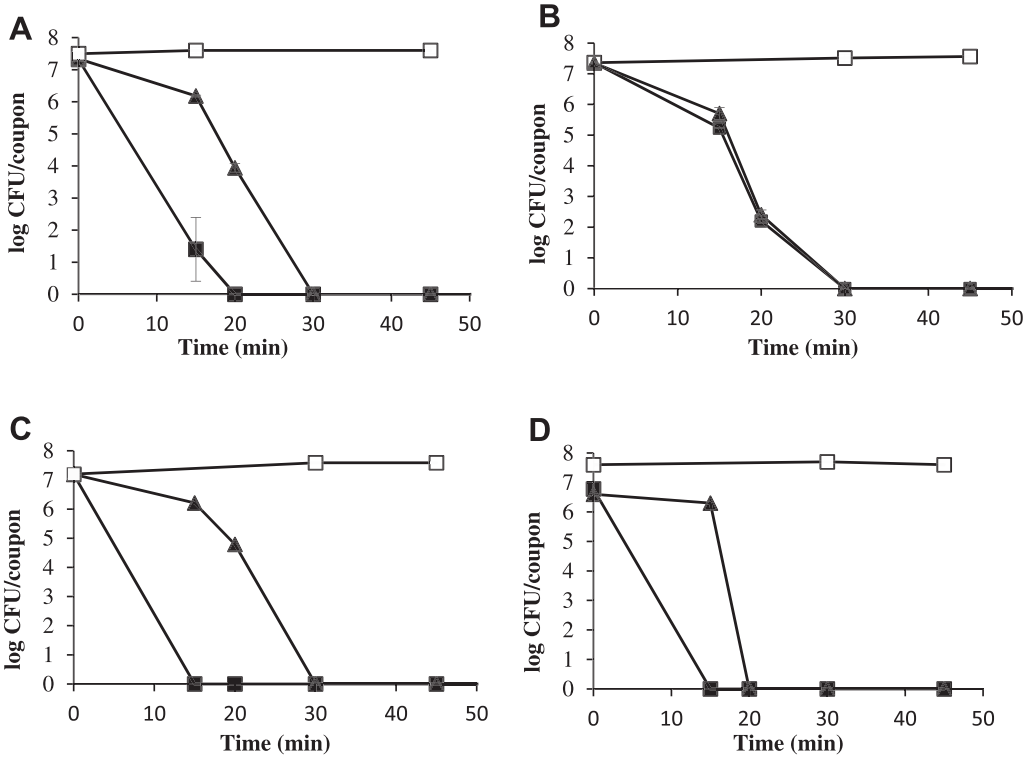

Fig. 6. Survival of *Salmonella* on copper coupon surfaces. The inoculum was suspended in 0.8% NaCl and applied to the surface as a drop under moist conditions. Results for *S.* Enteritidis (A), *S.* Typhimurium S9 (B), *S.* Typhimurium S19 (C), *S.* Typhimurium S20 (D) on C11000, 99.9% Cu (■), C26000, 70% Cu (▲), and stainless steel control (□).Values plotted at each sampling point are an average of 3 replicates. Error bars represent standard deviation from the mean.

In general, inhibitory effects decreased as copper content of the alloys decreased; however, this was not the case for all alloys. In particular, the alloy C75200, which has a higher copper content (65% Cu, 17% Zn, 18% Ni), was not more inhibitory to *Salmonella* than C28000, which has a lower copper content (60%Cu, 40% Zn) under some conditions. This could be attributed to the better corrosion resistance of C75200 (65.0% Cu), because of its content of 18% nickel. Better corrosion resistance of C75200 (65.0% Cu) means less cupric ions released into the cell suspension on the coupon, therefore reducing the bacterial inactivation. A previous study (Michels et al., 2005) found similar trends for the survival of *E. coli* O157:H7 on copper coupons. The cells survived longer on copper alloy coupons with high nickel content.

3.5. Survival of inoculum suspended in 0.8% NaCl on copper alloys

In order to compare the antimicrobial efficacy in the absence of organic compounds, coupons C11000 (99.9% Cu) and C26000 (70% Cu) were also tested using cells suspended in 0.8% NaCl solution. Survival rates were dramatically shortened with strains suspended in 0.8% NaCl and inoculated on copper alloys. Inocula spread over the surfaces and allowed to air-dry in slightly open petri dishes (Fig. 4) resulted in very rapid inactivation of all strains tested on C11000 (99.9% Cu) coupons. No survivors were detected for all strains after 1 min of exposure. Survival times on C26000 (70% Cu) coupons were prolonged with no viable cells detected after 3 min for *S.* Enteritidis and after 5 min for the resistant strains.

When the inocula were spread over the entire surface of copper alloys and kept moist in closed petri dishes (Fig. 5), survival times were decreased further with no survivors recovered after 3 min for all strains tested on C11000 (99.9% copper) coupons. Survival on 70% copper was prolonged for S19 and S20 with no viable cells detected at 5 min exposure. However, S9 showed a 4-log decrease in viable population after 10 min.

Using 25 μl of bacterial suspension placed as droplets in closed petri dishes (Fig. 6), on coupon C11000 (99.9% Cu), S19 and S20 had no survivors detected after 15 min, while S9 survived until no viable cells were recovered after 30 min of exposure. On coupon C26000 (70% Cu), survival of all strains was similar with no viable cells detected after 30 min.

The rapid inactivation rates of salmonellae observed when the cells were suspended in 0.8% NaCl suggest that the organic compounds in TSB may bind copper ions, decreasing the bioavailability and subsequently the overall toxicity to bacterial cells. A previous study (Noyce et al., 2006) demonstrated that adding liquid beef extract on copper coupons reduced the antimicrobial activity of all the copper alloys tested. These results suggest that copper-based food contact surfaces that are free from organic compounds or soil will likely be more bactericidal and thus may be effective antimicrobial surfaces. A recent study (Molteni et al., 2010) also reported that different media showed different copper dissolution. This may also be a reason for the varied inactivation rates observed between inocula suspended in TSB and 0.8% NaCl.

4. Conclusion

The main aim of this study was to assess the antimicrobial effects of copper alloys against *S. enterica*. Compared to stainless steel, all copper-containing alloys exhibited antimicrobial activity against *S. enterica*, with the activity increasing as the copper content of the alloys increased. Even the copper resistant strains only survived for short periods of time on the high copper content alloys. The copper alloys exhibited better kill effects under dry conditions. *Salmonella* cells were more sensitive to copper alloys in the absence of organic compounds. The results of this research will help the industry in understanding the antibacterial effects of copper and its potential as an alternative to stainless steel for food contact surfaces in food processing environments. Copper alloys showing promising results have potential for use in both production (feed handling equipment, feeders, and cage surfaces) and processing (food processing equipment, food handling and packaging areas). These potential applications merit further investigation.

Acknowledgments

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