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Inactivation of *Escherichia coli* O157:H7 during cooking of non-intact beef treated with tenderization/marination and flavoring ingredients

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ABSTRACT

This study examined the effect of tenderizing/marinating and flavoring ingredients on thermal inactivation of *Escherichia coli* O157:H7 in a lean ground beef model system, simulating non-intact products. Ground beef (3% fat) was inoculated with *E. coli* O157:H7 (5 strains; 6–7 log CFU/g), followed by mixing with nothing (control) or solutions of water, a mixture of flavoring agents (FA), 0.23% calcium chloride (CC) + FA, CC + FA + 0.3% acetic acid (AA), 0.5% sodium chloride (NaCl) + 0.25% sodium tripolyphosphate (STP), NaCl + STP + FA, NaCl + STP + 1.8% potassium lactate (PL), NaCl + STP + PL + FA, NaCl + STP + PL + AA, and NaCl + STP + PL + AA + FA. Samples (30 g) were extruded into tubes, stored (4 °C) overnight, and cooked to 60 °C (rare) or 65 °C (medium-rare) in a water bath. Cooking weight losses, and fat and moisture contents, water activity, pH, and total bacterial and *E. coli* O157:H7 populations were determined after inoculation, after storage, and after heating. Reductions of the pathogen at 60 °C in acid (AA)-treated samples were higher than reductions obtained in samples not treated with acid. Surviving pathogen counts at 65 °C in NaCl and STP-treated samples with no acid were higher ($P < 0.05$) than those of samples of all other tested treatments; however, the counts decreased to 0.7–1.6 log CFU/g when AA was added. Overall, the results of the study indicate that tenderizing/flavoring ingredient formulations combined with 0.3% AA (i.e., CC + FA + AA, NaCl + STP + PL + AA, and NaCl + STP + PL + AA + FA) enhanced destruction of *E. coli* O157:H7 during cooking of a non-intact beef product.

1. Introduction

Consumers consider tenderness, flavor, and juiciness as important attributes associated with the palatability of beef (George-Evins, Unruh, Marsden, & Kastner, 2000). Thus, the meat industry uses various tenderization techniques, such as aging, use of proteolytic enzymes, marination, electrical stimulation, flaking and forming, and mechanical tenderization to improve beef tenderness (Hajmeer, Ceylan, Marsden, & Phebus, 2000). In the United States, at least 18% of beef products available at the retail level have been mechanically-tenderized or injected with solutions for enhancement of tenderness and/or flavor (NCBA, 2006). A microbiological risk associated with injection of tenderization and marination solutions into beef muscle cuts is that these processes may lead to internalization of *Escherichia coli* O157:H7, or other foodborne pathogens, into the normally sterile deep tissues (Hajmeer et al., 2000; Heller et al., 2007; Sofos, Geornaras, Belk, & Smith, 2008). Studies by Sporing (1999) and Phebus, Thippareddi, Sporing, Marsden, & Kastner (2000) indicated that 3–4% of *E. coli* O157:H7 cells on the surface of beef subprimals were internalized into the tissue by blade tenderization. The internalized *E. coli* O157:H7 cells may survive and result in illness, ranging from mild bloody diarrhea to severe and life-threatening hemolytic-uremic syndrome (Belongia, MacDonald, & Parham, 1991), if such products are intentionally (consumer preference) or unintentionally (accidental) undercooked (Hajmeer et al., 2000; Heller et al., 2007; Sofos et al., 2008). Therefore, the United States Department of Agriculture Food Safety and Inspection Service expanded the definition of *E. coli* O157:H7 as an adulterant to include non-intact beef products other than ground beef (USDA-FSIS, 1999). In recent years, non-intact products other than ground beef have been associated with several recalls and outbreaks in the United States because of *E. coli*

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O157:H7 contamination (CDC, 2010; Laine et al., 2005; Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005; USDA-FSIS, 2003, 2004, 2005, 2007).

There are numerous reports in the literature on the use of chemical ingredients for enhancement of quality aspects or palatability traits of beef. Studies (Berge et al., 2001; Burke & Monahan, 2003) have shown that marination or injection of beef with organic acids (acetic, citric, lactic) enhances tenderness. Lawrence, Dikeman, Hunt, Kastner, & Johnson (2003) evaluated calcium ascorbate, calcium chloride, and calcium lactate for their effects on color, lipid oxidation, tenderness, and sensory attributes of beef strip loins. Sodium phosphate is commonly used by the industry to increase protein solubility and the water-binding ability of meat (Trout & Schmidt, 1986), and addition of sodium phosphate (as either pyrophosphate or hexametaphosphate) increased the tenderness of fresh beef (Streitel, Ockerman, & Cahill, 1977). Furthermore, injection of a brine solution containing sodium tripolyphosphate into pork *longissimus* increased juiciness and reduced Warner-Bratzler shear values, and also increased juiciness when injected into beef *semimembranosus* (Smith, Simmons, McKeith, Betchel, & Brady, 1984). Although both calcium chloride and sodium tripolyphosphate increase the tenderness of beef, they should not be included in the same mixture because the phosphates chelate calcium when in solution (Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2004). Moreover, studies by Eilers et al. (1994) and Morris, Theis, Miller, Acuff, & Savell (1997) showed that a 10% injection of 0.3 M calcium chloride had an adverse effect on palatability, imparting a bitter, metallic and sour taste to the cooked meat product. To compensate for the undesirable flavor characteristics related to chemical tenderizers, Scanga et al. (2000) suggested the use of chemical tenderizers in combination with marination and flavoring/seasoning agents (sodium chloride, hydrolyzed soy protein, maltodextrin, dried beef stock, spices) in order to enhance the palatability characteristics of beef.

Another microbiological concern associated with non-intact beef products that have been injected with tenderness and/or flavor-enhancing solutions is that the chemical ingredient(s) may interfere with thermal inactivation or increase the heat resistance of the internalized pathogen during cooking of these products (Sofos et al., 2008). Previous work by the authors (Mukherjee et al., 2008) screened individual ingredients used for tenderization or marination of beef (i.e., calcium ascorbate, calcium chloride, calcium lactate, potassium lactate, sodium chloride, acetic acid, and citric acid) for their effect on destruction during cooking (60 and 65 °C) of *E. coli* O157:H7 internalized in a lean ground beef model system (simulating non-intact products). In a study that followed (Yoon et al., 2009), logical combinations of two or three of the above ingredients were tested under the same conditions. Overall findings of these studies (Mukherjee et al., 2008; Yoon et al., 2009) were that destruction of *E. coli* O157:H7 during cooking was enhanced in organic acid-containing (especially acetic acid) treatments, and was generally not affected by any of the tested tenderizing salts, as compared to the control; however, once calcium salts were combined with an organic acid, reductions of the pathogen generally increased after cooking. The present study is a continuation of this work and includes testing of combinations of up to five ingredients, including sodium chloride plus sodium tripolyphosphate (ingredients commonly used by the industry in meat products), or calcium chloride, in combination with selected previously tested (Mukherjee et al., 2008; Yoon et al., 2009) tenderizing/marinating compounds, and a mixture of flavoring agents. Thus, the overall objective of this study was to evaluate various combinations of tenderizing/marinating and flavoring ingredients on thermal inactivation of *E. coli* O157:H7 in a lean ground beef model system. As previously reported (Mukherjee et al., 2008; Yoon et al., 2009), the ground beef model system was used to allow uniform inoculation, ingredient mixing, and heating of samples.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

E. coli O157:H7 strains ATCC43888, ATCC43889, ATCC43894, ATCC43895, and EO139 (strain EO139 was kindly provided by Dr. M. P. Doyle, University of Georgia, Griffin, GA) were individually cultured and subcultured in 10 ml of tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) at 35 °C for 24 h. The five cultures were then combined and centrifuged at 4629 × *g* for 15 min at 4 °C. Harvested cells were washed twice by centrifugation and resuspension in sterile phosphate-buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 L of distilled water), and then serially diluted in PBS to obtain a target level of 6–7 log CFU/g when 7 ml of inoculum was added to 700 g of ground beef.

2.2. Preparation, inoculation, and treatment of beef

Fresh beef knuckles (3% fat) were ground (0.95 cm and 0.16 cm plates; Hobart Mfg. Co., Troy, OH), and 700 g batches of the ground meat were inoculated (6–7 log CFU/g) with 7 ml of the *E. coli* O157:H7 inoculum mixture. The meat and inoculum were thoroughly mixed for 2 min in a bowl-lift stand mixer (speed setting of 2; KitchenAid®, Professional 600, St. Joseph, MI) followed by mixing for an additional 2 min with: (i) nothing (control), or solutions (39 ml) of (ii) sterile distilled water, (iii) a mixture of flavoring agents (FA; black pepper: 0.42 g, garlic powder: 0.875 g, onion powder: 1.05 g, old hickory smoked salt: 3.045 g, Worcestershire sauce: 10.5 ml), (iv) 0.23% wt/wt calcium chloride (CC; Fisher Scientific, Fair Lawn, NJ) + FA, (v) CC + FA + 0.3 % wt/wt acetic acid (AA; Fisher Scientific), (vi) 0.5% wt/wt sodium chloride (NaCl; Fisher Scientific) + 0.25% wt/wt sodium tripolyphosphate (STP; FMC Corporation, Philadelphia, PA), (vii) NaCl + STP + FA, (viii) NaCl + STP + 1.8% wt/wt potassium lactate (PL; Purac America, Inc., Lincolnshire, IL), (ix) NaCl + STP + PL + FA, (x) NaCl + STP + PL + AA, and (xi) NaCl + STP + PL + AA + FA. CC was not tested together with STP because, as indicated previously, phosphates chelate calcium when the two chemicals are present in the same solution (Lawrence et al., 2004). Samples (30 g) of the inoculated and treated beef were extruded into plastic tubes (2.5 cm diameter × 10 cm height; Nalgene, Nalge Nunc, Inc., Rochester, NY) with a caulking gun (Facilities Maintenance, Colorado State University, Fort Collins, CO), taking care to avoid formation of air pockets which would potentially interfere with heat transfer during cooking. The tubes containing the beef samples were individually covered with aluminum foil, and were stored at 4 °C overnight to simulate tenderization/marination of non-intact beef products.

2.3. Cooking of samples and microbiological analyses

The samples were heated to internal temperatures of 60 °C or 65 °C, simulating rare and medium-rare degrees of doneness of beef, respectively (Obuz, Dikeman, Erickson, Hunt, & Herald, 2004), in a circulating water bath set at 63 °C and 68 °C, respectively. The internal temperature of the samples was monitored during cooking, using type-K thermocouples (Pico Technology Ltd., Cambridge, UK) inserted into the center of the meat in the tubes, and real-time data recording software (PicoLog, Pico Technology Ltd.).