Antimicrobial activity of copper alone or in combination with ascorbic acid against E. coli O157:H7, Salmonella and Cronobacter spp. in laboratory medium

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Abstract
The aim of this study was to determine the effect of copper (50 ppm) alone or in combination of ascorbic acid (0.2%) on the survival and growth of E. coli O157:H7, Salmonella and Cronobacter sakazakii in laboratory medium (BHI broth). Bacterial growth was monitored during the incubation period for 8h at 37º C. Copper (50 ppm) or ascorbic acid (0.2%) alone slightly retarded the growth of all three tested bacteria. However, the growth of bacteria was significantly inhibited when Cu 50 ppm in combination with ascorbic acid 0.2% were added into BHI broth. These findings indicated that ascorbic acid in combination with copper sulphate, could be used as anti microbial agent for the growth of pathogens.

Introduction
Escherichia Coli O157:H7, Salmonella spp. and Cronobacter spp. are all pathogens that are most commonly known for their harmful effects on food and the people who consume such contaminated foods (Meng & Doyle, 1998). E. coli O157:H7 is found mainly in meats such as beef, raw milk, vegetables as well as dairy products (Hussain, 2007). Salmonella is commonly found in unpasteurized milk, poultry and egg products(Rodrigue et al. 1990). Cronobacter sakazakii is an emerging human pathogen that contaminates powdered infant formula (PIF) and has been associated with various cases of neonatal meningitis (Kim & Loesener 2008; Jang & Rhee, 2009). All of these bacteria can, and have caused death. However, these cases may be avoided with proper sterilization and preparation of food. With prior knowledge of the effects of copper and ascorbic acids on bacteria (Ibrahim et al. 2008; Tajkarimi et al. 2010), we developed an experiment that would further our studies and help our understanding of the matter. In the past numerous studies have been done using various treatments and measuring the impact they may have on the population growth and reductions of such bacteria. In our study, treatments such as copper (50 ppm), ascorbic acid (0.2%) and a combination of both were used. We used optical density readings and plate count methods to test our theory on which treatments would effectively reduce the bacterial population of all three bacterial populations. Our goal is to drastically reduce the bacterial level. Using a control, a broth with no treatment, as our reference, we compared the optical density of each of our bacteria and their treatments. We predicted the combination of both copper and ascorbic acid would have the greatest reduction in population than any of the other treatments.

Objective
To investigate the effect of lactic acid, acetic acid and copper (Cu) alone or in combination with ascorbic acid (Asc) against E. coli O157:H7 (43985), Salmonella Typhimurium ED405, and Cronobacter sakazakii 731 in laboratory media.

Methods

**Experimental chart**

- E. Coli O157:H7, Salmonella, and Cronobacter sakazakii strains
  - Individual Strains + BHI broth (12 hrs. 37º C)
  - Wash cells in peptone water (centrifuge 700 rpm, 10 min, 4 º C)
  - Adjusted culture concentration approximately 3.00 log CFU/ml
  - Inoculate 100 µl into treatments

**Cells were harvested by centrifugation**

- Vortex and incubate at 37º C for 8 hr

**O.D. readings (610 nm) and plating**

- Plate count to determine the bacterial population
- Serial dilutions and plating
- Turbidity observed
- O.D. readings using spectrophotometer

**Chemicals used**

- Media preparation
- Treatment solution (Filter sterilization)

**Results**

**Conclusions**

- This study demonstrates the ability of copper alone or in combination with lactic and acetic acid to inhibit the growth of tested foodborne pathogens.
- Our finding shows that combination of copper and ascorbic acid could be a good alternative to control these pathogens.
- Populations of E. coli O157:H7, Salmonella spp. and Cronobacter sakazakii reduced by 2.92, 3.59, and 3.0 log CFU/ml respectively when compared to their control population.
- Further studies are needed to evaluate the effect of these treatments in food model including juice and infant formula.

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References