Scientific Research Publishing

Food and Nutrition Sciences, 2021, 12, 392-405
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Antimicrobial Effects of Plant Compounds against Virulent *Escherichia coli* O157:H7 Strains Containing Shiga Toxin Genes in Laboratory Media and on Romaine Lettuce and Spinach

Javier R. Reyna-Granados[1,2], Lynn A. Joens[2], Bibiana Law[2], Mendel Friedman[3], Sadhana Ravishankar[2*]

[1]Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora, Ciudad Obregón, México
[2]School of Animal & Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA
[3]USDA-ARS Western Regional Research Center, Albany, CA, USA

Email: *sadhravi@arizona.edu

How to cite this paper: Reyna-Granados, J.R., Joens, L.A., Law, B., Friedman, M. and Ravishankar, S. (2021) Antimicrobial Effects of Plant Compounds against Virulent *Escherichia coli* O157:H7 Strains Containing Shiga Toxin Genes in Laboratory Media and on Romaine Lettuce and Spinach. *Food and Nutrition Sciences*, **12**, 392-405.
https://doi.org/10.4236/fns.2021.124030

Received: March 8, 2021
Accepted: April 19, 2021
Published: April 22, 2021

Copyright © 2021 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Escherichia coli strains produce Shiga-toxins Stx-1 and Stx-2 that contribute to their virulence. The objective was to evaluate antimicrobial activities of plant essential oils (oregano, cinnamon, lemongrass), their active components (carvacrol, cinnamaldehyde, citral) and plant-extracts (green tea polyphenols, apple skin, black tea, decaffeinated black tea, grapeseed and pomace extracts) against *E. coli* O157:H7 strains containing *Stx*-1 and *Stx*-2 genes, as determined by Multiplex Polymerase Chain Reaction, *in vitro* and on leafy greens. Antimicrobials at various concentrations in sterile PBS were added to bacterial cultures (~3 - 4 logs CFU/ml), mixed thoroughly, and incubated at 37˚C. Surviving bacteria were enumerated at 0, 1, 3, 5 and 24 h. The most effective essential oil (oregano oil; 0.5%) and plant extract (green tea; 3%) were evaluated against *E. coli* O157:H7 on romaine lettuce and spinach stored at 4˚C for 7 days. Microbial survival was a function of the concentration of antimicrobials and incubation times. All antimicrobials reduced bacterial population to below detection levels *in vitro*; however, essential oils and active com- ponents exhibited greater activity than plant extracts. Oregano oil and green tea reduced *E. coli* O157:H7 on lettuce and spinach to below detection. Plant- based antimicrobials have the potential to protect foods against *E. coli* O157: H7.

Keywords

E. coli O157:H7, Shiga Toxin Genes, Romaine Lettuce, Spinach, Inactivation, Essential Oils, Plant Extracts

1. Introduction

Escherichia coli O157:H7 is one of the most important foodborne pathogenic bacterium responsible for hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) in affected individuals. According to Marshall, *et al.* [1], *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* are the leading causes of multistate foodborne disease outbreaks in the United States.

According to Smith, *et al.* [2], STEC are foodborne pathogens that may cause diarrheal outbreaks and occasionally are associated with HUS. The disease in humans often arises from consuming contaminated food. *E. coli* O157:H7 has caused numerous foodborne outbreaks (via undercooked hamburger patties, apple cider, spinach, lettuce, etc.) and is associated with causing HUS in young children and the elderly. Virulent *E. coli* O157:H7 strains are known to produce the Shiga toxins Stx-1 and Stx-2. Rasooly, *et al.* [3] [4] found that orally fed Shiga toxin Stx-2 was highly toxic to mice, heat treatments (pasteurization) that destroy bacteria did not inactivate the toxin, and that freshly prepared apple juice inhibited the *in vitro* biological activity of the toxin. Beef cattle are one of the major reservoirs and illnesses have been reported to be contracted by visiting farms or eating undercooked hamburgers [5] [6] [7].

Foodborne pathogens in/on contaminated food can persist during storage. Thus, *E. coli* O157:H7 and *Listeria monocytogenes* survived during storage (4˚C - 8˚C) on ready-to-use packaged vegetables (lettuce, coleslaw mix, soybean sprouts); and growth occurred by 1.5 - 2 logs in 12 days [8]. Many researchers have investigated methods to prevent, control, or eradicate these pathogens in food. One such method is the use of natural antimicrobials derived from plant sources. Kiskó and Roller [9] studied the inactivation of *E. coli* O157:H7 using the plant compounds carvacrol and p-cymene. Kim, *et al.* [10] used carvacrol, limonene, nerolidol, and ß-ionone at various concentrations to inactivate *E. coli* O157:H7, *Salmonella* Typhimurium, *L. monocytogenes* and *Vibrio vulnificus*. The mechanism of action of these antimicrobial compounds may involve disruption of cell membranes [11] and altered protein synthesis or the inhibition of genes involved in the production of flagellin [12].

The purpose of the present study was to evaluate the antimicrobial activities of essential oils (oregano, cinnamon, and lemongrass), their active components (carvacrol, cinnamaldehyde, and citral), as well as plant extracts (green tea polyphenols, apple skin, black tea, decaffeinated black tea, golden grapeseed, and grape pomace) against *E. coli* O157:H7 *in vitro* and the most effective antimicrobials

on leafy greens.

2. Material and Methods

2.1. Bacterial Cultures, Culture Preparation and Media

E. coli O157:H7 strains 932 (clinical isolate from patients who had hemorrhagic colitis), ATCC 35150 (human feces isolate; acid resistant) and F4637 (sprout isolate) were used in this study. All three virulent isolates expressed genes for Shiga toxins 1 and 2 (*Stx*-1 and *Stx*-2). A cocktail of these three strains (in equal proportions) was prepared and used in all experiments. Cultures were prepared in tryptic soy broth with 0.6% yeast extract (TSB; Difco, Becton Dickinson, Sparks, MD). The cells were incubated overnight (18 to 20 h) at 37˚C to reach an optimal concentration of ~9 logs CFU/ml. After antimicrobial treatments, all viable organisms were enumerated by diluting in buffered peptone water (BPW; Difco, Becton Dickinson), plating on Cefixime-Tellurite-Sorbitol MacConkey agar (CT-SMAC; Difco, Becton Dickinson) and incubating at 37˚C for 18 - 24 h.

2.2. Plant Antimicrobials

The test compounds consisted of six essential oils and active components and six powder extracts. The essential oils of cinnamon, lemongrass, and oregano were obtained from Lhasa Karnak Herb Co. (Berkeley, CA). The essential oil components carvacrol (>98%), trans-cinnamaldehyde (99.5%), and citral (>96%) were obtained from Sigma-Aldrich Co. (St. Louis, MO). The powder extracts used were green tea polyphenols (≥95%, LKT Laboratories, Inc., Minnesota, MN), apple skin extract (Apple Poly™, standardized to 80% polyphenols/5% phloridzin, Apple Poly LLC, Morrill, NE), black tea extract, caffeinated and decaffeinated (hot water infused and freeze-dried English Breakfast tea from Coffee Bean Direct, Titusville, NJ), grapeseed extract (MegaNatural® Gold, >90% polyphenols, Polyphenolics Inc., Madera, CA), and grape pomace extract (MegaNatural® GSKE, >80% polyphenols and >0.075% anthocyanins, Polyphenolics Inc., Madera, CA). Initially all antimicrobials were tested for their antimicrobial activity *in vitro* in sterile phosphate-buffered saline pH 7.4 (PBS). The most effective antimicrobials from the essential oil and the plant extract groups were further tested by applying them directly onto foods implicated in *E. coli* O157:H7 outbreaks. Hence, oregano oil and green tea polyphenols were chosen to be tested on romaine lettuce and spinach.

2.3. Multiplex Polymerase Chain Reaction (PCR) for Detecting the Shiga-Toxin Genes in *E. coli* O157:H7 Strains

We wanted to select virulent strains of *E. coli* O157:H7 for the antimicrobial studies. Hence, nine strains were tested for the presence of Shiga-toxin *Stx*-1 and *Stx*-2 genes. Multiplex PCR was conducted to detect them using the protocol and primers designed by Blanco, *et al.* [13]. The Stx1 and Stx2 oligonucleotide se-

quence (5'-3') were: VT1-A forward CGCTGAATGTCATTCGCTCTGC (Eurofins, MVG Operon, Huntsville, AL) and VT1-B reverse C'GTGGTATAGCTAC TGTCACC (Eurofins); VT2-A forward CTTCGGTATCCTATTCCCGG (Eurofins) and VT2-B reverse CTGCTGTGACAGTGACAAAACGC (Eurofins). The nine *E. coli* O157:H7 isolates were 960212, 960218, F4546, F4637, and F5853 isolated from sprouts, 1388 from apple juice, 932 and ATCC 35150 from humans, and ATCC 43895 from beef. The DNA was extracted by suspending a loop of bacteria harvested from TSA in 250 µl of sterile water and incubating it at 100˚C for 5 min, using a thermocycler (Bio-Rad Hercules, CA) to release the DNA. This was used in Multiplex PCR. Multiplex PCR was determined with 150 ng of the primers; 0.2 mM of each deoxynucleotide (dATP, dGTP, dCTP, and dTTP) (Promega BioSciences, LLC. San Luis Obispo, CA); 10 mM Tris-HCl; 1.5 mM $MgCl_2$; 50 mM KCl (Promega BioSciences); and 1 U of DNA Taq polymerase (Promega BioSciences), and 7 µl of extracted DNA in a final volume of 30 µl. The PCR procedure of Blanco, et al. (2013) [13] was used. To visualize the amplicons, electrophoresis was performed by preparing 2% agarose gel (Difco, Becton Dickinson) in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA) (Promega BioSciences), and running at 130 V for 30 min. The gel was stained with ethidium bromide (Promega BioSciences). The size of DNA was analyzed by UV fluorescence using a 100 bp DNA ladder Molecular Marker (Promega BioSciences).

2.4. Antimicrobial Activities of Essential Oils, Their Active Components, and Plant Extracts against *E. coli* O157:H7 in PBS

The essential oils and their active components (oregano oil, cinnamon oil, lemongrass oil, carvacrol, cinnamaldehyde, and citral) and the plant powder extracts (green tea polyphenols, apple skin, black tea, decaffeinated black tea, grape pomace, and grapeseed) were dissolved in sterile PBS (Difco, Becton Dickinson). Serial dilutions were prepared at 0.3%, 0.2%, and 0.1% for the essential oil/active components and 4%, 3%, 2%, and 1% for the dehydrated plant extracts from stock solutions in micro-centrifuge tubes (90 µl). The test organism (10 µl; approximately 3 - 4 log CFU/ml) was added to each tube, mixed well, and sampled immediately (0 min). Surviving bacterial populations were also enumerated after incubation for 1, 3, 5 and 24 h. All samples were serially diluted in BPW, plated on CT-SMAC, and incubated for 18 - 24 h at 37˚C.

2.5. Antimicrobial Activities of Oregano Oil and Green Tea Polyphenols against *E. coli* O157:H7 on Romaine Lettuce and Spinach

Romaine lettuce and spinach leaves from local grocery stores were sectioned into 10-g pieces. The pieces were exposed to UV light under a biohood for 30 min. Lettuce and spinach samples were then placed into sterile plastic bags and inoculated by dipping in *E. coli* O157:H7 culture of ~6 log CFU/ml for 2 min. After inoculation, samples were dried for 1 h under a biohood. The inoculated leaf

samples were then immersed for 10 min in 0.5% oregano oil or 3% green tea solutions prepared as described above. After treatment, the samples were stored at 4˚C for 7 days. Survivors were enumerated on days 0, 1, 3 and 7. The samples were stomached (Seward, Ltd, London, UK) in 90 ml BPW at normal speed for 1 min. Samples were diluted in BPW, plated on CT-SMAC, and incubated at 37˚C for 18 to 24 h. Appropriate controls (uninoculated and untreated inoculated lettuce and spinach samples) were done for each test. All experiments were repeated three times.

2.6. Statistical Analysis

Each experiment was repeated three times and duplicate agar plates were used in each repeat giving 2 data points for each value, meaning duplicate numbers were obtained for each time point per repeat. An average of the duplicate values was taken for each repeat. The average values from the three repeats were then used to calculate the final mean and standard deviation.

3. Results and Discussion

3.1. Multiplex Polymerase Chain Reaction to Detect Shiga-Toxin Genes in *E. coli* O157:H7 Strains

As already mentioned, Shiga-toxin-producing *E. coli* is the leading cause of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), mainly in infants and the elderly. Virulent *E. coli* O157:H7 is characterized by the presence of *Stx*-1, *Stx*-2, intimin (*eae*-ξ), and *ehx*A genes [13].

Figure 1 shows the amplicons isolated from nine *E. coli* O157:H7 strains. All isolates contained both Shiga toxin genes 1 (516 bp) and 2 (302 bp), except ATCC 43895 (beef) that only showed the genes for Stx-1. One purpose of this study was to select the virulent isolates that had both *Stx*-1 and *Stx*-2 genes for

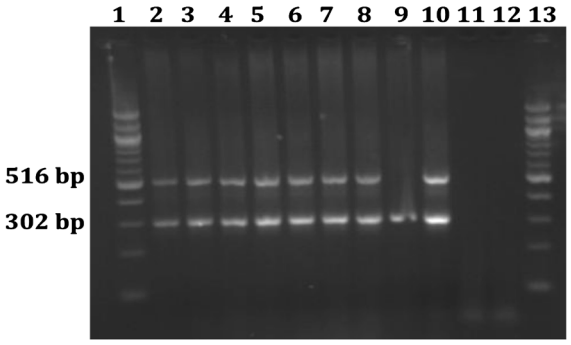

Figure 1. Agarose gel of 9 isolates of *E. coli* O157:H7. Submarine gel electrophoresis at 2% with 10 µl final reaction mixture in TBE buffer for 30 min at 130 Volts. Lines 1 and 13: 100 bp DNA ladder Molecular Marker, lines 2 to 10: template DNA for isolates of *E. coli* O157:H7 (2: 960212 isolate from sprouts, 3: 1388 from apple juice, 4: 932 from humans, 5: F4546 from sprouts, 6:960218 from sprouts, 7: F5853 from sprouts, 8: ATCC 35150 from humans, 9: ATCC 43895 from beef, and 10: F4637 from sprouts). Lines 11 and 12: negative controls (no template DNA). The amplicons on 302 bp represent the presence of Stx-1 and 516 bp the presence of Stx-2.

investigating the antimicrobial effect of plant compounds. Based on this analysis, the following *E. coli* O157:H7 strains were selected for evaluation: 932, ATTC 35150, and F4637, isolated from humans, humans, and sprouts, respectively. We do not know whether in addition to inactivating/inhibiting the growth of the bacteria, the plant-based antimicrobials also concurrently inhibit the toxicological properties of the toxins by modification of receptor-binding sites [14].

3.2. Antimicrobial Activity of Essential Oils, Their Active Components and Plant Extracts against *E. coli* O157:H7 in PBS

Figure 2 and Figure 3 show the results of the antimicrobial effect of each plant compound against *E. coli* O157:H7. Figure 2(a) and Figure 2(b) illustrate the

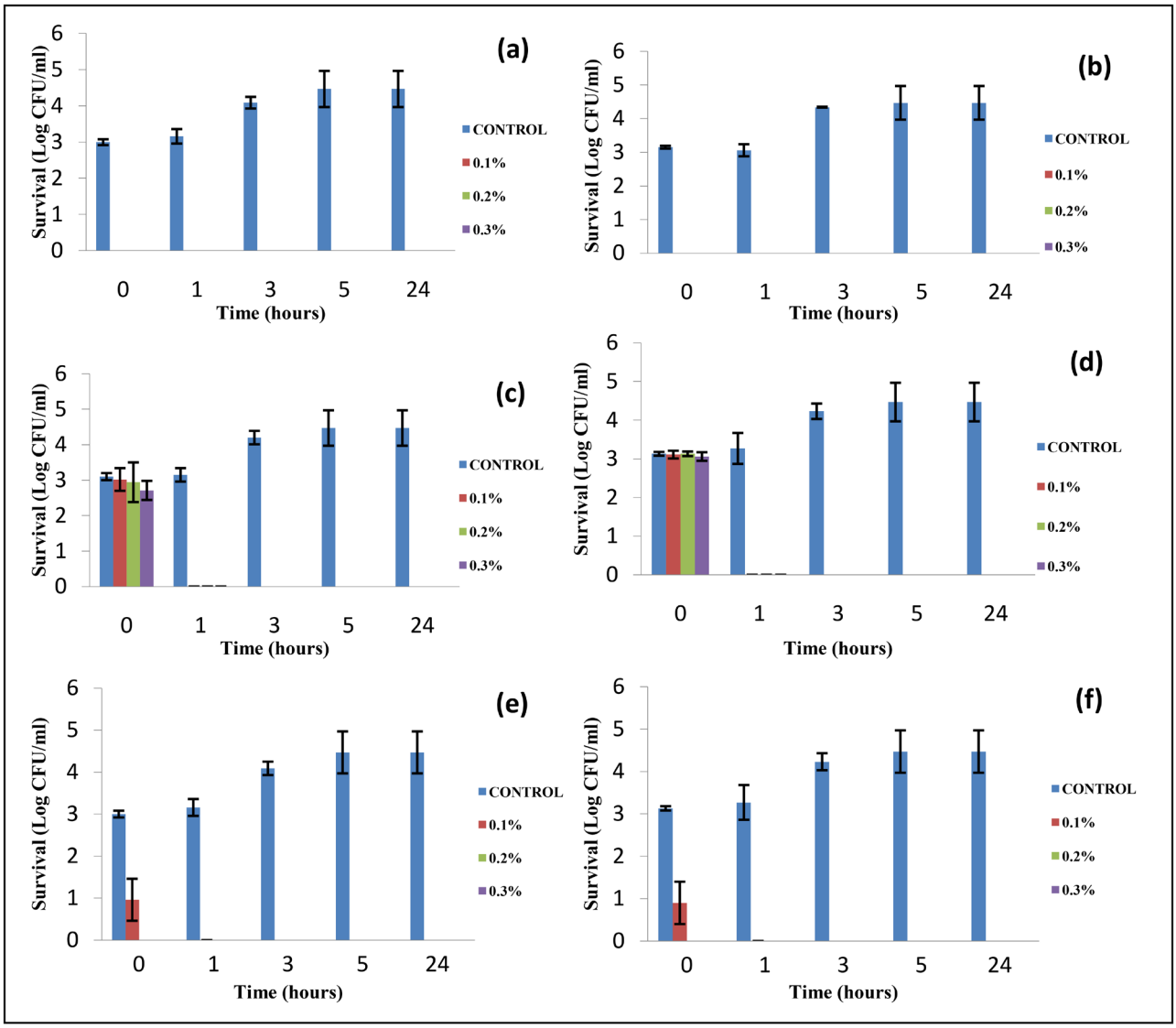

Figure 2. Antimicrobial activity of essential oils and their respective active compounds against a three strain-cocktail of *E. coli* O157:H7. (a) oregano oil, (b) carvacrol, (c) cinnamon oil, (d) cinnamaldehyde, (e) lemongrass oil, and (f) citral. Values plotted at each sampling time point are an average of three repeats with 2 replicates per repeat. Error bars represent standard deviation from the mean.

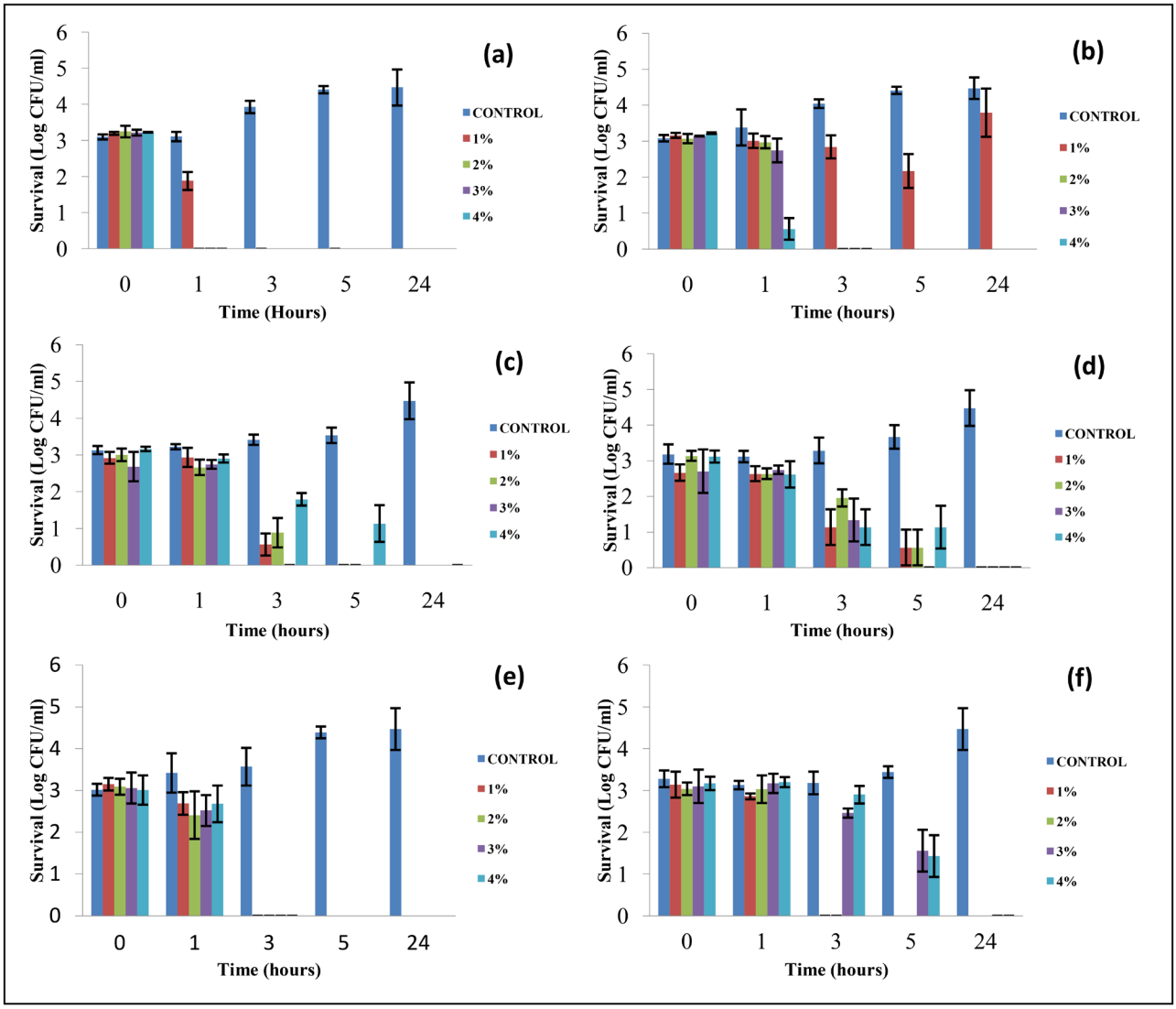

Figure 3. Antimicrobial activity of plant extracts against a three strain-cocktail of *E. coli* O157:H7 (a) green tea polyphenols, (b) apple skin extract, (c) black tea, (d) decaffeinated black tea, (e) grapeseed extract, and (f) grape pomace extract. Values plotted at each sampling time point are an average of three repeats with 2 replicates per repeat. Error bars represent standard deviation from the mean.

strong bactericidal activity of oregano oil and carvacrol, as evidenced by no detectable survivors at time 0 and thereafter, at all tested concentrations. Figure 2(c) and Figure 2(d) show that the bacteria exposed to cinnamon oil and cinnamaldehyde at different concentrations also did not survive (no detectable levels) starting at 1 h and thereafter. Figure 2(e) and Figure 2(f) depict the activity of lemongrass oil and citral, respectively; exposure to both these compounds resulted in no detectable survivors at time 0 at a concentration of ≥0.2%. A 2-log reduction was observed at a concentration of 0.1% at time 0 and no detectable survivors starting at 1 h and thereafter, respectively. The results show that the essential oils and their active compounds have the potential to inactivate *E. coli* O157:H7 rapidly and could be applied in foods for decontaminating them.

Figure 3(a) demonstrates the strong antimicrobial activity of green tea polyphenols as indicated by no detectable survivors at 1 h and thereafter for concentrations ≥ 2%, and for a 1% concentration by about a 1.5 log reduction and no detectable survivors at 1 and 3 h, respectively. Figure 3(b) depicts the bactericidal effect of apple skin extract that showed about a 2.5-log reduction after 1 h at 4%, and no detectable survivors starting at 3 h for 2%, 3%, and 4% concentrations, respectively. Black tea (Figure 3(c)) did not show any effect at 0 and 1 h at all concentrations, a >2-log reduction at 3 h at ≤2%, no detectable survivors at 3 h at 3%, and no detectable survivors at 24 h at all concentrations. Similar to black tea, decaffeinated black tea (Figure 3(d)) did not induce any change in the bacterial population at 0 and 1 h at all concentrations; but a ~1 - 2-log reduction was observed at all concentrations at 3 h, no survivors at 5 h at 3%, and finally no detectable survivors at 24 h at all tested concentrations. Figure 3(e) shows that exposure to grapeseed extract resulted in no detectable survivors at 3 h at all tested concentrations. Figure 3(f) shows that exposure of *E. coli* O157:H7 to grape pomace extract resulted in no detectable survivors at 3 and 5 h at ≤2% concentration, >1.5-log reduction at 5 h and no detectable survivors at 24 h, at 3% and 4% concentrations. One possible explanation for the better survival of *E. coli* O157:H7 at 3 and 4% concentrations of grape pomace extract at 3 and 5 h could be that the organism was able to utilize the nutrients available in the extract, which could also provide a protective effect on the pathogen. Verification of such an effect by conducting additional investigations is beyond the scope of this study; however, this observation provides additional area for future research.

Among the essential oils, oregano oil and carvacrol exhibited the best antimicrobial activity against *E. coli* O157:H7. Among the plant extracts, green tea polyphenols exhibited the highest antimicrobial activity. Black tea had better antimicrobial activity than decaffeinated black tea and grapeseed extract demonstrated better antimicrobial activity than grape pomace extract on *E. coli* O157:H7 *in vitro*.

3.3. Antimicrobial Activity of Oregano Oil and Green Tea Polyphenols against *E. coli* O157:H7 on Romaine Lettuce and Spinach

Figure 4 and Figure 5 show the antimicrobial activity of oregano oil at a concentration of 0.5% and green tea extract at 3% against *E. coli* O157:H7 on romaine lettuce and on spinach. Oregano oil (0.5%) on day 0 induced > 2 log reductions on romaine lettuce and >3 log reductions on spinach. For both leafy greens, no survivors were detected on day 1.

The results obtained from green tea were similar to those of oregano oil. At day 0, green tea (3%) induced a ~1.5 log reduction on lettuce and ~1 log reduction on spinach; no survivors were detected for both types of leafy greens on day 1. These results demonstrate that plant compounds can be used to decontaminate and protect produce during refrigerated storage.

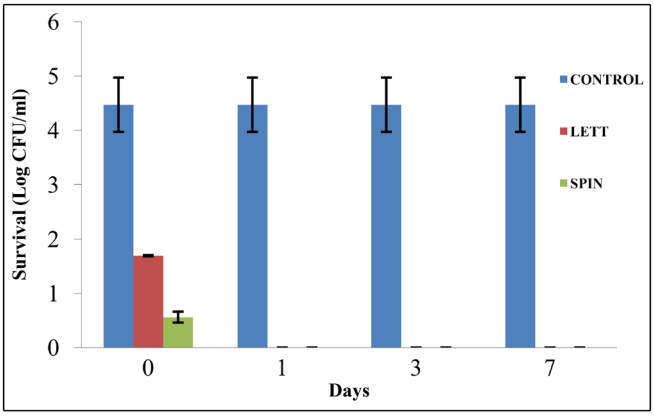

Figure 4. Antimicrobial activity of oregano oil against a three strain-cocktail of *E. coli* O157:H7 on romaine lettuce and spinach. LETT: romaine lettuce, SPIN; spinach, Values plotted at each sampling time point are an average of three repeats with 2 replicates per repeat. Error bars represent standard deviation from the mean.

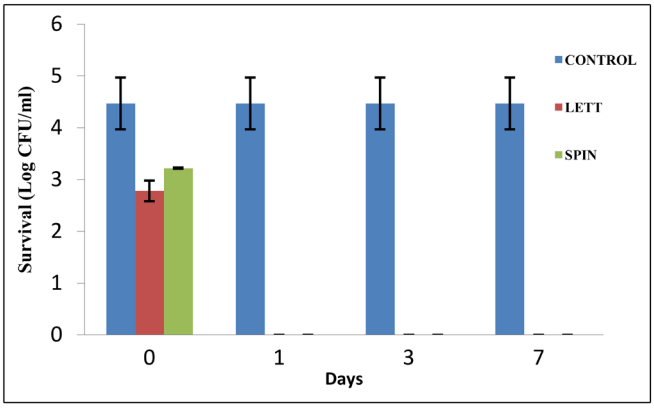

Figure 5. Antimicrobial activity of green tea polyphenols against a three strain-cocktail of *E. coli* O157:H7 on romaine lettuce and spinach. LETT: romaine lettuce, SPIN; spinach. Values plotted at each sampling time point are an average of three repeats with 2 replicates per repeat. Error bars represent standard deviation from the mean.

3.4. Related Antimicrobial Studies

There have been several related reports on the antimicrobial properties of plant essential oils and their bioactive compounds, as well as those of apple polyphenols, grape and tea compounds, that complement the current study.

Friedman, *et al.* [15] determined the quantitative antimicrobial activities of more than 100 essential oils and their bioactive compounds against four foodborne pathogens, expressed as bactericidal activity (BA50) values, defined as the concentration of the antimicrobial that inhibited 50% of the bacteria under the test conditions. This widely cited paper serves as a resource for selecting antimicrobials with high activities in the cell assays (*in vitro*) for evaluation against efficacy on contaminated salads and meats. An investigation by Moore-Neibel, *et al.* [16] examined the antimicrobial effectiveness of lemongrass essential oil on organic leafy greens, romaine and iceberg lettuces, and mature and baby spinach inoculated with *Salmonella* Newport and demonstrated the concentration- and

time-dependent inactivation of the pathogen. In another study on salad leaves, Todd, *et al.* [17] found that washing organic baby and mature spinach and iceberg and romaine lettuces with cinnamon oil inactivated antibiotic-resistant *Salmonella* Newport bacteria, indicating the potential of cinnamon oil as a practical treatment option. This was a positive progress because inhibiting the growth of antibiotic resistant pathogens is a major challenge for food microbiology [18].

Appearance of the food product can be an important factor in consumer choice. Yossa, *et al.* [19] demonstrated the efficacy of cinnamaldehyde-containing washes against *E. coli* O157:H7 and *Salmonella enterica* on iceberg lettuce without any effect on the color and texture of the leaves, maintaining their visual appearance for the consumer.

Poimenidou, *et al.* [20] reported that vinegar, lactic acid or oregano aqueous extract alone or in combination can serve as alternative washing solutions to chlorine against *E. coli* O157:H7 on fresh cut spinach and lettuce with acceptable appearance.

In an alternative strategy, Zhu, *et al.* [21] discovered that apple, carrot and hibiscus edible films containing the antimicrobials carvacrol and cinnamaldehyde inactivated *Salmonella* Newport on organic Romaine and Iceberg lettuce, and mature and baby spinach in sealed salad bags. Films with 3% carvacrol showed the highest reduction. A related study found these antimicrobial edible films also inactivated *E. coli* O157:H7 on organic leafy greens in sealed plastic bags [22]. The inactivation of the bacteria is most likely induced by vapors released from the antimicrobials incorporated into the films.

A similar approach has also been used for incorporating apple polyphenols into edible films to examine their effect on microbial activity and it was shown that the films inhibited the growth of *L. monocytogenes* but not *S. enterica* serotype Hadar [23] [24]. Human studies have shown other beneficial health activities for apple polyphenols, in which they ameliorated hyperglycemia [25] and protected against UV radiation-induced skin pigmentation [26].

Although not relevant to the present study, it is of interest that Wang, *et al.* [27] found that applying a mixture of acetic and lactic acids on lettuce reduced the presence of *E. coli* O157:H7 and *L. monocytogenes*, as well as aerobic mesophilic and psychrotrophic bacteria, coliforms, molds, and yeasts during storage.

Grape compounds have also previously been shown to have beneficial effects against microorganisms. For example, Peixoto, *et al.* [28] and Friedman [29] reported that the bioactive phenolic compounds in grape pomace, a byproduct of wine production composed of seeds, skin, and stems, exhibited antibacterial and other health benefits.

Black and green tea extracts, such as those used here, are often cited for their health benefits. Friedman, *et al.* [30] reported that both black tea theaflavins and green tea catechins inhibited the growth of *Bacillus cereus* at nanomolar levels and that some were more active than medicinal antibiotics; freshly prepared teas

were more active than day-old teas. Tea compounds also showed strong antiviral [31] and anti-parasitic trichomonad properties [32].

The results of the present study on salads and these related cited studies indicate that some of the test compounds can act as broad-spectrum antibiotics against nonresistant and resistant pathogens in cell assays (*in vitro*) and on contaminated leafy greens.

4. Conclusions

In conclusion, the findings of this study indicate that essential oils and their active components demonstrated better effectiveness as natural antimicrobials against *E. coli* O157:H7 than polyphenolic powder extracts, except for green tea and grapeseed extract, which showed strong activity. All the essential oils showed strong antimicrobial activity after 1 h (complete reduction with no detectable survivors) at all test concentrations and exposure times. The effectiveness of the antimicrobials was both concentration- and time-dependent. Exposure to oregano oil and carvacrol resulted in no detectable survivors at all concentrations at 0 min. Exposure to green tea and grapeseed extracts resulted in no detectable survivors at 3 h at all tested concentrations. All plant extracts exhibited storage time-dependent complete reduction with no detectable survivors after 24 h at all tested concentrations. Oregano oil (0.5%) and green tea polyphenols (3%) reduced the population of *E. coli* O157:H7 on contaminated lettuce and spinach to below detection limits.

As noted elsewhere [33], the general objective of the present and related studies by other investigators was to determine the antimicrobial effect of naturally occurring food-compatible plant compounds and extracts against bacterial foodborne pathogens. The results have shown that essential oils and bioactive compounds: (a) inactivated susceptible and resistant strains of *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *E. coli*, *L. monocytogenes*, *S. enterica*, *Staphylococcus aureus,* and *Mycobacterium avium* subspecies tuberculosis; (b) inactivated foodborne pathogens in apple juice, tomato juice, wines, in meat and poultry products, and on oysters and leafy greens; (c) simultaneously inhibited the growth of *E. coli* O157:H7 and the formation of carcinogenic heterocyclic amines in grilled beef patties; and (d) acted by an antimicrobial mechanism that seems to be largely governed by the disruption of cell membranes. The better survival of *E. coli* O157:H7 at high concentrations of some plant extracts such as grape pomace extract is an area that merits further investigation.

Acknowledgements

We thank Carol Levin for facilitating the preparation and formatting of the manuscript and Libin Zhu for submission of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Marshall, K.E., Nguyen, T.A., Ablan, M., Nichols, M.C., Robyn, M.P., Sundararaman, P., *et al.* (2020) Investigations of Possible Multistate Outbreaks of *Salmonella*, Shiga Toxin-Producing *Escherichia coli*, and *Listeria monocytogenes* Infections—United States, 2016. *Surveillance Summaries*, **69**, 1-14.

[2] Smith, A.M., Tau, N.P., Kalule, B.J., Nicol, M.P., McCulloch, M., Jacobs, C.A., *et al.* (2019) Shiga Toxin-Producing *Escherichia coli* O26:H11 Associated with a Cluster of Haemolytic Uraemic Syndrome Cases in South Africa, 2017. *Access Microbiology*, **1**, e000061.

[3] Rasooly, R., Do, P.M., Levin, C.E. and Friedman, M. (2010) Inhibition of Shiga Toxin 2 (Stx2) in Apple Juices and Its Resistance to Pasteurization. *Journal of Food Science*, **75**, M296-M301. https://doi.org/10.1111/j.1750-3841.2010.01615.x

[4] Rasooly, R., Do, P.M., Griffey, S.M., Vilches-Moure, J.G. and Friedman, M. (2010) Ingested Shiga Toxin 2 (Stx2) Causes Histopathological Changes in Kidney, Spleen and Thymus Tissues and Mortality in Mice. *Journal of Agricultural and Food Chemistry*, **58**, 9281-9286. https://doi.org/10.1021/jf101744z

[5] Hussein, H.S. (2007) Prevalence and Pathogenicity of Shiga Toxin-Producing *Escherichia coli* in Beef Cattle and Their Products. *Journal of Animal Science*, **85**, E63-E72. https://doi.org/10.2527/jas.2006-421

[6] Kamaruzzaman, E.A., Abdul Aziz, S., Bitrus, A.A., Zakaria, Z. and Hassan, L. (2020) Occurrence and Characteristics of Extended-Spectrum B-Lactamase-Producing *Escherichia coli* from Dairy Cattle, Milk, and Farm Environments in Peninsular Malaysia. *Pathogens*, **9**, Article No. 1007. https://doi.org/10.20944/preprints202009.0485.v1

[7] Ramos, S., Silva, V., Dapkevicius, M.d.L.E., Caniça, M., Tejedor-Junco, M.T., Igrejas, G., *et al.* (2020) *Escherichia coli* as Commensal and Pathogenic Bacteria among Food-Producing Animals: Health Implications of Extended Spectrum β-Lactamase (Esbl) Production. *Animals*, **10**, Article No. 2239. https://doi.org/10.3390/ani10122239

[8] Francis, G.A. and O'Beirne, D. (2001) Effects of Vegetable Type, Package Atmosphere and Storage Temperature on Growth and Survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Journal of Industrial Microbiology and Biotechnology*, **27**, 111-116. https://doi.org/10.1038/sj.jim.7000094

[9] Kiskó, G. and Roller, S. (2005) Carvacrol and *P*-Cymene Inactivate *Escherichia coli* O157:H7 in Apple Juice. *BMC Microbiology*, **5**, Article No. 36. https://doi.org/10.1186/1471-2180-5-36

[10] Kim, J., Marshall, M.R. and Wei, C.-I. (1995) Antibacterial Activity of Some Essential Oil Components against Five Foodborne Pathogens. *Journal of Agricultural and Food Chemistry*, **43**, 2839-2845. https://doi.org/10.1021/jf00059a013

[11] Dušan, F., Marián, S., Katarína, D. and Dobroslava, B. (2006) Essential Oils-Their Antimicrobial Activity against *Escherichia coli* and Effect on Intestinal Cell Viability. *Toxicology in Vitro*, **20**, 1435-1445. https://doi.org/10.1016/j.tiv.2006.06.012

[12] Burt, S.A., van der Zee, R., Koets, A.P., de Graaff, A.M., van Knapen, F., Gaastra, W., *et al.* (2007) Carvacrol Induces Heat Shock Protein 60 and Inhibits Synthesis of Flagellin in *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*, **73**, 4484-4490. https://doi.org/10.1128/AEM.00340-07

[13] Blanco, M., Blanco, J.E., Mora, A., Dahbi, G., Alonso, M.P., González, E.A., *et al.* (2004) Serotypes, Virulence Genes, and Intimin Types of Shiga Toxin (Verotoxin)-Producing *Escherichia coli* Isolates from Cattle in Spain and Identification of a New

Intimin Variant Gene (*Eae-ξ*). *Journal of Clinical Microbiology*, **42**, 645-651. https://doi.org/10.1128/JCM.42.2.645-651.2004

[14] Fraser, M.E., Fujinaga, M., Cherney, M.M., Melton-Celsa, A.R., Twiddy, E.M., O'Brien, A.D., *et al.* (2004) Structure of Shiga Toxin Type 2 (Stx2) from *Escherichia coli* O157: H7. *Journal of Biological Chemistry*, **279**, 27511-27517. https://doi.org/10.1074/jbc.M401939200

[15] Friedman, M., Henika, P.R. and Mandrell, R.E. (2002) Bactericidal Activities of Plant Essential Oils and Some of Their Isolated Constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection*, **65**, 1545-1560. https://doi.org/10.4315/0362-028X-65.10.1545

[16] Moore-Neibel, K., Gerber, C., Patel, J., Friedman, M. and Ravishankar, S. (2011) Antimicrobial Activity of Lemongrass Oil against *Salmonella enterica* on Organic Leafy Greens. *Journal of Applied Microbiology*, **112**, 485-492. https://doi.org/10.1111/j.1365-2672.2011.05222.x

[17] Todd, J., Friedman, M., Patel, J., Jaroni, D. and Ravishankar, S. (2013) The Antimicrobial Effects of Cinnamon Leaf Oil against Multi-Drug Resistant *Salmonella* Newport on Organic Leafy Greens. *International Journal of Food Microbiology*, **166**, 193-199. https://doi.org/10.1016/j.ijfoodmicro.2013.06.021

[18] Friedman, M. (2015) Antibiotic-Resistant Bacteria: Prevalence in Food and Inactivation by Food-Compatible Compounds and Plant Extracts. *Journal of Agricultural and Food Chemistry*, **63**, 3805-3822. https://doi.org/10.1021/acs.jafc.5b00778

[19] Yossa, N., Patel, J., Millner, P., Ravishankar, S. and Lo, Y.M. (2013) Antimicrobial Activity of Plant Essential Oils against *Escherichia coli* O157:H7 and *Salmonella* on Lettuce. *Foodborne Pathogens and Disease*, **10**, 87-96. https://doi.org/10.1089/fpd.2012.1301

[20] Poimenidou, S.V., Bikouli, V.C., Gardeli, C., Mitsi, C., Tarantilis, P.A., Nychas, G.-J., *et al.* (2016) Effect of Single or Combined Chemical and Natural Antimicrobial Interventions on *Escherichia coli* O157:H7, Total Microbiota and Color of Packaged Spinach and Lettuce. *International Journal of Food Microbiology*, **220**, 6-18. https://doi.org/10.1016/j.ijfoodmicro.2015.12.013

[21] Zhu, L., Olsen, C., McHugh, T., Friedman, M., Jaroni, D. and Ravishankar, S. (2014) Apple, Carrot, and Hibiscus Edible Films Containing the Plant Antimicrobials Carvacrol and Cinnamaldehyde Inactivate *Salmonella* Newport on Organic Leafy Greens in Sealed Plastic Bags. *Journal of Food Science*, **79**, M61-M66. https://doi.org/10.1111/1750-3841.12318

[22] Zhu, L., Olsen, C., McHugh, T., Friedman, M., Levin, C.E., Jaroni, D., *et al.* (2020) Edible Films Containing Carvacrol and Cinnamaldehyde Inactivate *Escherichia coli* O157:H7 on Organic Leafy Greens in Sealed Plastic Bags. *Journal of Food Safety*, **40**, e12758. https://doi.org/10.1111/jfs.12758

[23] Du, W.-X., Olsen, C.W., Avena-Bustillos, R.J., Friedman, M. and McHugh, T.H. (2011) Physical and Antibacterial Properties of Edible Films Formulated with Apple Skin Polyphenols. *Journal of Food Science*, **76**, M149-M155. https://doi.org/10.1111/j.1750-3841.2010.02012.x

[24] Espitia, P.J.P., Avena-Bustillos, R.J., Du, W.X., Chiou, B.S., Williams, T.G., Wood, D., *et al.* (2014) Physical and Antibacterial Properties of Açaí Edible Films Formulated with Thyme Essential Oil and Apple Skin Polyphenols. *Journal of Food Science*, **79**, M903-M910. https://doi.org/10.1111/1750-3841.12432

[25] Shoji, T., Yamada, M., Miura, T., Nagashima, K., Ogura, K., Inagaki, N., *et al.* (2017) Chronic Administration of Apple Polyphenols Ameliorates Hyperglycaemia in High-

Normal and Borderline Subjects: A Randomised, Placebo-Controlled Trial. *Diabetes Research and Clinical Practice*, **129**, 43-51. https://doi.org/10.1016/j.diabres.2017.03.028

[26] Shoji, T., Masumoto, S., Moriichi, N., Ohtake, Y. and Kanda, T. (2020) Administration of Apple Polyphenol Supplements for Skin Conditions in Healthy Women: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *Nutrients*, **12**, Article No. 1071. https://doi.org/10.3390/nu12041071

[27] Wang, J., Sun, Y., Tao, D., Wang, S., Li, C., Zheng, F., *et al.* (2019) Reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and Naturally Present Microbe Counts on Lettuce Using an Acid Mixture of Acetic and Lactic Acid. *Microorganisms*, **7**, Article No. 373. https://doi.org/10.3390/microorganisms7100373

[28] Peixoto, C.M., Dias, M.I., Alves, M.J., Calhelha, R.C., Barros, L., Pinho, S.P., *et al.* (2018) Grape Pomace as a Source of Phenolic Compounds and Diverse Bioactive Properties. *Food Chemistry*, **253**, 132-138. https://doi.org/10.1016/j.foodchem.2018.01.163

[29] Friedman, M. (2014) Antibacterial, Antiviral, and Antifungal Properties of Wines and Winery Byproducts in Relation to Their Flavonoid Content. *Journal of Agricultural and Food Chemistry*, **62**, 6025-6042. https://doi.org/10.1021/jf501266s

[30] Friedman, M., Henika, P.R., Levin, C.E., Mandrell, R.E. and Kozukue, N. (2006) Antimicrobial Activities of Tea Catechins and Theaflavins and Tea Extracts against *Bacillus cereus*. *Journal of Food Protection*, **69**, 354-361. https://doi.org/10.4315/0362-028X-69.2.354

[31] Friedman, M. (2007) Overview of Antibacterial, Antitoxin, Antiviral, and Antifungal Activities of Tea Flavonoids and Teas. *Molecular Nutrition & Food Research*, **51**, 116-134. https://doi.org/10.1002/mnfr.200600173

[32] Friedman, M., Tam, C.C., Cheng, L.W. and Land, K.M. (2020) Anti-Trichomonad Activities of Different Compounds from Foods, Marine Products, and Medicinal Plants: A Review. *BMC Complementary Medicine and Therapies*, **20**, Article No. 271. https://doi.org/10.1186/s12906-020-03061-9

[33] Friedman, M. (2017) Antimicrobial Activities of Plant Essential Oils and Their Components against Antibiotic-Susceptible and Antibiotic-Resistant Foodborne Pathogens. In: Rai, M., Zachino, S. and Derita, M.D., Eds., *Essential Oils and Nanotechnology for Treatment of Microbial Diseases*, CRC Press, Boca Raton, 14-38. https://doi.org/10.1201/9781315209241-2