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Synergistic effect of copper and lactic acid against *Salmonella* and *Escherichia coli* O157:H7: A review

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Abstract

The number of foodborne outbreaks due to bacterial pathogens such as *Salmonella* and *Escherichia coli* O157:H7 have raised concern about the safety of foods. Application of various natural antimicrobial compounds can provide opportunities for the control of pathogenic microorganisms and thus improves food safety and quality. Several researchers in the past have demonstrated antimicrobial properties of copper in different agricultural system. Recent works in our laboratory also showed synergistic effect of copper and lactic acid at low level. However, use of copper as antimicrobial agent in food systems still remains scarce. Our research group is interested in the use of such natural compounds to inhibit the growth of foodborne pathogens. We have used several natural compounds such as organic acids and plant extracts to control the growth of foodborne pathogens. We recently became interested in developing a novel natural system that is effective in controlling pathogenic microorganisms. This system consists of copper in combination with organic acids such as lactic acid. The use of copper at low concentration in combination with lactic acid has been shown to reduce if not eliminate the presence of foodborne pathogens. Therefore, the purpose of this review was to discuss and highlight the wide range of antimicrobial benefits of this new finding. This paper briefly covers possible antimicrobial mechanism of copper, synergistic effect and potential application in food products.

Key words: *Salmonella*, *Eshcerichia coli*, Copper, Lactic acid, Synergistic effect

Introduction

The Centers for Disease Control (CDC) estimate that there are 76 million cases of foodborne illness in the United States annually, with 14 million cases attributed to known pathogens (Doyle, 2000). *Salmonella* and *Escherichia coli* O157:H7 (*E. coli* O157:H7) are pathogens that are known to be major causes of foodborne outbreaks. The incidence of foodborne illness caused by *Salmonella*, one of the most common bacterial pathogen has remained unchanged during 2005-2009 (CDC, 2005, 2006, 2007, 2008, 2009). Similarly, enterohemorrhagic *E. coli* O157:H7 is another significant foodborne pathogen (Baskaran et al., 2010) responsible for several foodborne illnesses. *E. coli* O157:H7 is known to be major causes of hemorrhagic colitis and hemolytic-uremic syndrome (HUS) in the United States (Riley et al., 1983; Doyle, 1991). These pathogens are commonly found in a wide variety of raw meats, dairy products, vegetables, and water (Chang and Fang, 2007). The recent outbreak of the *E. coli* strain STEC O104:H4 in Germany is responsible for at least 48 deaths and has infected more than 4,000 people in Europe and the U.S. (Beutin, 2011). Therefore, Preventive measures taken during food processing are very important in controlling the spread of these pathogens. Numerous studies have investigated the efficacy of natural ingredients to control the presence of pathogenic microorganisms in food products. However, the food industry is always looking to use better alternatives that have strong antimicrobial activity in order to ensure safe wholesome food products. Weak organic acid such as lactic acid is environmentally friendly, naturally occurring and have generally recognized as safe (Brul and Coote, 1999). Even though, the current US–Canadian Recommended Dietary Allowance (RDA) for copper (Cu) is 9 mg/d for adult men and women, with a tolerable upper intake level (UL) of 10 mg/d for adults (Institute of Medicine, 2002), Cu is relatively nontoxic in most mammals, including humans (Scheinberg and Sternlieb, 1976; Linder, 1996). As excretion of Cu into the

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gastrointestinal tract regulates Cu retention in human body, more Cu is absorbed, turnover is faster and excess amount of Cu is excreted into the gastrointestinal tract. This excretion helps to regulate the total body Cu. This efficient homeostatic regulation of absorption and retention helps protect against Cu deficiency and toxicity indicating rare chances of chronic adverse effect (Institute of Medicine, 2002).

In this paper, we discussed antimicrobial activity of Cu alone or in combination with lactic acid against *Salmonella* and *E. coli* O157:H7. Since, both copper and lactic acid possess some antimicrobial properties; their synergistic effect could be significant in the fight against foodborne pathogens such as *Salmonella* and *E. coli* O157:H7.

2. Copper as an antimicrobial agent

Copper is very important trace metal that is essential to human health (Borkow and Gabbay, 2005). Cu is an essential element required for the activity of several biological enzymes. Cu as an antimicrobial agent has been approved and registered by the US Environmental Protection Agency (EPA) to reduce specific harmful bacteria linked to potentially deadly microbial infections (Theivasanthi and Alagar, 2011). Theivasanthi and Alagar (2011) have reported that not any bacterium has developed resistivity to Cu as with antibiotics. The biocidal activity of Cu has been known for several years and has been used in Bordeaux mixture for the protection of late season harvest grapes from fungi, such as *Coniella petrakii* and *Guignardia bidwellii* (Agrios, 1997). Microorganisms require low concentrations of Cu ions as cofactors for processing of metalloproteins and certain enzymes (Nies, 1999). Cu is an essential nutrient for humans, but, in high doses, Cu ions induce and inhibit growth in bacteria and have a toxic effect on most microorganisms (Faundez et al., 2004). Earlier studies have shown that metallic Cu and Cu alloys have antibacterial activity on *Salmonella* and *E. coli* O157:H7 and also inhibit the adhesion of bacteria on biofilm development (Ibrahim et al., 2008; Gyawali et al., 2011; Faundez et al., 2004). The addition of Cu to drinking glasses has been shown to reduce biofilm formation of *Streptococcus sanguis*, reducing the risk of oral infections. Sudha et al. (2009) found that water inoculated with *Salmonella typhi, E. coli*, and *V. cholera* after overnight storage in Cu pots was difficult to recover while there was significantly higher number of microbial growth in water stored in glass bottles. *E. coli* O157:H7 strain survives for much shorter periods on Cu and a brass known as Muntz metal than on stainless steel. Cu surfaces have an antimicrobial activity against *Salmonella enterica* and *Campylobacter jejuni* and have potential application as an inhibitory agent in different stages of food processing operations (Faundez et al., 2004). Beal et al. (2004) reported a 10- fold decrease in the D_{value} of *S. typhimurium* DT104:30 in a liquid pig feed with 150 mM Lactic acid and 50 ppm Cu. According to Ibrahim et al. (2008), slight growth inhibition of *Salmonella* spp. and *E. coli* O157:H7 was obtained with 50 ppm of Cu and there was significant inhibition observed with 100 ppm and 200 ppm Cu in carrot juice. A wide range of microorganisms has been shown to be susceptible to Cu including *Salmonella* and *E. coli* O157:H7. Recently, Delgado et al. (2011) reported that Cu nanoparticles can kill *E. coli* and has a great potential as antimicrobial agents.

3. Antimicrobial mechanisms of copper

The bactericidal action of Cu is dependent on the concentration of free ionic Cu (Menkissoglu and Lindow, 1991; Zevenhuizen et al., 1979). The way in which Cu acts on microorganisms is a complicated subject. However, a few of many proposed mechanisms include:

- The toxicity of Cu is largely due to its tendency to alternate between its cuprous - Cu (I), and cupric - Cu (II), oxidation states, differentiating Cu from other trace metals. Under aerobic conditions, this redox cycling leads to the generation of highly reactive hydroxyl radicals that readily and efficiently damage biomolecules, such as DNA, proteins, and lipids (Santo et al., 2008). As shown in Figure 1, free radicals produced from redox cycle can damage the cell integrity (Borkow and Gabbay, 2005).
- Cu reacts with proteins by combining –SH groups of enzymes and this leads to the inactivation of the proteins (Yoon et al., 2007). Cu ions bind to the sulfhydryl, amino and carboxyl groups of amino acids, thereby denaturing the proteins they compose. This makes enzymes and other proteins ineffective, compromising the biochemical process they control. Cell surface proteins necessary for transport of materials across cell membranes are also inactivated as they are denatured. Cu will bind with the phosphate groups that are part of the structural backbone of DNA molecules. This mechanism leads to the separation of the double helix and consequent destruction of the cell molecule (Meyer, 2001).
- Cu's toxicity is possibly due to the displacement of essential ions, which inactivate

enzymes and obstruct functional groups of proteins, producing free radicals from hydroperoxide compounds and thus affecting membrane integrity (Nies, 1999).

Based on all of these studies, it seems that it is Cu's electrochemical potential that enables its free Cu ion to affect the proteins and enzymes in microbes, thereby inhibiting their activity and giving Cu its antimicrobial characteristic.

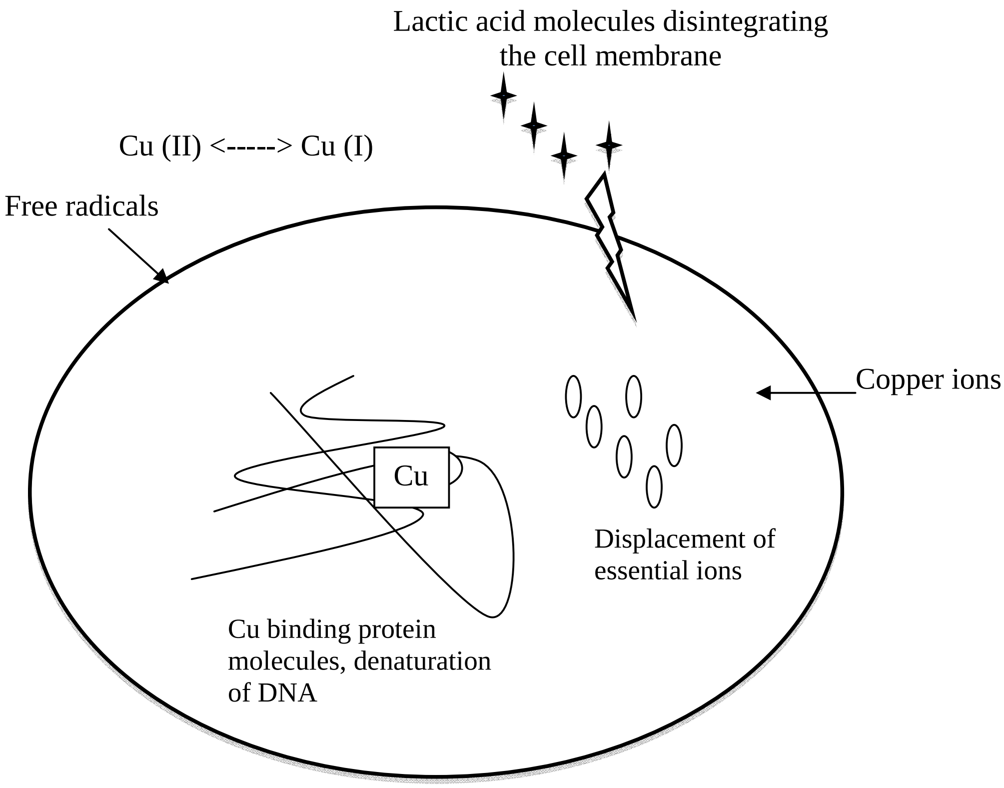

Figure 1. Schematic diagram showing mechanisms of toxicity of copper (Cu) to microorganisms.

4. Applications of copper

Currently Cu is used as a water purifier, algaecide, fungicide, nematocide, molluscicide, and antifouling and antibacterial agent (Borkow and Gabby, 2005). The efficacy of Cu and Cu alloys to inactivate hospital borne microbes is well-documented (Casey et al., 2010). For example, Cu alloys can replace touch surfaces at health-related facilities to reduce the microbial infection due to cross contamination. Methicillin-resistant *Staphylococcus aureus* (MRSA) is usually spread through direct contact via the hands of healthcare employees and it is extremely difficult to eradicate. Cu also has the potential to reduce MRSA, a serious public health threat that has defied nearly all antibiotics (Casey et al., 2010). Therefore, antimicrobial surfaces of Cu can play an important role in reducing contamination and limiting cross infection. Another important application of Cu is being used in food processing facilities**.** Foods are always in contact with various touch surfaces during processing. Cu and its alloys can eliminate foodborne pathogens due to their antimicrobial properties. The most common touch surface materials that are being used in the food processing industry, including slaughter houses are comprised of stainless steel which has no efficacy in destroying pathogenic microbes (Faundez et al., 2004). Despite the fact that stainless steel looks clean, it does not have any properties to combat *E. coli* O157:H7 and other harmful microbes and therefore can be a potential source of cross contamination (Lewis, 2005). It would be of greater benefit if these surfaces were replaced with Cu plates.

5. Lactic acid as an antimicrobial agent

Lactic acid is an effective antimicrobial agent and is classified by the U.S. Food and Drug Administration (FDA) as a Generally Recognized as Safe (GRAS) food additive (Sapers, 2005). The treatment solution pH and the degree of organic acid dissociation determine the antimicrobial effectiveness (Smulders, 1995; Tamblyn and Conner, 1997). Hence, inhibitory action is mainly due to the compound crossing the plasma membrane in the undissociated state. In a high acid environment (low pH), lactic acid remains undissociated and is in its most active antimicrobial form. Therefore, inhibition of growth by a weak acid preservative such as lactic acid is due to a number of actions including membrane disruption, inhibition of metabolic reactions, stress on intracellular pH homeostasis and the accumulation of toxic anions (Brul and Coote, 1999). Use of organic acids such as lactic acid is often used to decontaminate meat surface to reduce bacterial counts, as it is a natural meat compound produced during the post-mortem glycolysis (Pipek et al., 2005). Organic acids also have a great potential to serve as a practical means of decontamination as a sanitizing agent. Organic acids are the most commonly used chemical decontaminant for foods (Belk, 2001). Lactic acid is a biocidal to a wide range of microorganisms. However, *E. coli* O157:H7 is unusually tolerant to its antimicrobial properties and has survived up to 56 days in Tryptone Soy Broth (TSB) acidified to pH 4.7 (Conner and Kotrola, 1995). Similarly, Abdul-Raouf et al. (1993) determined that *E. coli* O157:H7 survived well in beef slurries with lactic acid. Doyle et al. (1999) reported that lactic acid (1.5%), hydrogen peroxide (0.1%), sodium benzoate (0.1%), and glycerol monolaurate (0.005%) alone on *E. coli* O157:H7 in 0.1% peptone water did not reduce bacterial population indicating that none of these chemicals could reduce bacterial population by 5.0 log CFU/ml. However, they found that lactic acid treatment provided the greatest biocidal effect against *E. coli* O157:H7 (Doyle et al., 1999). Studies have reported the decontamination effects of weak organic acid treatments on microorganisms in meats, especially in poultry and beef processing. According to Castillo et al. (2001), the treatment of 2% lactic acid reduced levels of *Salmonella typhimurium* and *E. coli* O157:H7 on inoculated carcass surfaces. Castillo et al. (2001) also reported that spraying 4% lactic acid on chilled carcasses in a commercial environment reduced aerobic plate counts by about 3 log. Lactic acid dips and spray washes of prechilled birds have also been used in the poultry industry, and the bacterial reduction was from 5.2 to 3.7 log CFU/g when poultry was immersed for 15 seconds in 1 or 2% lactic acid at pH 2.2 (Van der Marel et al., 1998). Lactic acid can also be used as a surface treatment on ready to eat (RTE) meats for the control of *Listeria monocytogenes* (Samelis et al., 2002; Barmpalia et al., 2004; Geornaras et al., 2006).

Lactic acid as a weak organic acid is successfully used as a sanitizer on food animal carcasses and may have the potential to reduce microorganisms on produce surfaces. A solution containing 1.5% lactic acid and 1.5% hydrogen peroxide as a 15 minute soak at 40 °C was reported to give a greater than 5 log reduction in the population of *E. coli* O157:H7, *Salmonella enteritidis*, and *L. monocytogenes* on spot inoculated apples, oranges, and tomatoes (Sapers, 2005). The populations of *S. typhimurium* and *E. coli* O157:H7 were not detected inside four tomatoes, which had been sprayed with purac lactic acid (250 ml of 2% lactic acid) at 5 °C. It was believed that the lack of detection of pathogen populations was mainly due to the fast bacterial reduction on the surface, which prevented the active pathogens from diffusing into the tomatoes (Ibarra-Sanchez et al., 2004). Similarly, there was a significantly lower number of *S. typhimurium* and *E. coli* O157:H7 on fresh cantaloupes with 2% lactic acid spray (Alvarado-Casillas, 2007). It was also documented that 0.5% of lactic acid eliminates *Yersinia enterocolitica* on shredded lettuce. The 5-minute dip wash treatment of 0.5% lactic acid showed a 2.5 log reduction of *Y. enterocolitica* (Escudero et al., 1999). Therefore, the use of lactic acid demonstrates a broad spectrum of antimicrobial activity against different kinds of foodborne pathogens including gram-negative pathogens such as *Salmonella* spp. and *E. coli* O157:H7. Organic acids have been tested with other antimicrobial agents to overcome bacterial acid resistance (Olasupo et al., 2004). Doyle et al. (1999) reported that the antimicrobial activity of organic acids can be increased or potentiated when combined with other food preservatives.

6. Synergistic effect of copper and lactic acid

Several examples of combination treatments have been reported to inactivate *Salmonella* and *E. coli* O157:H7 on different food products. Sometimes individual treatments show different modes of action and behave differently than when combined with other chemical agents. Weak organic acids such as lactic acid also show synergistic effect when combined with Cu. *E. coli* O157:H7 is tolerant to organic acids and can survive well in acidic food. It can adapt to acidic conditions and tolerate pH levels that would normally inactivate it (Eribo and Ashenafi, 2003). As a result of its resistivity to acidic media, lactic acid acts differently towards pathogens as compared to its combination effect with others antimicrobial agents and shows greater efficacy towards bacteria. Our study showed that lactic acid in combination with Cu sulfate could be used to inhibit the growth of pathogens. The effects of Cu alone, or in combination with lactic acid, on the growth of *Salmonella* and *E. coli* O157:H7 are shown in Table 1. In the control samples, the number of *Salmonella* increased from 3 log CFU/ml to 7.39 and 8.32 CFU/ml after 12 hr incubation in BHI and carrot juice respectively. With the addition of 50 ppm Cu, the growth of *Salmonella* was not significantly inhibited (P > 0.05). The number of cells reached 7.30 (BHI) and 8.23 CFU/ml (carrot juice). The addition of 0.2% of lactic acid produced a retarding effect on the growth of *Salmonella.* After 12 hr the number was 5.65 and 5.51 CFU/ml in BHI and carrot juice samples respectively. However, when a combination of both 50 ppm Cu and 0.2% lactic acid was used, a significant growth inhibition of *Salmonella* (P < 0.05) was observed. Similar results were also obtained with *E. coli* O157:H7 in both BHI and carrot juice (Table 1). The results showed that the combination of lactic acid and Cu produces a synergistic effect against *Salmonella* spp. and *E .coli* O157:H7 in laboratory medium (BHI) and carrot juice. The growth of both *Salmonella* and *E. coli* O157:H7 in the presence of both 50 ppm Cu and lactic acid 0.2% were significantly inhibited (P < 0.05).

Table 1. Survival and growth of mixture of 38 *Salmonella* strains and mixture of 6 *E. coli* O157:H7 strains in BHI broth and carrot juice in the presence of copper (Cu) alone or in combination with lactic acid (La) after incubation for 12 hr at 37 °C.

Treatments	Bacterial Population (Log CFU/ml)			
	BHI		Carrot Juice	
	Salmonella	*E. coli* O157:H7	*Salmonella*	*E. coli* O157:H7
Control	7.39	6.69	8.32	7.83
Copper (Cu 50ppm)	7.30	6.61	8.23	7.54
Lactic acid (La 0.2%)	5.65	4.70	5.51	5.73
Cu 50ppm + La 0.2%	2.44	1.89	2.79	2.37

Initial bacterial population was approximately 3.00 Log CFU/ml Replication (n=3), S.D. ± 0.21

The presence of Cu in acidic liquid food substrates significantly decreases the death rate of *S. typhimurium* DT104:30. It was found that the addition of 150 mM lactic and 50 ppm of Cu to liquid pig feed resulted in a 10-fold increase in the death rate (Beal et al., 2004). Russell (2008) also reported that an acidic copper sulfate-based commercial sanitizer was able to reduce *Salmonella* and *E. coli* during scalding and was thus able to extend the shelf life of broiler chicken carcasses. The combination of sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2) in the presence of copper sulfate ($CuSO_4$) has shown antifungal activity against *Penicillium digitatum* (Cerioni et al., 2009).

The use of Cu ion for decreasing microbial loads on fresh produce has proven to be deficient. However, several in vitro studies indicate that copper ion might be potentially useful as a produce sanitizer (Rodgers and Ryser, 2004). A study conducted by Jo and Rim (2007) showed that the addition of organic acid with silver ions enhanced the inhibitory effect on *E. coli* O157:H7 growth compared to individual treatments. Since, weak organic acids possess antimicrobial activity; the combined treatment with metal ions may be synergistic. Lactic acid not only reduces the pH of the medium; it also possesses permeabilizer properties and can freely pass across the plasma membrane and enter the cell, lactic acid can facilitate the entrance of Cu ions into the cell which produces a toxic effect. It can be assumed that the permeabilizing nature of lactic acid could enable the entry of Cu ions into the bacterial cells and therefore produce a synergistic effect against *E. coli* O157:H7 (Figure 1).

Our group also conducted another similar study on “antimicrobial activity of copper alone or in combination with lactic acid against different strains of *E. coli* O157:H7 in laboratory medium and on the surface of lettuce and tomatoes.” In this study, we tried to establish a concentration of Cu in combination with lactic acid that effectively inhibits the growth of *E. coli* O157:H7 and determine whether such concentration could be used to decontaminate *E. coli* O157:H7 populations on the produce surface. Figures 2(a) - 2(d) show the effect of Cu alone or in combination with lactic acid on the growth of four strains of *E. coli* O157:H7 in BHI broth. Average bacterial populations of four strains of *E. coli* O157:H7 after 8 hr of incubation at 37°C are presented in Table 2. In the control samples, the numbers of *E. coli* O157:H7 strains increased from an initial population of 3 log CFU/ml and reached an average of 9.93 log CFU/ml. With the addition of Cu at different concentrations, the growth of *E. coli* O157:H7 was not significantly inhibited ($P > 0.05$). Therefore, the concentration of Cu alone at 40 ppm did not significantly inhibit the tested strains. Growth in BHI broth containing 0.1% lactic acid was not significantly affected compared to growth in the control or BHI containing only Cu. *E. coli* O157:H7 grown in BHI broth containing 0.1% lactic acid in combination with 4 different concentrations of Cu showed slight inhibition on the growth of bacterial populations compared to the control and Cu alone samples. When *E. coli* O157:H7 was grown in BHI broth containing 0.2% lactic acid alone or in combination with 5 and 10 ppm Cu, reductions in the bacterial population of 2.91, 3.59 and 3.73 log CFU/ml compared to the control were not significantly different from each other. When a combination of 20 ppm Cu with 0.2% lactic acid was used, 4.37 log CFU/ml reduction in bacterial population was achieved compared to the control sample. Furthermore, with the addition of 40 ppm Cu to 0.2% lactic acid, the bacterial population was reduced by 5.03 log CFU/ml compared to the control (9.93 log CFU/ml). Therefore 20 and 40 ppm of Cu in combination with 0.2% of lactic acid is the most effective treatment to inhibit the growth of *E. coli* O157:H7 in BHI media. Table 2 shows a maximum of 5 log reduction in bacterial population achieved with combination of 40 ppm Cu and 0.2% lactic acid when compared to the control samples. This indicates that the combination of 40 ppm Cu and 0.2% lactic acid produces a significant effect ($P < 0.05$) against 4 different strains of *E. coli* O157:H7.

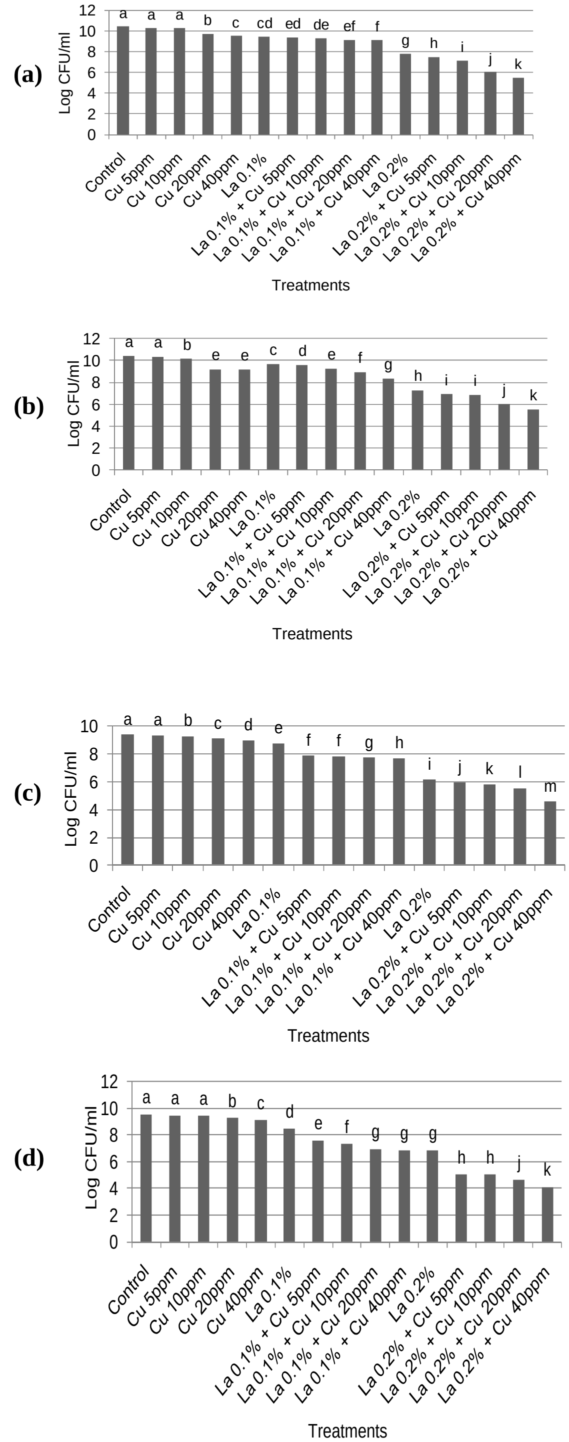

Figure 2. Populations of *E. coli* O157:H7 strains (a) strain H1730 (b) strain 43895+ (c) strain 43895- and (d) strain 86.24 in BHI broth containing copper (Cu) and lactic acid (La). Columns with the same letter are not statistically different ($P < 0.05$) according to the least significant different test.

Table 2. Populations of *E. coli* O157 strains (Log CFU/ml) grown in BHI broth containing different concentrations of copper (Cu) and lactic acid (La) after incubation for 8 hr at 37°C.

Treatments	Average
Control	$9.93^{a} \pm 0.55$
Cu 5ppm	$9.85^{a} \pm 0.53$
Cu 10ppm	$9.77^{a} \pm 0.48$
Cu 20ppm	$9.30^{ab} \pm 0.27$
Cu 40ppm	$9.19^{ab} \pm 0.22$
La 0.1%	$9.07^{abc} \pm 0.56$
La 0.1% + Cu 5ppm	$8.61^{bc} \pm 1.02$
La 0.1% + Cu10ppm	$8.42^{bc} \pm 1.01$
La 0.1% + Cu20ppm	$8.18^{bc} \pm 1.03$
La 0.1% + Cu40ppm	$7.99^{cd} \pm 0.95$
La 0.2%	$7.02^{de} \pm 0.70$
La 0.2% + Cu 5ppm	$6.34^{ef} \pm 1.08$
La 0.2% + Cu10ppm	$6.20^{ef} \pm 0.95$
La 0.2% + Cu20ppm	$5.56^{fg} \pm 0.64$
La 0.2% + Cu40ppm	$4.90^{gh} \pm 0.69$

Means (± standard deviation) within the same column followed by different letters are significantly different ($p < 0.05$). Initial bacterial population was approximately 3.00 Log CFU/ml.

These results showed slightly higher reduction in bacterial populations with combination of 40 ppm Cu and 0.2% lactic acid compared to our previous study with 50 ppm Cu and 0.2% lactic acid (Table 1). This difference observed could be due to the sterilization method of Cu ions. A preliminary study was conducted to determine the effect of sterilization method on the efficacy of Cu ions. When working with antimicrobial activity against pathogens such as *Salmonella* and *E. coli* O157:H7, we observed that the filter sterilized media (Cu) was more effective than autoclaved media (Cu). This indicates the sterilization process can influence the antimicrobial effects of Cu ions.

We also evaluated the impact of Cu and lactic acid on pH values to determine whether acid is the main inhibiting factor on the survival and growth of *E. coli* O157:H7. The pH levels of the treated BHI broth samples containing Cu and lactic acid were 7.10, 5.74, and 5.48 for 40 ppm Cu, 0.2% lactic acid and 40 ppm Cu with 0.2% lactic acid. These values indicate that individual or combined treatment had little effect on pH values. This leads us to conclude that acid is not the only factor influencing the survival activity of these pathogens. There might be some other mechanism involved in the inhibition of bacterial growth other than the acidic medium. It can be assumed that the permeabilizing nature of lactic acid could have enabled the entry of Cu ions into the bacterial cells, thereby producing a synergistic effect against such pathogens as demonstrated earlier in Figure 1. Recently, in a study conducted by our group has demonstrated the synergistic effects of Cu and lactic acid on the morphology of *E. coli* O157:H7. The significant reduction is cell size was observed when bacterial cells were treated with Cu in combination with lactic acid (Gyawali et al., 2011).

Based on the results obtained from these two studies in laboratory (BHI) media, another experiment was conducted to determine the potential of Cu and lactic acid solution to decontaminate produce surfaces. Tables 3 and 4 show the populations of *E. coli* O157:H7 on the surface of lettuce and tomato samples treated with Cu and lactic acid. Results showed that lactic acid and Cu alone did not effectively reduce the bacterial population. However, the treatment of lettuce and tomatoes with combination of Cu, lactic acid plus Tween 80 effectively reduced populations of *E. coli* O157:H7 from the produce samples. The populations of *E. coli* O157:H7 were reduced by 3.93 and 3.39 log in lettuce and tomato samples respectively when treated with 40 ppm Cu and 0.2% lactic acid with 0.1% Tween 80 (surfactant). Tween 80 also called polysorbate 80 is currently approved for various purposes such as an emulsifier in ice cream and custard products, a dispersing agent in pickle products and gelatin products, an emulsifier in shortenings and whipped toppings, and as a defoaming agent in the production of cottage cheese (Code of Federal Regulations, 2002). Cheng-An and Beuchat (1995) reported that a combination of 1% trisodium phosphate with 5% Tween 80 was more efficient in removing *Salmonella* spp. from chicken skin. There were about 1.1-1.2 log reductions of *E. coli* O157:H7 on the surfaces of strawberries when they were sanitized with 100 and 200 ppm of Tween 80 (Yu et al., 2001). The addition of surfactant like Tween 80 could have enhanced the lethality of copper and lactic acid solution by increasing surface contact of the sanitizer with the microbes by maximizing the release of the pathogen from inoculated vegetables. Therefore, the efficacy of treatment could be the result of the detachment of the cells to the surfaces rather than antimicrobial effect of Tween 80. It can be inferred that the use of Tween 80 could also enhance the efficacy of treatment solution in reducing the microbial populations on lettuce and tomatoes surfaces.

Table 3. Populations of mix 4 *E. coli* O157:H7 strains recovered from lettuce surface after treatment with copper (Cu) and lactic acid (La) with and without Tween 80.

Treatments	Populations (Log CFU/Lettuce Piece) of *E. coli* O157:H7	
	Without Tween 80	With Tween 80 (0.1%)
Control	$8.31^{a} \pm 0.014$	$7.46^{c} \pm 0.014$
La 0.2%	$6.67^{g} \pm 0.014$	$6.06^{h} \pm 0.007$
Cu 20 ppm	$7.65^{b} \pm 0.007$	$7.04^{e} \pm 0.014$
Cu 40 ppm	$7.19^{d} \pm 0.014$	$6.96^{f} \pm 0.014$
La 0.2%+Cu 20 ppm	$5.42^{i} \pm 0.014$	$4.54^{k} \pm 0.007$
La 0.2%+Cu 40 ppm	$5.08^{j} \pm 0.014$	$4.38^{l} \pm 0.007$

Means (± standard deviation) within the same column and row followed by different letters are significantly different ($p < 0.05$). Initial inoculum concentration was approximately 9 log CFU/ml.

Table 4. Populations of mix 4 *E. coli* O157:H7 strains recovered from tomato surface after treatment with copper (Cu) and lactic acid (La) with and without Tween 80.

Treatments	Populations (Log CFU/tomato) of *E. coli* O157:H7	
	Without Tween 80	With Tween 80 (0.1%)
Control	$7.01^{a} \pm 0.014$	$6.98^{a} \pm 0.021$
La 0.2%	$6.11^{f} \pm 0.014$	$6.01^{g} \pm 0.021$
Cu 20 ppm	$6.94^{b} \pm 0.007$	$6.24^{d} \pm 0.014$
Cu 40 ppm	$6.88^{c} \pm 0.014$	$6.15^{e} \pm 0.014$
La 0.2%+Cu 20 ppm	$4.81^{h} \pm 0.021$	$4.38^{j} \pm 0.007$
La 0.2% +Cu 40 ppm	$4.72^{i} \pm 0.014$	$3.62^{k} \pm 0.021$

Means (± standard deviation) within the same column and row followed by different letters are significantly different ($p < 0.05$). Initial inoculum concentration was approximately 9 log CFU/ml.

7. Conclusions

Even though copper and various organic acids and their derivatives have been considered as antimicrobial, their synergistic effect and potential use as an antimicrobial compound and mode of action have not been studied well. This paper reviewed the synergistic effects of copper and lactic acid as an antimicrobial agent against foodborne pathogens. The low concentration of copper alone may not be lethal enough to inhibit the growth of such pathogens. However, when both copper in combination with organic acid such as lactic acid was used, there was a significant inhibition in the growth of these pathogens. The inhibition of microbial growth could be due to the permeabilizing properties of acid which could have facilitated the entry of copper ions inside the cells thereby producing a toxic effect on microorganisms. Recent work in our laboratory has suggested that further research should explore the potential of the antimicrobial effect of copper and other natural ingredients including organic acids or essential oils against other foodborne pathogens (Gram positive and Gram negative bacteria) in different food systems. Some possibilities include adding low concentration of copper to several juices and even in dairy products. However, sensory analysis studies of foods are also needed to determine if such treatment would change the taste characteristics of the original food. Our work clearly demonstrated that copper in combination with lactic acid produces a synergistic inhibition effect on the microbial growth. Therefore, copper in combination with lactic acid has potential for controlling the growth of foodborne pathogens. This could be a novel and encouraging alternative approach to be used in food systems to help microbial safety within the food industry. This work also indicates the potential of copper/acid solution as a sanitizer for fresh fruits and vegetables as well as in decontamination of meat carcasses that could result in an extension of the shelf life of the meat products. This finding has wide range of implications for reducing outbreaks from cross contaminations of foodborne pathogens in the food processing industry. Since, copper has shown various antibacterial activities against foodborne pathogens in different food systems, copper could have additional medicinal properties as well. Therefore, copper could be used in future as therapeutic agent.

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References

Abdul-Raouf, U., L. Beuchat and M. Ammar. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Appl. Environ. Microbiol. 59:1999-2006.

Agrios, G. N. 1997. Plant Pathology, San Diego CA. Academic Press.

Alvarado-Casillas, S., S. Ibarra-Sanchez, O. Rodriguez-Garcia, N. Martinez-Gonzales and A. Castillo. 2007. Comparison of rinsing and sanitizing procedures for reducing bacterial pathogens on fresh cantaloupes and bell peppers. J. Food Prot. 70:655-660.

Barmpalia, I., I. Geornaras, K. Belk, J. Scanga, P. Kendall, G. Smith and J. Sofos. 2004. Control of *Listeria monocytogenes* on frankfurters with antimicrobials in the formulation and by dipping in organic acid solutions. J. Food Prot. 67:2456-2464.

Baskaran, S. A., M. A. R. Amalaradjou, T. Hoagland and K. Venkitanarayanan. 2010. Inactivation of *Escherichia coli* O157: H7 in apple juice and apple cider by trans-cinnamaldehyde. Int. J. Food Microbiol. 141(1-2):126-129.

Beal, J., S. Niven, A. Campbell and P. Brooks. 2004. The effect of copper on the death rate of *Salmonella typhimurium* DT104: 30 in food substrates acidified with organic acids. Lett Appl. Microbiol. 38:8-12.

Belk, K. 2001. Beef decontamination technologies. National Cattleman's Beef Association. Res. Tech. Serv. 1-8.

Beutin, L. 2011. Outbreak with aggregative EHEC O104:H4 in Germany: Specific characteristics and search for possible sources. In International Association for Food Protection, Milwaukee, WI.

Borkow, G. and J. Gabbay. 2005. Copper as a biocidal tool. Curr. Med. Chem. 12:2163-2175.

Brul, S. and P. Coote. 1999. Preservative agents in foods mode of action and microbial resistance mechanisms. Int. J. Food Microbiol. 50:1-17.

Casey, A., D. Adams, T. J. Karpanen et al. 2010. Role of copper in reducing hospital environment contamination. J. Hosp. Infect. 74:72-77.

Castillo, A., L. Lucia, D. Roberson, T. Stevenson, I. Mercado and G. Acuff. 2001. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. J. Food Prot. 64:58-62.

CDC, 2005. Preliminary FoodNet data on the incidence of infection with pathogenstransmitted commonly through food-10 states, United States, 2004. Morbidity and Mortality Weekly Rep., 54:352–356.

CDC, 2006. Preliminary FoodNet data on the incidence of infection with pathogenstransmitted commonly through food-10 states, United States, 2005. Morbidity and Mortality Weekly Rep. 55:392–395.

CDC, 2007. Preliminary FoodNet data on the incidence of infection with pathogenstransmitted commonly through food-10 states, 2006. Morbidity and Mortality Weekly Rep. 56:336–339.

CDC, 2008. Preliminary FoodNet data on the incidence of infection with pathogenstransmitted commonly through food-10 states, 2007. Morbidity and Mortality Weekly Rep. 57:366–370.

CDC, 2009. Preliminary FoodNet Data on the incidence of infection with pathogenstransmitted commonly through food-10 states, 2008. Morbidity and Mortality Weekly Rep. 58:333–337.

Cerioni, L., V. Rapisarda, M. Hilal, F. Prado and L. Rodriguez-Montelongo. 2009. Synergistic antifungal activity of sodium hypochlorite, hydrogen peroxide, and cupric sulfate against *Penicillium digitatum*. J. Food Prot. 72:1660-1665.

Chang, J. M. and T.J. Fang. 2007. Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. Food Microbiol. 24: 745-751.

Cheng-An, H. and L.R. Beuchat. 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. J. Food Prot. 58:19-23.

Code of Federal Regulations. 2002. Title 21, vol. 3. Polysorbate 80. 172:840. Available at:http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=172&showFR=1&subpartNode=21:3.0.1.1.3.9.

Conner, D. and J. Kotrola. 1995. Growth and survival of *Escherichia coli* O157: H7 under acidic conditions. Appl. Environ. Microbiol. 61:382-385.

Delgado, K., R. Quijadal, R. Palma and H. Palza. 2011. Polypropylene with embedded copper metal or copper oxide nanoparticles as a novel plastic antimicrobial agent. Lett. Appl. Microbiol. 53:50–54.

Doyle, M.P. 1991. *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. 12:289–301.

Doyle, M. P. 2000. Reducing foodborne disease. Food Technol. 54:130.

Doyle, M. P., K. S. Venkitanarayanan and T. Zhao. 1999. Inactivation of *Escherichia coli* O157:H7 by combinations of GRAS chemicals and temperature. Food Microbiol. 16:75-82.

Eribo, B. and M. Ashenafi. 2003. Behavior of *Escherichia coli* O157:H7 in tomato and processed tomato products. Food Res. Int. 36:823-830.

Escudero, M., L. Velázquez, M. Di Genaro and A. de Guzmán. 1999. Effectiveness of various disinfectants in the elimination of *Yersinia enterocolitica* on fresh lettuce. J. Food Prot. 62:665-669.

Faúndez, G., M. Troncoso, P. Navarrete and G. Figueroa. 2004. Antimicrobial activity of copper surfaces against suspensions of *Salmonella enterica* and *Campylobacter jejuni*. BMC Microbiol. 4:19.

Food and Nutrition Board. 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press.

Geornaras, I., P. Skandamis, K. Belk, J. Scanga, P. Kendall, G. Smith and J. Sofos. 2006. Postprocess Control of *Listeria monocytogenes* on commercial frankfurters formulated with and without antimicrobials and stored at 10°C. J. Food Prot. 69:53-61.

Gyawali, R., S. A. Ibrahim, S. H. A. Hasfa, S.Q. Smqadri and Y. Haik. 2011. Antimicrobial activity of copper alone and in combination with lactic acid against *Escherichia coli* O157: H7 in laboratory medium and on the surface of lettuce and tomatoes. J. Pathogens. doi:10.4061/2011/650968.

Ibarra-Sanchez, L., S. Alvarado-Casillas, M. Rodriguez-Garcia, N. Martinez-Gonzales and A. Castillo. 2004. Internalization of bacterial pathogens in tomatoes and their control by selected chemicals. J. Food Prot. 67:1353-1358.

Ibrahim, S., H. Yang and C. Seo. 2008. Antimicrobial activity of lactic acid and copper on growth of *Salmonella* and *Escherichia coli* O157:H7 in laboratory medium and carrot juice. Food Chem.109:137-143.

Institute of Medicine, 2002. Copper. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Food and Nutrition Board. National Academy Press, Washington, DC, pp. 224–257.

Jo, S. and A. Rim. 2007. Combined treatment with silver ions and organic acid enhances growth-inhibition of *Escherichia coli* O157:H7. Food Con. 18:1235-1240.

Lewis, A. 2005. Antimicrobial properties of copper alloys in food processing facilities- A white paper for the copper development Association Inc. and International Copper Association Inc.

Linder, M. C.1996. Copper. In: E. E. Ziegler and L. J. Filer (Eds.). Present knowledge of nutrition, 7th ed. Washington, DC: IL SI Press, 307-319.

Menkissoglu, O. and S. Lindow. 1991. Relationship of free ionic copper and toxicity of bacteria in solutions of organic compounds. Phytopathol. 81:1258-1263.

Meyer, W. 2001. Coping with resistance to copper/silver disinfection. Water Eng. Manage. 148:11.

Nies, D. 1999. Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol. 51:730-750.

Olasupo, N., D. Fitzgerald, A. Narbad and M. Gasson. 2004. Inhibition of *Bacillus subtilis* and *Listeria innocua* by nisin in combination with some naturally occurring organic compounds. J. Food Prot. 67:596-600.

Pipek, P., M. Houska, J. Jeleni'kova, K. Kyhos, K. Hoke and M. Sikulova. 2005. Microbial decontamination of beef carcasses by combination of steaming and lactic acid spray. J. Food Eng. 67(3):309-315.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Herbert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Engl. J. Med. 308:681–685.

Rodgers, S. and E. Ryser. 2004. Reduction of microbial pathogens during apple cider production using sodium hypochlorite, copper ion, and sonication. J. Food Prot. 67:766-771.

Russell, S. 2008. The effect of an acidic, copper sulfate-based commercial sanitizer on indicator, pathogenic, and spoilage bacteria associated with broiler chicken carcasses when applied at various intervention points during poultry processing. Poult. Sci. 87:1435.

Samelis, J., G. Bedie, J. Sofos, K. Belk, J. Scanga and G. Smith. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4C in vacuum packages. J. Food Prot. 65:299-307.

Santo, C., N. Taudte, D. Nies and G. Grass. 2008. Contribution of copper ion resistance to survival of *Escherichia coli* on metallic copper surfaces. Appl. Env. Microbiol.74:977.

Sapers, G., J. R. Gorny and A. E. Yousef. 2005. Microbiology of fruits and vegetables. Boca Raton Florida, CRC Press.

Scheinberg, I. H. and I. Sternlieb. 1976. Copper toxicity and Wilson's disease. In: A. S. Prasad (Ed.) Trace elements in human helath and disease, Vol 1. New York: Academic Press, 415-438.

Smulders, F. 1995. Preservation by microbial decontamination; the surface treatment of meats by organic acids, Vol. 253. Chapman and Hall, London.

Sudha, V., K. Singh, S. Prasad and P. Venkatasubramanian. 2009. Killing of enteric bacteria in drinking water by a copper device for use in the home: laboratory evidence. Soc. Trop. Med. Hyg. Trans. R. 103:819-822.

Tamblyn, K. and D. Conner. 1997. Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. J. Food Prot. 60:629-633.

Theivasanthi, T. and M. Alagar. 2011. Studies of Copper Nanoparticles Effects on Micro-organisms. Ann. Biol. Res. 2(3):82-87.

Van der Marel, G., J. Van Logtestijn and D. Mossel. 1988. Bacteriological quality of broiler carcasses as affected by in-plant lactic acid decontamination. Int. J. Food Microbiol. 6:31.

Yoon, K., J. Hoon Byeon, J. Park and J. Hwang. 2007. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. Sci. Total Environ. 373:572-575.

Yu, K., M. C. Newman, D. D. Archbold and T. R. Hamilton-Kemp. 2001. Survival of *Escherichia coli* O157:H7 on strawberry fruit amd reduction of the pathogen population by chemical agents. J. Food Prot. 64:1334-1340.

Zevenhuizen, L., J. Dolfing, E. Eshuis and I. Scholten-Koerselman. 1979. Inhibitory effects of copper on bacteria related to the free ion concentration. Microb. Ecol. 5:139-146.