https://doi.org/10.17221/258/2024-CJFS

Time-kill properties of citrus peel essential oils and constituents against foodborne pathogens

*Je-Hyuk Lee**

Department of Food and Nutrition, Kongju National University, Chungnam, Korea

**Corresponding author: leejeh21@kongju.ac.kr*

Citation: Lee J.H. (2025): Time-kill properties of citrus peel essential oils and constituents against foodborne pathogens. Czech J. Food Sci., 43: 161–169.

Abstract: Growth inhibition and time-kill properties of Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oils against foodborne pathogens were evaluated. Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oils prolonged the lag phase of *Bacillus cereus*, *Bacillus subtilis*, *Shigella sonnei*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* for > 24 h and extended the lag phase by 4–24 h against other food poisoning bacteria. Citrus fruit peel essential oil and their constituents after 12 and 24 h of incubation showed almost complete growth inhibition against all foodborne pathogens, except *Pseudomonas aeruginosa.* Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oils exhibited > 40% killing activity against *B. cereus*, *B. subtilis*, and *S. sonnei*, *V. parahaemolyticus*, and *V. vulnificus* after 12 and 24 h of incubation. Additionally, *B. subtilis* showed the highest microbial killing rate of over 16% per hour, followed by *Vibrio* sp. Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oil are anticipated to replace chemical preservatives against foodborne pathogens.

Keywords: Hallabong; Redhyang; Cheonhyehyang; antimicrobial activity; bacteriostatic activity; bactericidal

Food spoilage and proliferation of foodborne pathogens make it difficult to store food and cause serious hygienic problems. Generally, chemical preservatives are used to prevent spoilage and suppress the growth of foodborne pathogens during food storage. However, chemical preservatives have harmful effects on human health and contributes towards the emergence of resistant strains, raising concerns regarding their use (Bondi et al. 2017). Natural substances, which have inhibitory activity against food spoilage bacteria and foodborne pathogens and are safer for human health, are emerging as an alternative (Bondi et al. 2017).

Citrus essential oil contains approximately 400 compounds, of which approximately 1–15% are non-volatile and 85–99% are volatile constituents (Nannapaneni et al. 2009). Organic compounds in citrus peel essential oils include terpenes, aliphatic sesquiterpenes, oxygenated derivatives, and aromatic hydrocarbons (Merle et al. 2004). Terpenes in essential oils vary depending on the citrus variety and contain limonene, α-pinene, β-pinene, myrcene, linalool, and terpinene in different proportions (Mohamed et al. 2010). Citrus essential oils and their constituents, such as limonene and linalool, are generally recognised as safe (GRAS) natural substances that do not harm the human body (Ghosh et al. 2019). Citrus essential oils are rich in flavonoids (flavones, flavonols, and flavanones), terpenes, carotenes, and coumarins with antimicrobial activity and thus have antifungal and antibacterial activities (Upadhyay et al. 2010). The processing of citrus fruits produces waste, such as peels and pulp, which account for 40–50% of the wet fruit mass (Javed 2011). Accordingly, the treatment of large amounts of peel waste and the economic cost involved are emerging as additional problems, and the production and economic use of essential oils from citrus peels is one of the solutions to this problem (Javed 2011).

There have been many studies on the antibacterial properties of essential oils from citrus peels. However,

https://doi.org/10.17221/258/2024-CJFS

there has been no report on the antibacterial activity against food poisoning bacteria for essential oils from Hallabong, Redhyang, Cheonhyehyang, and orange, which are hybrid citrus fruits grown in Korea. The aim of this study was to investigate the growth inhibition and time-kill activity of Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oils against foodborne pathogens and to help predict the bactericidal mechanisms of food poisoning bacteria when using citrus essential oils in foods.

MATERIAL AND METHODS

Preparation of essential oil and reagents

The essential oils were extracted from Hallabong peels (*Citrus unshiu* Marc. × *Citrus sinensis* Osb.) × (*Citrus reticulata* Blanco)], Cheonhyehyang {[(*C. unshiu* × *C. sinensis*) × *C. reticulata*] × (*C. reticulate* × *C. sinensis*)}, Redhyang (Citrus hybrid 'Kanpei), and the navel orange (*C. sinensis* Osb.) using an essential oil extractor (EssenLab - PLUS, Hanil Labtech, Yangju, Korea) (Shin et al. 2022). Four orange hybrids (Hallabong, Redhyang, Cheonhyehyang, and orange) was authorised by Prof. Hyo-Gil Choi in Kongju National University (Shin et al. 2022). The essential oils were separated with 70% water vapor for 8 h and condensed as a supernatant in an essential oil-collecting tube. Carefully separated essential oils were dehydrated with anhydrous sodium sulphate for 24 h, and then placed in a dark glass bottle and stored at –20 °C. Limonene, linalool, γ-terpinene, and octanal, which were identified as major constituents by gas chromatography/mass spectrometry (GC/MS) analysis (Shin et al. 2022), were purchased from Sigma (Sigma-Aldrich Co., USA) and TCI (Tokyo Chemical Industry Co., Ltd., Japan). Streptomycin sulphate was purchased from Sigma-Aldrich, and other chemicals used were reagent grade.

Foodborne pathogens and culture condition

Foodborne pathogens used in this study were as follows: four gram-positive bacteria, *Bacillus cereus* KCCM 11204 (*B. cereus*), *Bacillus subtilis* KCCM 11316 (*B. subtilis*), *Listeria monocytogenes* KCCM 40307 (*L. monocytogenes*), and *Staphylococcus aureus* KCCM 12214 (*Staph. aureus*) and six gram-negative bacteria, *Pseudomonas aeruginosa* KCCM 11266 (*P. aeruginosa*), *Salmonella choleraesuis* KCCM 11806 (*Sal. choleraesuis*), *Salmonella enterica* CCARM 119 (*Sal. enterica*), *Shigella sonnei* KCCM 41282 (*Shi. sonnei*), *Vibrio parahaemolyticus* KCCM 11965 (*V. parahaemolyticus*), and *Vibrio vulnificus* KCCM 41665 (*V. vulnificus*). Foodborne pathogens were obtained from the Korean Culture Center of Microorganisms (KCCM) and Culture Collection of Antimicrobial Resistant Microbes (CCARM). *L. monocytogenes*, *V. parahaemolyticus*, and *V. vulnificus* were cultured in brain heart infusion medium (0.5% NaCl)(BD Difco, USA), nutrient broth (3% NaCl; BD Difco, USA), and trypticase soy broth (1.5% NaCl; BD Difco, USA), respectively. Nutrient broth (Difco, USA) was used for cultivation of other foodborne pathogens. Each foodborne pathogen was incubated for 12 h at 37 °C in a shaking incubator (SIF 600R, Lab Companion, Korea).

Determination of growth inhibition of foodborne pathogens by citrus peel essential oils and their constituents

Foodborne pathogen was inoculated so that the absorbance was approximately 0.1 (1×10^7 cells·mL^{-1}) at 600 nm in the culture medium containing citrus peel essential oils (30 mg·mL^{-1}) and its constituents (30 mg·mL^{-1}). While foodborne pathogen was cultured at 37 °C for 24 h in a shaking incubator (SIF 600R, Lab Companion, Korea), the growth inhibition of foodborne pathogen was determined by the absorbance at 4 h intervals using a plate reader (SPARK 10M, Tecan, Switzerland). Growth curves of foodborne pathogens were plotted, and the growth inhibitory effect was evaluated by the lag phase period and overall cell growth inhibition by citrus peel essential oil or its constituents.

Determination of time-kill activity against foodborne pathogens by citrus peel essential oils and their constituents

Time-kill activity (%) and the initial death rate (%·h^{-1}) against foodborne pathogens were determined by measuring absorbance according to incubation time using a culture medium containing citrus peel essential oils or ingredients (30 mg·mL^{-1}). Foodborne pathogens were inoculated so that the absorbance of the culture solution was approximately 0.7–0.9 ($1–2 \times 10^8$ cells·mL^{-1}) at 600 nm and then incubated at 37 °C for 24 h in a shaking incubator (SIF 600R, Lab Companion, Korea). The absorbance of the culture was measured at 600 nm at 4 h intervals using a plate reader (SPARK 10M, Tecan, Switzerland). Time-kill activity (%) was analysed after 12 and 24 h incubation by comparing the absorbance at beginning of culture and after incubation. The initial death rate (%·h^{-1}) was calculated as time-kill activity during the first 4 h of culture.

https://doi.org/10.17221/258/2024-CJFS

Statistical analysis

All antibacterial experiments were performed 4 times, and the results were analysed as the mean and standard deviation. For convenience, the average values of each experimental group were expressed in Tables 2, 3, and 4. The results were analysed statistically with one-way analysis of variance (ANOVA) and Duncan multiple comparisons to compare the differences between the presented results. The difference between the experimental groups was considered to be significant when *P*-value was below 0.05.

RESULTS AND DISCUSSION

Growth inhibition of foodborne pathogens by Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oils and their constituents

The growth inhibitory effect of citrus peel essential oil (30 mg·mL^{-1}) and their constituents (30 mg·mL^{-1}) on *V. parahaemolyticus* was shown in Figure 1. The growth of *V. parahaemolyticus* was inhibited by all citrus peel essential oils during 24 h of cultivation, and the lag phase on the cell growth curve lasted for more than 24 h. In addition, limonene and linalool showed a lag phase of more than 24 h, while there was no growth inhibition of γ-terpinene and octanal against *V. parahaemolyticus*.

The growth of most of the foodborne pathogens was inhibited after 24 h of cultivation in a medium containing Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oils and their constituents (Table 1). In particular, the growth of *B. cereus*, *B. subtilis*, *Shi. sonnei*, *V. parahaemolyticus*, and *V. vulnificus* were inhibited throughout the 24 h culture by all citrus peel essential oils, and the lag phase was prolonged for more than 24 h. Hallabong and Redhyang peel essential oils showed growth inhibition (lag phase) of more than 24 h against *L. monocytogenes*, *Staph. aureus*, *Sal. choleraesuis*, and *Sal. enterica* and showed a lag phase of 4–8 h for *P. aeruginosa*. Among the constituents of citrus peel essential oils, limonene, linalool, and γ-terpinene inhibited the growth of most foodborne pathogens (Table 1). In particular, linalool had a lag phase of approximately 4 to 24 h or more for all foodborne pathogens, and limonene showed growth inhibition of 4 to 24 h or more for all foodborne pathogens, except *Staph. aureus* and *P. aeruginosa*. In addition, γ-terpinene inhibited the growth of all foodborne pathogens, other than *Staph. aureus*, *Sal. choleraesuis*, and *V. parahaemolyticus.* Octanal extended the lag phase for approximately 16 h after the initiation of the culture only for *L. monocytogenes* and had no microbial growth inhibitory activity toward other food-poisoning bacteria.

Basil, sage, and thyme essential oils extended the lag phase and decreased the maximum specific growth rate of *Escherichia coli* (*E. coli*) (Gutierrez et al. 2008). It was also reported that the antibacterial activity of essential oil was due to the antibacterial activity of major constituents, such as linalool and camphor (Gutierrez et al. 2008). Nutmeg and thyme essential oils increased the lag phase of *B. cereus* and decreased the maximum specific growth rate (Valero and Salmerón 2003). Similar to the results of previous studies regarding the antimicrobial activity of essential oils, the antimicrobial activity of citrus peel essential oils against foodborne pathogens prolonged the lag phase and reduced the specific growth rate.

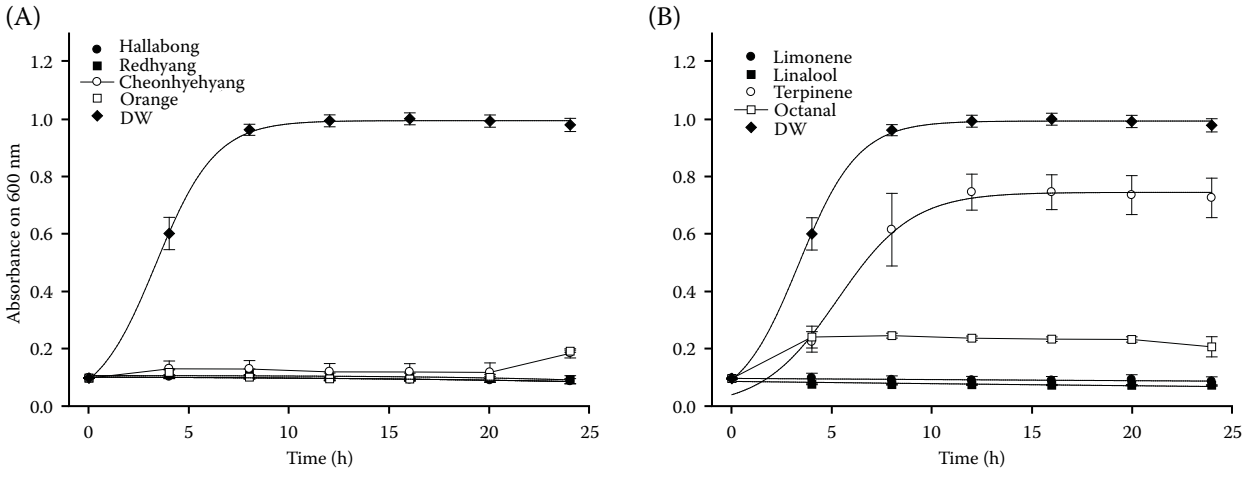

Figure 1. Growth inhibition of *Vibrio parahaemolyticus* by Hallabong, Redhyang, and Cheonhyehyang, and orange peel essential oils (A) and their constituents (B)

https://doi.org/10.17221/258/2024-CJFS

Table 1. Period of lag phase of foodborne pathogens by citrus peel essential oils and constituents (in h)

Foodborne pathogens	Essential oil (30 mg·mL^{-1})				Constituents of essential oil (30 mg·mL^{-1})					Antibiotics (30 mg·mL^{-1})
	Hallabong	Redhyang	Cheonhyehyang	orange	limonene	linalool	γ-terpinene	octanal	control	streptomycin
Bacillus cereus	> 24	> 24	> 24	> 24	> 24	16	> 24	–	–	> 24
Bacillus subtilis	> 24	> 24	> 24	> 24	16	16	12	–	–	> 24
Listeria monocytogenes	12	> 24	8	4	4	8	4	16	–	> 24
Staphylococcus aureus	> 24	> 24	4	4	–	8	–	–	–	> 24
Pseudomonas aeruginosa	4	8	–	–	–	4	4	–	–	> 24
Salmonella choleraesuis	> 24	> 24	–	20	20	> 24	–	–	–	> 24
Salmonella enterica	> 24	> 24	20	8	8	8	4	–	–	> 24
Shigella sonnei	> 24	> 24	> 24	> 24	> 24	> 24	8	–	–	> 24
Vibrio parahaemolyticus	> 24	> 24	20	20	> 24	> 24	–	–	–	> 24
Vibrio vulnificus	> 24	> 24	> 24	> 24	> 24	> 24	> 24	–	–	> 24

Growth inhibition of foodborne pathogens after 12 and 24 h incubation was expressed as a percentage compared to the control group (Table 2). Hallabong and Redhyang peel essential oil suppressed growth inhibition to approximately 80–100% against all foodborne pathogens, except *P. aeruginosa* at 12 and 24 h incubation. Cheonhyehyang and orange peel essential oils showed approximately 80–100% growth inhibitory activity at 12 and 24 h incubation against *B. cereus*, *B. subtilis*, *Sal. choleraesuis*, *Sal. enterica, Shi. sonnei*, *V. parahaemolyticus*, and *V. vulnificus. P. aeruginosa* had the lowest sensitivity, approximately 5.6–36.5%, to citrus essential oil. *P. aeruginosa* is resistant to chemical antibiotics because it has a cell wall with low permeability, enzymes that change antibiotics, and efflux pumps (Utchariyakiat et al. 2016). Additionally, *P. aeruginosa* was resistant to plant essential oils (Osho et al. 2010), with results similar to those in this study. *L. monocytogenes* and *Staph. aureus* showed a partial growth inhibition of approximately 9–60% by cheonhyehyang and orange peel essential oil. Limonene and linalool showed strong growth inhibition of approximately 80–100% against *B. cereus*, *B. subtilis*, *Sal. choleraesuis, Shi. sonnei*, *V. parahaemolyticus*, and *V. vulnificus*, and showed growth inhibitory activity of approximately 0–89% against other bacteria. γ-Terpinene and octanal showed strong growth inhibition against *B. cereus* and *V. vulnificus*, but not against other food-poisoning bacteria. Prolongation of the lag phase by citrus peel essential oils was observed for all foodborne pathogens, except *P. aeruginosa* (Table 1), and the cell growth inhibition rates at 12 and 24 h incubation reflected the results (Table 2). In addition, *L. monocytogenes, Staph. aureus*, *P. aeruginosa*, and *Sal. enterica*, which had less than 24 h of the lag phase, had reduced specific growth rate in the log phase (data not shown).

Das et al. (2020) reported that orange (*C. sinensis*) essential oil has antimicrobial activity that prolongs the lag phase of *L. monocytogenes* and lowers the growth rate. Also, mint, sage, and other vegetable essential

https://doi.org/10.17221/258/2024-CJFS

Table 2. Growth inhibition of foodborne pathogens by citrus peel essential oils and their constituents (in %)

Foodborne pathogens	Essential oil (30 mg·mL^{-1})				Component of essential oil (30 mg·mL^{-1})				Antibiotics (30 mg·mL^{-1})
	Hallabong	Redhyang	Cheonhyehyang	orange	limonene	linalool	γ-terpinene	octanal	streptomycin
Bacillus cereus	98.72^{a}/ 99.45^{a}	98.00^{a}/ 98.43^{a}	97.87^{ab}/ 98.50^{a}	98.33^{a}/ 99.09^{a}	98.20^{a}/ 99.67^{a}	100.00^{a}/ 54.97^{b}	95.37^{a}/ 95.21^{a}	83.13^{b}/ 82.16^{cd}	99.71^{a}/ 100.00^{a}
Bacillus subtilis	100^{a}/100^{a}	100^{a}/100^{a}	95.95^{ab}/ 87.63^{b}	99.86^{a}/ 81.41^{b}	85.63^{b}/ –	93.26^{ab}/–	99.01^{a}/–	56.49^{e}/ 30.34^{fg}	100^{a}/100^{a}
Listeria monocytogenes	99.38^{a}/ 13.51^{d}	99.38^{a}/ 100.00^{a}	32.35^{d}/ 9.45^{c}	26.56^{d}/ 19.35^{d}	21.92^{e}/ 12.64^{cd}	37.29^{d}/ 22.58^{c}	18.87^{cde}/ 12.76^{c}	89.08^{ab}/ 22.18^{g}	99.02^{a}/ 98.95^{b}
Staphylococcus aureus	94.66^{b}/ 95.35^{b}	91.73^{b}/ 85.41^{b}	60.44^{c}/ 25.89^{c}	53.23^{c}/ 36.54^{c}	44.56^{d}/ 23.75^{c}	42.62^{c}/–	11.00^{e}/ 0.04^{d}	50.73^{e}/ 32.29^{f}	98.26^{a}/ 100.00^{a}
Pseudomonas aeruginosa	5.57^{c}/ 19.89^{c}	36.54^{c}/ 12.96^{c}	12.45^{e}/ 19.50^{c}	17.53^{e}/ 26.60^{cd}	11.96^{e}/ 18.63^{cd}	20.80^{e}/ 14.38^{d}	16.80^{de}/ 13.82^{c}	71.97^{d}/ 64.28^{e}	98.13^{a}/ 100.00^{a}
Salmonella choleraesuis	97.34^{ab}/ 98.05^{ab}	98.07^{a}/ 98.92^{a}	87.27^{b}/ 85.99^{ab}	96.45^{a}/ 87.53^{b}	95.93^{a}/ 70.46^{b}	100^{a}/100^{a}	29.67^{bc}/ 32.16^{b}	73.95^{cd}/ 78.94^{cd}	97.48^{a}/ 98.34^{c}
Salmonella enterica	100^{a}/100^{a}	100^{a}/100^{a}	96.32^{ab}/ 20.21^{c}	82.41^{b}/ 25.26^{d}	68.41^{c}/ 9.27^{de}	89.03^{b}/ 16.21^{d}	35.42^{b}/ 6.88^{cd}	81.04^{bc}/ 75.80^{d}	99.12^{a}/ 100.00^{a}
Shigella sonnei	100^{a}/100^{a}	99.30^{a}/ 100.00^{a}	100^{a}/100^{a}	97.76^{a}/ 99.97^{a}	95.59^{a}/ 99.94^{a}	100^{a}/100^{a}	100.00^{a}/ 99.40^{a}	58.26^{e}/ 99.72^{a}	97.84^{a}/ 99.99^{a}
Vibrio parahaemolyticus	100^{a}/100^{a}	99.58^{a}/ 100.00^{a}	97.61^{ab}/ 95.47^{a}	100.00^{a}/ 94.83^{ab}	100^{a}/100^{a}	100^{a}/100^{a}	27.57^{bcd}/ 28.67^{b}	84.36^{b}/ 87.50^{bc}	100^{a}/100^{a}
Vibrio vulnificus	100^{a}/100^{a}	100^{a}/100^{a}	98.63^{a}/ 98.82^{a}	100^{a}/100^{a}	100^{a}/100^{a}	100^{a}/ 100^{a}	100^{a}/100^{a}	94.13^{a}/ 95.37^{ab}	100^{a}/100^{a}

The values are the growth inhibition (%) at 12 h incubation/ growth inhibition (%) at 24 h incubation; – not detected; each value represents an average; alphabetic letters with superscripts in the same column show statistically significant differences at $P < 0.05$ by Duncan's multiple range test

oils extended the lag phase and reduced the maximum specific growth rate (μ_{max}) in log phase of *B. cereus* (Valero and Salmerón 2003). The antibacterial properties of citrus peel essential oils against foodborne pathogens are similar to the antimicrobial activity of food spoilage bacteria and food poisoning bacteria using plant extracts and essential oils in previous studies.

Time-kill property against foodborne pathogens by Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oils and their constituents

The time-kill effect of citrus peel essential oil (30 mg·mL^{-1}) and their constituents (30 mg·mL^{-1}) for *V. parahaemolyticus* was shown in Figure 2. Citrus essential oil showed rapid microbial death of *V. parahaemolyticus* within approximately 5 h after start of incubation and continued to 24 h. Linalool had the highest killing activity against *V. parahaemolyticus*, followed by limonene, γ-terpinene, and octanal.

The time-kill activity against each foodborne pathogen was determined as the ratio of the decrease in absorbance at 12 and 24 h incubation from the absorbance at the time of inoculation (Table 3). Hallabong, Redhyang, Cheonhyehyang, and orange peel essential oil and ingredients showed bactericidal effects against all foodborne pathogens, except *L. monocytogenes*. Especially, the essential oils of Hallabong and Redhyang peel exhibited the most potent killing activity of 79.12% and 82.03% after 12 h incubation, and 81.36 and 84.45% after 24 h incubation against *B. subtilis*, respectively. Hallabong,

https://doi.org/10.17221/258/2024-CJFS

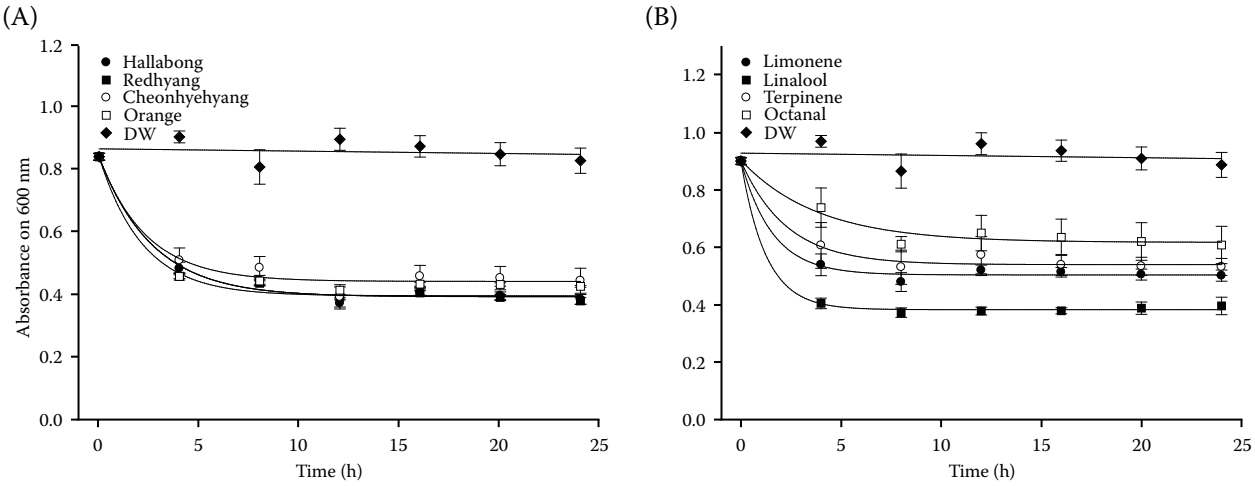

Figure 2. Time-kill kinetics of *Vibrio parahaemolyticus* by Hallabong, Redhyang, and Cheonhyehyang, and orange peel essential oils (A) and their constituents (B)

Table 3. Bacteriocidal activity of foodborne pathogens by citrus peel essential oils and their constituents (in %)

Foodborne pathogens	Essential oil (30 mg·mL^{-1})				Component of essential oil (30 mg·mL^{-1})				Antibiotics (30 mg·mL^{-1})
	Hallabong	Redhyang	Cheon-hyehyang	orange	limonene	linalool	γ-terpinene	octanal	streptomycin
Bacillus cereus	66.53^{b}/ 37.76^{d}	48.43^{c}/ 39.40^{c}	30.63^{bc}/ 37.96^{bc}	29.17^{e}/ 41.15^{d}	36.94^{bc}/ 29.40^{cd}	37.28^{g}/ 40.04^{e}	25.62^{cde}/ 43.53^{b}	34.96^{bc}/ 18.86^{cd}	34.69^{b}/ 39.12^{c}
Bacillus subtilis	79.12^{a}/ 81.36^{a}	82.03^{a}/ 84.45^{a}	31.62^{bc}/ 32.04^{c}	59.85^{a}/ 68.12^{a}	54.56^{a}/ 54.12^{a}	86.36^{a}/ 77.86^{a}	35.38^{bc}/ 42.11^{b}	54.11^{a}/ 24.95^{bc}	55.59^{a}/ 71.83^{a}
Listeria monocytogenes	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
Staphylococcus aureus	4.91^{f}/ 26.26^{e}	0.76^{f}/ 28.79^{d}	13.45^{e}/ 35.85^{bc}	4.64^{h}/ 31.05^{e}	12.21^{e}/ 27.25^{cd}	53.46^{cd}/ 46.76^{de}	14.19^{f}/ 26.01^{d}	15.17^{d}/ 14.82^{cd}	30.31^{b}/ 50.02^{b}
Pseudomonas aeruginosa	22.85^{e}/ 28.49^{e}	21.79^{e}/ 38.39^{c}	23.43^{cd}/ 37.71^{bc}	24.15^{f}/ 33.49^{e}	25.26^{d}/ 35.04^{bcd}	19.92^{h}/ 27.65^{f}	22.96^{def}/ 33.04^{c}	38.68^{b}/ 37.15^{a}	–/7.30^{fg}
Salmonella choleraesuis	37.42^{d}/ 22.70^{e}	38.52^{d}/ 24.25^{d}	40.31^{b}/ 25.22^{c}	34.95^{d}/ 22.14^{f}	44.64^{b}/ 26.47^{d}	49.65^{de}/ 50.37^{cd}	15.80^{ef}/ 3.36^{e}	15.04^{d}/ 24.07^{bc}	14.14^{c}/ 20.02^{e}
Salmonella enterica	20.82^{e}/ 10.92^{f}	19.87^{e}/ 12.09^{e}	20.70^{de}/ 1.28^{d}	19.75^{g}/ 12.88^{g}	24.06^{d}/ 9.05^{e}	44.97^{ef}/ 47.52^{de}	–/–	15.92^{d}/ 23.39^{bc}	–/4.40^{g}
Shigella sonnei	40.74^{d}/ 47.07^{c}	37.21^{d}/ 43.52^{c}	40.15^{b}/ 47.15^{ab}	40.04^{c}/ 43.48^{cd}	35.12^{c}/ 36.65^{bc}	44.14^{f}/ 49.24^{cd}	27.52^{bcd}/ 31.48^{cd}	0.21^{e}/ 10.83^{d}	10.04^{c}/ 4.68^{g}
Vibrio parahaemolyticus	56.18^{c}/ 56.64^{bc}	53.78^{bc}/ 55.24^{b}	53.71^{a}/ 47.50^{ab}	51.11^{b}/ 49.65^{c}	42.30^{bc}/ 44.35^{b}	58.02^{c}/ 56.05^{c}	36.39^{b}/ 40.97^{b}	27.85^{c}/ 32.49^{ab}	15.90^{c}/ 12.60^{ef}
Vibrio vulnificus	57.52^{c}/ 60.14^{b}	57.44^{b}/ 60.13^{b}	53.47^{a}/ 58.39^{a}	58.18^{a}/ 60.25^{b}	58.13^{a}/ 60.70^{a}	68.07^{b}/ 70.04^{b}	53.60^{a}/ 56.10^{a}	12.21^{d}/ 23.59^{bc}	18.38^{c}/ 31.15^{d}

The values are the bactericidal effect (%) at 12 h incubation/ bactericidal effect (%) at 24 h incubation; – not detected; each value represents an average; alphabetic letters with superscripts in the same column show statistically significant differences at $P < 0.05$ by Duncan's multiple range test

https://doi.org/10.17221/258/2024-CJFS

Redhyang, Cheonhyehyang, and orange peel essential oils showed approximately 37–60% of killing activity against *Shi. sonnei*, *V. parahaemolyticus*, and *V. vulnificus*. However, citrus peel essential oil and its constituents had relatively low killing activity of 0–53.46% after 24 h incubation against other foodborne pathogens and did not show killing activity against *L. monocytogenes*. In addition, limonene and linalool demonstrated potent killing activity against all foodborne pathogens, except *L. monocytogenes* and γ-terpinene, and octanal had relatively low killing activity.

The initial death rate against foodborne pathogens within the first 4 h incubation was investigated in a medium containing Hallabong, Redhyang, Cheonhyehyang, orange peel essential oil, and ingredients (each 30 mg·mL^{-1}) (Table 4). All citrus peel essential oils and their constituents, except for limonene, exhibited the initial death rate of > 10%·h^{-1} against *B. subtilis*, and redhyang peel essential oil showed the initial death rate of 11.08%·h^{-1} against *B. cereus*. In addition, citrus peel essential oils and limonene and linalool had the initial death rate of > 10%·h^{-1} against *V. parahaemolyticus* and *V. vulnificus*. γ-Terpinene had the initial death rate of 8.16 and 10.96%·h^{-1} against *V. parahaemolyticus* and *V. vulnificus*, respectively, and octanal had the initial death rate of 4.50%·h^{-1} only against *V. parahaemolyticus* but not against *V. vulnificus*. Among the constituents of essential oil, only linalool had the initial death rate of 13.9 and 11.47%·h^{-1} for *P. aeruginosa* and *Sal.*

Table 4. Bactericidal rate of foodborne pathogens by citrus peel essential oils and their components

Foodborne pathogens	Essential oil (30 mg·mL^{-1})				Component of essential oil (30 mg·mL^{-1})				Antibiotics (30 mg·mL^{-1})
	Hallabong	Redhyang	Cheonhyehyang	orange	limonene	linalool	γ-terpinene	octanal	streptomycin
Bacillus cereus	8.40^{c}	11.08^{b}	6.41^{d}	7.18^{d}	8.85^{abc}	15.49^{b}	4.21^{bcd}	6.36^{b}	10.36^{a}
Bacillus subtilis	16.79^{a}	18.03^{a}	16.03^{a}	18.81^{a}	3.21^{de}	20.57^{a}	17.23^{a}	10.66^{a}	3.92^{b}
Listeria monocytogenes	1.05^{e}	2.40^{e}	–	–	–	3.70^{e}	–	–	1.31^{de}
Staphylococcus aureus	–	–	1.83^{e}	0.67^{e}	0.93^{e}	7.65^{d}	1.75^{cd}	–	2.37^{cd}
Pseudomonas aeruginosa	–	–	–	–	–	13.73^{b}	–	7.38^{b}	–
Salmonella choleraesuis	8.71^{c}	9.31^{c}	9.35^{c}	9.59^{c}	9.63^{abc}	11.47^{c}	6.03^{bcd}	0.66^{d}	–
Salmonella enterica	6.22^{d}	5.91^{d}	5.46^{d}	6.12^{d}	5.39^{cd}	8.86^{d}	1.10^{cd}	–	–
Shigella sonnei	6.86^{cd}	6.40^{d}	7.00^{d}	7.08^{d}	6.18^{bcd}	8.38^{d}	3.82^{cd}	–	2.77^{bc}
Vibrio parahaemolyticus	10.70^{b}	11.43^{b}	9.93^{bc}	11.47^{b}	10.04^{ab}	13.77^{b}	8.16^{bc}	4.50^{c}	–
Vibrio vulnificus	12.21^{b}	12.13^{b}	11.14^{b}	12.82^{b}	12.24^{a}	15.36^{b}	10.96^{ab}	–	–

– not detected; each value represents an average; alphabetic letters with superscripts in the same column show statistically significant differences at $P < 0.05$ by Duncan's multiple range test

https://doi.org/10.17221/258/2024-CJFS

choleraseus, respectively, and had an initial death rate of approximately 3.70–20.57%·h^{-1} for all foodborne pathogens. *B. cereus*, *V. parahaemolyticus,* and *V. vulnificus* were the most sensitive to almost all essential oils and their constituents at the beginning of culture, whereas *Staph. aureus* and *P. aeruginosa* were the most insensitive to them. El-Tawab et al. (2017) reported that *Staph. aureus* was a microorganism that is difficult to control because it was resistant to antimicrobial agents. In particular, methicillin-resistant *Staph. aureus* was resistant to antibiotics and is difficult to remove, causing problems mainly due to infection in humans and dairy products (Virgin et al. 2009). *P. aeruginosa* and *Staph. aureus* in this study also had the lowest sensitivity to citrus essential oil, so the results were similar to previous reports.

The antibacterial mechanism of citrus essential oil is closely related to its chemical properties. Hydrophobic essential oils can easily penetrate, absorb, and deliver into bacterial and mitochondrial membrane with the lipid components, disrupting the cell structure and reducing the cell size of microorganisms (Zhang et al. 2017). Furthermore, it damages the cell wall and cell membrane, and reduces and aggregates the nuclear cytoplasm, thereby exhibiting a microbial killing effect. Essential oils in bacterial cells can especially damage mitochondrial membranes and generate free radicals in mitochondria to oxidise and damage lipids, proteins and DNA (Li et al. 2019). In addition, the essential oil impairs membrane integrity, causing abnormal release of nucleic acids and potassium ions in microbial cells, and exhibits antibacterial activity (Li et al. 2019).

The antibacterial activity of citrus peel essential oil against foodborne pathogens is due to the antimicrobial activity of the constituents of these oils. The volatile compounds in essential oils of citrus hybrid peel are generally similar in composition, only the proportions of the compounds differ. Therefore, it is highly probable that differences in antibacterial activities of Hallabong, Redhyang, Cheonhyehyang, and orange peels will differ depending on the content ratio of the compounds in essential oils. Although some microorganisms are insensitive to the antibiotic streptomycin, natural citrus peel essential oil has a broader spectrum of bactericidal against foodborne pathogens than streptomycin.

CONCLUSION

The essential oil in the peels of Korean citrus hybrids – Hallabong, Redhyang, and Cheonhyehyang, have the potential to be utilised as natural food preservatives exhibiting both bacteriostatic and bactericidal activities against various foodborne pathogens. However, further studies on shelf-life stability and safety evaluation are required to confirm their practical applicability. In addition, these citrus peel essential oils may serve as promising alternatives to chemical disinfectants in hygiene-related products such as hand sanitisers, soaps, cosmetics, and wet tissues.

REFERENCES

Bondi M., Lauková A., de Niederhausern S., Messi P., Papadopoulou C. (2017): Natural preservatives to improve food quality and safety. Journal of Food Quality, 2017: 1–3.

Das S., Vishakha K., Banerjee S., Mondal S., Ganguli A. (2020): Sodium alginate-based edible coating containing nanoemulsion of *Citrus sinensis* essential oil eradicates planktonic and sessile cells of food-borne pathogens and increased quality attributes of tomatoes. International Journal of Biological Macromolecules, 162: 1770–1779.

El Tawab A.A.A., Hassan W.M., AL Siraj S.S. (2017): Bacteriological and molecular studies of *Staphylococcus aureus* isolated from foods and human contact. Benha Veterinary Medical Journal, 33: 38–45.

Ghosh T., Srivastava S.K., Gaurav A., Kumar A., Kumar P., Yadav A.S., Pathania R., Navani N.K. (2019): A combination of linalool, vitamin C, and copper synergistically triggers reactive oxygen species and DNA damage and inhibits *Salmonella enterica* subsp. *enterica* serovar Typhi and *Vibrio fluvialis*. Applied and Environmental Microbiology, 85: e02487–18.

Gutierrez J., Barry-Ryan C., Bourke P. (2008): The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. International Journal of Food Microbiology, 124: 91–97.

Javed S., Javaid A., Mahmood Z., Javaid A., Nasim F. (2011): Biocidal activity of citrus peel essential oils against some food spoilage bacteria. Journal of Medicinal Plants Research, 5: 3697–3701.

Li Z.H., Cai M., Liu Y.S., Sun P.L., Luo S.L. (2019): Antibacterial activity and mechanisms of essential oil from *Citrus medica* L. var. *sarcodactylis*. Molecules, 24: 1577.

Merle H., Morón M., Blázquez M.A., Boira H. (2004): Taxonomical contribution of essential oils in mandarins cultivars. Biochemical Systematics and Ecology, 32: 491–497.

Mohamed A.A., El-Emary G.A., Ali H.F. (2010): Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in *Ehrlich ascites* carcinoma cells. Journal of American Science, 6: 820–826.

https://doi.org/10.17221/258/2024-CJFS

Nannapaneni R., Chalova V.I., Crandall P.G., Ricke S.C., Johnson M.G., O'Bryan C.A. (2009): *Campylobacter* and *Arcobacter* species sensitivity to commercial orange oil fractions. International Journal of Food Microbiology, 129: 43–49.

Osho A., Adetunji T., Fayemi S.O., Moronkola D.O. (2010): Antimicrobial activity of essential oils of *Physalis angulata* L. African Journal of Traditional, Complementary, and Alternative Medicines, 7: 303–306.

Shin S.D., Kim C.S., Lee J.H., (2022): Compositional characteristics and bacterial activity of essential oils in citrus peels . Food Science and Technology, 42: e95921.

Upadhyay R.K., Dwivedi P., Ahmad S. (2010): Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. Asian Journal of Medicine Sciences, 2: 152–158.

Utchariyakiat I., Surassmo S., Jaturanpinyo M., Khuntayaporn P., Chomnawang M.T. (2016): Efficacy of cinnamon bark oil and cinnamaldehyde on anti-multidrug resistant *Pseudomonas aeruginosa* and the synergistic effects in combination with other antimicrobial agents. BMC Complementary and Alternative Medicine, 16: 158.

Valero M., Salmerón M.C. (2003): Antibacterial activity of 11 essential oils against *Bacillus cereus* in Tyndallized carrot broth. International Journal of Food Microbiology, 85: 73–81.

Virgin J.E., Van Slyke T.M., Lombard J.E., Zadoks R.N. (2009): Short communication: Methicillin-resistant *Staphylococcus aureus* detection in US bulk tank milk. Journal of Dairy Science, 92: 4988–4991.

Zhang J., Ye K.P., Zhang X., Pan D.D., Sun Y.Y., Cao J.X. (2017): Antibacterial activity and mechanism of action of black pepper essential oil on meat-borne *Escherichia coli*. Frontier Microbiology, 7: 2094.

Received: December 13, 2024
Accepted: April 22, 2025
Published online: June 18, 2025