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Antimicrobial activity of oregano oil against antibiotic-resistant *Salmonella enterica* on organic leafy greens at varying exposure times and storage temperatures

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ABSTRACT

The objective of this study was to evaluate the effectiveness of oregano oil on four organic leafy greens (Iceberg and Romaine lettuces and mature and baby spinaches) inoculated with *Salmonella* Newport as a function of treatment exposure times as well as storage temperatures. Leaf samples were washed, dip inoculated with *S.* Newport (6-log CFU/ml) and dried. Oregano oil was prepared at 0.1, 0.3, and 0.5% concentrations in sterile phosphate buffered saline (PBS). Inoculated leaves were immersed in the treatment solution for 1 or 2 min, and individually incubated at 4 or 8 °C. Samples were taken at day 0, 1, and 3 for enumeration of survivors. The results showed that oregano oil was effective against *S.* Newport at all concentrations. *S.* Newport showed reductions from the PBS control of 0.7–4.8 log CFU/g (Romaine lettuce), 0.8–4.8 log CFU/g (Iceberg lettuce), 0.8–4.9 log CFU/g (mature spinach), and 0.5–4.7 log CFU/g (baby spinach), respectively. The antibacterial activity also increased with exposure time. Leaf samples treated for 2 min generally showed greater reductions (by 1.4–3.2 log CFU/g), than those samples treated for 1 min; however, there was minimal difference in antimicrobial activity among samples stored under refrigeration and abuse temperatures. This study demonstrates the potential of oregano oil to inactivate *S.* Newport on organic leafy greens.

1. Introduction

Non-typhoidal *Salmonella* is the leading cause of hospitalizations and deaths associated with foodborne pathogens in the United States (Scallan et al., 2011). Although this pathogen is traditionally associated with consumption of poultry, the number of fresh produce outbreaks caused by *Salmonella* is increasing. *Salmonella* Newport is a multi-drug resistant serovar that is among the four most commonly isolated serovars during the last 30 years (Centers for Disease Control and Prevention, 2007). This serovar has been associated with a number of fresh produce outbreaks in the United Kingdom and the United States (Berger et al., 2009).

Over the past decade, organic farming has been one of the fastest growing sectors in the U.S. agriculture, with increasing cropland acreages every year (United States Department of Agriculture (USDA): Economic Research Service, 2010). Organic foods are cultivated naturally using specific pest management, composting, and crop rotation practices. The USDA-National Organic Program (USDA-NOP) monitors organic food production (USDA-NOP, 2011), where use of chemicals is allowed on a limited basis. All chemicals and decontaminants must be approved by the USDA-NOP and they must be generally regarded as safe (GRAS) for human consumption (USDA-NOP, 2011).

With the advent of antibiotic resistance of certain foodborne pathogens, there has been a growing demand for alternative safe and natural antimicrobials for food products. Essential oils from herbs and spices have been known to exhibit antimicrobial properties. Numerous essential oils have shown antimicrobial activity *in vitro* against different foodborne pathogens such as *Escherichia coli*, *Campylobacter jejuni*, *Salmonella enterica*, and *Listeria monocytogenes*

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(Friedman et al., 2002; Olasupo et al., 2003). Extracts from clove were effective against *S.* Typhimurium and *E. coli* O157:H7 on fresh lettuce (Kim et al., 2011). Previous research has evaluated oregano and its active component carvacrol, both *in vitro* and on/in foods. For example, carvacrol and cinnamaldehyde exhibited antimicrobial activity *in vitro* against *C. jejuni* (Ravishankar et al., 2008) and antibiotic-resistant *S. enterica in vitro* and on celery and oysters (Ravishankar et al., 2010). Carvacrol vapor reduced the growth of *S.* Enteritidis in raw chicken (Burt et al., 2007). Oregano and carvacrol have been accepted as food additives and flavorings in the U.S. (United States Food and Drug Administration, 2011).

In an effort to discover new and natural antimicrobial treatments against *S.* Newport on organic produce, we previously evaluated the antimicrobial efficacy of apple, hibiscus, and olive formulations as well as lemongrass oil against this microorganism on leafy greens (Moore et al., 2011; Moore-Neibel et al., 2012). The objective of the present study was to extend these studies by evaluating the effectiveness of oregano oil on four different types of organic leafy greens inoculated with antibiotic-resistant *S.* Newport. The following factors that may affect the bacterial survival were evaluated: concentration of antimicrobial, exposure time, and storage temperature.

2. Materials and methods

2.1. Bacterial culture preparation and media

The strain used for this study was a multi-drug resistant *S. enterica* serovar Newport LAJ160311, with the JJPX01.0014 PulseNet PFGE profile. This strain was originally isolated from an oyster and obtained from Dr. Lynn Joens, University of Arizona, Tucson, Arizona (Brands et al., 2005a, 2005b). The culture was prepared by inoculating thawed cryo-preserved cells in 10 ml tryptic soy broth (TSB; Difco, Sparks, MD) and incubating overnight (18–22 h) at 37 °C. A fresh overnight culture (9 log CFU/ml) was prepared for each experiment. Cells from the overnight culture were diluted by suspending in buffered peptone water (BPW; Difco) to a concentration of 6-log CFU/ml for use in experiments.

2.2. Fresh produce and antimicrobials

The organic fresh produce used for this study was obtained from a local grocery store in Tucson, Arizona. Four different types of organic leafy greens were evaluated: Romaine lettuce, Iceberg lettuce, mature bunched spinach, and baby spinach. The four outer leaves of the Iceberg and Romaine lettuces were removed and discarded. Whole leaf samples (10 g ± 0.5 g each) were used for Romaine and Iceberg lettuces and multiple leaves (10 g ± 0.5 g total) were used for mature and baby spinach. The oregano oil (made from pure *Origanum vulgare*) was obtained from Lhasa Karnak Company (Berkeley, California).

2.3. Preparation of antimicrobial treatment solutions

Three dip solutions of oregano oil at 0.1%, 0.3%, and 0.5% (v/v) were prepared in sterile phosphate buffered saline (PBS, pH 7; sodium hydrogen phosphate dibasic, Fisher Chemical, Fairlawn, NJ; sodium phosphate monobasic, Chempure, Houston, TX; sodium chloride, Fisher Chemical, Fairlawn NJ). The various treatment solutions were mixed using a stomacher (Seward, London) at normal speed (230 rpm) for 30 s. Treatment solutions were prepared fresh and used immediately.

2.4. Antimicrobial activities of oregano oil against *S.* Newport on organic leafy greens

Organic leafy greens were thoroughly washed in deionized water and then weighed into 10 g samples as described previously. To reduce normal microflora, freshly washed samples were placed in a biohood under UV (254 nm) radiation for 30 min, 15 min on each side (data not shown). The samples were then dip-inoculated with *S.* Newport (6-log CFU/ml) for 2 min, removed from the solution using sterile forceps and dried for 30 min under a biohood. Control samples were taken after inoculation and after the 30 min drying time for determination of inoculum levels present on the leafy greens. Each test sample was then submerged in one of the antimicrobial treatment solutions (200 ml) for 1 or 2 min with manual gentle agitation and in PBS without antimicrobials. Immediately following treatment, samples were placed in individual stomacher bags and incubated at either 4 °C or 8 °C. Samples were collected immediately (day 0) and also following incubation, at day 1 and 3 for the enumeration of surviving *Salmonella*. Leaf samples (10 g) were pummeled in the stomacher at normal speed (230 rpm) in 90 ml BPW for 1 min. Enumeration of survivors following treatment was carried out by spread plating the serially diluted abovementioned suspensions on xylose-lysine-desoxycholate (XLD; Difco) agar. The plates were incubated at 37 °C for 24 h and the *Salmonella* colonies were counted. The experiments were repeated a total of three times.

2.5. Statistical analysis

Surviving microbial populations were converted to log CFU/g. Data were analyzed as a mixed effects model using PROC MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC) with TUKEY statement for pair-wise comparison. Interaction effects of leafy green, oregano concentration, contact time, and storage temperature were determined using 4-factor Factorial Analysis of Variance (ANOVA). All data were analyzed for normality and homogeneity of variance prior to ANOVA. In all cases, the level of statistical significance was considered to be $P < 0.05$.

3. Results

To determine whether there was any loss of organisms occurring during the inoculation and drying time, control leaf samples were processed after inoculation and before and after drying for 30 min. Bacterial levels were consistently ca. 5-log CFU/g on all leafy greens both before and after drying (data not shown) indicating no significant loss of organisms after the inoculation and drying processes.

3.1. Dose-dependent reductions in *S.* Newport populations treated with oregano oil as compared to the control

Leaves treated with 0.1, 0.3, and 0.5% oregano oil showed significant reductions for all leafy greens at each time point. It is important to note that no survivors (detection limit of 10 cells) were recovered in most samples treated with 0.5% oregano oil on days 0, 1, and 3 (Figs. 1–4). Romaine lettuce leaves treated with 0.1% oregano oil for 1 min and stored at 4 °C and 8 °C showed significant (0.8- and 0.9-log CFU/g) *Salmonella* reductions on day 3 as compared to the PBS control (Fig. 1) ($P < 0.05$). This reduction increased to 1.3-log CFU/g ($P < 0.05$) in Romaine samples treated for 2 min. Romaine leaf samples treated with 0.3% and 0.5% oregano oil for 1 and 2 min and stored at 4 °C and 8 °C showed significant reductions ranging from 4.2 to 4.7-log CFU/g on day 3 as compared to the control (Fig. 1) ($P < 0.05$). Similar to Romaine lettuce,