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Antibacterial Activity of Red Bell Pepper against *Escherichia coli* O157:H7 in Ground Beef

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Abstract

The objective of this study was to investigate the effect of red bell pepper on the survival and growth of Escherichia coli O157:H7 in ground beef. Four different strains of Escherichia coli O157:H7 (944, E0019, F4546, and Cider) were used in this study. Each strain was inoculated into 100g of ground beef to reach the initial inoculum level of approximately 3 log CFU/g. The inoculated ground beef was then mixed with red bell pepper, at the level of 5 % (W/W). Ground beef samples without treatment were used as controls. Samples were stored at 37 ℃, collected at 0 and 8 h, stomached for 120 seconds, serially diluted, and plated onto BHI agar plates. Plates were incubated at 37 ℃ for 24 hrs. Results showed that red bell pepper extract had a significant antibacterial activity against all 4 strains in ground beef. The red bell pepper extract reduced the population of Escherichia coli O157:H7 by approximately 1.2 log CFU/g in an average compared to the control sample. Our result suggests that red pepper could be used as an effective preservative against this pathogen in ground beef.

Key words: *Escherichia coli* O157:H7, ground beef, red pepper extract, inhibition.

Introduction

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial activity against foodborne pathogens (Deans and Ritchie, 1987; Lambert *et al.* 2001; Zhang *et al.* 2009). Plant essential oils and their components exhibit broad-spectrum activity against both Gram-negative and Gram-positive foodborne pathogens. Burt (2004) reported that several plant extracts and their essential oils have been used to inhibit the growth of or to reduce the number of, several foodborne pathogens such including *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes*. Consumers are now, paying closer attention to the risk of foodborne pathogens as well as the presence of artificial chemical preservatives used to control foodborne pathogens. Synthetic preservatives have been used in foods for decades; however, an increasing perception by consumers that synthetic compounds may lead to negative health consequences has led to a reduced acceptance of their use in foods (Ibrahim *et al.* 2006; Ibrahim *et al.* 2011). A number of studies have demonstrated that compounds existing in many spices also possess antimicrobial activity. As a result, consumers as well as the food industry are looking to use more natural food preservatives that have strong antimicrobial activity in order to ensure safe, wholesome food products. The antimicrobial effects of many plant extracts have been well studied. Natural compounds are developed from plants such as garlic, onions and peppers. These vegetables and herbs contain antimicrobial compound that inhibit microbial growth (Mau *et al.* 2002). This suggests that plant products have relatively high levels of antimicrobial agents and can be used to inhibit the growth of foodborne pathogens. Dorantes *et al.* (2000) have shown the inhibition of foodborne pathogens by *Capsicum annum* extracts. Similarly, the inhibitory effect of extract of *Capsicum annum* bell pepper against *Salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in minced beef meat has been reported (Careaga *et al.* 2003).
Many of the outbreaks caused by *E. coli* O157:H7 have been associated with eating undercooked ground beef (Bell *et al.* 1994). Spoilage microorganisms can readily grow in both fresh and precooked meat products. Although the antimicrobial effects of some herbs and spices have been well documented, only a few studies have been conducted to investigate the feasibility of using crude pepper as a potential antimicrobial agent for the preservation of ground

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beef and meat products (Ibrahim *et al*. 2009). Pepper extracts are currently widely used as spices in ground beef. Therefore, the objective of this study was to examine the antimicrobial activity of crude bell pepper extracts against four strains of *Escherichia coli* O157:H7 in ground beef.

Material and Methods

Bacterial strains. Four strains of *E. coli* O157:H7 (E0019, 944, cider, 4546) were used in this study. *E. coli* O157:H7 strains were obtained from North Carolina A &T State University food microbiology laboratory culture collection. These strains were maintained on tryptic soy agar (TSA, Difco Laboratories, Becton Dickinson, Sparks, MD, USA) slants at 4℃ and transferred to fresh tryptic soy broth (TSB) before use. Cultures of each strain were grown separately in TSB at 37℃ and transferred at 24 h intervals.

Preparation of crude pepper extract. To prepare the crude pepper extract, fresh red bell peppers were obtained from a local farmer's market in Greensboro, NC. The bell pepper was washed under running tap water, and blotted with a single-use paper towel. The pepper was chopped into pieces with a sterilized table knife. The pieces (100g) were then blended in a sterilized kitchen blender for 2-3 minutes to obtain a homogeneous blend. This blend was placed in 50 ml tubes and centrifuged at 5500 g for 45 min at 4 ℃. The supernatant was collected and filtered, using a 0.45 µm filter, then stored overnight at 4 ℃.

Survival and growth of E. coli O157:H7 in liquid medium. Overnight grown individual strains of *E. coli* O157:H7 were serially diluted to achieve 3.0 log CFU/ml inoculum level. A 0.1 mL of each strain was inoculated into 10 mL of BHI broth containing red pepper extract at different concentrations (0, 600, 800, and 1000 µL). Samples were then incubated at 37 ℃ for 8 hr. At the end of the incubation period, one mL of each sample was withdrawn and serially diluted in 0.1% peptone water. Appropriate dilutions were surface plated (100 µL) onto BHI agar. These plates were then incubated at 37 ℃ for 24 hr, and the colonies were counted to determine the bacterial population in each sample.

Antimicrobial assay by well diffusion method. A well-diffusion test was used to determine the antibacterial activity of crude red pepper extract. Batches of 100 ml of BHI agar medium (0.5% casein digest, 0.25% yeast extract, 0.1% dextrose and 1.5% agar) were prepared and sterilized at 12℃ for 15 min. The agar was placed in a water bath at 49℃, and allowed to cool, and each extract sample was then inoculated individually with a single strain of *Escherichia coli* O157:H7 to achieve an inoculum level of 5-6 log CFU/ml. The inoculated agar was then poured into Petri dishes and allowed to set. A well was created in the middle of each plate using a 1 cm cork borer. Into each well, different concentrations of pepper extract (0-1000 µL with 200 µL concentration increase for each plate) were placed containing each strain. The test was conducted in duplicate. The plates were incubated at 37℃ overnight and the zones of inhibition recorded.

Antibacterial activity in ground beef. The antibacterial activity of red bell pepper in ground beef was measured using samples of ground beef purchased from a local store (Greensboro, NC). Each sample was split into two 100 gram portions. The pepper was chopped into pieces with a sterilized table knife and added at 5% (W/W) to one portion of each ground beef sample. Ground beef samples without ground pepper served as control samples. Samples were inoculated with each of the *Escherichia coli* O157:H7 strains to achieve a final inoculum level of 3.00 log CFU/mL. The samples were then stored at 37 ℃ for 24 hours. A 0.1 ml aliquot of each sample was removed at the end of the incubation period, serially diluted in 0.1% peptone water and spread plated, in duplicate, on prepared BHI agar. The plates were then incubated at 37 ℃ for 24 hours, after which the colonies were counted to determine the bacterial populations in each sample.

Statistical analysis. The statistical analysis system (SAS Institute, Cary, NC) was used for the analysis of data with Duncan's multiple range to determine the significant differences ($P < 0.05$). At least three replications of each test were conducted.

Results and Discussion

Figure 1 shows the survival and growth of 4 different strains of *E. coli* O157:H7 in the presence of different concentrations of red pepper extract in BHI broth after 8 hr of incubation at 37 ℃. When *E. coli* O157:H7 strains were grown in BHI broth sample without extract, populations increased from 3.0 log CFU/mL to 8.02 log CFU/mL on average. The addition of extract at 600 µL reduced the bacterial populations to 7.11 log CFU/mL, indicating the antimicrobial effect of the pepper extract. Further addition of 600 and 1000 µl of extract reduced the bacterial population by 0.98-1.70 log CFU/mL when compared to the control sample.

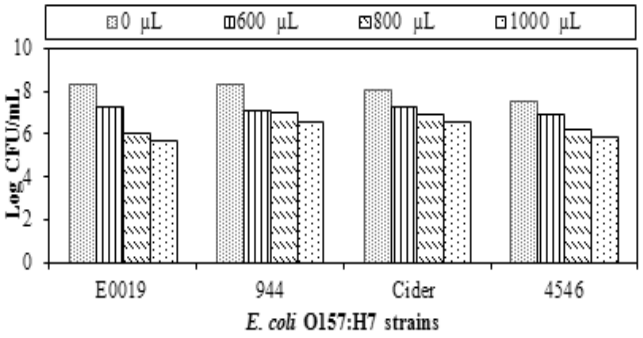

Figure 1. Inhibition of E. coli O157:H7 in the presence of red pepper extract in Brain Heart Infusion broth

Table 1. MIV and zone of growth inhibition for *E. coli* O157:H7 strains in the presence of red bell pepper extract on BHI agar plate

Strain	Inhibition zone (mm)	MIV (µL/mL)
E. coli O157:H7 (944)	8.5 ± 1.08	800
E. coli O157:H7 (cider)	7.0 ± 0.98	600
E. coli O157:H7 (E0019)	6.5 ± 1.10	600
E. coli O157:H7 (F4546)	6.0 ± 1.20	600

* Value of inhibition zone is expressed as mean ± standard deviation (n=3). MIV= minimum inhibitory volume.

The antimicrobial activity of red pepper extract against *E. coli* O157:H7 was also assessed using agar diffusion assay. Table 1 shows the MIV values for the pepper extract for the tested strains. MIV is defined as the lowest volume of red pepper extract that inhibited bacterial growth (Fig. 2).

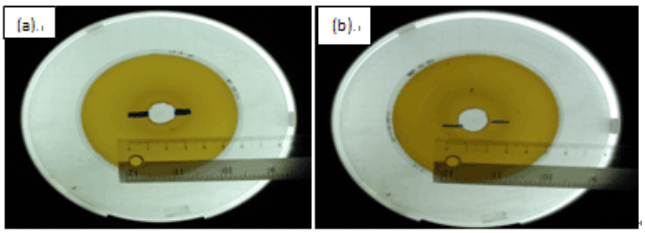

Figure 2. Zone of inhibition (mm) of *E. coli* O157:H7 in the presence of red bell pepper extract at (a) 600 µL and (b) 800 µL

Our results showed MIV values from 600-800 µL for the tested strains. The MIV for strain 944 was 800 µL whereas for strains cider, E0019, and F4546, the MIV was 600 µL. The largest zone of inhibition (8.5 mm) was observed with strain 944 followed by cider (7.0 mm), E0019 (6.5 mm), and F5546 (6.0 mm). From these results, it can be inferred that *E. coli* O157:H7 (944) was slightly more resistant to pepper extract compared to other strains. Similar results were also shown by Ibrahim et al. (2009, 2010). In addition, the authors reported the antimicrobial activity of chive and guava extract against Salmonella and E. coli O157:H7. The average MIV and MIC (minimum inhibitory concentration) of chive and guava extract ranged from 200 to 700 µL for Salmonella and E. coli O157:H7. Rahman et al. (2010) have also reported the antimicrobial activity of spices against food spoilage pathogens by using the disc diffusion test method. Thus, these studies would support the use of extracts of natural products, including herbs and spices, as potential natural ingredients to enhance food safety.

Table 2 shows the population of *E. coli* O157:H7 in ground beef samples treated with 5% (w/w) red bell pepper. When bacterial strains were inoculated in ground beef without pepper (control), population levels reached 7.84-8.33 log CFU/g from the initial population of 2.95-3.22 log CFU/g within 8 hr of incubation at 37 ℃. The addition of pepper reduced the bacterial population to 6.58-7.16 log CFU/g. Our results showed that red bell pepper inhibited the growth of all tested strains ($p < 0.05$) by 1.18 log CFU/g on average. Based on these results, red bell pepper at a minimum concentration level of 5% could be used as a natural ingredient to control the growth of foodborne pathogens including *E. coli* O157:H7.

Table 2. Population of *E. coli* O157:H7 in ground beef treated with 5% red bell pepper after incubation at 37 ℃ for 8 hr

	Bacterial population (Log CFU/g)		
Bacterial strains	Initial	Control	Treated
E. coli O157:H7 (944)	3.22 ± 0.21	7.90 ± 0.20^{a}	6.84 ± 0.13^{b}
E. coli O157:H7 (cider)	3.14 ± 0.26	8.32 ± 0.15^{a}	7.09 ± 0.17^{b}
E. coli O157:H7 (E0019)	3.10 ± 0.31	8.33 ± 0.23^{a}	7.16 ± 0.29^{b}
E. coli O157:H7 (F4546)	2.95 ± 0.27	7.84 ± 0.11^{a}	6.58 ± 0.18^{b}

* Mean values (n=3) in the same row followed by different letters are significantly different ($P < 0.05$).

Conclusion

Red bell pepper was effective against all four strains of *Escherichia coli* O157:H7. These findings showed that the red bell pepper had strong antimicrobial activity. Red bell pepper can be used in other fresh food and cooked foods and could also be added to fruit juices. However, further research is needed, especially sensory analysis, to determine if the addition of red bell pepper extract would change the taste of the original food. Since the food industry is looking toward naturally occurring antimicrobials agents to control the food borne pathogens, red pepper in combination with other natural ingredients could be explored as a natural preservative against *Escherichia coli* O157:H7 in ground beef. As a result, red bell pepper has the potential to satisfy consumer demand for a natural antimicrobial substance in food as an acceptable alternative to artificial preservatives.

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