



Short communication

Enhancing the thermal destruction of *Escherichia coli* O157:H7 in ground beef patties by trans-cinnamaldehyde

Mary Anne Roshni Amalaradjou^a, Sangeetha Ananda Baskaran^a, Ranjith Ramanathan^a, Anup Kollanoor Johny^a, Anu Susan Charles^a, Satyender Rao Valipe^a, Tyler Mattson^a, David Schreiber^a, Vijay K. Juneja^b, Richard Mancini^a, Kumar Venkitanarayanan^{a,*}

^a Department of Animal Science, University of Connecticut, Storrs, CT 06269, USA

^b U.S. Department of Agriculture, Agricultural Research Service, Microbial Food Safety Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

ARTICLE INFO

Article history:

Received 30 November 2009

Received in revised form

8 May 2010

Accepted 8 May 2010

Available online 9 June 2010

Keywords:

Escherichia coli O157:H7

Ground beef

Hamburger patties

Cooking

Trans-cinnamaldehyde

ABSTRACT

The effect of trans-cinnamaldehyde (TC) on the inactivation of *Escherichia coli* O157:H7 in undercooked ground beef patties was investigated. A five-strain mixture of *E. coli* O157:H7 was inoculated into ground beef (7.0 log CFU/g), followed by addition of TC (0, 0.15, and 0.3%). The meat was formed into patties and stored at 4 °C for 5 days or at –18 °C for 7 days. The patties were cooked to an internal temperature of 60 or 65 °C, and *E. coli* O157:H7 was enumerated. The numbers of *E. coli* O157:H7 did not decline during storage of patties. However, cooking of patties containing TC significantly reduced ($P < 0.05$) *E. coli* O157:H7 counts, by >5.0 log CFU/g, relative to the reduction in controls cooked to the same temperatures. The D -values at 60 and 65 °C of *E. coli* O157:H7 in TC-treated patties (1.85 and 0.08 min, respectively) were significantly lower ($P < 0.05$) than the corresponding D -values for the organism in control patties (2.70 and 0.29 min, respectively). TC-treated patties were more color stable and showed significantly lower lipid oxidation ($P < 0.05$) than control samples. TC enhanced the heat sensitivity of *E. coli* O157:H7 and could potentially be used as an antimicrobial for ensuring pathogen inactivation in undercooked patties. However detailed sensory studies will be necessary to determine the acceptability to consumers of TC in ground beef patties.

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1. Introduction

Escherichia coli O157:H7 is a major food-borne pathogen in the United States and cattle are the principal reservoir (Low et al., 2005). The majority of *E. coli* O157:H7 food-borne outbreaks have been associated with the consumption of undercooked ground beef patties (Rhee et al., 2003). The USDA has established a zero tolerance policy for *E. coli* O157:H7 in ground beef, and recommends that beef patties be cooked to an internal temperature of 71.1 °C (160 °F) to ensure inactivation of the pathogen. However, assured attainment of the recommended temperature may be difficult (D'Sa et al., 2000). Based on the degree of doneness, cooked patties can be classified as rare (60 °C/140 °F), medium-rare (65 °C/149 °F), medium (71 °C/160 °F) or well done (77 °C/170.6 °F) (Marksberry et al., 1993). A survey by the USDA on hamburger cooking practices revealed that 20% of the participants cooked patties rare or

medium rare (USDA, 1998b), which could result in the survival of *E. coli* O157:H7 present in the meat. Anyway, the use of thermometers to determine meat temperature during cooking of beef patties is limited (National Cattlemen's Beef Association, 1999) due to the inconvenience and uncertainty about the value of the procedure (USDA, 1998a). Instead most consumers determine the doneness of beef patties by observing the color and texture of the cooked meat (Rhee et al., 2003). However, premature browning in ground beef can lead to inadequate cooking by consumers, who can be misled by the color. Killinger et al. (2000) reported that the incidence of premature browning was 47% for patties formed from ground beef purchased from US retail stores. It would then be desirable to include an antimicrobial component in beef patties to ensure inactivation of *E. coli* O157:H7 in inadvertently undercooked meat.

The antimicrobial properties of several essential oils and their components have been demonstrated (Burt, 2004). Cinnamon is a spice widely used in many countries. Trans-cinnamaldehyde (TC), an aromatic aldehyde, is a major component of cinnamon extract. TC is classified as generally recognized as safe (GRAS) and is approved for use in foods. The antimicrobial activity of food-grade

* Corresponding author. Tel.: +1 (860) 486 0947.

E-mail address: kumar.venkitanarayanan@uconn.edu (K. Venkitanarayanan).

chemicals can be increased or potentiated when combined with heat (Venkitanarayanan et al., 1999). Juneja and Friedman (2008) reported that TC (0.5 and 1%) significantly increased the heat sensitivity of *E. coli* O157:H7 in ground beef. However the study did not investigate effect of TC on *E. coli* O157:H7 in refrigerated and frozen patties cooked to practically encountered undercooked temperatures (rare/medium rare). In addition, this study did not determine the effect of TC on meat color and lipid oxidation, two major attributes of meat acceptability. Therefore, the present study was undertaken to determine the antimicrobial efficacy of TC for inactivating *E. coli* O157:H7 in undercooked ground beef patties and its effect on the *D*-value of *E. coli* O157:H7 in ground beef cooked to 60 and 65 °C. In addition, the effect of TC on meat color and lipid oxidation during storage was assessed, to determine if incorporation of TC in the meat adversely affected its organoleptic qualities.

2. Materials and methods

2.1. Bacterial strains

A five-strain mixture of *E. coli* O157:H7 was used for the study. The strains were obtained from Dr. Michael P. Doyle at the Center for Food Safety, University of Georgia, Griffin, Georgia. Three strains were isolated from meat (E08, E10, E16), one strain (E06) was isolated from milk, and one strain (E22) was isolated from calf feces. All strains were induced for resistance to nalidixic acid (50 µg/ml) and rifampin (50 µg/ml). Each strain was cultured separately in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) supplemented with Nalidixic acid and Rifampin (Fischer Scientific, Pittsburgh, PA, USA) and incubated at 37 °C for 24 h. After three consecutive transfers, equal volumes of the cultures were combined and pelleted by centrifugation at 3600 × *g* for 12 min at 4 °C. The pellet was resuspended in 10 ml of phosphate buffered saline (PBS, pH 7.0) and the suspension was used as the inoculum. The numbers of *E. coli* O157:H7 in the individual cultures and the inoculum were determined by plating 100 µl of appropriate dilutions of each on tryptic soy agar (TSA; Difco, Becton Dickinson) plates containing nalidixic acid and rifampicin each at 50 µg/ml and incubating the plates at 37 °C for 24 h.

2.2. Preparation, inoculation and storage of beef patties

Fresh course ground beef (90% lean and 10% fat) was purchased locally. The beef was ground through a plate with 4.8 mm holes and the ground meat was separated into 135 50 g portions. The meat portions were randomly assigned to three treatments, namely control (0%), 0.15% and 0.3% TC (Sigma-Aldrich Chemical Co., St. Louis, USA). Each ground beef portion was inoculated with the five strain mixture of *E. coli* O157:H7 to an inoculation level of approximately 7.0 log CFU/g, followed by the addition of appropriate amounts of TC (0, 0.15 and 0.3%). The contents were mixed thoroughly and formed into patties (50 g ea, 60 × 15 mm). The patties were placed on foam trays (3 patties ea), overwrapped with oxygen-permeable fresh meat film (9.04 cc/m² at 73.4 °F over 24 h, E-Z Wrap Crystal Clear PVC Wrapping Film, Koch Supplies, Kansas City, MO, USA) and stored at 4 °C for 5 days or at –18 °C for 7 days.

2.3. Cooking

On each sampling day (0, 1, 3, 5 and 7), the inoculated beef patties (at 4 or –18 °C) were cooked individually in a double-sided George Foreman Lean Mean Grilling Machine (Salton Inc., Columbia, MO, USA) until the desired internal temperature (60 °C or 65 °C) was reached. Internal temperatures were continuously monitored using an Acutuff Model 34 Atkins 2 mm probe meat

thermometer (Koch Supplies, Kansas City, MO, USA) placed approximately in the geometric center of each patty. Patties were turned three times during cooking with the first turn at 30 °C, the second turn at 40 °C and the third turn at 50 °C, to provide uniform heat distribution on both sides of the patty during cooking.

2.4. Enumeration of surviving *E. coli* O157:H7

After cooking, patties were removed from the grill, immediately submerged individually in 100 ml sterile ice-cold PBS, and homogenized in a stomacher for 1 min. A volume of 100 µl of appropriately diluted meat homogenate was surface-plated on duplicate TSA plates supplemented with nalidixic acid and rifampicin each at 50 µg/ml. The bacterial colonies were enumerated after incubation of plates at 37 °C for 24 h. Each treatment also had uncooked controls that were stored at 4 or –18 °C for 5 or 7 days, respectively. At each sampling day these patties were removed from storage, placed in stomacher bags containing PBS for 15 min before stomaching and the surviving *E. coli* O157:H7 populations were determined without subjecting the patties to cooking. When *E. coli* O157:H7 was not detected by direct plating, samples were tested for surviving cells by enrichment for 24 h at 37 °C in 100 ml of TSB, followed by streaking on TSA with antibiotics. Representative colonies on TSA plates containing antibiotics were confirmed as *E. coli* O157:H7 using the *E. coli* O157 latex agglutination kit (Oxoid Ltd., Lenexa, KS, USA).

2.5. Estimation of *D*-values

The *D*-values of *E. coli* O157:H7 in control or TC-treated patties were determined according to Juneja et al. (1997). Ground beef patties inoculated with the 5-strain mixture of *E. coli* O157:H7 (7.0 log CFU/g) in plastic bags were mixed with a stomacher to ensure even distribution of the pathogen in meat. Thereafter, the meat samples in bags were sealed and then fully submerged in a temperature controlled water bath (model RTE-17, Digital Plus, NESLAB instruments, Inc., Newington, NH, USA). The temperature of the water bath was programmed to increase in a linear fashion to 60 or 65 °C. The internal temperature of the patties was continuously monitored by two copper-constantan thermocouples inserted at the center of two uninoculated bags prior to heat sealing. The thermocouple readings were measured and recorded using a Keithly-Metrabyte data logger Model DDL 4100 (Tauton, MA, USA) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the internal temperature. Come-up times were included as part of the total heating time. Three bags for each replicate were removed at designated time intervals with a sampling frequency of 1 min at 60 °C and 0.5 min at 65 °C. After removal, bags were immediately plunged into an ice-water bath and enumeration of surviving *E. coli* O157:H7 was performed as described above. Negative controls with bags containing uninoculated patties were included. The *D*-values were determined from the straight-line portion of the survival curves by plotting the log number of survivors against time for heating at each temperature (Excel Software, Microsoft Corporation, Redmond, WA, USA). To calculate the *D*-values by linear regression, only those survival curves with more than five values in the straight line portion of the plots descending more than 5 log cycles were used.

2.6. Meat color and lipid oxidation

The effect of TC on meat color was determined by measuring the reflectance spectra and *a** value of patties using a HunterLab Miniscan XE Plus spectrophotometer (HunterLab Associates, Reston,

VA, USA) with a 2.54 cm diameter aperture, illuminant A, and 10° standard observer (American Meat Science Association, 1991). At each time of color determination, reflectance spectra (from 400 to 700 nm, in 10 nm increments) and a^* values were measured at four random locations on each patty. The ratio of reflectance at 630 nm to 580 nm (R630/580) was used to determine the relative amounts of brown pigments as an indirect estimate of discoloration (Suman et al., 2008). The R630/580 decreases as discoloration increases. The effect of TC on lipid oxidation in control and treated ground beef patties (0.15 and 0.3% TC) during storage was determined using the thiobarbituric acid assay as previously reported (Yin et al., 1993).

2.7. Statistical analysis

Three patties per treatment per cooking temperature were used in this study. Each patty served as an experimental unit and the entire study was replicated three times. The design was a completely randomized $3 \times 2 \times 5$ factorial. Factors included 3 treatments (0%, 0.15% and 0.3% TC), 2 temperatures (60 °C and 65 °C), and 5 storage days (0, 1, 3, 5, and 7). Data were analyzed using the Proc Mixed subroutine of the Statistical Analysis Software (SAS Institute, 1987). Differences among means were detected at the 5% level using the Fisher's least significant difference (LSD) test. For the D -value estimation, duplicate samples were included and the study was repeated two times. Bonferroni mean separation test was used to determine significant differences ($P < 0.05$) among means for each D -value. The coefficient of multiple determination, defined as the square of the correlation coefficient, was used to estimate the proportion of variability in the response as explained by the regression of the logistic model used to analyze the inactivation kinetics of the bacteria.

3. Results

The average initial population of *E. coli* O157:H7 in the inoculated ground beef samples was 7.0 log CFU/g. The presence of TC at 0.15 or 0.3% did not result in any significant reduction ($P > 0.05$) in the pathogen load in the uncooked patties. Approximately 7.0 log CFU/g of *E. coli* O157:H7 still survived in the refrigerated and frozen patties at the end of the study (data not shown).

The antimicrobial effect of TC on *E. coli* O157:H7 in patties cooked to an internal temperature of 60 °C is depicted in Fig. 1A. Patties containing TC cooked to an internal temperature of 60 °C had greater than 5 log CFU/g significant reduction ($P < 0.05$) in *E. coli* O157:H7 counts. The control patties without TC that were cooked to 60 °C did not show a significant reduction ($P > 0.05$) in *E. coli* O157:H7 populations. A similar trend was observed in TC-treated and control patties stored at –18 °C for 7 days and cooked to 60 °C (data not shown).

At the internal cooking temperature of 65 °C, the control patties demonstrated a maximum decline of 3.0 log CFU/g in *E. coli* O157:H7 counts (Fig. 1B). However, greater than 5.0 log CFU/g reductions in *E. coli* O157:H7 counts were consistently observed in TC-treated, refrigerated (Fig. 1B) and frozen patties (data not shown). *E. coli* O157:H7 was completely inactivated (negative by enrichment) in patties containing 0.3% TC following cooking to 65 °C and 7 of refrigerated and frozen storage, respectively.

At 60 and 65 °C, the D -values of *E. coli* O157:H7 in TC containing patties were 1.85 and 0.08 min, respectively. The corresponding values for control patties were 2.70 and 0.29 min. TC had a significant effect ($P < 0.05$) on the color of raw beef patties. The a^* values of control and TC treated patties were 26.1 and 27.5 on day 0 and 14.1 and 19.7 on day 5 of storage, respectively. With respect to surface color, TC-treated patties had greater redness during storage compared with control samples. However, there was no difference in the a^* values between 0.15% and 0.30% TC-treated patties

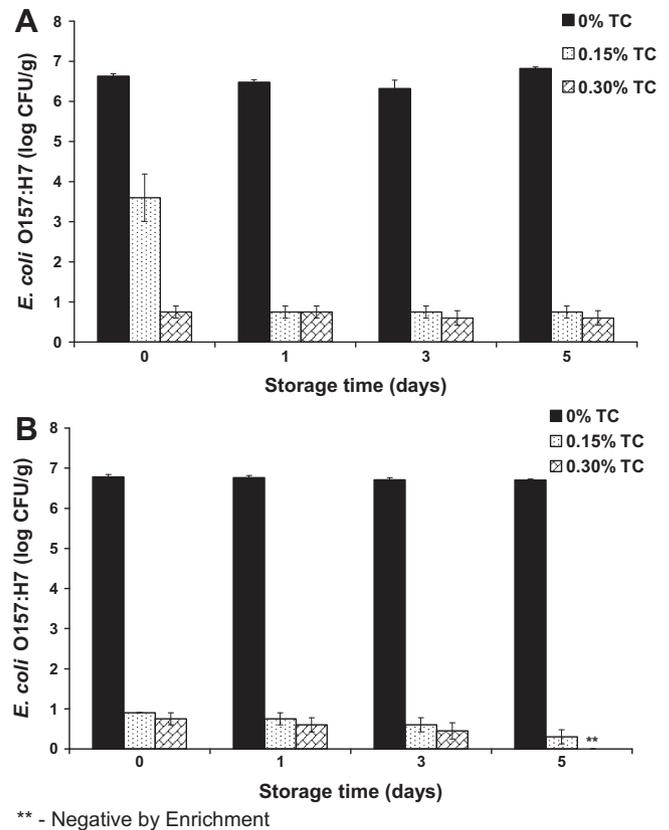


Fig. 1. A. Effect of trans-cinnamaldehyde on *E. coli* O157:H7 in ground beef patties stored at 4 °C for 5 days and cooked to an internal temperature of 60 °C. Data represent the mean of three determinations and three experiment repetitions. Bars represent standard error of the mean. B. Effect of trans-cinnamaldehyde on *E. coli* O157:H7 in ground beef patties stored at 4 °C for 5 days and cooked to an internal temperature of 65 °C. Data represent the mean of three determinations and three experiment repetitions. Bars represent standard error of the mean.

(data not shown). Increased redness values (a^*) in TC-treated patties were also indicated by a greater R630/580 nm ratios (less brownish discoloration) compared to control samples (data not shown). The progression of lipid oxidation following addition of TC to ground beef patties is shown in Fig. 2. TC-treated patties consistently had significantly lower ($P < 0.05$) TBARS values than the control patties throughout storage.

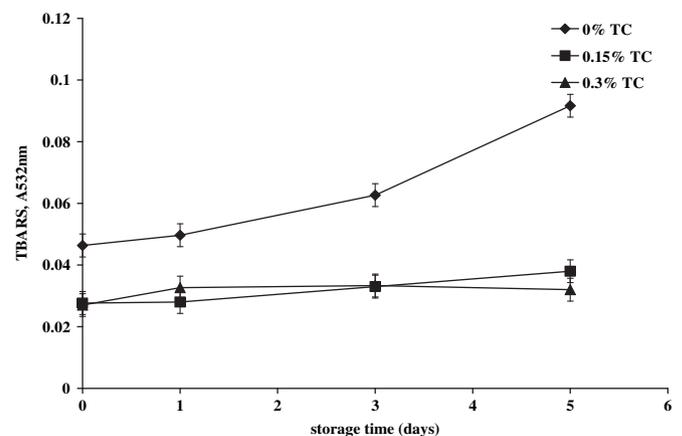


Fig. 2. Lipid oxidation (TBARS) in trans-cinnamaldehyde treated ground beef patties. Data represent the mean of three determinations and three experiment repetitions. Bars represent standard error of the mean.

4. Discussion

Although TC exerted little antimicrobial activity on *E. coli* O157:H7 in patties stored at 4 or -18°C , the combination of TC and cooking brought about reductions in counts of *E. coli* O157:H7 that were substantially greater than the reductions in patties without TC cooked to the same temperatures. TC decreased the heat resistance of *E. coli* O157:H7 in beef patties, as evident from the smaller *D*-values for the pathogen in patties with TC as compared to patties without TC. Similar results were reported by Juneja and Friedman (2008), for *E. coli* O157:H7 in ground beef patties cooked to temperatures between 55 and 62.5°C . Knight and Mckellar (2007), likewise observed that addition of cinnamon oil decreased the heat resistance of *E. coli* O157:H7 in apple cider.

Numerous studies have reported a relationship between lipid oxidation and oxymyoglobin oxidation, where byproducts of lipid oxidation can trigger meat discolorization (Chan et al., 1997; Monahan et al., 2005; Yin and Faustman, 1993). Thus the improved color of TC-treated patties could be attributed to the reduced lipid oxidation in meat brought about by the antioxidant effect of TC.

The antimicrobial activity of lipid-soluble TC is attributed to its effects on the bacterial cell membrane which include inhibition of glucose uptake (Gill and Holley, 2006). Shibasaki and Kato (1978) reported that heating makes bacterial plasma membrane more fluid, thereby increasing the antimicrobial activity of lipid-soluble small molecules.

Results of this study indicate that TC enhanced the inactivation of *E. coli* O157:H7 in undercooked ground beef patties. In addition, TC-treated patties had greater color stability and lower TBARS values than untreated patties. Therefore, trans-cinnamaldehyde could potentially be used as an antimicrobial additive for ensuring the inactivation of *E. coli* O157:H7 in undercooked ground beef patties. However, detailed sensory studies will be necessary to determine the consumer acceptability of patties that contain TC.

Acknowledgement

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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