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Antibacterial Activities of Plant Essential Oils and Their Components against *Escherichia coli* O157:H7 and *Salmonella enterica* in Apple Juice

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We evaluated 17 plant essential oils and nine oil compounds for antibacterial activity against the foodborne pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juices in a bactericidal assay in terms of % of the sample that resulted in a 50% decrease in the number of bacteria (BA_{50}). The 10 compounds most active against *E. coli* (60 min BA_{50} range in clear juice, 0.018−0.093%) were carvacrol, oregano oil, geraniol, eugenol, cinnamon leaf oil, citral, clove bud oil, lemongrass oil, cinnamon bark oil, and lemon oil. The corresponding compounds against *S. enterica* (BA_{50} range, 0.0044−0.011%) were Melissa oil, carvacrol, oregano oil, terpeineol, geraniol, lemon oil, citral, lemongrass oil, cinnamon leaf oil, and linalool. The activity (i) was greater for *S. enterica* than for *E. coli*, (ii) increased with incubation temperature and storage time, and (iii) was not affected by the acidity of the juices. The antibacterial agents could be divided into two classes: fast-acting and slow-acting. High-performance liquid chromatography analysis showed that the bactericidal results are related to the composition of the oils. These studies provide information about new ways to protect apple juice and other foods against human pathogens.

KEYWORDS: Antibacterial activities; apple juice; essential oils; food safety; *Escherichia coli* O157:H7; *Salmonella enterica*; HPLC

INTRODUCTION

Pathogenic strains of *Escherichia coli* such as O157:H7 and *Salmonella enterica* have been linked to foodborne illnesses, including some induced by contaminated apple juice (*1*). For example, numerous outbreaks of diarrhea and hemolytic uremic syndrome have been associated with the consumption of infected apple cider or apple juice (*2*−*6*). Pathogens may contaminate the juice by using apples that have fallen to the ground, by using cow manure to fertilize the orchards, or by improperly washing apples before processing (*7*, *8*). It is hypothesized that pathogens can adhere to and persist on the surface of apples. It has been shown that *E. coli* can enter deeper into the tissues of apples through the calyx during temperature changes associated with processing and as result of bruising and that these pathogens can be present on apples prior to harvesting them from trees (*9*−*12*). These considerations suggest the need to protect apple juice against contamination by pathogens.

E. coli O157:H7 appears to be the main organism implicated in these outbreaks, presumably because it is more tolerant of the acid pH of the juice than are other pathogens. Thus, although control strains of *E. coli* failed to grow in the juice or cider, O157:H7 grew well in both unpasteurized and pasteurized apple juice (*13*). In addition, *E. coli* O157:H7 survived better in preservative-free apple cider than did *Salmonella typhimurium*. *E. coli* O157:H7 in apple juice is reported to be susceptible to inactivation by pulsed electric fields (*14*), irradiation (*15*−*17*), organic acid (*18*, *19*), and heat (*20*−*24*) including that produced by microwaves (*25*). Some spoilage organisms in apple juice resist heat inactivation (*26*).

The use of heat and irradiation with fruit juices can both kill bacteria that are present and induce compositional and other changes in the juice (*16*, *27*−*29*). These effects in juices and the widespread production and consumption of unpasteurized apple juices, especially those designated organic juices, suggest the need to develop additional effective, food compatible, safe formulations to protect the juice and the consumer against contamination by pathogens.

Antimicrobial activities of essential oils have been extensively studied; for a review, see ref *30*. Previously, we used a bactericidal assay to test a large number of plant essential oils, oil constituents, and phenolic compounds against four human pathogens that may contaminate food (*31*−*33*). In the present study, we evaluate several parameters that influence bactericidal activities against *E. coli* O157:H7 and *S. enterica* of a selected group of active test substances in commercial clear Mott's apple juice with ascorbic acid (MAJA), in commercial cloudy Mott's apple juice without ascorbic acid (MAJ), and in freshly prepared

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juices from four apple varieties. The parameters, which were expected to influence bactericidal activity, were examined as follows: (i) pH effects, (ii) concentration effects, (iii) time−temperature effects, (iv) nature of antibacterial agent, and (e) source of apple juice.

MATERIALS AND METHODS

Test Compounds. Plant essential oils were purchased from Yerba Buena Company (Berkeley, CA); U. Ravid provided the Melissa oil. All other compounds came from Sigma (St. Louis, MO). The apples (Arkansas Black, Empire, Fuji, and Gala) and commercial apple juices used were obtained from a local store: Mott's brand "with 120% of vitamin C of daily requirement" and Mott's cloudy apple juice without ascorbic acid.

Citric Acid, Malic Acid, and Phosphate Buffers. The pH of 1% solutions of citric and malic acids in cloudy and clear apple juices was measured after dilution by one-third with apple juice (pH = ~3.0 for all four solutions). This initial dilution corresponds to the procedure used to prepare antibacterial assay mixtures. The diluents used were MAJ and MAJA (both pH 3.7) in series diluted by one-fourth. A second set of solutions was adjusted to pH 3.7 with 1 N NaOH prior to dilutions, and this pH was maintained throughout this dilution series. The pH in the unadjusted samples of MAJ or MAJA with citric or malic acids ranged from ~pH 3.0 to 3.5 from the highest to the lowest concentrations of the dilution series. Phosphate-buffered saline (PBS, pH 7.0) was prepared by mixing dibasic sodium phosphate and monobasic sodium phosphate (100 mM) at a 2:1 ratio, diluting by half with H_2O, and adding NaCl (150 mM).

Preparation of Fresh Apple Juices. The apples were juiced using an Omega (Harrisburg, PA) model 9000 centrifugal batch juicer. The apples were washed in lukewarm tap water and patted dry. They were then cut into pieces without removing any of the skin or core and inserted into the juicer. The juicer was fitted with a coarse paper filter supplied by Omega. The juice was collected and centrifuged at 10000 rcf for 10 min. The supernatant was filtered through a 0.45 μM nylon membrane (Sigma-Aldrich) with a 1.2 μM glass microfiber prefilter (Whatman, United Kingdom) under vacuum, changing the filters as they became clogged. The juices were stored under refrigeration.

Sources of Bacteria. The following bacteria were used in this study (*31*): the Food and Drug Administration provided *E. coli* O157:H7 (strain SEA18B88), isolated from apple juice associated with an outbreak of human contamination. *S. enterica* serovar Hadar (strain MH136) was isolated from ground turkey.

Preparation of Samples for Bactericidal Assays. A shaking method was used to prepare suspensions of oils in apple juices. The shaking method was performed in 10 mL Erlenmeyer flasks containing 4.95 mL of juice and 50 μL of oil. The flask was shaken by hand for 5 s, the procedure was repeated three times, and ~0.5 mL was transferred into a sterile 1.9 mL microcentrifuge tube for dilution. A sample (200 μL) of the oil suspension was drawn immediately after shaking and added to 600 μL of apple juice for a one-fourth dilution. The dilution was shaken before adding another 200 μL of this sample to the next tube in the dilution series.

Bactericidal Assays. The previously described bactericidal assay (*31*−*33*) was adapted for apple juice. Briefly, *E. coli* or *S. enterica* organisms stored on streaked plates were subcultured by streaking onto Luria−Bertani agar (LB) plates (Difco, Sparks, MD). The plates were incubated at 37 °C for 24 h. Isolated colonies from the plate were harvested by sterile loop and suspended in 5 mL of LB broth in a 15 mL sterile plastic tube. The tube was capped and incubated with shaking (150 rpm) at 37 °C for 18 h.

A 1 mL sample of an 18 h broth culture of *E. coli* or *S. enterica* was added to a 1.9 mL microfuge tube, and the bacteria were pelleted by centrifugation in a microfuge at 14000 rpm for 1 min. After the supernatant was removed, 1 mL of sterile PBS was added to the pellet and the pellet was resuspended by gentle aspiration in and out of a transfer pipet. The optical density (OD) was then determined at 620 nm. The OD of the sample was adjusted to ~0.8 by dilutions with PBS. The samples produced colony-forming units (CFU) of ~150 per lane on the square plates used for counting. The sample (10 μL) was added to 990 μL of PBS (1:100); 40 μL of the 1:100 sample was then added to 10 mL of juice.

Juice (100 μL) with or without the test substance was added to each well in a 96 well tissue culture microtiter plate (Nalge, Nunc, Rochester, NY) followed by the addition of 50 μL of bacteria suspension. In a typical experiment used, six wells each were used for the negative control (apricot oil), six wells each were used for a positive control (cinnamaldehyde or carvacrol), and four wells each were used for six test compounds in the clear or cloudy juices.

The wells on the plate were covered with a SealPlate cover (Marsh, Rochester, NY) and sealed on the outside edges with Parafilm to minimize any effects of volatile oils on other wells. The microtiter plates were sampled immediately (21 °C) or incubated with gentle shaking. In time−course studies, a microtiter plate with a matched control and dilutions of a test substance was prepared near each incubation time (~5, 30, 60, or 120 min).

Following incubation, a 20 μL sample from each well was spotted at the top of a square plate with grids containing LB agar for *E. coli* or *S. enterica*. The plates were then placed uncovered for 10 min in a biological safety hood until the sample liquid dried. Covered *E. coli* or *S. enterica* plates were incubated overnight at 37 °C. The colonies of *E. coli* or *S. enterica* strains on LB agar were then counted after 18−24 h. The experiments were done in duplicate using two pellets processed from one species. CFUs were enumerated for each streak using a colony counter.

Bactericidal Activities (BA_{50} Values). Bactericidal activities are defined as the % of test compound that kills 50% of the bacteria under the test conditions, determined as follows: Each compound was tested at a series of six dilutions, typically from 0.00065 to 0.67% in the reaction mixture. The CFU values from all experiments were transferred to a Microsoft Excel 8.0 Spreadsheet. The number of CFU from each dilution was matched with the average control value to determine the percent of bacteria killed per well. Each of the dose−response profiles (% test compound vs % bactericidal activity) was examined graphically, and the BA_{50} values were estimated by a linear regression (*34*). The lower the BA_{50} or the higher the $1/BA_{50}$ value, the higher the activity.

High-Performance Liquid Chromatography (HPLC) of Plant Essential Oils. Selected essential oils analyzed by HPLC for their content of bactericidal compounds were cinnamon, clove bud, lemongrass, bitter orange, orange Mandarin, sweet orange, lemon, tangerine oil, lime oil, grapefruit oil, and palmarosa. The HPLC system consisted of a Beckman 110B pump, a Thermo Separation Products AS3500 Autosampler (loop size 100 μL), and a UV 3000HR scanning detector with both deuterium and tungsten lamps. The system was controlled by Thermo Separation Products PC1000 System Software. The following conditions were used for the analysis of most of the oil components. A Supelco LC-ABZ column was used (250 mm × 4.6 mm plus a 2 cm precolumn). The particle size of the column packing was 5 μM. The eluent consisted of 50% acetonitrile, 50 mM ammonium phosphate, and 0.05% phosphoric acid, pH 3.1. The eluent was degassed once before use. The flow rate of the pump was 1 mL/min, and the sample volume injected was 20 μL. The absorption was monitored at three wavelengths at the λ_{max} of the compounds of interest: perillaldehyde, 235 nm; citral, 240 nm; cinnamaldehyde; and eugenol, 280 nm. A calibration curve was run daily using duplicate injections. For limonene, we used a Hypercarb column by ThermoQuest with an eluent consisting of 60% acetonitrile, 40 mM ammonium phosphate, and 0.05% phosphoric acid, pH 3.1. The absorbance was monitored at 195 nm; all other conditions were the same.

HPLC Method for Miscibility of Carvacrol and Cinnamaldehyde in Apple Juice. Neat carvacrol or cinnamaldehyde was pipetted into 50 mL of room temperature Mott's clear apple juice in a 60 mL separatory funnel. The funnel was vigorously shaken for 1 min. The funnel was allowed to sit for ~24 h. A sample was drawn off the bottom for carvacrol (less dense than water) and off the top for cinnamaldehyde (denser than water). The levels of carvacrol and cinnamaldehyde were determined by HPLC as described earlier. Concentrations of 0.025 and 0.05% were analyzed directly. The levels of 0.1% and higher were diluted 1:10 with apple juice before HPLC analysis. A standard curve was run by dissolving carvacrol or cinnamaldehyde in 50% ethanol.

Table 1. Effect of Concentration and Time/Temperature on Bactericidal Activities (BA_{50} Values) of Essential Oils and Oil Compounds against *E. coli* O157:H7 and *S. enterica* in Cloudy and Clear Mott's Apple Juices and in Freshly Prepared Juices from Arkansas Black, Empire, Gala, and Fuji Apples

	BA_{50} value[a] for *E coli*			BA_{50} value for *S. enterica*		
	5 min; 21 °C	60 min; 37 °C	120 min; 37 °C	5 min; 21 °C	60 min; 37 °C	120 min; 37 °C
			concentration			
carvacrol 0.67%;[b] MAJ[c]	0.058 ± 0.04[d]	0.0088 ± 0.0006	0.0080 ± 0.0002	0.046 ± 0.06	0.0068 ± 0.0001	0.0060 ± 0.0001
carvacrol 0.067%; MAJ	0.038 ± 0.004	0.0082 ± 0.0006	0.0096 ± 0.001	0.023 ± 0.02	0.0089 ± 0.0006	0.0084 ± 0
carvacrol 0.67%; MAJA[e]	0.11 ± 0.006[f]	0.023 ± 0.002[f]	0.022 ± 0.003[f]	0.048 ± 0.02[f]	0.0062 ± 0.001[f]	0.0049 ± 0.0002
carvacrol 0.067%; MAJA	0.039 ± 0.004[f]	0.0095 ± 0.0007[f]	0.0088 ± 0.0007[f]	0.026 ± 0.01[f]	0.0025 ± 0.0003[f]	0.0022 ± 0.0004
cinnamaldehyde 0.67%; MAJ	0.40 ± 0.1	0.088 ± 0.01	0.032 ± 0.01	0.38 ± 0.08	0.023 ± 0.004	0.015 ± 0.01
cinnamaldehyde 0.067%; MAJ	>0.067	0.037 ± 0.0007	0.035 ± 0.005	>0.067	0.021 ± 0.02	0.012 ± 0.008
cinnamaldehyde 0.67%; MAJA	>0.67	0.11 ± 0.004	0.079 ± 0.01	>0.67	0.019 ± 0.001	0.016 ± 0.003
cinnamaldehyde 0.067%; MAJA	>0.067	0.039 ± 0.002	0.037 ± 0.0007	>0.067	0.0086 ± 0.0005	0.0082 ± 0.002
			apple varieties			
carvacrol; ABAJ;[g] 0.13%	0.036 ± 0.02[h]	0.019 ± 0.001[h]	0.020 ± 0.001[h]	0.014 ± 0.005[h]	0.0051 ± 0.0004[h]	0.0044 ± 0.0008[h]
carvacrol; ABAJF;[g] 0.13%	0.021 ± 0.001[h]	0.018 ± 0.001	0.013 ± 0.001	0.022 ± 0.001	0.0050 ± 0.0001	0.0052 ± 0.0002
cinnamaldehyde; ABAJ; 0.34%	0.24 ± 0.05[h]	0.053 ± 0.004[h]	0.093 ± 0.08[h]	0.18 ± 0.03[h]	0.047 ± 0.003[h]	0.031 ± 0.02[h]
cinnamaldehyde; ABAJF; 0.34%	0.29 ± 0.01	0.050 ± 0.002	0.049 ± 0.001	0.23 ± 0.02	0.049 ± 0.002	0.049 ± 0.001
carvacrol; EMAJ;[i] 0.13%	0.020 ± 0	0.012 ± 0.01	0.0062 ± 0.001	0.018 ± 0.003	0.0053 ± 0.0006	0.0051 ± 0.0001
carvacrol; EMAJF;[i] 0.13%	0.023 ± 0.003	0.011 ± 0.007	0.0099 ± 0.006	0.019 ± 0.003	0.0052 ± 0.0001	0.0056 ± 0.0001
cinnamaldehyde; EMAJ; 0.34%	0.24 ± 0.035	0.044 ± 0.002	0.046 ± 0.0007	0.20 ± 0.02	0.050 ± 0.002	0.047 ± 0.006
cinnamaldehyde; EMAJF; 0.34%	0.22 ± 0.01	0.046 ± 0.004	0.048 ± 0.001	0.18 ± 0	0.042 ± 0	0.015 ± 0.002
carvacrol; FJAJ;[j] 0.13%	0.020 ± 0.003	0.020 ± 0.002	0.016 ± 0	0.017 ± 0.002	0.019 ± 0.0007	0.011 ± 0.004[h]
carvacrol; FJAJF;[j] 0.13%	0.031 ± 0.004	0.015 ± 0.001	0.016 ± 0.004	0.018 ± 0.002	0.010 ± 0.003	0.0073 ± 0.002
cinnamaldehyde; FJAJ; 0.34%	0.21 ± 0.02	0.056 ± 0.003	0.048 ± 0.002	0.19 ± 0	0.048 ± 0.001	0.046 ± 0.004[h]
cinnamaldehyde; FJAJF; 0.34%	0.29 ± 0.09	0.054 ± 0.006	0.045 ± 0.003	0.17 ± 0.007	0.045 ± 0.002	0.048 ± 0.003
carvacrol; GLAJ;[k] 0.13%	0.043 ± 0.06[l]	0.0097 ± 0.007[l]	0.0095 ± 0.007[l]	0.018 ± 0.003[l]	0.0054 ± 0.0005[l]	0.0051 ± 0.0006[l]
carvacrol; GLAJF;[k] 0.13%	0.022 ± 0.002	0.011 ± 0.003	0.0072 ± 0.001	0.020 ± 0	0.0050 ± 0.0006	0.0050 ± 0.0004
cinnamaldehyde; GLAJ; 0.34%	0.20 ± 0.02[l]	0.050 ± 0.002[l]	0.037 ± 0.01[l]	0.20 ± 0.02	0.050 ± 0.002	0.036 ± 0.01
cinnamaldehyde; GLAJF; 0.34%	0.21 ± 0.007	0.053 ± 0.003	0.044 ± 0.0007	0.19 ± 0.02	0.045 ± 0.003	0.016 ± 0.002
carvacrol; MAJ; 0.13%	0.027 ± 0.008[m]	0.013 ± 0.01[n]	0.0075 ± 0.002[o]	0.023 ± 0.004[o]	0.0057 ± 0.001[h]	0.0039 ± 0.002[h]
carvacrol; MAJF; 0.13%	0.020 ± 0.001	0.012 ± 0.005	0.0067 ± 0.0001	0.020 ± 0	0.0046 ± 0.0002	0.0049 ± 0.0002
cinnamaldehyde; MAJ; 0.34%	0.29 ± 0.10[p]	0.067 ± 0.025[q]	0.039 ± 0.01[r]	0.22 ± 0.11[o]	0.021 ± 0.01[h]	0.016 ± 0.005[h]
cinnamaldehyde; MAJF; 0.34%	0.19 ± 0.02	0.032 ± 0.01	0.020 ± 0.003	0.18 ± 0.01	0.016 ± 0.004	0.011 ± 0.001

[a] BA_{50}, % test substance causing 50% kill of bacteria. [b] Percent in well. [c] MAJ, Mott's apple juice; cloudy, pH 3.7. MAJF, filtered MAJ. [d] $n = 2$, unless otherwise indicated. [e] MAJA; clear, pH 3.7. [f] Percent in well. [g] ABAJ, Arkansas Black apple juice; ABAJF, filtered Arkansas Black apple juice. [h] $n = 4$. [i] EMAJ, Empire apple juice; EMAJF, filtered EMAJ. [j] FJAJ, Fuji apple juice; FJAJF, filtered FJAJ. [k] GLAJ, Gala apple juice; GLAJF, filtered GLAJ. [l] $n = 6$. [m] $n = 16$. [n] $n = 14$. [o] $n = 8$. [p] $n = 12$. [q] $n = 11$. [r] $n = 7$.

RESULTS AND DISCUSSION

Apricot oil was used as the negative control because it was inactive in both clear and cloudy juices. Because of our extensive experience with carvacrol and cinnamaldehyde, these two compounds were used as positive controls. **Tables 1** and **2** list the experimental BA_{50} values for 26 essential oil/oil compounds. To facilitate quick comparison of activities for practical use, the calculated $1/BA_{50}$ values are plotted as bar graphs in **Figures 1−4**. These bar graphs illustrate the relative potencies of the test substances against the two pathogens in clear and cloudy apple juices.

Effect of pH. Because the acid pH of apple juice could contribute to the antibacterial activities of the test compounds, especially with *S. enterica* (*35*), we carried out a series of studies designed to define this parameter (results not shown). Both *E. coli* and *S. enterica* organisms survived exposure up to 2 h to apple juice solutions whose pH was adjusted with either citric or malic acid to pH 3.7, respectively. *E. coli* also survived exposure to pH 2.8−3.0, whereas there was a low level of kill of *S. enterica* ($BA_{50} = 0.31-0.39$) in the lower pH citric and malic acid solutions. The acid pH of the apple juices does not seem to significantly contribute to bactericidal activities.

Concentration−Solubility Effects. We used two stock solutions to evaluate the effect of concentration of active carvacrol and cinnamaldehyde on bactericidal activity (**Table 1** and **Figure 1**): 0.67 and 0.067%. The lower concentrations of both compounds were miscible with apple juice and highly bactericidal. Thus, the 60 min BA_{50} value for *E. coli* (0.0088) resulting from a dilution series of 0.67% suspension of carvacrol in cloudy apple juice is similar to the corresponding value of a 0.067% dilution series of carvacrol solutions in the cloudy juice (0.0082). Surprisingly, the corresponding *E. coli* BA_{50} value for the 0.67% cinnamaldehyde dilution series (0.088) is about two times higher (less active) than the BA_{50} values from the 0.067% dilution series (0.037). These observations suggest that solubility/miscibility of the active compound in juice are key factors influencing activity.

In the case of suspensions, it appears that the amount miscible with the juice exerts the major influence on activity. Similar observations were noted with the clear juice, where in terms of % stock solution: *E. coli* BA_{50}, for carvacrol = 0.67:0.023 and 0.067:0.0095, and for cinnamaldehyde = 0.67:0.11 and 0.067:0.039, respectively (**Table 1**). The lower concentrations used in this study are similar to the observed miscibilities of carvacrol and cinnamaldehyde discussed below.

Activities in Cloudy and Clear Commercial and in Fresh Juices. Tables 1 and **2** and **Figures 1−4** show that with some exceptions, the activities were lower in the cloudy as compared to the clear juice. Some of the antibacterial agent may become adsorbed to the surface of the apple pulp in the cloudy juice, possibly lowering its effective concentration in the juice.

The 60 min *E. coli* BA_{50} values of carvacrol in fresh juices prepared from four apple varieties (Arkansas Black, Empire, Fuji, Gala) ranged from 0.0097 for Gala apple juice (GLAJ) to

Table 2. Bactericidal Activities (BA_{50} values) of Oils and Oil Compounds against *E. coli* O157:H7 and *S. enterica* in Cloudy (MAJ) or in Clear (MAJA) Mott's Apple Juices Incubated for 5 min at 21 °C and for 30 and 60 min at 37 °C

	BA_{50} value[a] for *E coli*			BA_{50} value for *S. enterica*		
oil/oil compound	~5 min; 21 °C	30 min; 37 °C	60 min; 37 °C	~5 min; 21 °C	30 min; 37 °C	60 min; 37 °C
apricot oil; MAJ[b]	>0.67[c,d]	>0.67	>0.67	>0.67	>0.67	>0.67
apricot oil; MAJA[e]	>0.67[f]	>0.67[f]	>0.67[f]	>0.67	>0.67	>0.67
bergamot oil; MAJ	>0.67	0.26 ± 0.19	0.28 ± 0.19	0.54[g]	0.096 ± 0.006	0.078 ± 0.004
bergamot oil; MAJA	0.58[g]	0.20 ± 0.16[f]	0.21 ± 0.16[f]	0.33 ± 0.25	0.028 ± 0.004	0.027 ± 0.001
carvacrol; MAJ	0.027 ± 0.008[h]	0.014 ± 0.004[i]	0.013 ± 0.01[j]	0.023 ± 0.004[k]	0.0063 ± 0.001[i]	0.0057 ± 0.001[i]
carvacrol; MAJA	0.033 ± 0.02[k]	0.017 ± 0.01[i]	0.018 ± 0.01[l]	0.017 ± 0.006[m]	0.0055 ± 0.002[m]	0.0045 ± 0.0006[m]
cinnamaldehyde; MAJ	0.29 ± 0.10[n]	0.10 ± 0.002[i]	0.067 ± 0.025[o]	0.22 ± 0.11[k]	0.027 ± 0.004	0.021 ± 0.001[i]
cinnamaldehyde; MAJA	0.27 ± 0.23[j]	0.097 ± 0.002	0.094 ± 0.04[p]	0.12 ± 0.006	0.092 ± 0.05	0.018 ± 0.02[f]
cinnamon bark oil; MAJ	>0.67	0.096 ± 0.001	0.097 ± 0.005	0.38 ± 0.07	0.039 ± 0.003	0.030 ± 0.0007
cinnamon bark oil; MAJA	0.40 ± 0.04[i]	0.099 ± 0.005	0.089 ± 0.008[j]	0.22 ± 0.18[l]	0.024 ± 0.003	0.014 ± 0.01[j]
cinnamon Cassia oil; MAJ	0.46 ± 0.01	0.097 ± 0.007	0.092 ± 0.006	0.14 ± 0.04	0.024 ± 0.007	0.025 ± 0.003
cinnamon Cassia oil; MAJA	0.46 ± 0.08[j]	0.098 ± 0.004	0.10 ± 0.03[j]	0.21 ± 0.08[j]	0.023 ± 0.001	0.018 ± 0.005[f]
cinnamon leaf oil; MAJ	0.13 ± 0.03	0.094 ± 0.005	0.098 ± 0.0007	0.10 ± 0.007	0.025 ± 0.007	0.026 ± 0
cinnamon leaf oil; MAJA	0.26 ± 0.18[j]	0.092 ± 0.001	0.065 ± 0.03[j]	0.076 ± 0.03[l]	0.021 ± 0.002	0.010 ± 0.01[l]
citral; MAJ	0.49 ± 0[q]	0.10 ± 0.01	0.098 ± 0.02[l]	0.31 ± 0.19[q]	0.027 ± 0.0007	0.025 ± 0.003[j]
citral; MAJA	0.20 ± 0.15[l]	0.10 ± 0.003	0.071 ± 0.02[l]	0.088 ± 0.03[l]	0.0047 ± 0.003	0.0084 ± 0.003[j]
clove bud oil; MAJ	0.27 ± 0.16	0.10 ± 0.002	0.098 ± 0.004	0.11 ± 0.003	0.026 ± 0.004	0.026 ± 0.001
clove bud oil; MAJA	0.26 ± 0.19[j]	0.091 ± 0.003	0.075 ± 0.002[j]	0.10 ± 0.005[j]	0.024 ± 0.002	0.019 ± 0.007[j]
eugenol; MAJ	0.15 ± 0.05[j]	0.089 ± 0.007[j]	0.061 ± 0.003[j]	0.076 ± 0.004	0.025 ± 0.002	0.023 ± 0.001
eugenol; MAJA	0.29 ± 0.22[j]	0.060 ± 0.03[l]	0.050 ± 0.02[k]	0.11 ± 0.007[j]	0.021 ± 0.001	0.012 ± 0.002
geraniol; MAJ	0.11 ± 0.01[f]	0.057 ± 0.05[f]	0.026 ± 0.004[f]	0.10 ± 0.007	0.023 ± 0	0.020 ± 0
geraniol; MAJA	0.089 ± 0.02[l]	0.027[g]	0.025 ± 0.006[m]	0.031 ± 0.006[j]	0.0048 ± 0	0.0069 ± 0.004[l]
grapefruit oil; MAJ	>0.67	0.25 ± 0.19	0.12 ± 0.04	0.45[g]	0.057 ± 0.04	0.064 ± 0.04
grapefruit oil; MAJA	>0.67	0.25 ± 0.19	0.16 ± 0.07	0.26 ± 0.10	0.018 ± 0.01	0.014 ± 0.009
lavender oil; MAJ	>0.67	0.25 ± 0.21	0.26 ± 0.23	0.30 ± 0.18	0.11 ± 0.006	0.10 ± 0.001
lavender oil; MAJA	>0.67	0.23 ± 0.18	0.25 ± 0.21	0.32 ± 0.02	0.025 ± 0.002	0.020 ± 0.001
lemon oil; MAJ	>0.67[k]	0.12 ± 0.01[k]	0.11 ± 0.01[k]	0.51 ± 0.16[p]	0.022 ± 0	0.021 ± 0
lemon oil; MAJA	>0.67[f]	0.19 ± 0.08[f]	0.093 ± 0.06[r]	0.24 ± 0.19[r]	0.018 ± 0.01[j]	0.0081 ± 0.01[s]
lemongrass oil; MAJ	>0.67	0.094 ± 0.01	0.098 ± 0.007	0.36 ± 0.01	0.027 ± 0.001	0.028 ± 0.004
lemongrass oil; MAJA	0.12 ± 0.01[j]	0.096 ± 0.004	0.079 ± 0.02[j]	0.11 ± 0.01[j]	0.0074 ± 0.0002	0.0097 ± 0.007[j]
lime oil; MAJ	>0.67	0.25 ± 0.23	0.23 ± 0.21	0.35 ± 0.21	0.062 ± 0.04	0.058 ± 0.04
lime oil; MAJA	>0.67	0.27 ± 0.23	0.23 ± 0.21	0.38 ± 0.07	0.038 ± 0.02	0.017 ± 0.01
linalool; MAJ	0.32 ± 0.09	0.25 ± 0.22	0.24 ± 0.19	0.13 ± 0.04	0.10 ± 0.001	0.10 ± 0.004
linalool; MAJA	0.42 ± 0.04	0.22 ± 0.18	0.25 ± 0.25	0.11 ± 0.002	0.020 ± 0.01	0.011 ± 0.001
linalyl acetate; MAJ	>0.67	>0.67	>0.67	>0.67	>0.67	>0.67
linalyl acetate; MAJA	>0.67	0.54[g]	0.50[g]	>0.67	0.031 ± 0.004	0.026 ± 0.003
Melissa oil; MAJ	>0.67	0.28 ± 0.16	0.27 ± 0.20	>0.67	0.058 ± 0.04	0.059 ± 0.04
Melissa oil; MAJA	>0.67	0.26 ± 0.18	0.24 ± 0.22	0.56[g]	0.0083 ± 0.002	0.0044 ± 0.003
orange bitter oil; MAJ	>0.67	0.23 ± 0.20	0.11 ± 0.02	>0.67	0.035 ± 0.008	0.037 ± 0.008
orange bitter oil; MAJA	0.44 ± 0.04[j]	0.26 ± 0.21	0.19 ± 0.13[j]	0.30 ± 0.21[j]	0.016 ± 0.006	0.019 ± 0.02[j]
orange Mandarin oil; MAJ	>0.67	0.11 ± 0.01	0.10 ± 0.001	0.44 ± 0.007	0.086 ± 0.006	0.062 ± 0.02
orange Mandarin oil; MAJA	0.42 ± 0.02[j]	0.25 ± 0.19	0.19 ± 0.13[l]	0.23 ± 0.16	0.022 ± 0.001	0.017 ± 0.01
orange sweet oil; MAJ	>0.67	0.12 ± 0.04	0.070 ± 0.04	>0.67	0.097 ± 0.002	0.087 ± 0.01
orange sweet oil; MAJA	>0.67	0.25 ± 0.21	0.26 ± 0.23	0.58[g]	0.034 ± 0.02	0.023 ± 0.01
oregano Spanish oil; MAJ	0.089 ± 0.02[j]	0.016 ± 0.007[j]	0.016 ± 0.009[j]	0.027 ± 0.0007	0.0060 ± 0.0001	0.0066 ± 0.0005
oregano Spanish oil; MAJA	0.076 ± 0.04[m]	0.024 ± 0.001[l]	0.023 ± 0.001[k]	0.017 ± 0.009[l]	0.0062 ± 0.0006	0.0060 ± 0.0008
tangerine oil; MAJ	>0.67	0.25 ± 0.24	0.23 ± 0.21	>0.67	0.068 ± 0.05	0.064 ± 0.04
tangerine oil; MAJA	>0.67	0.27 ± 0.26	0.29 ± 0.26	>0.67	0.062 ± 0.05	0.037 ± 0.02
terpinene; MAJ	>0.67	>0.67	>0.67	>0.67[j]	0.097 ± 0.01[j]	0.083 ± 0.04[l]
terpinene; MAJA	>0.67	>0.67	>0.67	>0.67[j]	0.080 ± 0.03[j]	0.059 ± 0.04
terpinen-4-ol; MAJ	0.39 ± 0.007	0.27 ± 0.22	0.10 ± 0	0.23 ± 0.14[j]	0.098 ± 0.009	0.087 ± 0.03[j]
terpinen-4-ol; MAJA	0.38 ± 0.03	0.089 ± 0.001	0.097 ± 0.02	0.11 ± 0.005	0.0056 ± 0.002	0.0064[g]

[a] BA_{50}, % test substance causing 50% kill of bacteria. [b] MAJ; cloudy; pH 3.7. [c] Values of >0.67 indicate inactivity. [d] $n = 2$, unless otherwise indicated. [e] MAJA; clear; pH 3.7. [f] $n = 3$. [g] $n = 1$. [h] $n = 16$. [i] $n = 4$. [j] $n = 14$. [k] $n = 8$. [l] $n = 6$. [m] $n = 10$. [n] $n = 12$. [o] $n = 11$. [p] $n = 5$. [q] $n = 9$. [r] $n = 7$. [s] $n = 13$.

0.02 for Fuji juice (FJAJ). The corresponding range for cinnamaldehyde was from 0.044 for Empire juice (EMAJ) to 0.056 for FJAJ. For *S. enterica*, the values for carvacrol ranged from 0.019 for FJAJ to 0.0054 for GLAJ, and for cinnamaldehyde, they ranged from 0.047 for Arkansas Black juice to 0.050 for both EMAJ and GLAJ (**Table 1**). In some cases, apple variety may have influenced antibacterial activities of carvacrol and cinnamaldehyde tested under the same conditions.

Effect of Incubation Temperature and Time on Bactericidal Activities. Figure 3 shows that bactericidal activity increased with incubation temperature at the three temperatures tested (4, 21, and 37 °C) and with time at the three time periods tested (5, 60, and 120 min). The antibacterial activity at 37 °C was in most cases about three times greater than at the lower temperatures. Both *E. coli* and *S. enterica* were viable when incubated in a refrigerator for up to 2 h.

Also noteworthy is the fact that the data on the temperature dependence of activity revealed that carvacrol, cinnamaldehyde, citral, and thyme oil kill pathogens at a refrigeration temperature (4 °C). This observation suggests that by adding a few drops of one of these compounds, consumers may be able to protect unpasteurized apple juice stored in a refrigerator against contamination by human pathogens such as *E. coli* and *Salmonella*. Expectations are that these natural antimicrobials will continue to inactivate the pathogens in the juice stored for longer periods in a refrigerator.

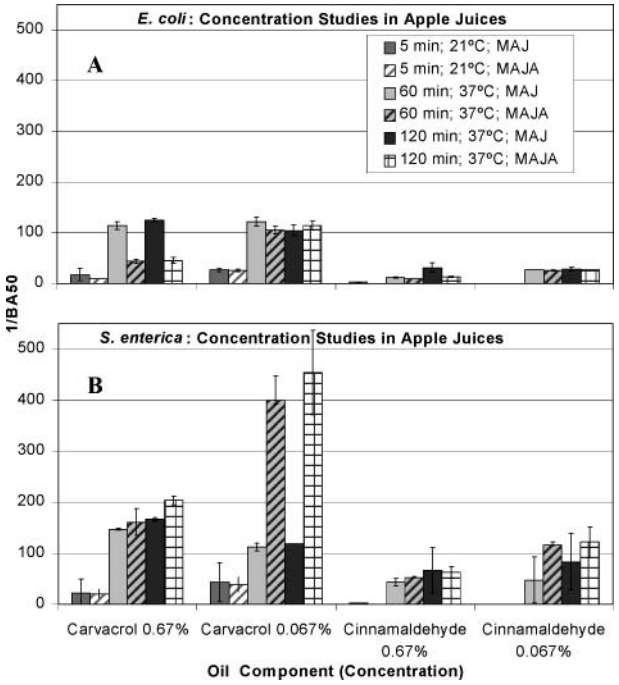

Figure 1. Effect of concentration of carvacrol and cinnamaldehyde on bactericidal activities ($1/BA_{50}$ values calculated from 60 min BA_{50} values listed in **Table 1**) in cloudy MAJ and clear MAJA.

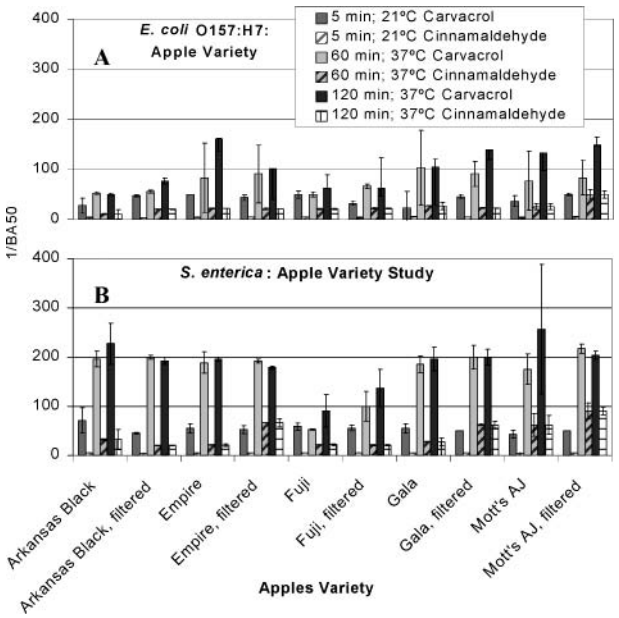

Figure 2. Time/temperature dependence of bactericidal activities ($1/BA_{50}$ values calculated from 60 min BA_{50} values listed in **Table 1**) of carvacrol and cinnamaldehyde in unfiltered and filtered apple juices prepared from four varieties of apples.

For both *E. coli* and *S. enterica*, the data show that (i) carvacrol, cinnamaldehyde, geraniol, linalool, and terpinen-4-ol killed the bacteria almost on contact (5 min); (ii) inactivation increased with time of contact; and (iii) most test compounds were effective after 30 or 60 min (**Tables 1** and **2**). The rapid death of the bacteria induced by some of the compounds during the first few minutes suggests that the test substances consist of two types of bactericidal agents: fast-acting and slow-acting. The fast-acting compounds could be used in the home to disinfect apple juice prior to consumption.

Another useful observation is that a number of sensory compatible citrus oils (grapefruit, lemon, lime, bitter orange, orange Mandarin, sweet orange, and tangerine) were bactericidal against both pathogens, especially at 37 °C. Expectations are that organoleptic studies will show that these oils as well as cinnamon oil would be compatible with apple juice and possibly other juices in terms of sensory properties including flavor, taste, and color. Because these oils are derived from citrus products and cinnamon oil is already used in food (*36*), it may be safe to include them in small amounts in apple or other juices.

For *S. enterica* (with a 60 min BA_{50} range for clear juice from 0.0044 to 0.059, **Table 2**), the data show similar trends but much greater activities than with *E. coli* (corresponding BA_{50} range from 0.018 to 0.50). At 5 min and 21 °C, the most

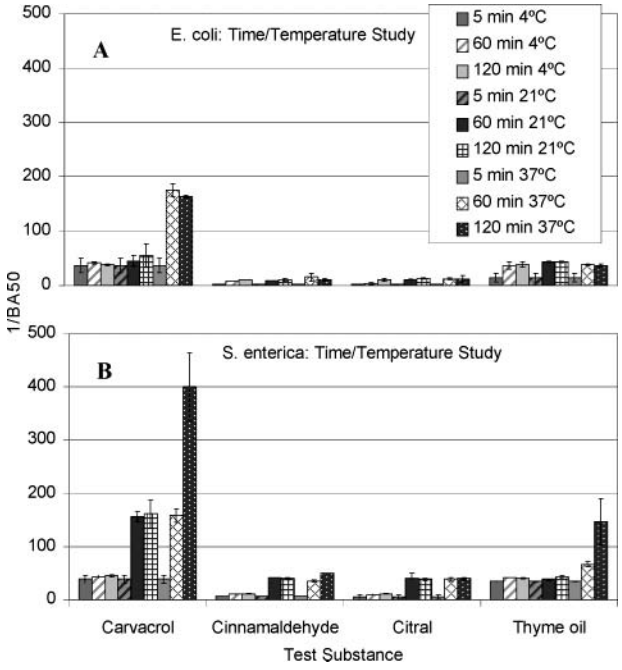

Figure 3. Time/temperature dependence of bactericial activities ($1/BA_{50}$ values calculated from BA_{50} values not shown in **Table 1**) of carvacrol, cinnamaldehyde, citral, and thyme oil in cloudy MAJ.

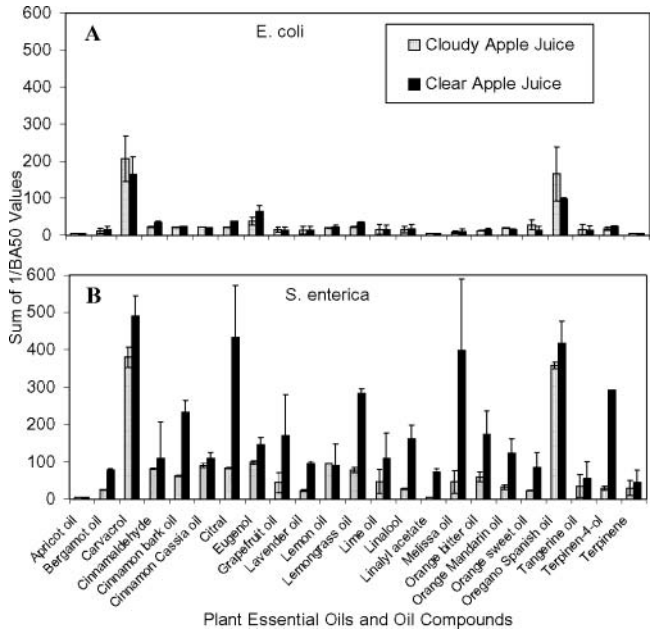

Figure 4. Plots of sum of reciprocal values of bactericidal activities (sum of all $1/BA_{50}$ calculated from BA_{50} values shown in **Table 2**) of active oils and oil compounds against *E. coli* and *S. enterica* in cloudy and clear apple juices.

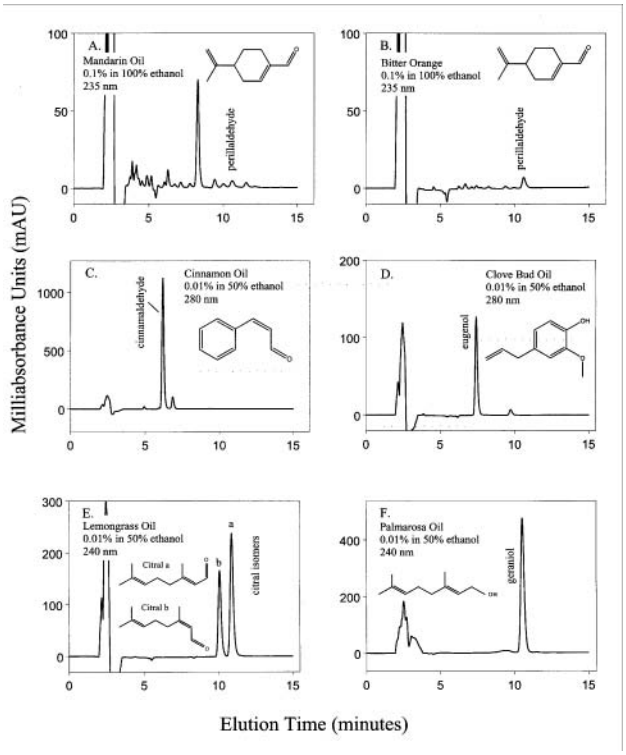

Figure 5. HPLC of 6 bactericidal compounds evaluated in this study: eugenol (clove oil), cinnamaldehyde (cinnamon oil), citral, two isomers (lemongrass oil), and geraniol.

effective antibacterial agent was carvacrol, whereas the citrus oils or oil constituents were less active. Activities of the citrus oil increased significantly at 60 min and 37 °C. A striking result is the nearly 200-fold greater activity of Melissa oil against *S. enterica* ($BA_{50} = 0.0044$) as compared to *E. coli* ($BA_{50} = 0.24$) (**Table 2**).

Comparison of Activities of Essential Oils and Oil Compounds. Figure 5 depicts HPLC chromatograms of 6 essential oils. These and HPLC data for additional five oils show that clove oil contained 86.5% eugenol; cinnamon oil, 85.7% cinnamaldehyde; lemongrass oil, 86.1% citral; palmarosa oil, 85.0% geraniol; bitter orange oil, 87.1% limonene and 0.06% perillaldehyde; orange Mandarin oil, 33.8% limonene and 0.1% perillaldehyde; and sweet orange, tangerine, grapefruit, lemon, and lime oils, 90.0, 82.9, 82.6, 61.6, and 38.5% limonene, respectively.

The observed similarities of BA_{50} values among some of the oil compounds and the oils from which they are derived (e.g., cinnamaldehyde/cinnamon oil; citral/lemongrass oil; eugenol/clove oil) may be due to the fact that these oils consist of about 86% active oil compounds. It is unknown how antibacterial activity is affected by the large variation in limonene content of the citrus oils mentioned above as well as the presence of other constituents in these oils, which were not measured in this study.

The activities of cinnamaldehyde and cinnamon bark, cinnamon leaf, and cinnamon (Cassia) oils were essentially identical. From an economic standpoint, the data suggest that the use of inexpensive synthetic cinnamaldehyde may be preferable to the use of the plant-derived oils. This is also true for carvacrol, the major constituent of oregano oil, and possibly also for other active components of essential oils.

Miscibility of Carvacrol and Cinnamaldehyde in Clear Apple Juice. Figure 6 shows the relationship of added cinnamaldehyde or carvacrol to the ultimate concentrations found in

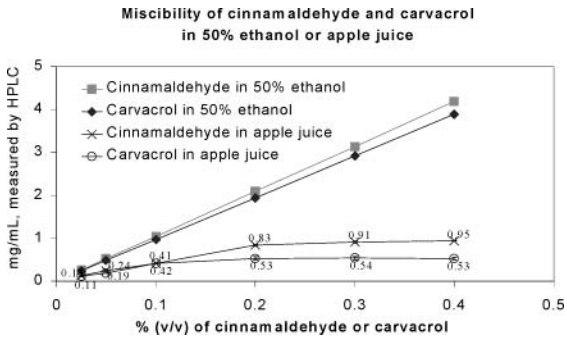

Figure 6. Miscibility of cinnamaldehyde and carvacrol determined by measuring the absorbance of HPLC peaks as compared to absorbances of known solutions of the test compounds in 50% ethanol (internal standard).

the final solutions measured by HPLC. At all levels of added cinnamaldehyde and carvacrol, the final amount measured in clear apple juice was less than in 50% ethanol used as a reference. For both carvacrol and cinnamaldehyde, apple juice containing 0.025−0.10% of the essential oil appeared clear. Higher concentrations appeared cloudy. The maximum miscibility of carvacrol in apple juice is about 0.5 mg/mL, and for cinnamaldehyde, it is between 0.8 and 1 mg/mL. A related study describes the solubility/stability of chlorogenic acid in apple juice (*37*).

In conclusion, selected plant essential oils and oil compounds were found to be bactericidal against *E. coli* O157:H7 and *S. enterica* in apple juice. The activity against *S. enterica* was greater than against *E. coli*. Our findings complement earlier cited efforts to protect apple juice against contamination by human pathogens as well as reported antimicrobial effectiveness of essential oils in other foods including carrots (*38*), salads (*39*), and meat, poultry, and fish (*40*−*44*). The results suggest some areas for further studies with some of the food compatible compounds. Some possibilities include adding active oils or their active components during a stage in the production and storage of apple juice and/or adding a few drops of one of the oils to unpasteurized juice before consumption and monitoring the effects on the microbial flora including pathogens.

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LITERATURE CITED

(1) Deliganis, C. V. Death by apple juice: The problem of foodborne illness, the regulatory response, and further suggestions for reform. *Food Drug Law J.* **1998**, *53*, 681−728.

(2) Koodie, L.; Dhople, A. M. Acid tolerance of *Escherichia coli* O157: H7 and its survival in apple juice. *Microbios* **2001**, *104*, 167−175.

(3) Parish, M. E. Coliforms, *Escherichia coli* and *Salmonella* serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. *J. Food Prot.* **1998**, *61*, 280−284.

(4) Parish, M. E. Public health and nonpasteurized fruit juices. *Crit. Rev. Microbiol.* **1997**, *23*, 109−119.

(5) Steele, B. T.; Murphy, N.; Rance, C. P. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. *J. Pediatr.* **1982**, *101*, 963−965.

(6) Besser, R. E.; Lett, S. M.; Weber, J. T.; Doyle, M. P.; Barrett, T. J.; Wells, J. G.; Griffin, P. M. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *J. Am. Med. Assoc.* **1993**, *269*, 2217−2220.

(7) Hancock, D. D.; Rice, D. H.; Herriott, D. E.; Besser, T. E.; Ebel, E. D.; Carpenter, L. V. Effects of farm manure-handling practices on *Escherichia coli* O157 prevalence in cattle. *J. Food Prot.* **1997**, *60*, 363−366.

(8) Buchanan, R. L.; Edelson, S. G.; Miller, R. L.; Sapers, G. M. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. *J. Food Prot.* **1999**, *62*, 444−450.

(9) Janisiewicz, W. J.; Conway, W. S.; Brown, M. W.; Sapers, G. M.; Fratamico, P.; Buchanan, R. L. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Appl. Environ. Microbiol.* **1999**, *65*, 1−5.

(10) Liao, C. H.; Sapers, G. M. Attachment and growth of *Salmonella Chester* on apple fruits and in vivo response of attached bacteria to sanitizer treatments. *J. Food Prot.* **2000**, *63*, 876−883.

(11) Riordan, D. C.; Sapers, G. M.; Hankinson, T. R.; Magee, M.; Mattrazzo, A. M.; Annous, B. A. A study of U.S. orchards to identify potential sources of *Escherichia coli* O157:H7. *J. Food Prot.* **2001**, *64*, 1320−1327.

(12) Kenney, S. J.; Burnett, S. L.; Beuchat, L. R. Location of *Escherichia coli* O157:H7 on and in apples as affected by bruising, washing, and rubbing. *J. Food Prot.* **2001**, *64*, 1328−1333.

(13) Roering, A. M.; Luchansky, J. B.; Ihnot, A. M.; Ansay, S. E.; Kaspar, C. W.; Ingham, S. C. Comparative survival of *Salmonella typhimurium* DT 104, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in preservative-free apple cider and simulated gastric fluid. *Int. J. Food Microbiol.* **1999**, *46*, 263−269.

(14) Evrendilek, G. A.; Zhang, Q. H.; Richter, E. R. Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* 8739 in apple juice by pulsed electric fields. *J. Food Prot.* **1999**, *62*, 793−796.

(15) Fan, X.; Thayer, D. W. γ-Radiation influences browning, antioxidant activity, and malondialdehyde level of apple juice. *J. Agric. Food Chem.* **2002**, *50*, 710−715.

(16) Zegota, H.; Zegota, A.; Bachmann, S. Effect of irradiation and storage on patulin disappearance and some chemical constituents of apple juice concentrate. *Z. Lebensm. Unters. Forsch.* **1988**, *187*, 321−324.

(17) Buchanan, R. L.; Edelson, S. G.; Snipes, K.; Boyd, G. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. *Appl. Environ. Microbiol.* **1998**, *64*, 4533−4535.

(18) Liao, C.-H.; Schollenberger, L. M.; Phillips, J. G. Lethal and sublethal action of acetic acid on *Salmonella in vitro* and on cut surfaces of apple slices. *J. Food Sci.* **2003**, *68*, 2793−2798.

(19) Reinders, R. D.; Biesterveld, S.; Bijker, P. G. Survival of *Escherichia coli* O157:H7 ATCC 43895 in a model apple juice medium with different concentrations of proline and caffeic acid. *Appl. Environ. Microbiol.* **2001**, *67*, 2863−2866.

(20) Folsom, J. P.; Frank, J. F. Heat inactivation of *Escherichia coli* O157:H7 in apple juice exposed to chlorine. *J. Food Prot.* **2000**, *63*, 1021−1025.

(21) Linton, M.; McClements, J. M.; Patterson, M. F. Inactivation of *Escherichia coli* O157:H7 in orange juice using a combination of high pressure and mild heat. *J. Food Prot.* **1999**, *62*, 277−279.

(22) Ryu, J. H.; Beuchat, L. R. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. *Int. J. Food Microbiol.* **1998**, *45*, 185−193.

(23) Splittstoesser, D. F.; McLellan, M. R.; Churey, J. J. Heat resistance of *Escherichia coli* O157: H7 in apple juice. *J. Food Prot.* **1996**, *59*, 226−229.

(24) Ingham, S. C.; Uljas, H. E. Prior storage conditions influence the destruction of *Escherichia coli* O157:H7 during heating of apple cider and juice. *J. Food Prot.* **1998**, *61*, 390−394.

(25) Kozempel, M. F.; Annous, B. A.; Cook, R. D.; Scullen, O. J.; Whiting, R. C. Inactivation of microorganisms with microwaves at reduced temperatures. *J. Food Prot.* **1998**, *61*, 582−585.

(26) Shearer, A. E.; Mazzotta, A. S.; Chuyate, R.; Gombas, D. E. Heat resistance of juice spoilage microorganisms. *J. Food Prot.* **2002**, *65*, 1271−1275.

(27) Ekasari, I.; Bonestroo, M. H.; Jongen, W. M. F.; Pilnik, W. Mutagenicity and possible occurrence of flavonol aglycones in heated orange juice. *Food Chem.* **1989**, *31*, 289−294.

(28) Friedman, M.; Wilson, R. E.; Ziderman, I. I. Effect of heating on mutagenicity of fruit juices in the Ames test. *J. Agric. Food Chem.* **1990**, *38*, 740−743.

(29) Scaman, C. H.; Jim, V. J.; Hartnett, C. Free galactose concentrations in fresh and stored apples (*Malus domestica*) and processed apple products. *J. Agric. Food Chem.* **2004**, *52*, 511−517.

(30) Kalemba, D.; Kunicka, A. Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.* **2003**, *10*, 813−829.

(31) Friedman, M.; Henika, P. R.; Mandrell, R. E. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* **2002**, *65*, 1545−1560.

(32) Friedman, M.; Henika, P. R.; Mandrell, R. E. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* **2003**, *66*, 1811−1821.

(33) Friedman, M.; Buick, R.; Elliott, C. Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetaive cells and spores, *Escherichia coli*, and *Staphylococcus areus*. *J. Food Prot.* **2004**, *67*, 1774−1778.

(34) SAS. *SAS Online Doc*; SAS Institute: Cary, NC, 1999.

(35) Jonge, R.; Ritmeester, W. S.; Leusden, F. M. Adaptive responses of *Salmonella enterica* serovar *Typhimurium* DT104 and other *S. typhimurium* strains and *Escherichia coli* 0157 to low pH environments. *J. Appl. Microbiol.* **2003**, *94*, 625−632.

(36) Friedman, M.; Kozukue, N.; Harden, L. A. Cinnamaldehyde content in foods determined by gas chromatography−mass spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 5702−5709.

(37) Friedman, M.; Jürgens, H. S. Effect of pH on stability of plant phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 2101−2110.

(38) Valero, M.; Salmeron, M. C. Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *Int. J. Food Microbiol.* **2003**, *85*, 73−81.

(39) Koutsoumanis, K.; Lambropoulou, K.; Nychas, G. J. A predictive model for the nonthermal inactivation of *Salmonella enteritidis* in a food model system supplemented with a natural antimicrobial. *Int. J. Food Microbiol.* **1999**, *49*, 63−74.

(40) Kim, J. M.; Marshall, M. R.; Cornell, J. A.; Preston, J. F., III; Wei, C. I. Antibacterial activity of carvacrol, citral, and geraniol against *Salmonella typhimurium* in culture medium and on fish cubes. *J. Food Sci.* **1995**, *60*, 1364−1368, 1374.

(41) Tsigarida, E.; Skandamis, P.; Nychas, G. J. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 degrees C. *J. Appl. Microbiol.* **2000**, *89*, 901−909.

(42) Skandamis, P. N.; Nychas, G. J. Effect of oregano essential oil on microbiological and physicochemical attributes of minced meat stored in air and modified atmospheres. *J. Appl. Microbiol.* **2001**, *91*, 1011−1022.

(43) Mejlholm, O.; Dalgaard, P. Antimicrobial effect of essential oils on the seafood spoilage microorganism *Photobacterium phosphoreum* in liquid media and fish products. *Lett. Appl. Microbiol.* **2002**, *34*, 27−31.

(44) Giannenas, I.; Florou-Paneri, P.; Papazahariadou, M.; Christaki, E.; Botsoglou, N. A.; Spais, A. B. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. *Arch. Tierernaehr.* **2003**, *57*, 99−106.

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