



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.

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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; Vatter 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; Vatter 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).

2.6. Total RNA extraction

Bacterial suspension was filtered through a No. 1 filter paper. Aliquots (1 ml, 0.5 ml, and 0.25 ml) of bacterial suspension were sampled from the control experiment at 0 min, 30 min, and 60 min. Similarly, 1.5 ml aliquots of bacterial suspension were sampled from the treatment experiments at both 30 min and 60 min. Cell suspension samples were transferred into 1.5 ml centrifuge tubes and centrifuged at $5000 \times g$ at 4°C for 5 min. Total RNA was extracted by the RNeasy[®] Mini kit (QIAGEN, Valencia, CA, USA) according to manufacturer's instructions. Briefly, the bacterial pellet was resuspended in 100 μl of lysozyme-containing TE buffer by vortexing and incubated at room temperature (25°C) for 5 min. Three hundred and fifty microliters of buffer RLT was added to the sample and mixed thoroughly by vortexing. The sample was centrifuged at $8000 \times g$ for 2 min. RNA was precipitated with 250 μl ethanol (100%), purified with the RNeasy mini column (QIAGEN), washed with RPE buffer and finally eluted with 30 μl of RNase-free water. RNA quality was assessed by gel electrophoresis (1% agarose plus EtBr). RNA yield was determined by Quanti-iT[™] RNA assay kit (Molecular Probes, Eugene, OR, USA) using the Qubit[™] fluorometer (Invitrogen, Carlsbad, CA, USA). RNA samples were purified further with RQ1 DNase (Promega, Madison, WI, USA).

2.7. Quantitative real-time PCR (qPCR) analysis

RNA was reverse transcribed by the iScript[™] cDNA synthesis kit (Bio-Rad) following the manufacturer's instructions. Briefly, 4 μl of $5 \times$ iScript reaction cocktail was mixed with 1 μl iScript reverse transcriptase and 1 μg RNA sample at a total volume of 20 μl . The synthesis reaction was performed in the MyCycler[™] PCR machine (Bio-Rad) at 25°C for 5 min, 42°C for 30 min, and 85°C for 5 min. The cDNA samples were stored at -20°C .

Genes associated with cell wall and membrane biogenesis [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] were selected for qPCR expression analysis. The reference gene was 16s ribosomal RNA (*rrsB*). Gene-specific primers were designed based on the genomic sequences of NC 002655 available at the National Center for Biotechnology Information (NCBI, 2006). Sequences of the gene-specific primers are listed in Table 1.

Specificity and efficiency of each gene-specific primer were determined by amplifying a dilution series of cDNA using iQ[™] multicolor real-time PCR Detection System (Bio-Rad, Hercules, CA). Amplification of the specific target cDNA was performed in a reaction cocktail comprised of the following: 5 μl cDNA (2.5 ng), 1 μl forward primer (2 μM), 1 μl reverse primer (2 μM), 10 μl Absolute[™] QPCR SYBR Green Fluorescein Mixes (ABgene, Surrey, UK), and 3 μl nuclease free water. Amplification was performed with an initial denaturation at 95°C for 15 min, followed by 50 cycles of amplification with the following parameters: step-denaturation at 95°C

for 15 s, primer annealing and synthesis 55°C for 30 s with a single fluorescence measurement, extension step (72°C for 5 min), denature step (95°C for 1 min), then annealing (55°C for 1 min), and melting curve program (55 – 95°C with a temperature increasing of $0.5^\circ\text{C}/\text{cycle}$). Transcript abundance was expressed as relative expression values, i.e. ratio = $(E_{\text{target}})^{\Delta\text{CP}(\text{control} - \text{sample})} / (E_{\text{ref}})^{\Delta\text{CP}(\text{control} - \text{sample})}$ (Pfaffl, 2001; Pfaffl et al., 2002).

2.8. Statistics

The experiment utilized a randomized complete block design with time and concentration as factors. Experiments were individually repeated three times. The number of cells was transformed into log CFU/g before analysis by Proc GLM (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as $P < 0.05$. Differences among treatments were examined for the level of significance by least significant difference (LSD).

For sensory evaluation study, data were analyzed by SYSTAT, version 11.0, using the GLM procure with the Sensory SIMS 2000 information management system. Means were compared by the Duncan's multiple range test with a significance level of 0.05.

3. Results

3.1. Antimicrobial effect of cranberry concentrate in ground beef

The mean level of background microbiota in fresh control ground beef (without pathogen inoculation) was 3.5 log CFU/g. This level was typical of fresh ground beef (Tamplin, 2002). The pH of ground beef samples was approximately 5.6. The pH of the cranberry concentrate itself was 2.2. The pH of ground beef with 2.5%, 5%, and 7.5% (w/w) cranberry concentrate was 4.9, 4.7, and 4.6, respectively. The population number (total aerobic bacteria) recovered from TSA plates was mainly inoculated *E. coli* O157:H7 and natural background microbiota. Total aerobic bacteria grew approximately 1.5 log CFU/g in the control ground beef samples from day 0 to day 5, while they remained stable in the samples supplemented with 2.5% and 5% of cranberry concentrate and had approximately 1 log CFU/g reduction in the 7.5% treatment. Compared to the control on day 5, ground beef samples supplemented with cranberry concentrate (2.5%, 5%, and 7.5%) resulted in differences of 1.5 log, 1.8 log, and 2.7 log CFU/g, respectively ($P < 0.05$) (Fig. 1A). Time and concentration had a synergistic effect on the reduction of total aerobic bacteria ($P < 0.05$).

E. coli O157:H7 in control ground beef samples did not grow during the storage period. Cranberry concentrate (2.5%, 5%, and 7.5%) reduced *E. coli* O157:H7 population by 0.4 log, 0.7 log, and 2.4 log CFU/g, respectively, by day 5 ($P < 0.05$) (Fig. 1B). The inhibition effect of cranberry concentrate increased with time and concentration. Time and concentration had a synergistic effect on the reduction of *E. coli* O157:H7 ($P < 0.05$).

Table 1
Gene primer sequences used in the study.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Gene product ^b
<i>rrsB</i> ^a	TGCAAGTCGAACGGTAACAG	AGTTATCCCCCTCCATCAGG	16s Ribosomal protein
<i>osmY</i>	ACGTTGCGACGCTAAAGAA	CATGACGGGAAGGGACG T	Hyperosmotically induced periplasmic protein
<i>hdeA</i>	GCTTCTTCTGCCAGTTGTGAGCAA	AGCCAGGAAATCTTACAGGTCCA	Acid induced periplasmic chaperone
<i>ompC</i>	ACTGGTGGTCTGAAATACGACGC	GAAGTTCTGTGCTTTGTTCGCCCA	Acid induced outer membrane protein
<i>slp</i>	AACCTGTGGGATTACGGCTATGGT	AGGTGTTACTGACTCACCGCATT	Outer membrane lipoprotein induced by stationary phase and starvation
<i>cfa</i>	TTCAAAGACACGCTGCGTATTGCC	ATCAAGCATGCGGCTGAACAAGTC	Cyclopropane fatty acyl phospholipid synthase

^a *rrsB* primer sequence was from a published paper (Leverton and Kaper, 2005). Other primers were designed in this study.

^b From NCBI.

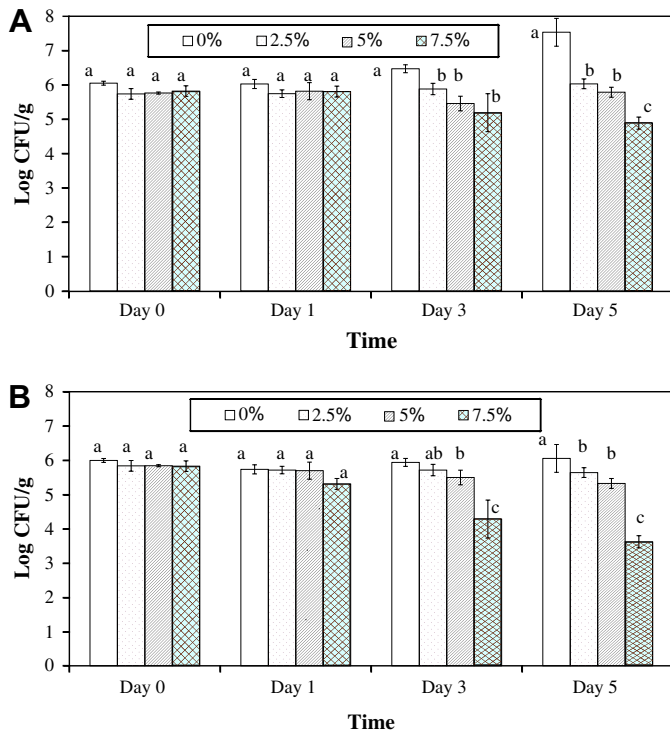


Fig. 1. Antimicrobial effects of cranberry concentrate (0%, 2.5%, 5%, and 7.5% w/w) on (A) total aerobic bacteria and (B) *Escherichia coli* O157:H7 in ground beef at 4 °C during 5-day storage. Bars with different letters in the same sampling time indicate significant differences ($P < 0.05$). Data were presented as average \pm standard deviation (error bar), $n = 3$.

3.2. Acceptability

Fifty persons were enrolled for the sensory evaluation of the burger products. Most panelists were university faculty and students. There was no significant difference for appearance and texture between the four samples. No difference in flavor was found among 0%, 2.5% and 5% cranberry concentrate burgers. Flavor and overall acceptability ratings of burgers with 7.5% cranberry concentrate were lower than the control burgers ($P < 0.05$). Burgers with 7.5% cranberry concentrate were neither liked nor disliked by the panelists probably because the flavor was a little sour. For overall acceptability, burgers with 2.5% cranberry concentrate had the highest score among all four kinds of burgers. Even burgers with 5% cranberry concentrate were not significantly different from control burgers (0%) for overall acceptability (Table 2).

3.3. Gene expression analysis by qPCR

The specificity of primers (Table 1) was checked by agarose gel electrophoresis and melting curve analysis. All primers showed a single amplification product with an expected length in the agarose gel and a single peak in the melting curve graph. The sizes of amplified *cfa*, *hdeA*, *ompC*, *osmY*, *rrsB*, and *slp* cDNA fragments were 123 bp, 97 bp, 114 bp, 111 bp, 104 bp, and 90 bp, respectively (Fig. 2). In addition, the melting curve analysis by real-time PCR also showed a specific melting peak for each of these five genes. The melting temperature of *cfa*, *hdeA*, *ompC*, *osmY*, *rrsB*, and *slp* was 83.5 °C, 82 °C, 83.5 °C, 85 °C, 85 °C, and 83 °C, respectively. The efficiency for *cfa*, *hdeA*, *ompC*, *osmY*, *rrsB*, and *slp* was 1.82, 1.86, 1.91, 1.85, and 1.88, respectively.

Relative expression of the genes surveyed was corrected for efficiency (Fig. 3). Gene expression levels in *E. coli* O157:H7 were altered by cranberry concentrate (2.5%). Relative to the control

Table 2

Acceptability of cooked burgers with different levels of cranberry concentrate.

Attribute	0%	2.5%	5%	7.5%
Appearance	6.68 ^a	7.1	6.78	6.7
Flavor	6.5	6.6	6.08	5.06
Texture	6.4	6.46	6.44	5.9
Overall	6.4	6.78	6.1	5.42

^a Means followed by different letters in the same row among the four treatments (0%, 2.5%, 5%, 7.5% w/w cranberry concentrate) are statistically different (Duncan's multiple range test, $P < 0.05$). Nine-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely) was used.

samples, pronounced reduction in transcript abundance was observed for *slp*, *hdeA*, and *cfa* in response to treatment with cranberry extract. Reduction in transcript abundance was also observed for *ompC* relative to control levels but not as pronounced as the downregulation of *slp*, *hdeA* and *cfa* expression. In contrast, *osmY* transcript level was not significantly affected by the cranberry treatment. Expression of *slp* was upregulated 3.6- and 3.4-fold, respectively, at 30 min and 60 min in the control sample, while it was expressed with only 0.6-fold at 30 min and 0.4-fold at 60 min in the supplemented sample. Expression of *hdeA* was upregulated significantly with 8.5-fold at 30 min and 5.4-fold at 60 min in the control samples. In the samples supplemented with cranberry concentrate, *hdeA* is suppressed and silent. Gene of *cfa* expressed 1.5- and 2.0-fold in the control samples at 30 min and 60 min, respectively. However, the expression levels were only 0.6- and 0.9-fold in the samples treated with cranberry concentrate at 30 min and 60 min. For *ompC*, the expression levels in the treatment samples were slightly lower in the control samples. *OsmY* was expressed in an almost similar quantity in both control and treatment samples.

4. Discussion

Our previous studies showed that cranberry concentrate can inhibit the growth of major foodborne pathogens *in vitro* (Wu et al., 2008). In the present study, we have demonstrated cranberry concentrate reduces *E. coli* O157:H7 inoculated in ground beef after 5-day storage ($P < 0.05$). Burgers supplemented with cranberry concentrate (2.5%, 5%, and 7.5% w/w) are accepted by the sensory evaluation panelists.

According to our previous study by transmission electron microscopy, exposure of bacterial cells to cranberry concentrate resulted in morphological damage such as loss of the structural integrity of the wall, membrane and intracellular matrix (Wu et al., 2008). To investigate the molecular basis of this response, the

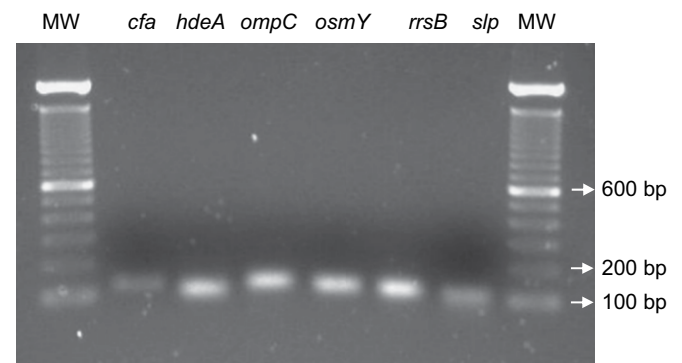


Fig. 2. Specificity of primers confirmed by the electrophoresis analysis on 1.5% agarose gel.

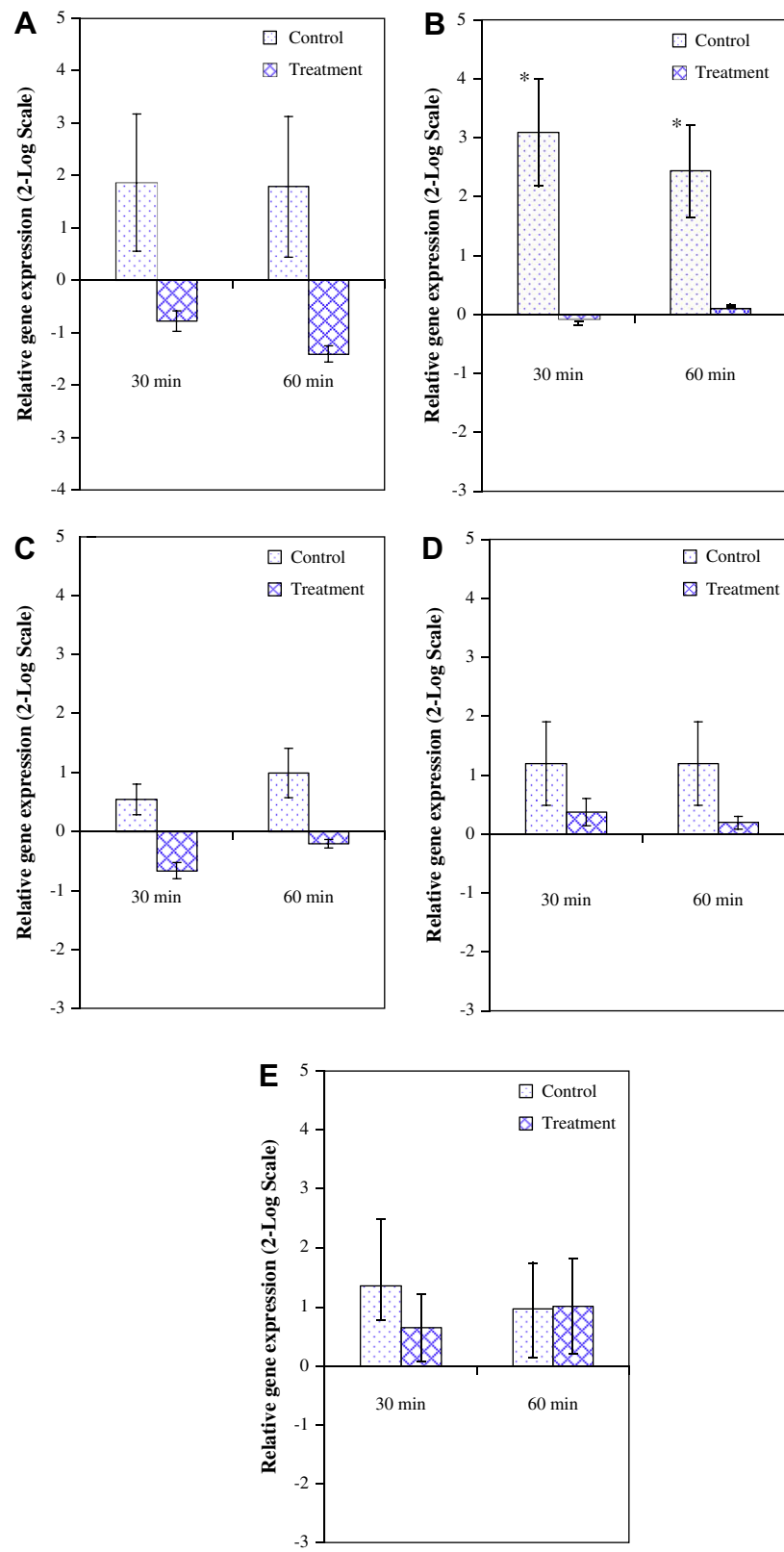


Fig. 3. Relative expression of (A) *slp*, (B) *hdeA*, (C) *cfa*, (D) *ompC*, and (E) *osmY* genes. Transcript abundance at different time points normalized with respect to 0 min control with *rrsB* gene as reference. C: control. T: cranberry concentrate treatment 2.5% v/v. Bars, ± 1 standard error. * Indicates significant differences compared to 0 min control mRNA level, $P < 0.05$ ($n = 3$).

expression of genes related to cell wall and membranes biogenesis was investigated. In the control sample, *E. coli* O157:H7 grew steadily (Fig. 4). This may require a high quantity of fatty acid available for cell membrane synthesis. The gene of *cfa* (cyclopropane fatty acyl phospholipid synthase) could be expressed correspondingly to catalyze the synthesis of fatty acid depending on the requirement. In the treatment sample, *cfa* was downregulated. Correspondingly, *E. coli* O157:H7 growth was inhibited and maintained a stagnant level in the treatment samples (Fig. 4).

hdeA is a gene encoding a single domain α helical hns-dependent expression protein A and exists in the periplasmic space. *hdeA* is known as a chaperone that protects acid effects to the *E. coli* cells (Masuda and Church, 2003). This gene was highly expressed in the control cells but significantly downregulated after 30 min and 60 min treatment with the cranberry extract ($P < 0.05$). The pH of treatment sample was 4.6, which did not cause induction of the expression of *hdeA* in this experiment. The mechanism of downregulated *hdeA* expression of *E. coli* which was treated with cranberry concentrate needs to be explored. Further analysis of a larger set of genes involved in acid protection is required to view a clearer picture of how this mechanism is affected by cranberry extract treatment.

ompC is a gene encoding an outer membrane protein. Like *hdeA*, this gene was highly expressed under control condition but was downregulated by cranberry extract. *slp* is another outer membrane protein which can be induced by carbon starvation during the stationary phase. Expression of this gene was decreased by addition of cranberry extract. *osmY* encodes an osmotically induced periplasmic protein. The lack of detectable expression of this gene in both the control and treated cells indicates that cranberry extract did not cause significant alteration in the osmotic condition of the cells to affect the expression of the *osmY* gene.

The observed downregulation of genes required for growth-related physiological processes is in support of the hypothesis that treatment with cranberry extract has a negative impact on cell growth and viability. Thus, the gene expression data presented here provide an additional layer of information at the molecular level that further substantiates the growth-inhibitory effects of cranberry extract to *E. coli* O157:H7 as indicated by previous analysis.

These results suggest that the effects of cranberry concentrate on the growth inhibition of foodborne pathogens may be at the molecular level rather than only physical damage. The results support our finding that the growth and viability of pathogens were inhibited (Wu et al., 2008). We hypothesize that cranberry concentrate may interact with the cell outer membrane first. By disruption of the outer membrane, it then enters inside the cell and

inhibits the transcription of genes. This may prevent the synthesis of proteins that are required for bacterial growth. The inhibition of gene transcription may happen in a short time period.

Cranberry concentrate contains organic acids, anthocyanins and non-anthocyanin polyphenolic compounds (Hong and Wrolstad, 1986; Kim and Lee, 2005). Organic acids of cranberry concentrate are such as citric acid, quinic acid, and malic acid. The phenolic acids in cranberry concentrate include benzoic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid and ursolic acid (Chen et al., 2001). Wen et al. (2003) found that phenolic acids can have antimicrobial effect against *Listeria monocytogenes*; the effect was pH dependent. According to our previous study (Wu et al., 2008), low pH of the cranberry concentrate (mainly contributing by the organic acids) plays an important role in inhibiting pathogens. However, our pH effect analysis also indicated that at the same pH level, cranberry concentrate showed greater antibacterial effects than the acidic solution, implying that other bioactive compounds such as phenolics in the cranberry concentrate may also contribute to the antimicrobial actions (Wu et al., 2008).

Researchers explored inhibition of *E. coli* O157:H7 in ground beef using acid or natural compounds. Allanson et al. (2000) found 0.37 log and 2.29 log CFU/g reduction of *E. coli* O157:H7 in ground beef samples with 1% and 2% soluble polyactic acid (SPLA), respectively, after 7 days storage at 4 °C. The pH of ground beef samples with 1% and 2% SPLA was approximately 4.2 and 3.9. Smith et al. (2005) used lactic acid bacteria to inhibit *E. coli* O157:H7 in the ground beef and found the treatment reduced 3 log CFU/g of *E. coli* O157:H7 when compared with the control after 5 days of storage at 5 °C. A 1 log CFU/g reduction was observed after 9 days of storage at 4 °C by adding grape extract (1%) or pine bark extract (1%) to ground beef (Ahn et al., 2004). In the present study cranberry concentrate showed similar inhibition effects on *E. coli* O157:H7, while 7.5% cranberry concentrate resulted in higher reductions (2.4 log CFU/g) compared with lower concentrations.

Ground beef is a potentially hazardous food which can harbor pathogenic microorganisms and permit their growth or the production of toxins if temperature and time are not controlled. Effective methods to prevent and eliminate potential *E. coli* O157:H7 contaminations in ground beef are essential for the food industry and consumers. The present study indicated that cranberry concentrate at the tested concentrations did not cause significant negative impact on the organoleptical characteristics of burgers and also possessed antimicrobial effects. The addition of cranberry concentrate to ground beef may serve as an additional hurdle to control potential *E. coli* O157:H7 outbreaks associated with ground beef. Based on the inhibition of growth-related genes shown in the study and our previous viable cell and ultrastructural analysis, the cranberry concentrate studied is proven as an antimicrobial agent.

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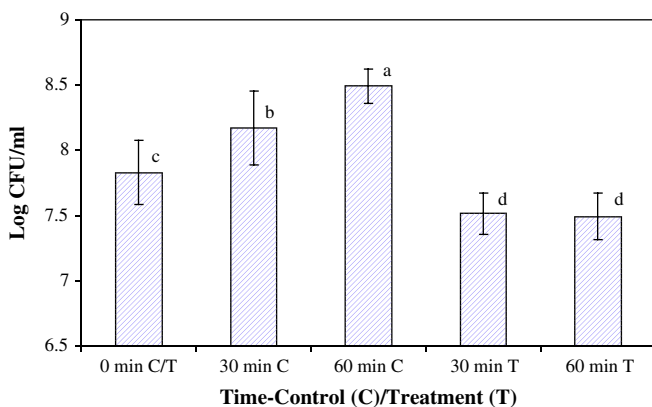


Fig. 4. Growth of *E. coli* O157:H7 in the control (2.5% v/v water in BHI) and the treatment (2.5% v/v cranberry concentrate in BHI) at 37 °C. C: control. T: treatment (2.5% v/v cranberry concentrate). Bars, ± 1 standard error. Bars with different letters indicate significant differences, $P < 0.05$ ($n = 3$).

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