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Evaluation of Carvacrol for the Control of *Escherichia coli* O157 on Cattle Hide and Carcass Cuts

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Abstract

The antimicrobial activity of carvacrol against *Escherichia coli* O157 was assessed on cattle hide and beef carcass cuts. Carvacrol (0, 10, 20, and 30 mg/mL) was applied using a spray bottle to cattle hide and beef carcass cuts, inoculated with a cocktail of *E. coli* O157 (5–6 $\log_{10}$ CFU/cm^2) and left in contact for 10 min. Following treatment, hide and carcass cuts were sampled using a swab method, and *E. coli* O157 were enumerated by plate counts on selective media. Carvacrol (30 mg/mL) significantly ($p < 0.05$) reduced inoculated *E. coli* O157 (1.4 and 1.58 $\log_{10}$ CFU/cm^2) compared to the no wash and water wash (0 mg/mL) controls on both carcass cuts and hide respectively. This preliminary study shows that carvacrol has the potential to control *E. coli* O157 on bovine hide and carcass cuts, but further research with larger scale trials is needed.

Introduction

Escherichia coli O157 is a foodborne pathogen and a major concern for public health and the food industry worldwide. Ruminants, particularly cattle, are recognized as a major reservoir of *E. coli* O157 (Armstrong *et al.*, 1996), and it has previously been reported that *E. coli* O157 can be shed in feces at levels up to $>10^7$ CFU/g (Chase-Topping *et al.*, 2007). Contaminated beef products have been implicated in a number of outbreaks over the years (Elder *et al.*, 2000; McEvoy *et al.*, 2003). Interest in assessing the potential use of essential oils (EOs) and their components as natural antimicrobials for control of pathogens such as *E. coli* O157 has increased due to consumer demand for natural minimally processed foods (Benchaar *et al.*, 2008; Fisher and Phillips, 2006; Gutierrez *et al.*, 2008). Carvacrol is one of the major active components of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) EOs. Previous research carried out by these authors showed carvacrol significantly reduced *E. coli* O157 under various environmental conditions encountered in the food industry. In addition, carvacrol was shown to reduce inoculated *E. coli* O157 in a model bovine rumen system, but the concentrations used were shown to effect the natural fermentation in the rumen, suggesting that it would not be suitable for *in vivo* application in cattle and alternative applications should be investigated (Rivas *et al.*, 2010). The objective of the present study was to evaluate the ability of carvacrol to reduce inoculated *E. coli* O157 on cattle hide and carcass cuts.

Materials and Methods

Bacterial strains and control reagents

Four toxigenic *E. coli* O157 strains (380-94, T6H11, J21, and 13C1T3) were obtained from the Teagasc Food Research Centre, Ashtown culture collection. All strains were made resistant to nalidixic acid (50 μg/mL) and streptomycin sulphate (1000 μg/mL), as outlined by Park (1978) to aid in the recovery of the inoculated strains.

Preparation of inoculum and carvacrol

All bacterial cultures were inoculated into 10 mL of tryptone soya broth (TSB; Merck, Whitehouse Station, NJ) and incubated overnight at 37°C. The overnight *E. coli* O157 cultures were centrifuged at 8000 × *g* for 10 min and washed in 10 mL of phosphate-buffered saline (PBS) prior to use. A cocktail of *E. coli* O157 strains were prepared in PBS (total volume 20 mL) to achieve a final concentration of 4–5 $\log_{10}$ CFU/cm^2 or 5–6 $\log_{10}$ CFU/cm^2 to hide and carcass surfaces, respectively.

A stock solution of carvacrol (purchased as a liquid from Sigma-Aldrich, St. Louis, MO) in ethanol (v/v; ratio of 90:10) was prepared to make an emulsification to aid in application, and diluted in sterile water (50 mL) to achieve a final concentration of 10, 20, or 30 mg/mL carvacrol.

Hide and carcass samples

Cattle hide (approximately 2 × 2 m) and carcass pieces (20 × 20 cm; from pre-chilled carcasses) were obtained either

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during in-house processing at Teagasc Food Research Centre, Ashtown or from a local abattoir (from the slaughter line), and transported immediately to the laboratory for processing. The conditions of the hides obtained were dry and moderately dirty. The carcass cuts were obtained from the brisket area of the carcass. The hide or carcass cuts did not undergo any pre-treatments or washes at the abattoir prior to collection. The large sections of hide were cut into smaller 30×30 cm pieces, and one piece was used for each treatment. Each hide and carcass piece was pegged onto a stainless steel board, and smaller sections on each hide (20×20 cm) and carcass (15×15 cm) piece were marked with a permanent marker. The prepared inoculum of *E. coli* O157 was gently poured onto the marked areas and spread evenly using a sterile plastic plate spreader. Hide and carcass pieces were then left to dry for 1 h in a laminar flow hood to allow for bacterial attachment. The volume of inoculum that was applied dried substantially on both hide and carcass pieces prior to treatment. Inoculated hide and carcass pieces were treated with either a 50-mL wash containing 10, 20, and 30 mg/mL carvacrol or with 50 mL of sterile distilled water only. Treatments were applied to marked areas using a hand-held pressure spray bottle and left in contact for 10 min. A piece of hide and carcass inoculated with the marked *E. coli* O157 cocktail but not treated with any washes (no wash) was used as a control. A hide and carcass piece not inoculated or treated was tested for the presence of enteric bacteria that possess the same antibiotic-resistant profile as the marked *E. coli* O157 strains applied in the study. The hide and carcass pieces were held at room temperature throughout the experiment. The assays for all treatments were performed in triplicate ($n = 3$) with separate hide and carcass pieces and inoculum cocktails.

Sampling and enumeration of E. coli *O157*

The swab samples for microbiological analysis were performed on the hide and carcass portions in accordance with Regulation (EC) 2073/2005. The swab was then placed into sterile stomacher bags (Seward Laboratory, London, UK) and diluted in 90 mL of modified TSB containing streptomycin sulfate (1000 μg/mL) and nalidixic acid (50 μg/mL; mTSB-nas) and stomached for 2 min. The swab samples were performed in duplicate using different areas of each treated sample. The enumeration of *E. coli* O157 was immediately performed as outlined by McGee *et al.* (2004), where all stomached swab and excision samples were serially diluted in MRD and 0.1-mL aliquots were spread plated in duplicate onto tryptic soya agar (TSA; Oxoid, Basingstoke Hampshire, UK) and incubated at 37°C for 2 h. Plates were then overlaid with sorbitol MacConkey agar containing streptomycin sulphate (1,000 μg/mL) and nalidixic acid (50 μg/mL; SMAC-nas) and incubated at 37°C for 48 h.

Statistical analysis

All counts for all experiments were $\log_{10}$ transformed, and one-way analysis of means and comparisons of means (Tukey's method) were performed on all data sets using Minitab 12.1 (Minitab Inc., Minneapolis, MN).

Results and Discussion

This study evaluated the ability of carvacrol to reduce *E. coli* O157 inoculated on cattle hide and carcass cuts. On carcass cuts, carvacrol treatment at concentrations of 10 and 20 mg/mL did not significantly ($p > 0.05$) reduce *E. coli* O157 compared to the no wash and water wash (0 mg/mL) controls (Fig. 1). However, treatment with 30 mg/mL carvacrol yielded a 1.4 $\log_{10}$ CFU/cm^2 reduction in *E. coli* O157 compared to no wash and water wash controls. On hide, carvacrol treatment at concentrations of 10 and 20 mg/mL did not significantly reduce *E. coli* O157 compared to the no wash and water wash (0 mg/mL) controls (Fig. 1). However, a concentration of 30 mg/mL carvacrol significantly ($p < 0.05$) reduced *E. coli* O157 by 1.58 $\log_{10}$ CFU/cm^2 in comparison to the no wash and water wash controls. There are no other published studies to date that have investigated the use of carvacrol on hide or carcass matrixes. Many published studies focused on the use of organic acids such as lactic acid and acetic acid on beef carcass and hide, where reductions of 0.7–3.2 $\log_{10}$ CFU/cm^2 of *E. coli* O157 following treatments of 10–100 mg or mg/mL have been reported (Carlson *et al.*, 2008; Cutter, 1994, 1999; Cutter and Rivera-Betancourt, 2000; King *et al.*, 2005). This study demonstrates that carvacrol at concentrations of 30 mg/mL can reduce inoculated *E. coli* O157 on carcass and hide pieces, and may potentially be used as an alternative control strategy to reduce *E. coli* O157 in the food

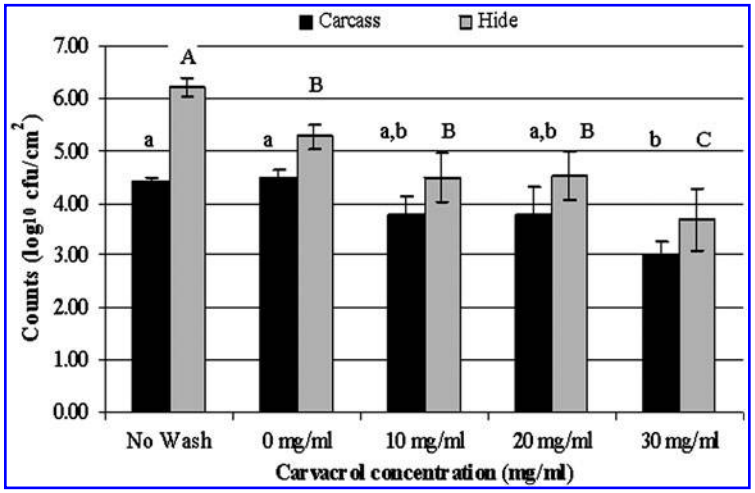

FIG. 1. Number of surviving *Escherichia coli* O157 following exposure to carvacrol on beef carcass cuts and cattle hide. Values noted with the same letter are not significantly ($p > 0.05$) different. Upper case is used for hide samples, and lower case is used for carcass samples.

chain. However, this experiment was performed as "proof of concept," and the results shown warrant further investigation using realistic, larger scale experiments prior to any commercial consideration.

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Disclosure Statement

No competing financial interests exist.

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