COPPER AND LACTIC ACID SOLUTION REDUCES ESCHERICHIA COLI O157:H7 INOCULATED ON LEAFY GREENS

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Abstract: *We evaluated the efficacy of copper alone (Cu) or in combination with lactic acid (LA) in decontaminating leafy greens (cilantro, parsley, and dill) that were inoculated with Escherichia coli O157:H7. Samples were individually submerged in an approximately 8 log CFU/ml cocktail suspension consisting of 3 E. coli O157:H7 strains. Inoculated samples were air dried under a biosafety hood for 2 h before exposure to various treatment solutions. Individual samples were then treated with water (control), copper, lactic acid alone or with a combination of Cu/LA acid solution. Our results indicated that a reduction of at least 2.0 CFU/g of E. coli O157:H7 was achieved when a combination of 50 ppm Cu with 0.2% LA was applied. These results demonstrated that this combination treatment could be useful in improving the microbial safety of cilantro, parsley, and dill.*

Introduction

Disinfection is one of the most important processing steps affecting the safety of fresh produce. Washing fresh produce with tap water may remove soil and other debris, but it has a limited effect on surface microorganisms. A variety of disinfectants such as chlorine have been used to reduce the bacterial populations on fruits and vegetables. Besides their potential toxicity, these disinfectants cannot completely remove or inactivate microorganisms on fresh produce (Deza et al., 2003). Thus, there is a need for alternative sanitizers to be used for the disinfection of fresh produce including leafy greens. Consumers as well as the food industry are also looking for the use of natural ingredients that can help to ensure the safety of food products.

Organic acid is generally recognized to be a safe chemical for use in foods and has been widely used to control the growth of pathogenic bacteria in foods (Tajkarimi et al., 2011). Lactic acid is successfully used as a sanitizer on animal carcasses and may also have the potential to reduce microorganisms on produce surfaces. Copper (Cu) ion in low concentrations is an essential micronutrient and a vital cofactor for the processing of certain enzymes. However, higher concentrations of Cu can cause the inhibition, or even death, of microorganisms. Thus, there is a great potential for Cu to be used as an antimicrobial agent to inhibit foodborne pathogens. The antimicrobial activity of Cu alone or in combination with LA on laboratory medium and carrot juice has been reported (Ibrahim et al., 2008). Our previous study also showed that Cu in combination with LA significantly reduced the *E. coli* O157:H7 population on the surface of lettuce and tomatoes (Gyawali et al., 2011). Therefore, in this study we tested the effect of Cu and acid on leafy greens. The objective of this study was to investigate the antimicrobial effect of Cu and LA alone or in combination against *Escherichia coli* O157:H7 on leafy greens.

Material and Methods

Bacterial strains

Three individual strains of *E .coli* O157:H7 (H1730, E0019, F4546) grown in BHI broth were mixed together to produce a mixture of *E. coli* O157:H7. The cells of this mixture were harvested by centrifugation at 8, 000 rpm for 10 min at 4°C. The supernatant was decanted, and the cell pellet was resuspended in 500mL of sterile peptone water (0.1%, w/v) to give a cell number of at least 8 log CFU/ml.

Sample preparation and inoculation

Leafy greens (cilantro, dill, and parsley) were purchased from a local grocery store (Greensboro, NC) on the day of the experiments. Samples were washed with tap water, dried, and individually dipped into a bacterial solution for one-hour. After inoculation, samples were dried under the biological safety cabinet for 2 hr to facilitate the attachment of bacteria.

Treatment solution

Batches of 90 ml deionized distilled water were mixed with Cu (CuSO4·5H2O) and lactic acid (LA) alone or in combination to obtain 50 ppm and 0.2% solution respectively. A combination of Cu with LA was also prepared at the same concentrations. An additional 90 ml of distilled water without treatment was prepared as a control. Samples were filter sterilized using 0.2-μm Nalgene filter (NalgeNunc International, Rochester, NY, USA) before being tested for antimicrobial effects.

Treatment procedure

After air drying, 10 g of each sample were individually immersed in sterile plastic bags containing each treatment solution (control, 50 ppm Cu, 0.2% LA, or Cu/LA solution) for approximately 3 min. After treating the samples, each sample was then transferred into new sterile stomacher bags containing 90 ml of peptone water and then homogenized for 1 min at 200 rpm.

Bacterial population count

After homogenization, solution from each treated bag was 10-fold serially diluted in peptone water, and 0.1 ml of appropriate diluents was spread-plated onto BHI agar medium. The plates were incubated at 37°C for 24 h and colonies were counted and calculated as log CFU/g.

Results and Discussion

Table 1 shows the effect of Cu and LA solution against the *E. coli* O15:H7 population on cilantro, dill, and parsley. The average initial level of *E. coli* O157:H7 was 7.17 log CFU/g (7.16, 7.12, and 7.23 log CFU/g for cilantro, dill, and parsley respectively). Washing the samples (cilantro, dill, and parsley) with the control treatment (distilled water) reduced the cell population to only 7.01 CFU/g on average, which indicates that water treatment is not effective in decontaminating leafy greens. However, when 50 ppm Cu was used as a treatment solution, the average cell reduction was 0.97 log CFU/g. Higher cell reduction was achieved when samples were treated with Cu in combination with LA ($p < 0.05$). The *E. coli* O157:H7 population was then reduced by 1.7, 2.17, and 2.25 log CFU/g on cilantro, dill, and parsley respectively. On average, this combination treatment reduced the levels of *E. coli* O157:H7 by 2.04 log CFU/g.

Washing with regular tap water, chlorine, peroxyacetic acid, acidified sodium chlorite, hydrogen peroxide, ozone, or brush and spray washers has not always been effective enough to reduce the bacterial population in produce (Ganesh et al., 2011). For example, the most commonly used washing treatment in the produce industry is chlorine. Foley et al. (2004) reported that the use of chlorine at 200 ppm reduced pathogenic microflora by approximately 1.5-2 logs on lettuce, cilantro, and parsley. The efficacy of the combination treatment that we used in our study was found to be superior compared to chlorine treatment. Moreover, these commonly used sanitizing solutions in the fresh produce industry have raised numerous safety concerns related to humans and the environment. In this study, our treatment exposure time was approximately 3 min. It is expected that higher cells reductions could be achieved by increasing the treatment time.

Conclusion

Our results showed that a Cu and LA solution has an antimicrobial effect against *E. coli* O157:H7. An average pathogen reduction of 2.04 log CFU/g was achieved on the surface of leafy greens that were treated with Cu/LA combination. This combination treatment could be thus be useful for improving the microbial safety of fresh produce, including leafy greens.

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Table 1 - Antimicrobial effect of copper and lactic acid solution against E. coli O157:H7 population (Log CFU/g)

Treatments	Leafy greens			Average population
	Cilantro	Dill	Parsley	
Control	7.12 ± 0.04	6.91 ± 0.24	6.99 ± 0.26	7.01[A]
Cu	6.56 ± 0.01	6.03 ± 0.06	6.02 ± 0.09	6.20[B]
LA	6.08 ± 0.06	5.22 ± 0.26	6.07 ± 0.07	5.79[C]
Cu + LA	5.46 ± 0.32	4.95 ± 0.02	4.98 ± 0.04	5.13[D]

All experiments were conducted twice. Comparisons between treatments were analyzed by SAS 9.2 (SAS Inst., Cary, NC). *P* values less than 0.05 were considered statistically significant.

Values are means ± standard deviation. Values with different letters within a column are significantly different ($p < 0.05$).

Initial population was 7.16, 7.12, and 7.23 log CFU/g for cilantro, dill, and parsley respectively. (Average initial population=7.17 log CFU/g)

IMPACT OF SWEET POTATO AND METAL IONS ON THE GROWTH AND ENZYMATIC ACTIVITY OF LACTOBACILLUS REUTERI ATCC 55730

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Abstract *The objective of this study was to determine the effect of sweet potatoes and metal ions on the growth and enzymatic activity of Lactobacillus reuteri ATCC 55730. Extract from baked sweet potatoes was supplemented with 4 g/L each of beef extract, yeast extract, and proteose peptone #3 to form the sweet potato medium (SPM). The growth and enzymatic activity of L. reuteri ATCC 55730 in SPM was then compared to standard MRS. The enzymatic activity of L. reuteri ATCC 55730 including α–glucosidase, β-glucosidase, acid phosphatase, and phytase was determined spectrophotometrically using a corresponding substrate. Our results showed similar growth patterns for L. reuteri ATCC 55730 growing in SPM and MRS. Final bacterial population reached 8.59±0.21 and 8.55±0.31 log CFU/mL in SPM and MRS respectively after 16 h of incubation at 37ºC. L. reuteri ATCC 55730 grown in SPM showed a 103.5, 74.5, and 43.6 % increase in β-glucosidase, acid phosphatase, and phytase activity*