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Determination of bactericidal efficacy of essential oil extracted from orange peel on the food contact surfaces

Chia-Min Lin [a,*], Shane-Rong Sheu [b], Shu-Chen Hsu [c], Yung-Hsiang Tsai [a]

[a] Department of Seafood Science, National Kaohsiung Marine University, No.142, Haijhuan Rd., Nanzih District, Kaohsiung City 81143, Taiwan
[b] Department of Automation and Control Engineering, Far East University, No.49, Chung Hua Rd., Hsin-Shih Township, Tainan County 744, Taiwan
[c] Department of Beverage and Food Management, Far East University, No.49, Chung Hua Rd., Hsin-Shih Township, Tainan County 744, Taiwan

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ABSTRACT

Essential oil was extracted from peels of sweet orange fruits by supercritical technique and the resulting compounds were analyzed by gas chromatography. After emulsifying with tween-20 in sterile water, the antibacterial efficacy against *Vibrio parahaemolyticus, Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus*, which were inoculated on the surfaces of samples of stainless steel and plastic cutting board pieces, was determined. Sterile water was used as control and two commercial detergents, A and B, were used as comparative agents. Compared with control, more than 5-log reduction was obtained against *V. parahaemolyticus* at 1% and *S. typhimurium* and *E. coli* at 2.5% of the essential oil. No significant reduction ($P \geq 0.05$) was obtained against *S. aureus* even at 5%. Antibacterial activities of the essential oil and detergent B were significantly higher ($P < 0.05$) than detergent A and control. In addition, significantly higher ($P < 0.05$) reductions were obtained on stainless steel than plastic cutting boards. Our results showed that the extracted orange oil could effectively inactivate *V. parahaemolyticus*, *S. typhimurium*, and *E. coli* but not *S. aureus*, on the food contact surfaces.

1. Introduction

Citrus essential oil mainly exists in fruit peels which are usually discarded as waste. Thus, citrus essential oil could be manufactured at a more affordable price than other plant essential oils, since it is the largest sector of the world production of essential oil (Tirado, Stashenko, Combariza, & Martinez, 1995). Besides being used as a fragrance, citrus essential oils have been reported to possess antibacterial activities against *Escherichia coli, E. coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes,* and *Vibrio vulnificus* in media (Kim, Marshall, & Wei, 1995) and to *S. typhimurium* on fish cubes (Kim, Marshall, Cornell, Preston, & Wei, 1995). Essential oils of lemon, orange, and bergamot were also demonstrated to possess bactericidal effect against *Campylobacter jejuni*, *E. coli* O157:H7, *L. monocytogenes, Bacillus cereus, Staphylococcus aureus* (Fisher & Phillips, 2006) and *Acrobacter butzlei* (Fisher, Rowe, & Philips, 2007) in media and on foods. In addition, antifungal activities against *Penicillium digitatum, Penicillium italicum* (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998), and yeast, *Saccaromyces cerevisiae* (Belletti et al., 2004), have been reported. Those reports also demonstrated low water solubility of citrus essential oil could be overcome by mixing it with an emulsifier, such as tween-20 (Fisher & Phillips, 2006; Kim, Marshall, & Wei, 1995; Kim & Shin, 2004).

Several methods such as water, steam, organic solvent extraction, as well as cold pressing have been used to obtain essential oils from citrus fruit peels (Lin, 2005). Supercritical CO_2 extraction is a technique which can extract essential oil at lower temperature to avoid potential damage to desired compounds at high temperatures. Citrus essential oil extracted by supercritical technique has been demonstrated to be an effective method to extract essential oil form citrus fruit peels (Espinosa, Diaz, & Brignole, 2000; Gao & Li, 2005; Mira, Blasco, & Subirats, 1996).

According to the Department of Health, the highest occurring cause for foodborne outbreaks in Taiwan is cross-contamination, which mostly occurs on the surfaces of kitchen utensils (Bureau of Food Safety, 2008). The United State (FDA, 2008) and Europe (Beumer & Kusumanigrum, 2003) also emphasize that avoiding cross-contamination is the key procedure to prevent the occurrence of foodborne illnesses. Pathogenic bacteria could be transferred between raw materials, cloth, hands, and food contact surfaces to create cross-contamination (de Boer & Hahné, 1990; Scott & Bloomfield, 1990). Currently, the most common practice to eliminate bacteria on the food contact surfaces is washing with detergent at elevated temperatures. Nonetheless, it was reported

* Corresponding author. Tel.: +886 7 361 7141x3614; fax: +886 7 364 0634.
E-mail address: cmlin@mail.nkmu.edu.tw (C.-M. Lin).

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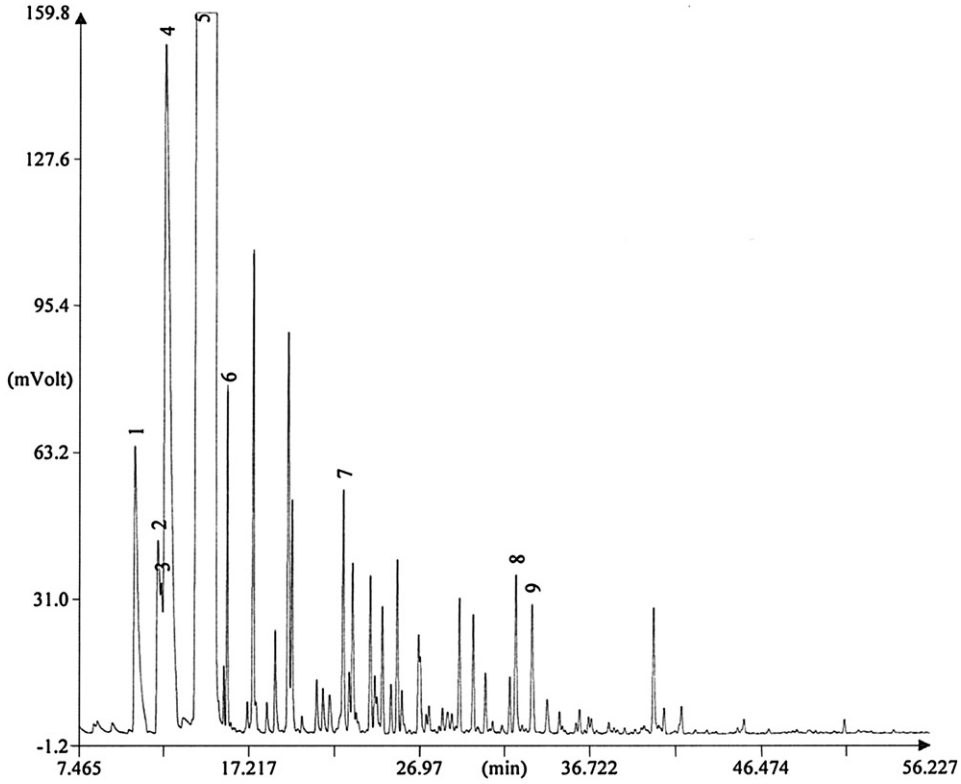

Fig. 1. GC chromatograms obtained from analysis of orange essential oil. Number corresponds to (1) α-terpineol, (2) β-pinene, (3) myrcene, (4) linalool, (5) limonene, (6) neryl acetate, (7) α-pinene, (8) γ-terpinene, (9) geranyl acetate.

that *S. typhimurium*, *S. enteritidis*, *C. jejuni*, and *E. coli* could survive after regular washing processes (Mattick et al., 2003). That study also showed that bacteria could survive on the surfaces of cutting boards and stainless steel after washing with detergent, as well as in the detergent solution. In addition, most commercial detergents and sanitizers are artificially synthetic compounds, which have a negative impact on the environment. Furthermore, some of those detergents could be converted to become environmental hormones, which are known to impact negatively on human health (Saouter, Pittinger, & Feijtel, 2001; Sonnenschein & Soto, 1998). Nowadays, consumers have negative feeling towards to artificial synthetic compounds and more positive ones towards using natural compounds. Thus, an effective sanitizer, composed of natural compounds, is needed to ensure inactivation of microorganisms on the food contact surfaces and thereby reducing its environmental impact. Citrus essential oil, which can be produced from agricultural waste and possesses high antibacterial ability, could be an excellent candidate for a sanitizer used on the food contact surfaces to reduce the occurrence of cross-contamination. In addition, applying citrus essential oils to foods usually adversely affects its organoleptic characteristics (Fisher & Philips, 2008). Therefore, it would be more practical to apply essential oils onto the food contact surfaces rather than introducing them into the food systems. Hence, the objective of this research is to extract citrus essential oil, from peels of orange fruits by supercritical CO_2 technique and to determine the bactericidal efficacy of the extracted oil on the food contact surfaces.

Table 1
Chemical constitution of sweet orange essential oil determined by GC.

No. of peak	Chemical	Retention time (min)	Concentration	
			Relative ratio (%)	Absolute concentration (mg/g)
1	α-terpineol	10.7	1.01	4.11
2	β-pinene	12.0	0.49	1.59
3	myrcene	12.2	0.25	1.05
4	linalool	12.5	3.49	11.6
5	limonene	14.5	88.4	350
6	neryl acetate	16.0	0.35	1.15
7	α-pinene	22.6	0.36	1.39
8	γ−terpinene	32.5	0.27	1.10
9	geranyl acetate	33.4	0.22	1.05

2. Materials and methods

2.1. Preparation of orange fruits and extraction of essential oil

Sweet orange fruits (*Citrus sinensis* var. Liucheng) were purchased from a wholesale market in Shinhua Township, Tainan County, Taiwan. After purchasing, the fruits were stored at 4 °C, then washed and peeled within 3 days. The orange peels were diced into 1 × 1 cm pieces and stored at −20 °C before extraction. The peel pieces were vacuum dried, frozen in liquid nitrogen, then ground into powder. 100 g of powder was placed into the supercritical extractor, designed by Dr. Shane-Rong Sheu at Far East University. The extraction parameters are as followings: temperature, 323 K; pressure, 10 MPa; flow rate of CO_2, 3.5 kg/h; time, 90 min.

2.2. Analysis of essential oil components

Components of the extracted essential oil were analyzed by a gas chromatograph (GC, Shimadzu GC-2014, Kyoto, Japan) connected to a flame ionization detector (FID-2014). Standard chemicals and DB-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film)

Table 2
Reduction of bacterial populations ($\log_{10}$ CFU/mL)[1] in PBS (for *V. parahaemolyticus*) or BPB (for the other 3 bacteria) after being treated with various concentrations of essential oil.

Bacteria	Concentration of oil[2]				
	1%	2.5%	5%	7.5%	10%
V. parahaemolyticus	3.75 ± 0.54^{aA}	4.34 ± 1.06^{aAB}	5.48 ± 0.84^{aB}	5.35 ± 0.53^{aB}	5.86 ± 0.89^{aB}
E. coli	0.12 ± 0.64^{bA}	2.95 ± 1.84^{bB}	4.45 ± 1.24^{bBC}	5.06 ± 0.84^{aC}	5.77 ± 1.26^{aC}
S. typhimurium	0.13 ± 0.63^{bA}	2.95 ± 1.13^{bB}	4.69 ± 1.07^{bC}	5.26 ± 1.03^{aC}	5.86 ± 1.36^{aC}
S. aureus	0.42 ± 0.75^{bA}	2.67 ± 0.86^{bB}	3.55 ± 1.26^{bBC}	3.86 ± 1.06^{bC}	3.96 ± 1.22^{bC}

[1] Means and standard deviations were obtained from triplicate studies.
[2] Bacterial populations with the same lower case or capital letters within the same column or row, respectively, are not significantly different ($P = 0.05$).

were purchased from Sigma (St. Louis, MO, USA) and Agilent Technology (Folsom, CA, USA), respectively. The parameters of GC analysis were based on Lin (2005): nitrogen was used as a carrier gas; the flow rate was 1.0 mL/min; the initial temperature was 75 °C; the rate of temperature increase was 2 °C/min; the injection temperature was 250 °C. The quantity of the essential oil components was determined by comparing the peak area of standards and the test samples.

2.3. Bacterial species and incubation

The three most frequently occurring foodborne pathogens in Taiwan, *Vibrio parahaemolyticus* O4:K12, *Salmonella enterica* subsp. *enterica* serovar *typhimurium* ATCC 14028, and *S. aureus* ATCC 12600, were used. Nonpathogenic *E. coli* ATCC 11775, a common sanitary indicator, was also tested. All bacteria stocks were stored at −80 °C in tryptic soy broth (TSB) with 15% glycerol. Before testing, the bacteria stocks were streaked onto tryptic soy agar (TSA) to observe colony characteristics and uniformity. A well-isolated single colony was picked up and incubated for two consecutive 24-h intervals in TSB at 37 °C. Three percent of NaCl was added into the medium of *V. parahaemolyticus* during incubation because of its halophilic characteristic. After incubation, the bacteria were collected by centrifugation at 5000*g* for 10 min at 4 °C, and then suspended in Butterfield phosphate buffer (BPB, pH 7.2). *V. parahaemolyticus* was suspended in phosphate buffer saline (PBS, pH 7.4). During antibacterial activity tests, optical density at 600 nm (OD_{600}) of bacterial suspension was maintained at 1.0, at which point the bacterial population was equal to approximately 10^9 CFU/mL, according to our preliminary study.

Table 3
Reduction of bacterial populations ($\log_{10}$CFU/piece)[1] on the surfaces of the pieces of stainless steel and cutting boards, when being suspended in milk, treated with detergent A, B, or 1% (for *V. parahaemolyticus*) or 5% (for the other 3 bacteria) of the essential oil.

Surface/bacteria	Treatment[2,3]		
	Detergent A	Detergent B	Essential oil
Stainless steel			
V. parahaemolyticus	2.58 ± 0.67^{bA}	5.37 ± 1.06^{bB}	4.82 ± 1.13^{bB}
E. coli	0.07 ± 0.06^{aA}	0.74 ± 0.69^{aA}	0.61 ± 0.05^{aA}
S. typhimurium	0.39 ± 1.26^{aA}	0.88 ± 2.02^{aA}	0.49 ± 1.56^{aA}
S. aureus	0.17 ± 1.69^{aA}	0.39 ± 2.01^{aA}	0.59 ± 2.06^{aA}
Cutting board			
V. parahaemolyticus	2.04 ± 1.61^{bA}	4.87 ± 2.02^{bB}	4.49 ± 2.23^{bB}
E. coli	0.37 ± 2.01^{aA}	1.24 ± 1.56^{aA}	0.90 ± 1.34^{aA}
S. typhimurium	0.15 ± 1.69^{aA}	0.34 ± 2.02^{aA}	0.68 ± 1.43^{aA}
S. aureus	0.23 ± 1.67^{aA}	1.01 ± 1.36^{aA}	1.27 ± 1.03^{aA}

[1] Means and standard deviations were obtained from triplicate studies.
[2] Using the same surface, bacterial populations with the same lower case or capital letters within the same column or row, respectively, are not significantly different ($P = 0.05$).
[3] Difference between the same treatment for the same bacterial species on different surface are not significant ($P = 0.05$

2.4. Emulsification of orange essential oil

Tween-20 was added at the final concentrations of 1 or 5% as an emulsifier. The orange oil containing tween-20 was emulsified with a homogenizer at 13,500 or 24,000 rpm for 5 min (Kim, Marshall, & Wei, 1995; Kim & Shin, 2004).

2.5. Antibacterial activity of the orange oil in the buffers

The antibacterial efficacy of the orange oil was first determined in a buffer. The bacteria were suspended in BPB or PBS at 10^7 CFU/mL, and then mixed with the emulsified oil. The final concentrations of orange oil were at 0.5, 1%, 2.5%, 5%, 7.5%, or 10%. After shaking for 2 min, the mixture was serially diluted (1:10) in BPB or PBS. One mL of dilutant at a suitable dilution was placed into a Petri-dish, and then mixed with approximately 12–15 mL of TSA. Duplicate plates were used for each dilution and bacterial populations were determined by the average of the colony number after incubation at 37 °C for 24 h. Buffer without essential oil was used as a control. The lowest concentration showing bactericidal activity was used for the following test of antibacterial activity on the food contact surfaces. A preliminary study was also conducted to evaluate the bactericidal efficacy of the emulsifier. In the study, buffers containing tween-20 at 1 or 5% were tested by using the same procedures and bacterial spices described previously.

2.6. Antibacterial activity of the orange oil on the food contact surfaces

Professional grade of plastic cutting board (450 × 300 × 20 mm, made with heat-resistant polypropan) and stainless steel (grade SUS 304 qualified with good hygiene practice, GHP, of Taiwan) were sliced into pieces of 6 × 6 cm before the experiment. The pieces of the plastic cutting board and stainless steel were then used as models for the food contact surfaces. All pieces of cutting board and stainless steel were autoclaved at 121 °C for 15 min before the experiment. The test bacteria were incubated and centrifuged as previously described, and then the bacterial cells were re-suspended in 5% peptone or sterile full milk (grade A, fat content 3.0–3.8%). These two materials were used to simulate that bacterial contamination with a light or a heavy organic load, respectively, during cross-contamination. One hundred μL of bacterial suspension, containing approximately 10^7 CFU, was placed onto the surface of the cutting board or the stainless steel pieces by a micropipette in a laminar flow. The 100 μL bacterial suspension was delivered in 40–50 drops evenly distributed on the surface. After air-drying for 30–40 min in a laminar hood at room temperature, the pieces were placed into a sterile plastic bag containing 250 mL of treatment solution. Applying concentrations of essential oil on the food contact surfaces were based on the results of bactericidal tests in buffer, which showed the essential oil was more effective to *V. parahaemolyticus* than other three bacteria.

Table 4
V. parahaemolyticus populations ($\log_{10}$ CFU/piece)[1] on the surfaces of the pieces of stainless steel, cutting boards or in the treatment solutions, after being treated with detergent A, B, essential oil, or control.

	Treatment[2]				
	Detergent A	Detergent B	Essential oil		Control
			1%	2.5%	
Stainless steel					
Surface	2.89 ± 1.27^{b}	1.07 ± 1.02^{c}	0.85 ± 0.13^{c}	0.98 ± 0.643^{c}	5.43 ± 0.46^{a}
Solution	2.55 ± 0.05^{b}	ND[3c]	ND[3c]	ND[3c]	1.22 ± 0.46^{a}
Cutting board					
Surface	3.75 ± 1.88^{b}	ND[3c]	1.10 ± 2.23^{c}	ND[3c]	6.33 ± 0.46^{a}
Solution	3.68 ± 1.88^{b}	0.42 ± 0.03^{c}	ND[3c]	ND[3c]	1.45 ± 0.56^{a}

[1] Means and standard deviations were obtained from triplicate studies.
[2] Data in the same row attached with the same letter on the surface of same material are not significantly different ($P = 0.05$).
[3] ND: not detectable.

Thus, lower concentrations were used for *V. parahaemolyticus* than the three bacterial species. The concentrations of essential oil were at 1% and 2.5% for *V. parahaemolyticus*; 2.5% and 5% for *S. typhimurium* and *E. coli*; 5% and 10% for *S. aureus*. After shaking for 1 min, the pieces of cutting board and stainless steel were taken out of the plastic bag, then placed into a sterile plastic bag containing 250 mL BPB or PBS. After vigorously hand rubbing for 2 min, 1 mL of BPB or PBS was serially diluted (1:10) and the bacterial population was determined by mixing TSA agar as previously described. The remaining treatment solution was also decimally diluted and bacterial populations were determined. In addition, 2 mL of the remaining solution was added into 50 mL of TSB, then incubated at 37 °C for 24 h to check for the existence of any viable bacteria. Since 250 mL of treatment solution was used for each piece of stainless steel or cutting board, bacterial population of each piece were obtained by timing the populations obtained by plate count ($\log_{10}$ CFU/mL) and the 250 mL treatment solution ($\log_{10}$ CFU/mL × 250 mL/piece = $\log_{10}$CFU/piece).

2.7. Comparative sanitizers

Two commercial kitchen detergents, brand A and B, were chosen as comparative sanitizers for this study. Detergent A, a popular household detergent in Taiwan, contains artificial chemicals only and its major components are linear alkylbenzene sulfonate (LAS) and sodium lauryl ether sulphate (SLES) (pH 7 ± 1). Detergent B is made of natural plant liquid soap, calcium carbonate, sodium citrate, water and lemon extract (pH 10 ± 1). Sterile tap water was used as a negative control. Thus, in total, 4 treatments were tested, essential oil, detergent A, detergent B, and negative control.

2.8. Statistical analyses

Experiment was designed based on the principle of the completely randomized design. Four replicates were used for each kind of surface in each treatment. Thus, there were four pieces of plastic cutting board or stainless steel for each solution. Each bacteria species was tested in triplicate. Each piece was considered as a single sample. Means and standard deviations were determined by combining data of the same bacterial species treated with the same solution. All statistical analyses were done with the SPSS statistical analysis program (version 12). One-way ANOVA and Duncan's test for multiple comparisons were used to determent significant differences ($P = 0.05$) between treatments.

3. Results

3.1. GC analysis of essential oil components

Nine major compounds were detected by GC in the orange essential oil (Fig. 1). The most abundant compound was limonene (88.4%). Other compounds, except for linalool (3.49%) and α-terpineol (1.01%), were all less than 1% of the total composition (Table 1).

3.2. Antibacterial activity of the orange essential oil in the buffers

The efficacy of orange essential oil against *V. parahaemolyticus* was significantly higher than the other three bacteria ($P < 0.05$), particularly at a low concentration (1%). For the other three bacteria, higher concentrations (2.5 or 5%) were needed to achieve more than 1-log reduction. However, significantly higher

Table 5
S. typhimurium populations ($\log_{10}$ CFU/piece)[1] on the surfaces of the pieces of stainless steel, cutting boards or in the treatment solutions, after being treated with detergent A, B, essential oil, or control.

Surface	Treatment[2]				
	Detergent A	Detergent B	Essential oil		Control
			2.5%	5%	
Stainless steel					
Surface	5.56 ± 0.28^{a}	0.86 ± 1.34^{b}	0.85 ± 0.13^{c}	0.28 ± 0.69^{c}	5.53 ± 0.66^{a}
Solution	2.44 ± 0.32^{a}	ND[3c]	0.46 ± 0.12^{c}	ND[3c]	2.53 ± 0.66^{a}
Cutting board					
Surface	5.29 ± 0.55^{a}	1.04 ± 1.22^{b}	1.15 ± 0.68^{b}	0.98 ± 1.08^{b}	5.53 ± 0.66^{a}
Solution	2.75 ± 1.28^{a}	ND[3b]	0.15 ± 0.13^{b}	ND[3b]	2.53 ± 0.66^{a}

[1] Means and standard deviations were obtained from triplicate studies.
[2] Data in the same row attached with the same letter on the surface of same material are not significantly different ($P = 0.05$).
[3] ND: not detectable.

Table 6
E. coli populations ($\log_{10}$ CFU/piece)[1] on the surfaces of the pieces of stainless steel, cutting boards or in the treatment solutions, after being treated with detergent A, B, essential oil, or control.

	Treatment[2]				
	Detergent A	Detergent B	Essential oil		Control
			2.5%	5%	
Stainless steel					
Surface	6.42 ± 0.22^{a}	ND[3c]	1.78 ± 0.71^{c}	0.98 ± 0.64^{c}	6.12 ± 0.17^{a}
Solution	2.55 ± 0.05^{a}	ND[3b]	0.88 ± 0.11^{b}	ND[3b]	2.22 ± 0.46^{a}
Cutting board					
Surface	5.90 ± 0.19^{a}	3.04 ± 1.08^{b}	2.33 ± 0.46^{c}	ND[3d]	6.12 ± 0.17^{a}
Solution	2.68 ± 1.88^{a}	0.42 ± 0.03^{c}	0.55 ± 0.02^{c}	ND[3c]	2.22 ± 0.46^{a}

[1] Means and standard deviations were obtained from triplicate studies.
[2] Data in the same row attached with the same letter on the surface of same material are not significantly different ($P = 0.05$).
[3] ND: not detectable.

reductions were not obtained at higher concentrations, such as 7.5 and 10% (Table 2). Bactericidal activity was not observed when tween-20 was used without essential oil. In addition, no significant differences of bactericidal efficacy were observed when the orange oil was emulsified with different speeds and tween-20 concentrations (data not shown).

3.3. Antibacterial activity of the orange oil on the food contact surfaces

When bacteria were suspended in full milk, less than 1-$\log_{10}$CFU/piece of reduction was obtained except the surface inoculated with *V. parahaemolyticus* (Table 3). In addition, no significant difference between treatment solutions was obtained, except for *V. parahaemolyticus*, when the bacteria were suspended in full milk (data not shown). When bacteria were suspended in peptone, antibacterial effects were obtained from all bacterial species. Comparing with control, 5-$\log_{10}$CFU/piece of reduction was obtained on stainless steel for *V. parahaemolyticus, S. typhimurium*, and *E. coli* (Table 4, 5, 6) but *V. parahaemolyticus* was treated with a lower concentration than *S. typhimurium*, and *E. coli* (1% vs. 2.5%). The lowest reduction was obtained from the treatment to inactivate *S. aureus* (Table 7). Less than 1 − $\log_{10}$CFU/piece of difference was observed between control and all three sanitizers, on both the surfaces of stainless steel and cutting board. In addition, no significant differences ($P \geq 0.05$) were obtained for the surfaces inoculated with *S. aureus* treated with the essential oil, detergents, or control. Comparing between sanitizers, significantly higher reductions ($P < 0.05$) were obtained from treatments of essential oil and detergent B than ones of detergent A and control. Bacterial populations were 1–2 log higher ($P < 0.05$) on the surfaces of the cutting board than on stainless steel, but no significant differences ($P \geq 0.05$) were observed in the solutions used to treat the stainless steel or cutting board samples. The bacterial populations of all tested bacteria, except for *V. parahaemolyticus*, were significantly higher on the surface and treatment solutions when full milk was used as carrier rather than peptone. When 5% peptone was used, viable bacterial cells could not be recovered in some treatment solutions of detergent B and the essential oil despite using broth enrichment.

4. Discussion

Staphylococcus aureus was the most resistant and *V. parahaemolyticus* is the most susceptible bacterial species in this study. Though some previous studies showed Gram negative bacteria were usually more resistant to citrus essential oils than Gram positive (Burt, 2004), another study showed *S. aureus* was more resistant than *L. monocytogenes* and *B. cereus* but about the same as *E. coli* O157 and *C. jejuni* when treated with citrus essential oil (Fisher & Phillips, 2006). Fisher and Phillips (2006), Fisher and Philips (2008) also suggested that the antibacterial effects of citrus essential oil were not uniform between bacteria. They depended on the compounds and the bacteria tested. In addition, *S. aureus* was demonstrated to possess significantly higher survival ability than *S. enteritidis* and *C. jejuni* on the surface of stainless steel (Kusumanigrum, Riboldi, Hazeleger, & Beumer, 2003) and was the most commonly isolated pathogen in domestic refrigerators (Jackson, Blair, McDowell, Kennedy, & Bolton, 2007). Those results indicated that *S. aureus* was able to survive in an adverse environment and this survival ability could be the reason why *S. aureus* showed higher resistance against the orange essential oil than other three bacteria in this study. Though there is no previous report for the antibacterial efficacy of citrus essential oil against *V. parahaemolyticus*, Kim, Marshall, and Wei (1995) reported *V.*

Table 7
S. aureus populations ($\log_{10}$ CFU/piece)[1] on the surfaces of the pieces of stainless steel, cutting boards or in the treatment solutions, after being treated with detergent A, B, essential oil, or control.

	Treatment[2]				
	Detergent A	Detergent B	Essential oil		Control
			5%	10%	
Stainless steel					
Surface	5.42 ± 0.09^{a}	5.31 ± 0.11^{a}	5.30 ± 0.14^{a}	4.98 ± 0.64^{c}	5.43 ± 0.43^{a}
Solution	2.55 ± 0.23^{a}	1.38 ± 0.12^{a}	1.46 ± 0.10^{a}	1.24 ± 0.86^{a}	1.62 ± 0.36^{a}
Cutting board					
Surface	5.37 ± 0.15^{a}	5.06 ± 0.28^{a}	5.36 ± 0.08^{a}	5.36 ± 0.08^{a}	5.43 ± 0.43^{a}
Solution	1.78 ± 1.03^{a}	1.42 ± 0.63^{a}	1.56 ± 0.82^{a}	1.62 ± 0.36^{a}	1.62 ± 0.36^{a}

[1] Means and standard deviations were obtained from triplicate studies.
[2] Data in the same row attached with the same letter on the surface of same material are not significantly different ($P = 0.05$).

vulnificus was the most susceptible bacterial species among the tested bacteria, including *E. coli*, *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes*. It has also been reported that *V. parahaemolyticus* is more susceptible to environmental stresses than *E. coli* or *Salmonella* species (Joseph, Colwell, & Kaper, 1983). Hence, it was no surprise that we obtained the highest reduction from the treatment of *V. parahaemolyticus*.

It has been demonstrated in several reports that antibacterial efficacy varied between different carrying materials (Fisher, Rowe, & Phillips, 2007; Kusumanigrum et al., 2003; Kusumanigrum, van Putten, Rombouts, & Beumer, 2002; Lee, Cartwright, Grueser, & Pascall, 2007; Mattick et al., 2003). Usually, bacteria were more resistant to antimicrobial agents in suspension with a higher organic load. In our study, full milk and peptone represented heavy and light organic loads, respectively, and the results showed antibacterial efficacy was much lower, when bacteria were suspended in milk, than in peptone. These results are similar to previous studies in which bacteria were also suspended in milk (Kusumanigrum et al., 2003; Lee et al., 2007; Mattick et al., 2003). The high fat and protein contents of full milk could offer good protection to bacteria and interact with the antibacterial agents. Comparing different surfaces, it has been reported that sanitizers were more effective on smooth than on rough surfaces (Kusumanigrum et al., 2002, 2003; Lee et al., 2007; Mattick et al., 2003). Our results also showed that a higher reduction was obtained on a smooth surface, such as stainless steel, rather than on a rough surface, such as a cutting board.

Most commercial detergents emphasize their sanitary effectiveness on the contact surfaces, but seldom mention the possibility of the remaining bacteria in the washing solution. Our results showed that bactericidal activities of the essential oil and detergent B were significantly higher ($P < 0.05$) than that of the detergents made of artificial surfactant such as detergent A. However, detergents made of artificial surfactants are commonly used in household and commercial establishments. The inability of those detergents to inactivate bacteria in the washing solution could create a potential hazard because the solutions are usually discarded carelessly. In addition, it is common to wash all utensils in a large container filled with detergent solution. Therefore, if the detergent solution cannot inactivate the pathogen effectively, surviving bacteria in the solution could be a potential reservoir for cross-contamination.

The results of this study demonstrated that the extracted orange essential oil is an effective agent to inactivate bacteria on the food contact surfaces. This is the first published data related to inactivating pathogenic bacteria on the food contact surfaces with orange essential oil, which can be mass produced from regular agricultural waste.

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References

Belletti, N., Ndagijimana, M., Claudio, S., Guerzoni, M. E., Lanciotti, R., & Gardini, F. (2004). Evaluation of the antimicrobial activity of citrus essences on *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry, 52*, 6932–6938.

Beumer, R. R., & Kusumanigrum, H. (2003). Kitchen hygiene in daily life. *International Biodeterioration & Biodegradation, 51*, 299–302.

de Boer, E., & Hahné, M. (1990). Cross-contamination with *Campylobacter jejuni* and *Salmonella* spp. from raw chicken products during food preparation. *Journal of Food Protection, 53*, 1067–1068.

Bureau of Food Safety, the Department of Health, Taiwan (2008). http://food.doh.gov.tw, 2008/12.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology, 94*, 223–253.

Caccioni, D. R., Guizzardi, M., Biondi, D. M., Renda, A., & Ruberto, G. (1998). Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. *International Journal of Food Microbiology, 43*, 73–79.

Espinosa, S., Diaz, S., & Brignole, E. A. (2000). Optimal design of supercritical fluid processes. *Computers and Chemical Engineering, 24*, 1301–1307.

Fisher, K., & Philips, C. (2008). Potential antimicrobial uses of essential oils in foods: is citrus the answer? *Trends in Food Science & Technology, 19*, 156–164.

Fisher, K., & Phillips, C. A. (2006). The effect of lemon, orange, and bergamot essential oils and their components on the survival of *Campylobacter jejuni, Escherichia coli* O157, *Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. *Journal of Applied Microbiology, 101*. 1232–1240.

Fisher, K., Rowe, C., & Philips, C. A. (2007). The survival of three strains of *Acrobacter butzleri* in the presence of lemon, orange and bergamot essential oils and their components in vitro and on food. *Letters in Applied Microbiology, 44*, 495–499.

Food and Drug Administration, the US. Department of Health and Human Service, (2008). http://www.fda.gov/. 12/2008.

Gao, Y., & Li, B. (2005). Extraction of refined oil from citrus peel by supercritical CO_2 fluid. *Journal of University of Shanghai for Science and Technology, 27*, 207–210.

Jackson, V., Blair, I. S., McDowell, D. A., Kennedy, J., & Bolton, D. J. (2007). The incidence of significant foodborne pathogens in domestic refrigerators. *Food Control, 18*, 346–351.

Joseph, S. W., Colwell, R. R., & Kaper, J. B. (1983). *Vibrio parahaemolyticus* and related halophilic vibrios. *Critical Review in Microbiology, 10*, 77–123.

Kim, J. M., Marshall, M. R., Cornell, J. A., Preston, J. F., & Wei, C. I. (1995). Antibacterial activity of carvacrol, citral, and geraniol against *Salmonella typhimurium* on culture medium and on fish cubes. *Journal of Food Science, 60*, 1364–1374.

Kim, J., Marshall, M. R., & Wei, C.-I. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry, 46*, 1739–1745.

Kim, Y. S., & Shin, D. H. (2004). Volatile constituents from leaves of *Callicarpa japonica* (Thumb.) and their antibacterial activities. *Journal of Agricultural and Food Chemistry, 52*, 781–787.

Kusumanigrum, H. D., van Putten, M. M., Rombouts, F. M., & Beumer, R. R. (2002). Effects of antibacterial dishwashing liquid on foodborne pathogens and competitive microorganisms in kitchen sponges. *Journal of Food Protection, 65*, 61–65.

Kusumanigrum, H. D., Riboldi, G., Hazeleger, W. C., & Beumer, R. R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology, 85*, 227–236.

Lee, J., Cartwright, R., Grueser, T., & Pascall, M. A. (2007). Efficiency of manual dishwashing conditions on bacterial survival on eating utensils. *Journal of Food Engineering, 80*, 885–891.

Lin, Y. H. (2005). Study of constitution of pummelo peel oil and antibacterial activities of its' emulsion for *S. aureus* and *E. coli*. Master thesis. Keelung, Taiwan: the Department of Food Science, National Taiwan Ocean University.

Mattick, K., Durham, K., Domingue, G., Jørgensen, F., Sen, M., Schaffner, D. W., et al. (2003). The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food. *International Journal of Food Microbiology, 85*, 213–226.

Mira, B., Blasco, M., & Subirats, S. (1996). Supercritical CO_2 extraction of essential oils from orange peel. *Journal of Supercritical Fluids, 9*, 238–243.

Saouter, E., Pittinger, C., & Feijtel, T. (2001). Aquatic environmental impact of detergents: from simple to more sophisticated models. *Ecotoxicology and Environmental Safety, 50*, 153–159.

Scott, E., & Bloomfield, S. F. (1990). The survival and transfer of microbial contamination via cloths, hands and utensils. *Journal of Applied Bacteriology, 68*, 271–278.

Sonnenschein, C., & Soto, A. M. (1998). An updated review of environmental estrogen and androgen mimics and antagonists. *Journal Steroid Biochemistry & Molecular Biology, 65*, 143–150.

Tirado, C. B., Stashenko, E. E., Combariza, M. Y., & Martinez, J. R. (1995). Comparative study of Colombian citrus oils by high-resolution gas chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography A, 697*, 501–513.