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Application of cranberry concentrate (*Vaccinium macrocarpon*) to control *Escherichia coli* O157:H7 in ground beef and its antimicrobial mechanism related to the downregulated *slp*, *hdeA* and *cfa*

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ABSTRACT

The possible use of cranberry concentrate (CC) as a natural food preservative was studied by examining its antimicrobial effect on the growth of *Escherichia coli* O157:H7 inoculated in ground beef, its organoleptical effect on beef patties, and its antimicrobial mechanism on the gene regulation level.
Inoculated ground beef was added with CC and stored at 4 °C for 5 days. Bacteria were detected on day 0, 1, 3, and 5. Cranberry concentrate (2.5%, 5%, and 7.5% w/w) reduced total aerobic bacteria 1.5 log, 2.1 log, and 2.7 log CFU/g and *E. coli* O157:H7 0.4 log, 0.7 log, and 2.4 log CFU/g, respectively, when compared to the control on day 5.
Fifty panelists evaluated the burgers supplemented with CC. No differences in appearance, flavor, and taste were found among burgers with 0%, 2.5%, and 5% CC.
The expression of *E. coli* O157:H7 cyclopropane fatty acyl phospholipid synthase (*cfa*), hypothetical protein (*hdeA*), outer membrane porin protein C (*ompC*), hyperosmotically inducible periplasmic protein (*osmY*), and outer membrane protein induced after carbon starvation (*slp*) genes with or without CC (2.5% v/v) treatment was investigated by quantitative real-time PCR. Compared to the control, *slp*, *hdeA*, and *cfa* were markedly downregulated, *ompC* was slightly downregulated, while *osmY* was slightly affected.

1. Introduction

Burgers are one of the most popular meat products in the United States. They are consumed extensively in school, restaurants, and individual households in U.S. However, this product was linked to several outbreaks caused by *Escherichia coli* O157:H7 contamination. From 1992 to 1993, a multi-state *E. coli* O157:H7 outbreak due to consumption of hamburgers from one restaurant chain occurred, resulting in more than 500 laboratory-confirmed infections and four associated deaths (U.S. FDA, 2006). In 2002, approximately 19 million pounds of raw ground beef was recalled because of *E. coli* O157:H7 contamination (U.S. CDC, 2002). *E. coli* O157:H7 can survive in healthy cow guts and may contaminate beef when cows are slaughtered. Frenzen et al. (2005) estimated that the annual cost of illness due to *E. coli* O157 from all sources of infection was $405 million, including $370 million for premature deaths, $30 million for medical care, and $5 million for lost productivity. It is important to ensure product safety to prevent possible outbreaks.

American cranberries (*Vaccinium macrocarpon*) contain many bioactive compounds that have antioxidant, anti-mutagenic, anti-hypercholesterolemic and other beneficial health properties such as preventing urinary tract infections (Cunningham et al., 2004; Vattem et al., 2005; Neto, 2007). Phenolic phytochemicals in the cranberries are now known to have potential for inhibition of development and progression of cancer and cardiovascular diseases (Reed, 2002; Vattem et al., 2005). We have previously reported the antibacterial activity of American cranberry (*V. macrocarpon*) concentrate against commonly occurring foodborne pathogens *in vitro* (Wu et al., 2008). The potential application of cranberry concentrate at low concentrations in ground beef as an additional hurdle to prevent possible *E. coli* O157:H7 contamination has not been previously reported. Consumers today tend to choose food products that are natural, safe, and with multi-health benefits. Burgers with cranberry concentrate may be a product that can meet consumers' requirements. However, a sensory evaluation study is required to know if consumers accept the organoleptical properties of this product.

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Studies that aim to understand the possible antimicrobial mechanism by natural plant products with molecular technology are rare. According to our previous study by transmission electron microscopy, cranberry concentrate damaged bacterial cell walls and membranes (Wu et al., 2008). As an initial effort to investigate the antimicrobial mechanism of cranberry extract on the molecular level, an analysis of the expression of genes that are expressed as outer membrane protein (*ompC* and *slp*), and periplasmic membrane protein (*osmY*) and cell wall phospholipid synthesis (*cfa*), cell envelope associated protein (*hdeA*) by quantitative real-time PCR was performed in the present study.

The objectives of this study were to (1) investigate antimicrobial effects of cranberry concentrate (*V. macrocarpon*) on *E. coli* O157:H7 in ground beef, (2) conduct a sensory evaluation of burgers supplemented with cranberry concentrate, and (3) study the antimicrobial mechanism of cranberry concentrate on *E. coli* O157:H7 on the gene regulation level.

2. Methods

2.1. Materials

Fresh, raw, 90% lean ground beef was purchased from a local grocery store in Old Town, Maine, USA. Cranberry concentrate was obtained from Ocean Spray (Rapids, WI, USA) and stored at 4 °C. Tryptic soy agar (TSA; Difco, Becton Dickinson and Co., Sparks, MD, USA) was used for enumerating total viable aerobic bacteria in the ground beef. MacConkey sorbitol agar (MSA; Difco) plates with a cefixime tellurite supplement (Dynal Inc., Lake Success, NY, USA) were overlaid with approximately 7 ml TSA agar twice to make thin agar layer (TAL) plates. TAL plates were used to enumerate both injured and uninjured *E. coli* O157:H7 since sublethally injured cells if any might not be able to recover on the selective media (Wu and Fung, 2003).

2.2. Microbiological analysis

Two strains of *E. coli* O157:H7 (ATCC 35150 and 43888) were used in the study. Cultures were maintained on TSA slant at 4 °C. Before the experiment, pathogen identity was confirmed by Gram stain, biochemical tests, and RIM latex agglutination test (Remel Inc., Lenexa, KS, USA). *E. coli* O157:H7 strains were inoculated to 10 ml brain heart infusion (BHI, Difco, Sparks, MD, USA) broth individually at 37 °C for 18–24 h. They were then cultured again in 100 ml BHI broth individually and allowed to grow for another 18–24 h. The broth was centrifuged at 15,300 × *g* for 20 min at 4 °C to obtain the pellet. The supernatant was discarded, and the pellet was washed with 0.1% peptone water (Bacto, Sparks, MD, USA) and suspended with 100 ml of 0.1% peptone water (approximately 9 log CFU/ml). Two strains were mixed, diluted once (approximately 8 log CFU/ml), and used to inoculate ground beef samples. Four hundred grams of ground beef was dispensed into four 100 g samples in sterile filter stomacher bags. One ml of the culture was inoculated into each of these samples to get approximately 6 log CFU/g. The major bacteria in the ground beef were the inoculated *E. coli* O157:H7. The ground beef was then mixed thoroughly by hand and held in a laminar hood for 1 h to make cells disperse in the samples. Cranberry concentrates (0%, 2.5%, 5%, and 7.5% w/w) were then mixed with the inoculated samples. Each treatment was divided into 25 g portions in four aseptic filter stomacher bags and kept at 4 °C.

Microbiological analysis was done on day 0, 1, 3, and 5. On each day the 25 g samples were mixed with 100 ml sterile 0.1% peptone water and stomached for 2 min with a stomacher (Lab Blender 400, Tekmar, Cincinnati, OH, USA). Serial dilutions were prepared and 100 μl of appropriate diluents were surface plated in duplicate onto TSA and TAL plates. Fresh ground beef without inoculation was also analyzed for the background microbiota level using the same procedure. All plates were incubated at 37 °C for 24–48 h and presumptive colonies were counted. Randomly selected presumptive colonies were confirmed by Gram stain test and commercial diagnostic kits.

2.3. pH measurement

Fresh ground beef with cranberry concentrate (0%, 2.5%, 5%, and 7.5% w/w) was checked for pH. Briefly, a 10-g portion of sample with 90 ml distilled water in a filter stomach bag was blended by a stomacher machine for 2 min. The pH of the suspension was then measured with a pH meter (Fisher Scientific, Pittsburgh, PA, USA).

2.4. Sensory evaluation

Ground beef was mixed with cranberry concentrate (0%, 2.5%, 5%, 7.5% w/w) and then ground in a meat grinder (Hobart Manufacturing Co., Troy, OH). Patties (100 g) were formed using a burger mold (Univex, Salem, NH, USA) and cooked on a commercial broiler (EmberGlo, Chicago, IL, USA). The approximate cooking time for 0%, 2.5%, 5%, and 7.5% patties to reach the internal temperature of 160 °F was 13 min, 11 min, 10 min, and 9 min, respectively. A thermometer was used to monitor the internal temperature.

The test was approved by the University of Maine College of Natural Sciences, Forestry and Agriculture Committee for the Protection of Human Subjects. Fifty panelists of at least 18 years old were recruited by fliers and the University's First Class electronic communication platform. People allergic to either beef or cranberries were not allowed to participate. Panelists were asked to make appointments at 20 min intervals. Patties were cooked according to a schedule of the appointments. Cooked burgers were cut into four pieces and kept at about 55 °C in a food warmer. Each sample was served in a small china vessel which was labeled with a three digit numerical code. The serving order of all these four samples was randomly set up by computer software Sensory SIMS 2000 information management system (Sensory Computer Systems, Morristown, NJ, USA). Evaluations were conducted in the sensory evaluation laboratory, which is equipped with 12 individual workstations. Panelists answered the questionnaire with computers linked to a server housing the Sensory SIMS 2000 information management system. Panelists were asked to rate the appearance, flavor, texture and overall acceptability of each of the four samples using a nine-point hedonic test (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) (Peryam and Pilgrim, 1957). Each panelist received all four samples simultaneously on a single tray. Panelists were asked to drink water before evaluating each sample.

2.5. Cell growth and treatment for real-time PCR study

E. coli O157:H7 cells were grown for 18 h and diluted with 0.1% peptone water to approximately 7 log CFU/ml. One hundred milliliters of BHI was inoculated with 0.1 ml of bacterial suspension to get approximately 4 log CFU/ml. The cells were incubated in a 37 °C water bath and shaken at 150 rpm. The optical density (OD) was monitored at 600 nm by a spectrometer (SmartSpec Plus Spectrophotometer, Bio-Rad, Hercules, CA). At OD = 0.4, the cranberry concentrate (2.5% v/v) was added to one bottle as a treatment. Sterile distilled water (2.5% v/v) was added to another bottle as a control. Viable cells counts were enumerated by serial dilutions and plating on petrifilm (3 M, St. Paul, MN) in a time-course study (0 min, 30 min, and 60 min).