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Inhibitory activity of plant-derived antimicrobials against spoilage microorganism, *lactobacillus brevis*, on organic leafy greens

Abstract

Essential oils (EO) of oregano and cinnamon and their primary derivatives, carvacrol and cinnamaldehyde, respectively, were evaluated against Lactobacillus brevis during flume-tank washing of organic leafy greens. The organic leafy greens tested were, baby spinach, romaine lettuce, and iceberg lettuce. Organic leafy greens, inoculated with L. brevis (6logsCFU/ml), were washed with antimicrobial treatments at 0.5% concentration in phosphate buffered saline (PBS). Hydrogen peroxide, distilled water (DW), and PBS were used as controls. Post-treatment wash water was also tested for surviving bacteria. Surviving bacterial populations were determined on leafy greens stored at 4C on day 0, 1, and 3. Significant reductions ($P<0.05$) in bacterial populations were observed immediately after the application of antimicrobials and during subsequent storage of all leafy greens. Oregano EO and carvacrol were the most effective and reduced the L. brevis populations immediately to undetectable levels on all leafy greens. Cinnamaldehyde reduced the bacterial populations between 2.9 to 4.8logsCFU/g on romaine and iceberg lettuce, and 1.2 to 1.9logCFU/g on baby spinach. A reduction of 1.0 to 1.5logCFU/g was observed by day 3 on all the leafy greens treated with cinnamon EO. Oregano EO and carvacrol were further tested at a lower concentration of 0.1% and 0.3%. Complete reduction in bacterial populations was observed immediately on leafy greens washed with the antimicrobials at 0.3% concentration. At 0.1%, both antimicrobials reduced the populations to undetectable levels on iceberg and by 1.7-2.7logsCFU/g on baby spinach and 2.8-3.1logsCFU/g on romaine lettuce. Post-treatment wash water did not show any bacterial growth for any of the treatments except cinnamon EO, DW, and PBS. Essential oils and their derivatives have the potential to inhibit spoilage microorganisms such as L. brevis on organic leafy greens during flume-washing.

Keywords: spoilage bacteria, lactobacillus brevis, inhibition, plant-derived antimicrobials, essential oils, organic leafy greens, flume-tank washing

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Abbreviations: EO, essential oils; PBS, phosphate buffered saline; DW, distilled water; FAO, food and agricultural organization; WHO, world health organization; USDA-NOP, united states department of agriculture-national organic program; BPW, buffered peptone water; PBS, phosphate buffered saline; MIC, minimum inhibitory concentration

Introduction

Fresh or minimally-processed produce has become an important part of the regular diet, as a result of consumer's quest for health and life style changes. Consumption of fresh produce has increased dramatically (by 30%) in the past few decades.[1] This trend has also been observed towards organic fresh produce consumption, including leafy greens. Increased consumption and large scale production of organic fresh produce has been accompanied with the challenge to control microbial contamination. The Food and Agricultural Organization (FAO) and the World Health Organization (WHO) identified leafy vegetables as the highest priority commodity in terms of fresh produce microbial safety.[2] Despite modern processing technologies, it is estimated that about 20% of all fruits and vegetables produced each year is lost to spoilage.[1] Some of the spoilage bacteria isolated from fresh produce are, *Pseudomonas, Erwinia, Bacillus, Xanthomonas, Flavobacterium* and lactic acid bacteria.[3–9] Lactobacilli are among the common food-spoilage microorganisms that result in economic losses to the fresh-produce industry.

Several factors could contribute to the presence and growth of microbes on fresh produce. Microorganisms can be introduced to the crop at any stage during pre- and post-harvest handling, processing, packaging, distribution, and storage.[1] There is well-documented evidence that leafy greens can contain high levels of post-harvest microbial contamination,[1,2,10–13] leading to spoilage. Fresh cut leafy greens can also be contaminated by spoilage microorganisms through contact by people or equipment during processing or after passing through processing steps.[1] Shredding and slicing steps in fresh-cut produce processing were shown to result in increased aerobic plate counts.[1–3] on cut cabbage, lettuce, and onions.[5,14] Additionally, in comparison to conventionally grown produce, organic produce has been shown to possess high levels of microbial contamination.[15] Controlling the growth of spoilage and pathogenic microorganisms on organic fresh produce, during processing, transportation and storage, can be challenging. Organic producers and processors must follow the regulations set by the United States (US) Department of Agriculture-National Organic Program (USDA-NOP) to get organic product certification.[16] Chemical sanitizers are routinely used during

flume-tank washing of conventionally-grown leafy greens; however, USDA-NOP regulations prohibit the use of synthetic sanitizers during organic produce processing. Chlroine (4ppm, residual), hydrogen peroxide, acetic acid, ozone, and peroxyacetic acid are some of the sanitizers approved for use during flume-tank washing of organic fresh produce;[17] of which hydrogen peroxide is the most commonly used. Several studies have reported that the antimicrobial efficacy of hydrogen peroxide depends on the type of bacteria or produce.[10,18–22] Washing of the produce with hydrogen peroxide can also result in browning of certain produce such as lettuce and mushrooms[23] and therefore, may not be appropriate for all produce types.

The USDA-NOP regulations, coupled with the limited availability of effective sanitizers, have driven the need to investigate other antimicrobial alternatives for organic fresh produce.[24] Plant-derived or natural antimicrobials have been reported to be effective against food borne pathogens as well as food-spoilage microorganisms. Essential oils (EO) of oregano and cinnamon exhibited inhibitory activity on leafy greens against *Pseudomonas* spp. (25,26), *E. coli* O157:H7 (27,28), and *Salmonella enterica* Newport.[29,30] Primary constituents of oregano and cinnamon EO, cravacrol and cinnamaldehyde, respectively, also showed antimicrobial activity against *Listeria monocytogenes*[31] *E. coli* O157:H7[20] antibiotic-resistant *Campylobacter jejuni*[32] and *S. Newport*.[26,33] Few studies have also reported the effectiveness of oregano EO, cinnamon EO, carvacrol, and cinnamaldehyde against lactic acid bacteria *in vitro*.[34,35] However, very little is known about the effectiveness of essential oils and their derivatives on organic leafy greens against spoilage-causing lactic acid bacteria. In the present study, the antimicrobial efficacy of oregano EO, cinnamon EO, carvacrol, and cinnamaldehyde was evaluated against *Lactobacillus brevis* on organic leafy greens, for use in flume-tank washing and subsequent 3-day storage.

Materials and methods

Two experiments were conducted to test the effectiveness of oregano and cinnamon EO and their primary derivatives, carvacrol and cinnamaldehyde, respectively, against *L. brevis* on organic leafy greens. In experiment 1, all the plant-derived antimicrobials were evaluated at 0.5% (v/v) concentration, along with controls; and in experiment 2, oregano EO and carvacrol were tested at a concentration of 0.1% and 0.3% (v/v).

Bacterial culture preparation

A culture of *Lactobacillus brevis* ATCC® 27646™ (isolated from lettuce leaves) was used in this study. Cryo-preserved cells were revived by transferring a swab from the frozen stock culture to de man, Rogossa, Sharpe broth (MRSB; RPI corp., Prospect, IL) and incubating at 37°C for 18-24h. From the resulting culture, 100µl was then subcultured twice into MRSB, and maintained at 4°C on MRS agar (MRSA; Acumedia, Lansing, MI). A single colony was inoculated into 9ml MRSB, and incubated at 37°C for 18-22h. One ml from the resultant culture was transferred to 9ml MRSB and incubated at 37°C for 18-20h to obtain an overnight culture. The overnight culture was further diluted in buffered peptone water (BPW; Acumedia, Lansing, MI) to obtain a population of 6logsCFU/ml, which was used as the dip-inoculum for leafy greens.

Antimicrobial treatment preparation

Plant-derived antimicrobials tested in the study were, oregano and cinnamon EO (NOW Foods, Bloomingdale, IL) and their respective primary constituents, carvacrol (Sigma-Aldrich Corp., St Louis, MO) and cinnamaldehyde (Fisher Scientific, Pittsburgh, PA). On the day of the experiment, antimicrobial wash treatments were prepared at a concentration of 0.1, 0.3, and 0.5% (v/v) in phosphate buffered saline (PBS: sodium chloride; Fisher Scientific, NJ, USA; potassium chloride, sodium phosphate monobasic, and sodium phosphate dibasic; Sigma-Aldrich, MO, USA). Common wash treatments in the organic produce industry, hydrogen peroxide (3%) and sterile distilled water, along with PBS, were used as the controls for comparisons with the tested antimicrobials.

Organic leafy greens preparation

The organic leafy greens tested in the study were, baby spinach, romaine and iceberg lettuce. The leafy greens were bought on the day of the experiment from local grocery stores in Stillwater, OK, transported on ice, and stored at 4°C until use. All the leafy greens were washed thoroughly for 2minutes under running tap water to remove any dirt, soil, or organic matter. Outer layers of romaine and iceberg lettuce were discarded, and the core of iceberg lettuce removed, before separating the leaves and cutting them into 1.5sq. inch pieces, using sterile scalpel. Whole leaves of baby spinach (approximately 1.5 to 2.0sq. inch leaves) were used.

Antimicrobial treatment of organic leafy greens

Approximately 500g of leafy greens were prepared as described above, transferred to a sterile plastic tub, and washed three times in sterile distilled water for 2minutes each, using gentle back-and-forth motion. Leafy green samples were then transferred to a bio-safety cabinet and exposed to UV light for 30minutes (15minutes on each side of the leaf), to reduce background micro flora. A 20g sample was set aside after UV exposure to serve as the un-inoculated, negative control. The remaining leafy greens were dipped for 2minutes in 4800ml of the *L. brevis* inoculum (6logsCFU/ml). Leaves were then removed from the inoculum using sterile forceps and placed for 30minutes under the bio-safety hood to facilitate bacterial attachment. A 20g sample of the inoculated leafy greens was set aside to be used as the inoculated positive control, and the remaining leafy greens separated into 20g samples. Each of these samples was then washed in the appropriate antimicrobial treatment (200ml each) for 2minutes using a gentle back-and-forth motion. After washing, leaves were removed from the treatment solution and excess liquid shaken off before placing them into a sterile Whirl-Pak™ bag (NASCO, Fort Atkinson, WI). Treated leaves were stored at 4°C for 3days and surviving *L. brevis* populations enumerated on days 0, 1, and 3. Day 0 sampling was conducted immediately after the leafy greens were treated with antimicrobials. For sampling, a 5g sample from each stored sample was transferred to a sterile Whirl-Pak™ bag containing 45ml of BPW and stomached at 230rpm for 1minute. The resulting solution was serially diluted in BPW and appropriate dilutions plated on MRSA. Surviving *L. brevis* colonies (CFU/g) were counted after 40-48h of incubation at 37°C. Wash water, resulting from all treatments, was also enumerated for any *L. brevis* growth by plating on MRSA, immediately following treatment. Additionally, to recover any injured bacteria, the post-treatment wash waters were enriched in MRSB for 24 and 48h at 37°C and plated on MRSA.

Statistical analysis

All experiments were repeated 3 times with separate batches of each leafy green. The *L. brevis* populations recovered at each sampling period were converted to $\log_{10}$ CFU/g. Statistical analysis

was conducted using PROC GLM in SAS v9.4 software (SAS Inst., Cary, NC, U.S.A) to determine analysis of variance (ANOVA) with significant difference ($P<0.05$) among treatments. Means were separated using Duncan's multiple range test.

Results and discussion

Results of the study for each type of organic leafy greens treated with different antimicrobial treatments, along with controls, are shown in (Tables 1-6). Bacterial population data for negative control is not shown as no *L. brevis* colonies were recovered for any of the experiments conducted.

Table 1 Antimicrobial effects of oregano and cinnamon essential oils, carvacrol and cinnamaldehyde at 0.5% concentration against *Lactobacillus brevis* on baby spinach at 4^{0}C

Treatment	Surviving *L. brevis* population* (Log_{10} CFU/g)			Log reductions (Log_{10} CFU/g)		
	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC[1]	4.74±0.27^a	4.58±0.11^a	4.83±0.17^a	-	-	-
PBS[2]	3.42±0.19^b	3.51±0.26^{b,c}	3.72±0.23^b	1.32	1.07	1.11
DW[3]	2.64±0.63^c	2.58±0.36^c	2.46±0.38^c	1.07	1.06	0.84
HP[4]	2.83±0.19^c	3.09±0.42^c	2.98±0.36^c	1.91	1.49	1.85
Oregano EO[5]	ND±0.00^d	ND±0.00^d	ND±0.00^d	4.74	4.58	4.83
Cinnamon EO	3.72±0.19^b	3.67±0.29^b	3.48±0.21^{b,c}	1.02	0.91	1.35
Carvacrol	ND±0.00^d	ND±0.00^d	ND±0.00^d	4.74	4.58	4.83
Cinnamaldehyde	3.54±0.47^b	3.27±0.42^{b,c}	2.92±0.76^c	1.20	1.31	1.91

[1]PC, inoculated positive control (Unwashed Leafy Greens)
[2]PB, phosphate buffered saline
[3]DW, distilled water
[4]HP, hydrogen peroxide
[5]EO, essential oil
*Values represent average mean of three replications. Standard deviation (±SD) for surviving *L. brevis* population (Log_{10} CFU/g) is presented following mean value. Day 0 indicates the reduction occurred immediately after the treatment. Mean values with superscript a, b, c, etc. provide evidence of significant difference ($P<0.05$), where different letters represent statistical significance between treatments for the same sampling day.

ND, no growth detected.

Table 2 Antimicrobial effects of carvacrol, cinnamaldehyde, and oregano and cinnamon essential oils at 0.5% concentration against Lactobacillus brevis on romaine lettuce at 40C

Treatment	Surviving *L. brevis* population (Log_{10} CFU/g)*			Log reductions (Log_{10} CFU/g)		
	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC[1]	3.91±0.06^a	4.12±0.02^a	4.35±0.03^a	-	-	-
PBS[2]	2.69±0.11^b	2.96±0.02^b	3.02±0.02^b	1.22	1.16	1.33
DW[3]	3.22±0.05^b	3.16±0.10^b	3.07±0.15^b	1.43	1.64	1.55
HP[4]	ND±0.00^c	ND±0.00^d	1.52±0.05^d	3.91	4.12	2.83
Oregano EO[5]	ND±0.00^c	ND±0.00^d	ND±0.00^f	3.91	4.12	4.35
Cinnamon EO	2.37±0.21^b	2.82±0.03^b	2.80±0.01^c	1.54	1.30	1.55
Carvacrol	ND±0.00^c	ND±0.00^d	ND±0.00^f	3.91	4.12	4.35
Cinnamaldehyde	0.49±0.85^c	0.91±0.79^c	1.42±0.03^e	3.42	3.21	2.93

[1]PC, inoculated positive control (Unwashed Leafy Greens)
[2]PBS, phosphate buffered saline
[3]DW, distilled water
[4]HP, hydrogen peroxide
[5]EO, essential oil
*Values represent average mean of three replications. Standard deviation (±SD) for surviving *L. brevis* population (Log_{10} CFU/g) is presented following mean value. Day 0 indicates the reduction occurred immediately after the treatment. Mean values with superscript a, b, c, etc. provide evidence of significant difference ($P<0.05$), where different letters represent statistical significance between treatments for the same sampling day.

ND, no growth detected

Table 3 Antimicrobial effects of carvacrol, cinnamaldehyde, and oregano and cinnamon essential oils at 0.5% concentration against Lactobacillus brevis on iceberg lettuce at 40C

Treatment	Surviving *L. brevis* population (Log_{10} CFU/g)*			Log reductions (Log_{10} CFU/g)		
	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC[1]	4.80±0.04[a]	4.85±0.07[a]	4.71±0.12[a]	-	-	-
PBS[2]	3.37±0.29[b]	3.31±0.16[b]	3.24±0.11[c]	1.43	1.54	1.47
DW[3]	3.16±0.06[b]	3.23±0.04[b]	3.24±0.02[c]	1.26	1.35	1.45
HP[4]	ND±0.00[c]	ND±0.00[c]	1.71±0.09[d]	4.8	4.85	3.0
Oregano EO[5]	ND±0.00[c]	ND±0.00[c]	ND±0.00[f]	4.8	4.85	4.71
Cinnamon EO	3.41±0.11[b]	3.36±0.10[b]	3.50±0.12[b]	1.39	1.49	1.21
Carvacrol	ND±0.00[c]	ND±0.00[c]	ND±0.0s[f]	4.80	4.85	4.71
Cinnamaldehyde	ND±0.00[c]	ND±0.00[c]	1.30±0.09[e]	4.80	4.85	3.41

[1]PC, inoculated positive control (Unwashed Leafy Greens)

[2]PBS, phosphate buffered saline

[3]DW, distilled water

[4]HP, hydrogen peroxide

[5]EO, essential oil

*Values represent average mean of three replications. Standard deviation (±SD) for surviving *L. brevis* population (Log_{10}CFU/g) is presented following mean value.

Day 0 indicates the reduction occurred immediately after the treatment. Mean values with superscript a, b, c, etc. provide evidence of significant difference ($P<0.05$), where different letters represent statistical significance between treatments for the same sampling day.

ND, no growth detected

Table 4 Antimicrobial effects of oregano essential oil and carvacrol at 0.1 and 0.3% concentrations against Lactobacillus brevis on baby spinach at 40C

Treatment	Survivig *L. brevis* population (Log_{10}CFU/g)*			Log reductions (Log_{10}CFU/g)		
	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC[1]	3.71±0.81[a]	3.64±0.76[a]	3.3±0.89[a]	-	-	-
PBS[2]	2.66±0.90[a,b]	2.62±0.90[a,b]	2.62±0.50[a]	1.05	1.02	0.68
Oregano EO[3] 0.1%	1.990.55±[b]	1.41±0.82[b,c]	1.17±0.60[b]	1.72	2.23	2.13
Oregano EO 0.3%	ND±0.00[c]	ND±0.00[d]	ND±0.00[c]	3.71	3.64	3.30
Carvacrol 0.1%	2.05±0.80[b]	0.95±0.91[c,d]	0.83±0.81[b,c]	1.66	2.69	2.47
Carvacrol 0.3%	ND±0.00[c]	ND±0.00[d]	ND±0.00[c]	3.71	3.64	3.30

1PC, inoculated positive control (Unwashed Leafy Greens)

2PBS, phosphate buffered saline

3EO, essential oil

*Values represent average mean of three replications. Standard deviation (±SD) for surviving L. brevis population (Log10CFU/g) is presented following mean value.

Day 0 indicates the reduction occurred immediately after the treatment. Mean values with superscript a, b, c, etc. provide evidence of significant difference ($P<0.05$), where different letters represent statistical significance between treatments for the same sampling day.

ND, no growth detected

Table 5 Antimicrobial effects of oregano essential oil and carvacrol at 0.1 and 0.3% concentrations against Lactobacillus brevis on romaine lettuce at 40C

Treatment	Surviving *L. brevis* population (Log_{10} CFU/g)*			Log reductions (Log_{10} CFU/g)		
	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC[1]	4.65±0.09[a]	4.80±0.05[a]	4.62±0.19[a]	-	-	-
PBS[2]	3.24±0.01[b]	3.16±0.09[b]	3.13±0.08[b]	1.41	1.64	1.49
Oregano EO[3] 0.1%	1.58±0.02[c]	1.82±0.04[c]	1.77±0.23[c]	3.07	2.98	2.85
Oregano EO 0.3%	ND±0.00[d]	ND±0.00[d]	ND±0.00[d]	4.65	4.80	4.62
Carvacrol 0.1%	1.87±0.10[c]	1.78±0.16[c]	1.68±0.07[c]	2.78	3.02	2.94
Carvacrol 0.3%	ND±0.00[d]	ND±0.00[d]	ND±0.00[d]	4.65	4.80	4.62

[1]PC, inoculated positive control (Unwashed Leafy Greens)

[2]PBS, phosphate buffered saline

[3]EO, essential oil

*Values represent average mean of three replications. Standard deviation (±SD) for surviving *L. brevis* population (Log_{10}CFU/g) is presented following mean value. Day 0 indicates the reduction occurred immediately after the treatment. Mean values with superscript a, b, c, etc. provide evidence of significant difference ($P<0.05$), where different letters represent statistical significance between treatments for the same sampling day.

ND: No growth detected

Table 6 Antimicrobial effects of oregano essential oil and carvacrol at 0.1 and 0.3% concentrations against Lactobacillus brevis on iceberg lettuce at 40C

Treatment	Surviving *L. brevis* population (Log_{10} CFU/g)*			Log reductions (Log_{10} CFU/g)		
	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC[1]	4.42±0.08[a]	4.58±0.02[a]	4.69±0.02[a]	-	-	-
PBS[2]	3.15±0.07[b]	3.22±0.05[b]	3.23±0.04[b]	1.27	1.36	1.46
Oregano EO[3] 0.1%	1.22±0.09[c]	ND±0.00[c]	ND±0.00[c]	3.20	4.58	4.69
Oregano EO 0.3%	ND±0.00[e]	ND±0.00[c]	ND±0.00[c]	4.42	4.58	4.69
Carvacrol 0.1%	1.11±0.08[d]	ND±0.00[c]	ND±0.00[c]	3.31	4.58	4.69
Carvacrol 0.3%	ND±0.00[e]	ND±0.00[c]	ND±0.00[c]	4.42	4.58	4.69

[1]PC, inoculated positive control (Unwashed Leafy Greens)

[2]PBS, phosphate buffered saline

[3]EO, essential oil

*Values represent average mean of three replications. Standard deviation (±SD) for surviving *L. brevis* population (Log_{10}CFU/g) is presented following mean value.

Day 0 indicates the reduction occurred immediately after the treatment. Mean values with superscript a, b, c, etc. provide evidence of significant difference ($P<0.05$), where different letters represent statistical significance between treatments for the same sampling day.

ND, no growth detected

Antimicrobial efficacy of essential oils and derivatives at 0.5% concentration

Compared to the positive control, at 0.5% concentration, the essential oils and their derivatives reduced *L. brevis* populations significantly ($P<0.05$) on all the leafy greens (Tables 1-3). Of these, oregano EO and its primary constituent, carvacrol, were the most effective on all organic leafy greens tested. These antimicrobials immediately (day 0) reduced *L. brevis* populations on all the organic greens, with no detectable microbial populations over 3-day storage. On iceberg and romaine lettuce, cinnamaldehyde also showed significant reductions (3.2 to 4.9logsCFU/g) in *L. brevis* populations on day 0 and 1, however an increase in bacterial population was observed on day 3 (Tables 2, Table 3). Cinnamon EO reduced *L. brevis* populations between 0.9 to 1.6logCFU/g over 3days on all three leafy greens. Hydrogen peroxide was able to reduce *L. brevis* populations between 1.5 to 4.8logsCFU/g on days 0, and 1, but an increase in *L. brevis* growth was observed by day 3. No *L. brevis* was recovered from any of the post-treatment wash waters except PBS and cinnamon EO (data not shown), indicating that antimicrobials were bactericidal.

The antimicrobial effectiveness of cinnamon EO and cinnamaldehyde was also found to vary with the type of leafy green, as well as storage time. These antimicrobials were more effective on romaine and iceberg lettuce, compared to baby spinach (Table 1). Treatment with cinnamaldehyde reduced *L. brevis* populations by 1logCFU/g on baby spinach, by 3.4logsCFU/g on romaine lettuce and to undetectable levels on iceberg lettuce by day1. A similar trend was observed with cinnamon EO, however, these reductions were not maintained on romaine and iceberg lettuce, where slight increase in *L. brevis* populations was observed. The mechanism by which essential oils or their primary constituents exert antimicrobial activity is yet to be completely understood. However, these antimicrobials are known to exhibit inhibitory activity as a result of their strong

hydrophobic capacities.[36] Carvacrol, a phenolic compound, possesses a hydroxyl group and acts by disrupting the structural integrity of the cell membrane, leading to the efflux of cellular components, finally causing cell death.[37,38] Cinnamaldehyde, an aldehyde, acts by affecting cell protein synthesis, by inhibiting cellular metabolism, potentially by serving as ATPase inhibitor, and also by disrupting integrity of the cell-wall surface.[39,40] In this study, oregano EO and carvacrol were more effective than cinnamon EO and cinnamaldehyde which could be explained by the difference in their chemical structures and the mechanism of action on the bacterial cell. Carvacrol may be more effective due to the presence of a free hydroxyl group and interaction with the cell surface of *L. brevis*. Differences in efficacy of cinnamon EO and cinnamaldehyde on the different types of leafy greens could be explained by the different types of surfaces of leafy greens used in the study. Previous studies with antimicrobial compounds and plant extracts also reported varied antimicrobial efficiency with different leafy green surfaces.[20,41] The trend observed in the current study was also similar to that obtained in a previous study,[42] where *E. coli* O157:H7 survivors were least susceptible to antimicrobial plant extracts on baby spinach.

In the present study, hydrogen peroxide also exhibited antimicrobial efficacy on day 0 and 1 but was not able to maintain it over 3 days. Similar results were found in other studies against *E. coli* O157:H7[20,42] and *S. Newport*.[41] Hydrogen peroxide is an oxidizing agent and exhibits antimicrobial activity by generating cytotoxic oxidizing molecules with the help of available peroxidase enzyme.[43] The antibacterial efficacy of hydrogen peroxide can therefore largely depend on the availability of peroxidase in the food matrix.[44,45] These findings suggest that traditionally used hydrogen peroxide may not be effective in controlling the growth of spoilage microorganisms on organic produce stored for more than a day.

Antimicrobial efficacy of oregano essential oil and carvacrol at 0.1% and 0.3% concentrations

Results from experiment 1 were used to determine the most effective essential oil and its derivative at 0.5% concentration and tested further at lower (0.1% and 0.3%) concentrations. Compared to the positive control, oregano EO and carvacrol at 0.1% significantly ($P<0.05$) reduced *L. brevis* populations (1.7 to 4.7logsCFU/g) on all the leafy greens. Efficacy of these antimicrobials increased with increased concentration where the two exhibited better inhibitory activity at 0.3% than at 0.1%. At 0.3% concentration, both, oregano EO and carvacrol, immediately reduced *L. brevis* populations to undetectable levels on all the leafy greens (Tables 4-6). Similar results were observed in the studies using carvacrol and oregano EO against *E. coli* O157:H7,[20] *S.* Newport;[33] and *C. jejuni*.[32] In the present study, 0.1% was not as effective as 0.3% of oregano EO or carvacrol but still exhibited significant ($P<0.05$) reductions in *L. brevis* populations. Results of the current study are similar to other studies on the effects of oregano EO and carvacrol against spoilage microorganisms. Gutierrez et al.[25] reported the effectiveness of oregano EO against *Pseudomonas* on lettuce and against *Lactobacillus* in beef extract. An *in vitro* study conducted by Gunn[46] revealed the minimum inhibitory concentration (MIC), and minimum lethal concentration of carvacrol to be 0.1%, and 0.2% respectively, against *Pseudomonas* and lactic acid bacteria.

As observed in experiment 1 for other antimicrobials at 0.5%, the effects of the two antimicrobials at 0.1% concentration varied with the type of leafy green. Both, 0.1% oregano EO and carvacrol exhibited higher efficacy in iceberg lettuce followed by romaine lettuce and baby spinach. While a 1.7logCFU/g reduction was observed on baby spinach on day 0, *L. brevis* populations were reduced by about 3logsCFU/g on romaine and iceberg lettuce. These reductions were maintained in spinach and romaine lettuce, whereas no bacterial populations were recovered by day 1 and day 3 on iceberg lettuce for both, oregano EO and carvacrol (Table 4-6). Treatment with 0.3% oregano EO and carvacrol reduced the *L. brevis* populations immediately to undetectable levels on all leafy greens which were maintained over 3days (Tables 4-6). This pattern of varying effectiveness of antimicrobials with different produce types has also been observed in similar studies against food borne pathogens. In a previous study by Denton et al.,[20] 0.1% carvacrol reduced *E. coli* O157:H7 populations to undetectable levels on iceberg lettuce, while on romaine lettuce and baby spinach, 3.7 and 2.2logsCFU/g reductions were observed on day 0, respectively. These results indicate that the type of fresh produce could influence the antimicrobial efficacy of essential oils and their derivatives.

Conclusion

Plant-derived essential oils and their derivatives have the potential to inhibit spoilage microorganisms, such as *L. brevis,* on organic leafy greens and can be used as alternatives to traditional sanitizers by organic produce industry. Further investigation is required to test the organoleptic properties and effectiveness of these sanitizers against other spoilage microbes.

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Conflict of interest

No financial or conflict of interest declared.

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