pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 40, No. 3, pp. 267~279 (2025)
https://doi.org/10.13103/JFHS.2025.40.3.267

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Research note

유기산, 티몰 및 초음파의 병행 처리에 의한 팽이버섯에서 *Escherichia coli* O157:H7와 *Listeria monocytogenes*의 제어 효과

박사랑 · 이상우 · 윤세경 · 조현동 · 김인용 · 윤재현*
국립순천대학교 식품영양학과

Control of *Escherichia coli* O157:H7 and *Listeria monocytogenes* Inoculated in Enoki Mushrooms (*Flammulina velutipes*) by Combined Treatments with Organic Acids, Thymol, and Ultrasound

Sa-Rang Park, Sang-Woo Lee, Se-Gyeong Yoon, Hyun-Dong Cho, In-Yong Kim, Jae-Hyun Yoon
Department of Food and Nutrition, Sunchon National University, Suncheon, Korea

(Received January 31, 2025/Revised May 2, 2025/Accepted May 13, 2025)

ABSTRACT - The aim of this study was to investigate the antibacterial effects of ultrasound (US) together with organic acids (OAAs) and thymol in controlling *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated in enoki mushrooms (*Flammulina velutipes*). Enoki mushrooms, which were inoculated, corresponding to each of the bacterial populations of $10^{5\text{-}6}$ CFU/g, were treated with varying concentrations of OAAs, minimum inhibitory concentrations (MICs=0.107 mg/L) of thymol and/or 20 kHz US for 15 min at 25°C. Approximately≤1.7-log reductions in the viable number of *E. coli* O157:H7 and *L. monocytogenes* were achieved after individual treatments with 3% OAA, 2×MIC thymol, and 20 kHz US at 25°C for 15 min. In particular, dipping in 3% lactic acid (LA)+2×MIC thymol incorporated with 20 kHz US at 25°C for 15 min exhibited an additive effect, resulting in >3 log reductions against *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms. In addition, there was no significant (P>0.05) difference in the color parameters of enoki mushrooms before and after combined treatments with LA+thymol+US. US appears to be related to the increased accessibility of a formulation of LA and thymol close to *E. coli* O157:H7 cells, which was accompanied by the disintegration of cell membranes.

Key words: Enoki mushroom (*Flammulina velutipes*), Food-borne pathogenic bacteria, Organic acid, Thymol, Ultrasound

Although limited data are available that focus on the prevalence of *Escherichia coli* O157:H7 present in edible mushrooms, enterohemorrhagic *E. coli* (EHEC) was detected in 4 (4.0%) out of a total of 100 mushroom samples[1]. Furthermore, a 2016-2020 outbreak of *Listeria monocytogenes* infections associated with enoki mushrooms (*Flammulina velutipes*) imported from the Republic of Korea caused a total of 31 hospitalizations and four deaths in the USA[2]. Ok et al.[3] reported that *L. monocytogenes* predominated in enoki mushrooms (12.5%) collected from rural grocery stores located in the Republic of Korea, 2022. Similarly, the proportion of enoki mushrooms which were found to be positive for *L. monocytogenes* was 10% ($\frac{5}{50}$)[4]. Lee et al.[5] also have determined the emergence of *L. monocytogenes* recovered from online market-purchased enoki mushrooms in the Republic of Korea. All results give a clear indication on the relevant consumption of edible mushrooms within the food safety issues.

Escherichia coli O157:H7 has been recognized as one of the etiological agents in causing food-borne outbreaks worldwide with principal manifestations, including hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombocytopenic purpura (TPP)[6]. *L. monocytogenes* is a high risk vector for the life-threatening disease, so-called as listeriosis with

*Correspondence to: Jae-Hyun Yoon, Department of Food and Nutrition, Sunchon National University, Suncheon 57922, Korea
Tel: +82-61-750-3651, Fax: +82-61-750-3650
E-mail: nevbge999@gmail.com

Copyright © The Korean Society of Food Hygiene and Safety. All rights reserved. The Journal of Food Hygiene and Safety is an Open-Access journal distributed under the terms of the Creative Commons Attribution Non-Commercial License(http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution,and reproduction in any medium, provided the original work is properly cited.

high fatality rates of 20-30%, resulting in abortion, encephalitis, meningitis, septicemia, and stillbirth with at-risk-groups, including infants, children, pregnant women, and the elderly (>65 years) or immunocompromised individuals[7].

L. monocytogenes is highly widespread and ubiquitous in the natural environments and easily found in food, soil, and water, as well as in the clinical and healthcare facilities[8]. *L. monocytogenes* is a facultatively anaerobic, catalase-positive, gram-positive, nonspore-forming, and rod-shaped bacterium, which is divided into four evolutionary lineages (I-V) and 13 serotypes[9]. Specifically, $\frac{1}{2}$a, $\frac{1}{2}$b, and 4b are mainly responsible for 95% of the reported human listeriosis cases and outbreaks[10]. In the case of highly susceptible groups of the population, newborns, infants and toddlers (less than 5 years), the elderly (more than 65 years), and individuals with compromised immune systems are at the greatest risk of becoming sick from listeriosis infections which are characterized by symptoms, such as encephalitis, meningitis, and septicemia, with a high case fatality rate of up to 20-30%[11].

Edible mushrooms can be contaminated with *Bacillus cereus*, *E. coli*, EHEC, *L. monocytogenes*, *Salmonella*, and *Staphylococcus aureus* during all farm-to-consumer stages where good hygienic practices remain a challenge[1,12-14]. Otherwise, edible mushrooms are no longer considered low risks (i.e. a stable and safe product) in terms of food safety and public health. In particular, enoki mushrooms that have completed a growing cycle undergo no further washing or processing steps before packaging and consumption. It should be also noted that enoki mushrooms are often consumed raw (in their form of salads and sandwiches), based on the increased consumer's preference for natural and nutritious diets[15]. While the cleaning and disinfection procedures are not standardized across mushroom production facilities, chemical agents, such as sodium hypochlorite, have been exploited not only to remove attached casing soil particles, but also to reduce initial microbial loads on the surface of mushroom products[16,17]. A variety of thermal processes (i.e. drying and cooling) have been frequently utilized to prevent the growth of spoilage and/or pathogenic microorganisms and retard the quality deterioration (i.e. enzymatic reactions) in edible mushrooms, but can have a negative impact on the textural and nutritional properties of mushroom products to be treated[18]. So far, previous studies have been conducted considering the attempt to investigate the effectiveness of chemical or natural antimicrobials and the application of new technologies in ensuring the food quality and safety[19-22]. However, along with the fact that consumers are being concerned about consuming foods, such as fruits, mushrooms, and vegetables, that may contain the residue of chemical preservatives, the usage of some aqueous disinfectants/sanitizers, such as chlorine, hydrogen peroxide, and trisodium phosphate, alone have not been always successful in controlling food-borne pathogens inoculated in fruits and vegetables due in part to the failure of the solutions to reach the inaccessible rough regions, such as stomata, which would possess a few of adherent and/or internalized cells of the organisms. In addition, the application of new technologies, such as a 405-nm light-emitting diode (LED) illumination[23], ozone in the gaseous state[24], ultrasound[23,25], and ultraviolet-C[26,27], alone would be less likely to either reduce or eliminate both the pathogenic bacteria and the natural microbiota of edible mushrooms. Previously, we determined that dipping in a mixture of 3% LA and 0.1% nisin incorporated with a 40-kHz US operation exerted marked synergistic effects for reducing the viable numbers of *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms by approximately ≥5 log-reductions[25]. Moreover, both *E. coli* O157:H7 and *L. monocytogenes* were undetectable in enoki mushrooms stored at 5°C for up to 5 d after combined treatments with LA+nisin+US, concomitantly with the destruction of cell membrane-associated structures with the formation of local pores[25]. However, there are limited studies regarding the bacteriostatic or bactericidal efficacy of combined treatments with organic acids (OAAs), essential oils (EOs), and a low intensity-pulsed ultrasound (US) for inactivating food-borne pathogenic bacteria inoculated in edible mushrooms at a room temperature. This study, therefore, focused on new and more effective methods for the sake of evaluating the potent applicability of an OAA and EO dip incorporated with a 20-kHz US operation in a concurrent trial, which aimed at improving the postharvest quality of enoki mushrooms.

Alternatively, along with the fact that public concern and impact over synthetic chemicals have drawn considerable attention and request to the use of naturally derived substances to control the proliferation of pathogenic microorganisms, OAAs and EOs represent a suite of natural antimicrobial agents that have been already widely used in the food industry. The antibacterial actions of OAAs are primarily due to the cytoplasmic acidification,

which disrupts key intracellular processes, such as DNA replication and protein synthesis[28]. Thymol, a natural monoterpene found in oregano and thyme, exhibits a broad spectrum of antibacterial activities[29]. Furthermore, US (i.e. pressure waves with a frequency of 20 kHz or more) has the potential to be used for the control of pathogenic bacteria inoculated in fresh fruits and vegetables, and the US-driven cavitation bubbles oscillate, grow, and collapse asymmetrically, thereby generating a very high temperature[30]. Comprehensively, combined treatments show potent bacteriostatic or bactericidal effects, which are greater than those observed after individual treatments as long as OAA, thymol, and US function through nonoverlapping pathways for inactivating microorganisms. This study, therefore, was undertaken to compare/characterize the effectiveness of different combinations of OAAs, such as acetic acid (AA), citric acid (CA), lactic acid (LA), and malic acid (MA), thymol, and US in reducing or eliminating the number of *E. coli* O157:H7 and *L. monocytogenes* inoculated on the surface of enoki mushrooms.

Materials and Methods

Preparation of bacterial inoculums

E. coli O157:H7 ATCC 43889 and *L. monocytogenes* ATCC 19114 were obtained from the bacterial culture collections of Chung-Ang University (Anseong, Korea) and used in this study. Bacterial stocks of *E. coli* O157:H7 ATCC 43889 or *L. monocytogenes* ATCC 19114 were maintained in tryptic soy broth (TSB; Difco® Laboratories Inc., Detroit, MA, USA) or TSB supplemented with 0.6% yeast extract (TSBYE; Difco® Laboratories Inc.) at -80°C and then activated in TSB or TSBYE, following 24 h of incubation at 37°C. Individually, the overnight culture (1 mL) of *E. coli* O157:H7 ATCC 43889 and *L. monocytogenes* ATCC 19114 grown in TSB and TSBYE, respectively, were placed in a sterile microtube, centrifugated at 8,000 × *g* for 3 min, washed twice in 0.1 M phosphate buffer saline (PBS; pH 7.1±0.1), and resuspended in 1 mL of PBS. On the day before the following experiments, enoki mushrooms were purchased from a grocery store located in Sucheon, Korea and used without undergoing any processing. One mL of each of the bacterial suspensions was spot-inoculated on the surface of enoki mushrooms weighing 100 g, corresponding to 10^{5-6} CFU/g, and the samples were air-dried in a laminar biosafety hood at 25°C for 30 min.

Determination of MICs

A stock solution containing 10% thymol (Sigma-Aldrich® Co., St Louis, MO, USA) was completely dissolved in 99% ethanol (Sigma-Aldrich® Co.) and then diluted in sterile deionized water (DW) to yield a final concentration of 1%. All the solutions were sterilized through a 0.22-μm pore size polycarbonate membrane filtration (Millipore Ltd., Mississauga, Canada).

Briefly, 100 μL of 10% thymol was mixed with 2×TSB (100 μL) in a 96-well plate at a 1:1 ratio to give a final concentration of 0.5%. Then, 100 μL of 0.5% thymol was added to the next well containing 100 μL of TSB (column 1), and serial two-fold dilutions were applied to other rest wells from columns 2 to 30, resulting in a final volume of 100 μL. Each of the wells containing varying concentrations of thymol was inoculated with 10 μL of the cultured strains of *E. coli* O157:H7 or *L. monocytogenes* and incubated at 37°C for 24 h. The optical densities of the bacterial solutions were measured by using a Multiskan™ Go microplate spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 595 nm. The MIC was determined as the lowest concentration of a compound at which optical densities at 595 nm were less than 0.4.

Individual or combined treatments

OAAs were prepared according to an instruction provided by manufacturer, including AA (DAEJUNG Chemicals & Metals Co., Ltd., Siheung, Korea), CA (DAEJUNG Chemicals & Metals Co., Ltd.), LA (DAEJUNG Chemicals & Metals Co., Ltd.), and MA (DAEJUNG Chemicals & Metals Co., Ltd.).

Enoki mushrooms (weighing 100 g) contaminated with *E. coli* ATCC 43889 or *L. monocytogenes* ATCC 19114 were dipped in 0.25, 0.5, 0.75, 1, 2 or 3% AA, CA, LA or MA at 25°C for 0, 5, 10 or 15 min. In the same manner, 100 g of enoki mushrooms was transferred to a sterile glass beaker and immersed in 250 mL of MIC (= 0.107 mg/L) or 2×MIC thymol at 25°C for 0, 5, 10 or 15 min. Furthermore, a portion (25 g) of enoki mushrooms carrying *E. coli* O157:H7 or *L. monocytogenes* was immersed in the cleaner basket of the WiseClean® ultrasonic processor with 20 kHz and 290-300 W (Witeg Labortechnik GmbH, Wertheim, Germany) filled with 250 mL of sterile DW and, the samples were sonicated at 20 kHz US at 25°C for 0, 5, 10 or 15 min.

As stated above, the samples weighing 25 g was placed inside the US cleaner tank in which 250 mL of I)3% LA,

II)2×MIC thymol or III)a mixture of 3% LA and 2×MIC thymol was filled and sonicated at 20 kHz US at 25°C for 0, 5, 10 or 15 min All the formulations used alone or in combination were sterilized, following a membrane filtration with a 0.22-μm pore size polycarbonate syringe filter (Millipore Ltd.) before use.

Enumeration

After individual or combined treatments, 25 g of each of the enoki mushroom samples treated was transferred to a stomacher bag containing 100 mL of Dey-Engley neutralizing (DE) broth (Difco® Laboratories Inc.) to minimize the residual effect of all the sanitizing solutions and homogenized via a BagMixer® 400 stomacher (INTERSCIENCE Lab. Inc., St Nom la Bretèche, France) for 1 min. Bacterial solutions (1 mL) were serially diluted in 9 mL of 0.1% peptone water (PW; Difco® Laboratories Inc.), and the 100-μL diluent carrying *E. coli* ATCC 43889 and *L. monocytogenes* ATCC 19114 was spread on selective media, sorbitol MacConkey agar (SMAC; Oxoid™ Ltd., Basingstoke, UK) and Oxford agar (Oxoid™ Ltd.) supplemented with the Oxford Listeria selective supplement (Oxoid™ Ltd.), respectively, in order to count the viable number of these bacteria inoculated in enoki mushrooms. All agar plates were incubated at 37°C for 24-48 h.

A propidium iodide (PI) uptake analysis

In order to identify the inactivation-associated mechanisms, PI (Sigma-Aldrich® Co.) was utilized to evaluate the cell membrane damage of *E. coli* O157:H7 cells before and after individual or combined treatments with 3% LA, 2×MIC thymol, and/or 20 kHz US at an ambient temperature for 5, 10 or 15 min. A stock solution containing 1 mg/ mL PI was dissolved in sterile DW and stored in the dark at 4°C. After individual or combined treatments, the cells of *E. coli* O157:H7 were centrifugated at 12,000 × g for 3 min at 4°C, washed three times in 0.1 M PBS, and then inoculated into 0.1 M PBS to achieve an optical density at 600 nm (OD_{600}) of 0.2. After centrifugation at 10,000 × *g* at 4°C for 3 min, the resultant supernatants were discarded, and PI was added to the cell pellets at its final concentration of 2.9 μM, followed by 15-20 min of incubation in the dark. Thus far, each of the samples were washed three times with 0.1 M PBS and resuspended in 0.1 M PBS. Fluorescence intensities of each sample were measured using a microplate reader at an excitation wavelength of 493 nm and an emission wavelength of 630 nm. The fluorescent intensity of the treated cells was subtracted from that of the untreated cells, and the resultant data were normalized against the OD_{600} of the cell suspension as follows:

$$(F_{treated}/OD_{600})/(F_{untreated}/OD_{600}),$$

where $F_{treated}$ is the fluorescence value of bacterial cells exposed to each of the treatments, $F_{untreated}$ is the fluorescence value of the untreated cells.

A transmission electron microscopy (TEM)

After individual or combined treatments, each of the bacterial fluids (35 mL) containing *E. coli* ATCC 43889 centrifugated at 12,000 ×g for 3 min, rinsed in 0.1 M PBS (pH 7.0) three times, and were resuspended in 0.1 M PBS. The cell fluids then were prefixed in 2% paraformaldehyde overnight at 4°C. Each of the bacterial solutions was washed in 0.1 M PBS, postfixed in 1% osmium tetroxide, and were serially dehydrated by 30%, 50%, 70%, 95%, and 100% ethanol solutions, following the infiltration with 2 mL of epoxy resin. Polymerization of the resins was performed at 60°C for 24 h. The resins were cut (section: approximately 120 nm thickness) and were photographed with a JEOL JEM 1200 EX transmission electron microscope (JEOL USA Inc., Peabody, MA, USA). The prefixed samples were used for carrying out the TEM assay according to a method described in a study conducted by Yoon et al.[23].

Measurement of color parameters

The thee *L**, a*, and b* values of each sample (weighing 25 g) were measured after individual or combined treatments with LA, thymol, and US at 25+2°C for 15 min using a UltraScan® PRO colorimeter (HunterLab. Co., Ltd., Reston, VA, USA). Each of the enoki mushroom samples was measured ten times at different locations, and *L**, a*, and b* values were averaged.

Analysis

Nontreated enoki mushroom samples were used as a negative control in this study, and all the experiments were repeated three times with duplicate plates. The obtained results were expressed as the mean±standard deviation. Significant ($P<0.05$) differences among the treated and nontreated groups were determined using the analysis of variances (ANOVA) with the Duncan's multiple range test (SAS Institute Inc., Cary, NC, USA).

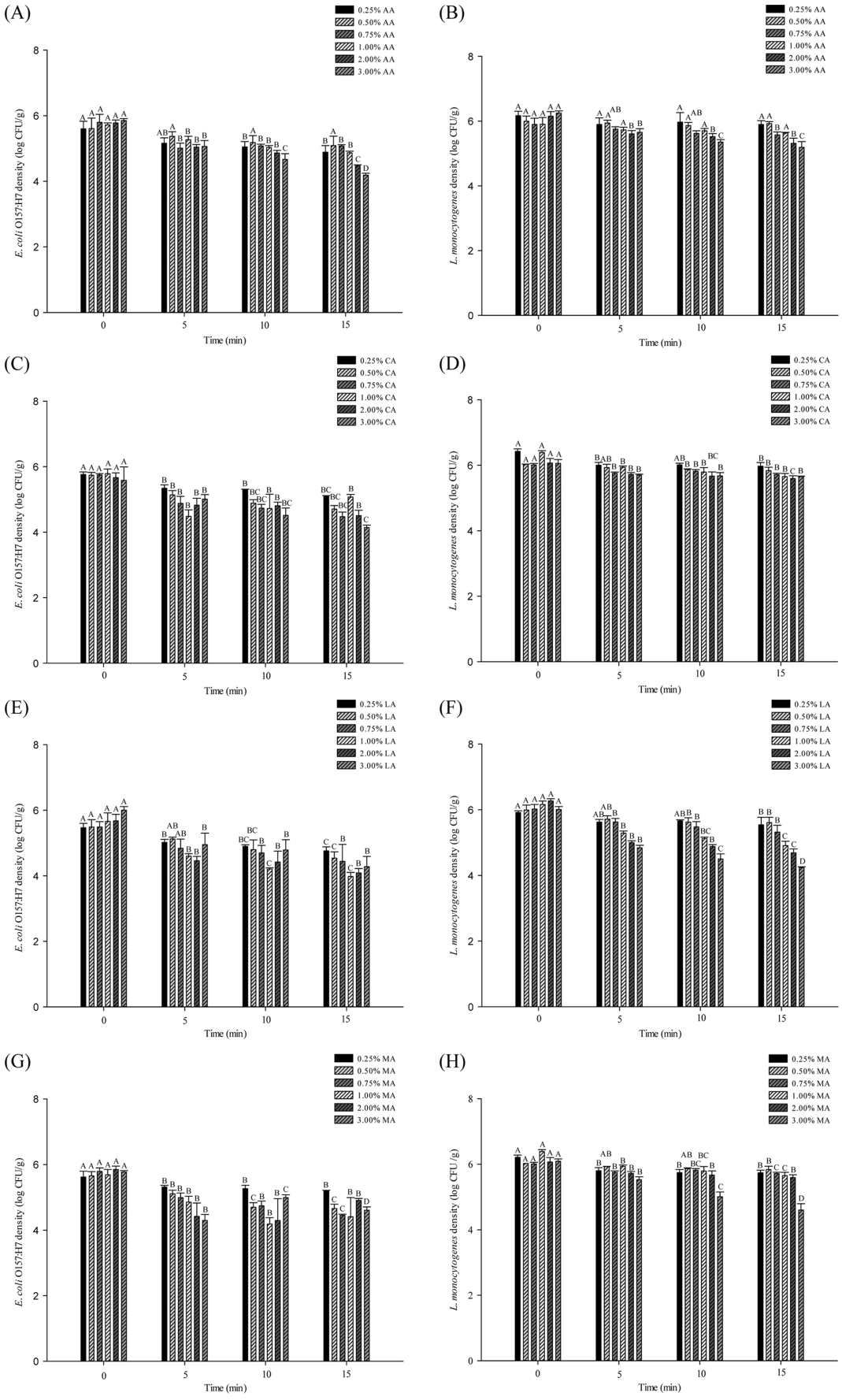

Fig. 1. Fate of (A, C, E, and G) *E. coli* O157:H7 ATCC 43889 and (B, D, F, and H) *L. monocytogenes* ATCC 19114 inoculated in enoki mushrooms before and after dipping in 0.25, 0.5, 0.75, 1, 2 or 3% (A-B) AA, (C-D) CA, (E-F) LA or (G-H) MA at 25°C for 0, 5, 10 or 15 min. $^{A-D}$Mean values with different uppercase letters within the entire groups denote significant ($P<0.05$) differences.

Results and Discussion

Fig. 1 represents the survival of *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms before and after dipping in AA, CA, LA or MA at 25°C for up to 15 min. When *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms were subjected to individual treatments with 0.25-3% AA, CA, LA or MA at 25°C for 15 min, less than 1.66, 1.43, 1.68 or 1.28 log-reductions were observed albeit the log-reduction levels of these bacteria were positively proportional to the increase of the OAA's concentrations. Particularly, dipping in 3% LA at 25°C for 15 min showed a notable difference in antibacterial effects compared to the others, with the maximum reduction of *E. coli* O157:H7 and *L. monocytogenes* by 1.68 and 1.97 log CFU/g, respectively.

Fig. 2 shows the survival of *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms before and after individual treatments with MIC (=0.107 mg/L) or 2×MIC thymol dipping or 20 kHz US operation at 25°C for up to 15 min. Treatment of *E. coil* O157:H7 inoculated in enoki mushrooms with MIC or 2×MIC thymol dipping gave less than 1.48- or 2.01-log reductions of this bacterium. Similarly, *L. monocytogenes* inoculated in enoki mushrooms was decreased by 0.99 or 1.02 log CFU/g after dipping in MIC or 2×MIC thymol at 25°C for 15 min. Additionally, no significant ($P>0.05$) differences between the initial loads and the survivor populations of *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms were observed after 15 min of 20 kHz US operation at 25°C.

Effect of a mixture of 3% LA and 2×MIC (=0.107 mg/L) thymol for reducing or eliminating of *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms is shown in Fig. 3A. As a result, the simultaneous application of 3% LA and 2×MIC thymol led to a higher log-reduction of *E. coil* O157:H7 (2.45 log CFU/g) and *L. monocytogenes* (2.16 log CFU/g) than did either 3% LA or 2×MIC thymol alone.

Fig. 3B and 3C summarize the log-reduction of *E. coil*

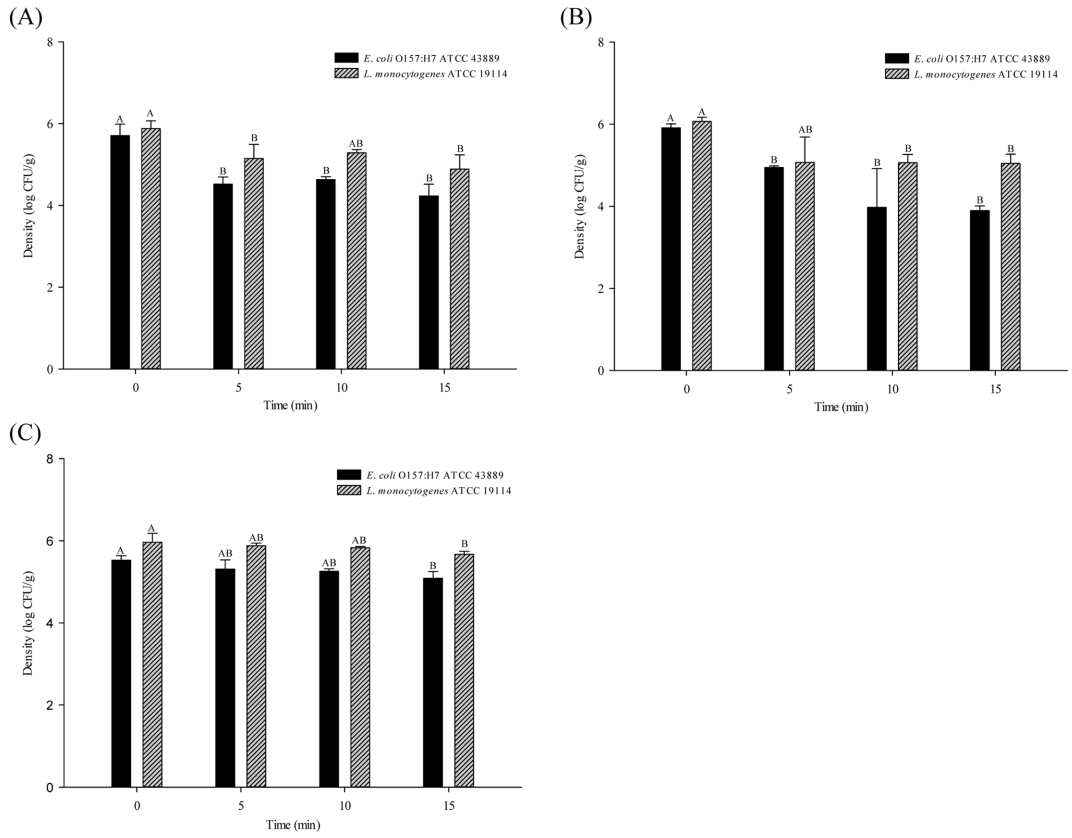

Fig. 2. Fate of *E. coli* O157:H7 ATCC 43889 and *L. monocytogenes* ATCC 19114 inoculated in enoki mushrooms before and after individual treatments with (A) MIC (=0.107 mg/L) or (B) 2×MIC thymol dipping or (C) 20 kHz US at 25°C for 0, 5, 10 or 15 min. $^{A-D}$Mean values with different uppercase letters within the entire groups denote significant ($P<0.05$) differences.

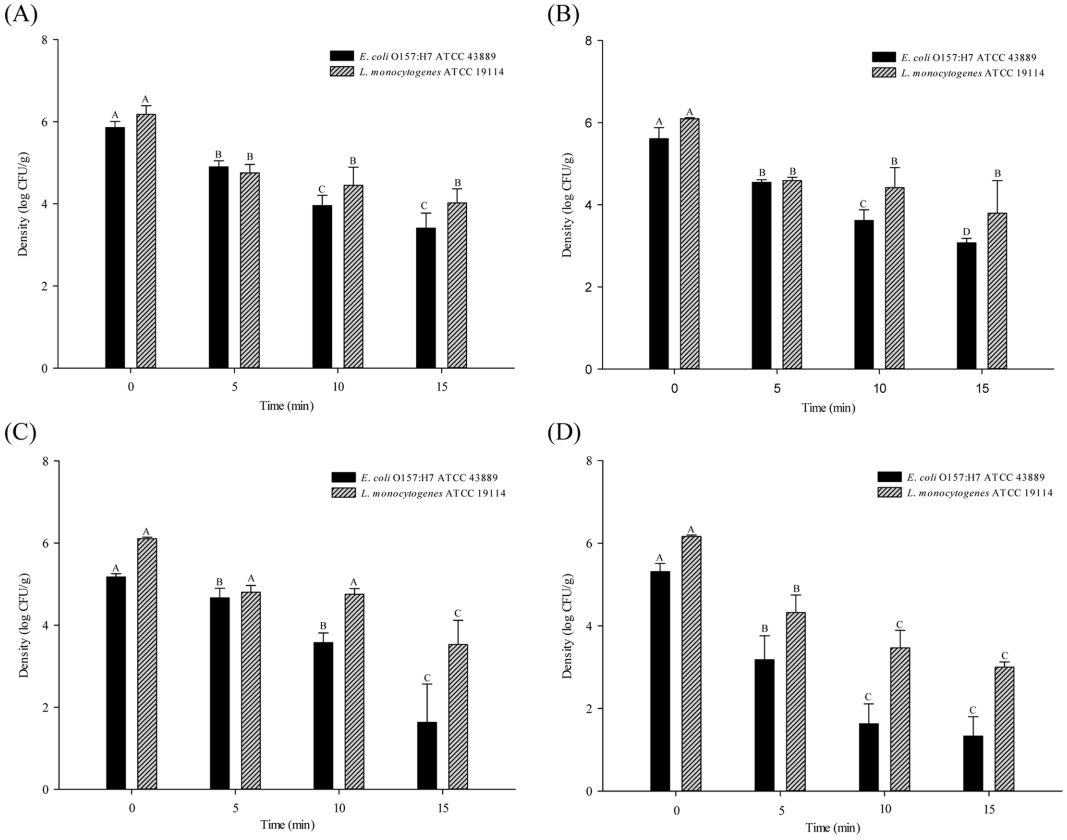

Fig. 3. Fate of *E. coli* O157:H7 ATCC 43889 and *L. monocytogenes* ATCC 19114 inoculated in enoki mushrooms before and after dipping in (A) 3% LA+2×MIC thymol, (B) 3% LA incorporated with 20 kHz US, (C) 2×MIC thymol incorporated with 20 kHz US, and (D) 3% LA+2×MIC thymol incorporated with 20 kHz US at 25°C for 0, 5, 10 or 15 min. $^{A-D}$Mean values with different uppercase letters within the entire groups denote significant (P<0.05) differences.

O157:H7 and *L. monocytogenes* in enoki mushrooms treated with 3% LA or 2×MIC thymol dipping incorporated with 20 kHz US at 25°C for 0, 5, 10 or 15 min. The higher lethal activity for killing *E. coil* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms was achieved, ranging from 2.58 to 3.55 log CFU/g, after combined treatments with 2×MIC thymol+20 kHz US at 25°C for 15 min as compared with that (log-reductions: 2.30-2.54 log CFU/g) of 3% LA+20 kHz US.

Effect of a mixture of 3% LA and 2×MIC thymol together with 20 kHz US for controlling *E. coli* O157:H7 and *L. monocytogenes* inoculated on enoki mushrooms is depicted in Fig. 3D. There were significant (P<0.05) reductions of *E. coil* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms after dipping in 3% LA or 2×MIC thymol incorporated with 20 kHz US at 25°C for 15 min, which was decreased by approximately 3.17 and 3.98 log CFU/g, respectively.

Fig. 4. displays the membrane damage of *E. coli* O157:H7 cells obtained by measuring PI uptake values after

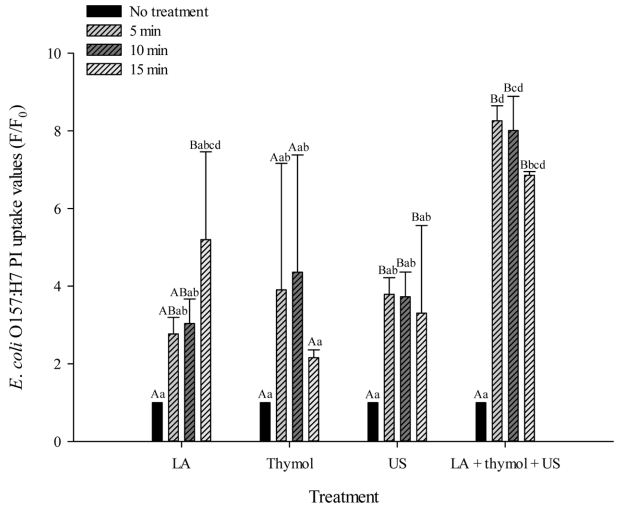

Fig. 4. Measurement of PI uptake values of *E. coli* O157:H7 after individual or combined treatments with 3% LA, 2×MIC thymol, 20 kHz US or 3% LA+2×MIC thymol+20 kHz US. $^{A-B}$Mean values with different uppercase letters within each of the groups (i.e. I)LA, II)thymol, III)US or IV)LA+thymol+US) denote significant (P<0.05) differences. $^{a-d}$Mean values with different lowercase letters within the entire groups denote significant (P<0.05) differences.

individual or combined treatments with 3% LA, 2×MIC thymol, 20 kHz US or 3% LA+2×MIC thymol+20 kHz US. Individually, LA-, thymol-, and US-treated groups did not show significant (P>0.05) differences in PI uptake values. By contrast, the PI uptake value of *E. coli* O157:H7 cells were 8.261 (5 min), 8.012 (10 min) or 6.860 (15 min) after dipping in a mixture of 3% LA and 2×MIC thymol incorporated with 20 kHz US significantly (P<0.05) higher than that of 3% LA, 2×MIC thymol or 20 kHz US alone.

TEM-based images of *E. coli* O157:H7 before and after individual or combined treatments with LA, 2×MIC thymol, and/or US at 25°C for 15 min are depicted in Fig. 5. The untreated control cells of *E. coli* O157:H7 were shown to retain a dense cytoplasm with an intact membrane. By contrast, *E. coli* O157:H7 cells displayed an enlarged peripheral layer and shrunken cytoplasm after dipping in 3% LA at 25°C for 15 min. The cells of *E. coli* O157:H7 treated with 2×MIC thymol showed a less dense cytoplasm than the untreated control cells. The cell envelope of *E. coli* O157:H7 treated with a mixture of 3% LA and 2×MIC thymol was obviously scattered from its original form, which was accompanied by the disorganization of the intracellular structures. Particularly, a concurrent trial of 3% LA, 2×MIC thymol, and US contributed to less molecular crowding and unorganized cytoplasm alongside the appearance of cytoplasm aggregation and breakage of cell membrane.

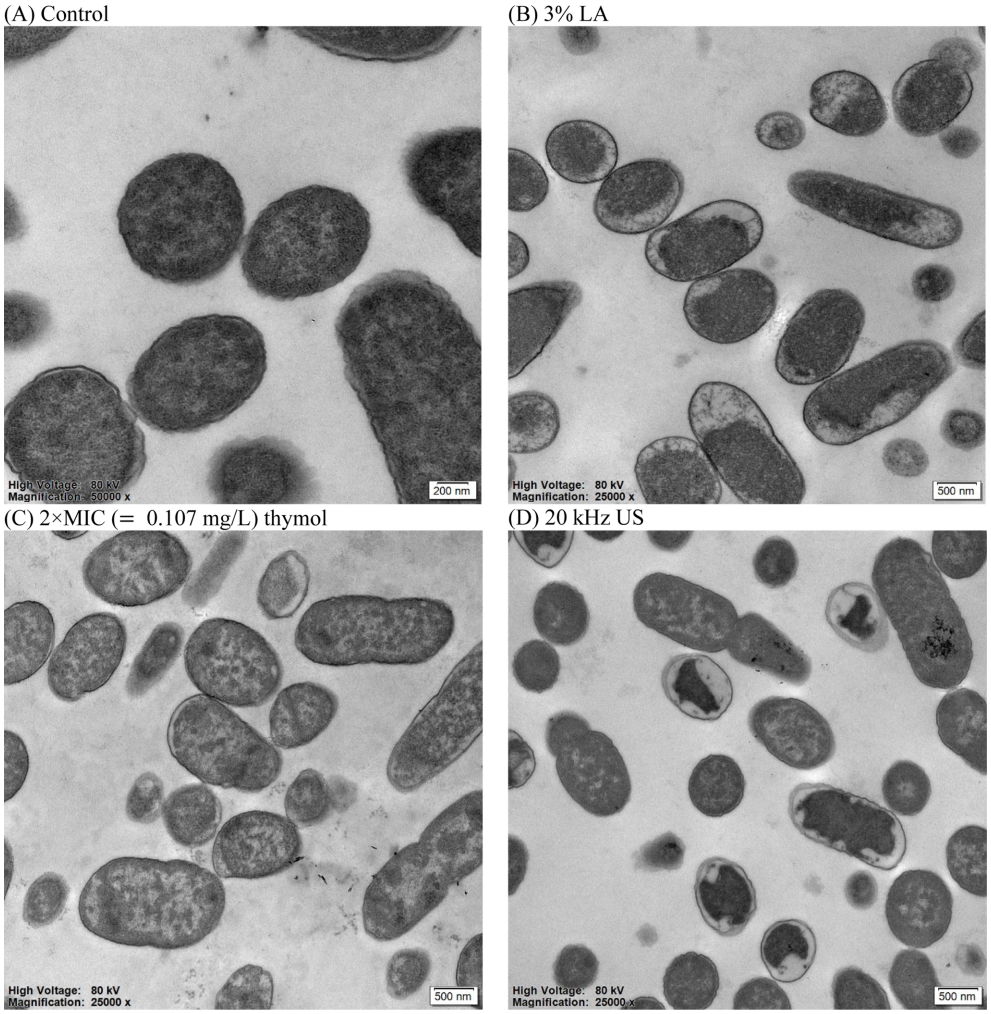

Fig. 5. TEM-based images of *E. coli* O157:H7 (A) before and after individual or combined treatments with (B) 3% LA, (C) 2×MIC thymol, (D) US, (E) 3% LA+2×MIC thymol, (F) 2×MIC thymol+US, (G) 3% LA+US, and (G) 3% LA+2×MIC thymol+US at 25 °C for 15 min.

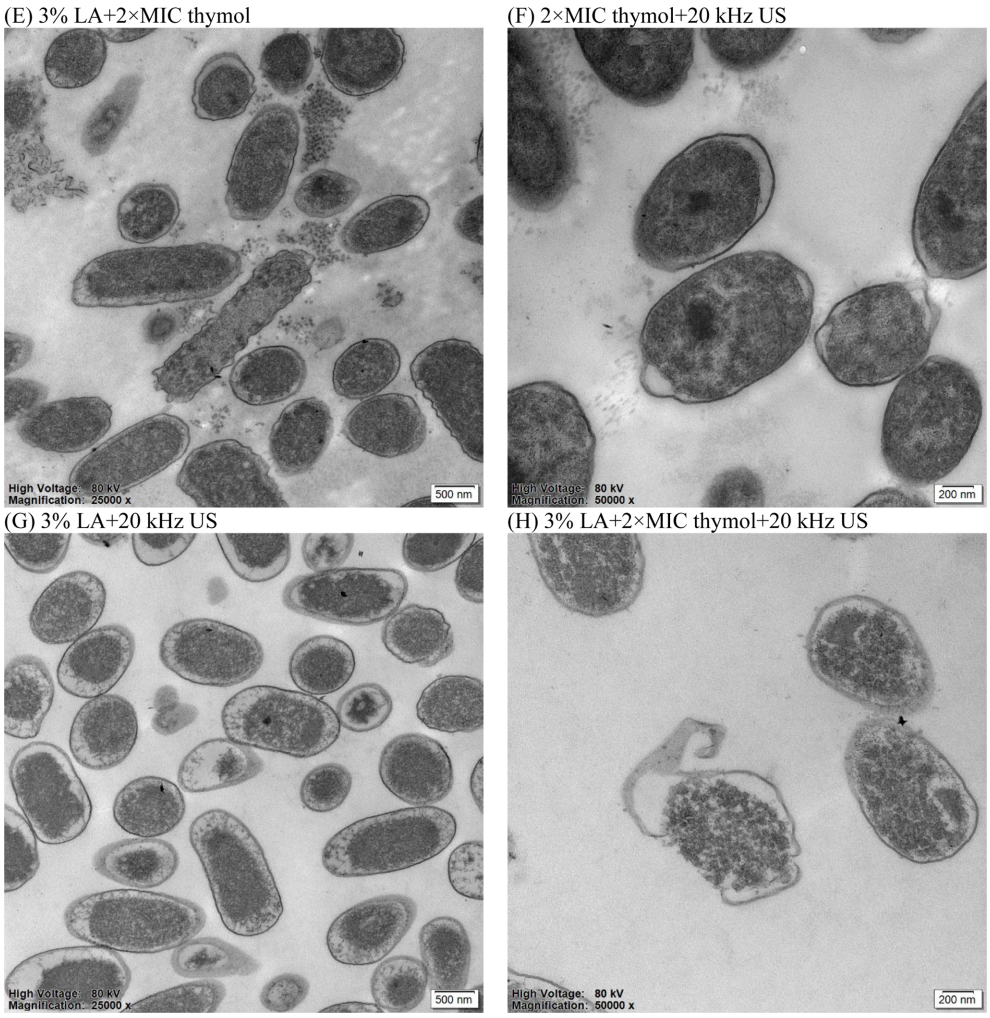

Fig. 5. (Continued) TEM-based images of *E. coli* O157:H7 (A) before and after individual or combined treatments with (B) 3% LA, (C) 2×MIC thymol, (D) US, (E) 3% LA+2×MIC thymol, (F) 2×MIC thymol+US, (G) 3% LA+US, and (G) 3% LA+2×MIC thymol+US at 25 °C for 15 min.

As shown in Table 1, there was no significant (P>0.05) difference in the pH levels between "3% LA" and "3% LA+2×MIC thymol (+20 kHz US)". Particularly, we observed that dipping in a mixture of 3% LA and 2×MIC thymol with or without the incorporation of 20 kHz US at 25°C had the lowest pH levels, ranging from 1.76 to 1.77, immediately after used to decontaminate enoki mushrooms carrying *E. coli* O157:H7 or *L. monocytogenes*. Thus, it should be plausible that 1) the mechanistic nature of LA for reducing or eliminating *E. coli* O157:H7 and *L. monocytogenes* would be closely attributed to its effects in lowering the pH of solvents; 2) while we found no significant (P>0.05) difference in the initial pH levels between "3% LA (pH 1.87)" and "3% LA+2×MIC thymol+20 kHz US (pH 1.77)", the latter was significantly (P<0.05) more effective for controlling *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms than the former, which means that the use of "3% LA+2×MIC thymol+20 kHz US" may act more mechanistically over OAAs (i.e. the cytoplasmic acidification) through other different pathways to inactivate food-borne pathogenic bacteria. Additionally, the L*, a*, and b* values indicate that dipping in LA and/or thymol with or without the incorporation of US did not significantly (P>0.05) affect the color of enoki mushrooms (Table 2).

It is noteworthy that either reducing or eliminating major food-borne pathogenic microorganisms present in food products before the consumption is considered to be the best optimal choice to curtail public health risks closely related to food safety issues. In this study, as

shown in Fig. 1, dipping in 3% OAAs (AA, CA, MA, and LA) had only marginal antibacterial effects, showing by 0.19-1.68 log-reductions in the population of *E. coli* O157:H7 and *L. monocytogenes* inoculated on enoki mushrooms, which is in line with Yoon and Lee[23]. Decreases in the pH of the foods (to that of or below the pK_a of the acids) promote the transition of a greater concentration of protonated acids, which can penetrate into the cells and adversely affect intracellular activities, such as DNA replication and protein synthesis by either impairing transport of nutrients or causing the leakage of internal metabolites to the outside[31,32].

Although the usage of 2×MIC thymol alone was not effective for inactivating *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms, showing by less than 1.5-log reductions, earlier studies have shown that thymol can exert a potent antibacterial effect against a wide variety of pathogenic bacteria[13,33]. Thymol has been demonstrated for its ability to alter the membrane-related characteristics, including membrane potential and membrane fluidity[34]. Specifically, thymol was shown to depolarize the cell membrane of *E. coli* O157:H7 and *S. aureus*, which was accompanied by the corresponding perturbance in the electrical potential of bacterial cells[34]. Tian et al.[35] also highlighted a thymol-induced depolarization (i.e. a significant decrease in the membrane potential) arisen out of a significant the increase in the fluorescence unit due to the partitioning of the DiBAC4 probe into the cell membrane of *Cronobacter sakazakii* cells, which was in good agreement with Gu, Lan, and Xie[36], and Wang et al.[37]. Both a conspicuous dissipation in the membrane potential and a loss of the membrane electric potential were highly correlated with the disintegration of cell membranes[38]. Taken together, thymol can disintegrate the cell membrane of microorganisms, concomitantly with the leakage of intracellular materials (i.e. ATP, ion, and proton), the loss of membrane potentials, and the impairment of energy metabolisms[39]. Although the cell membrane of *E. coli* O157:H7 had no marked damage/injury upon its treatment with thymol at 2×MIC, we postulated that thymol would appear to increase the cell membrane permeability, helping OAAs to cross the disintegrated cell membrane, which was in line with the corresponding increase in the PI uptake capacity and reduction of cell's integrity and viability once *E. coli* O157:H7 was subjected to combined treatments with thymol, LA, and US.

Table 1. Effect of 15 min of dipping with or without US incorporation at 25°C on the pH levels of different antibacterial solutions used to decontaminate enoki mushrooms

Treatment	Before (0 min)	After (15 min)
3% AA	2.17+0.05^{Cb}	2.69+0.07^{Ba}
3% CA	1.76+0.02^{Db}	2.08+0.04^{Ca}
3% LA	1.87+0.01^{Db}	2.11+0.04^{Ca}
3% MA	1.86+0.01^{Db}	2.01+0.04^{Ca}
2×MIC thymol	6.47+0.23^{Bb}	6.64+0.17^{Aa}
20 kHz US	6.75+0.29^{Aa}	6.82+0.46^{Aa}
3% LA+2×MIC thymol	1.76+0.00^{Db}	2.30+0.01^{Ca}
3% LA+20 kHz US	1.86+0.01^{Db}	2.01+0.04^{Ca}
2×MIC thymol+20 kHz US	6.25+0.09^{Bb}	6.55+0.03^{Aa}
3% LA+2×MIC thymol+20 kHz US	1.77+0.01^{Db}	2.15+0.04^{Ca}

$^{A-D}$Means in the same row with different lowercase letters denote significant (P<0.05) differences.
$^{a-b}$Means in the same column with different lowercase letters denote significant (P<0.05) differences.

Table 2. Color parameters of enoki mushrooms after dipping in 3% LA, 2×thymol or a mixture of 3% LA and 2×thymol with or without the incorporation of 20 kHz US at 25 °C for 15 min

Treatment	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)
Control (no treatment)	27.27±0.44^{A}	3.84±0.38^{A}	10.91±0.20^{A}
3% LA	28.70±2.56^{AB}	3.80±0.77^{A}	13.02±0.80^{AB}
2×MIC thymol	20.61±3.08^{C}	7.59±2.32^{B}	15.83±3.15^{B}
20 kHz US	29.97±1.80^{AB}	3.39±0.54^{A}	12.29±0.51^{A}
3% LA+2×MIC thymol	32.01±2.24^{B}	2.69±0.90^{A}	13.20±1.14^{AB}
3% LA+20 kHz US	29.69±1.55^{AB}	3.31±0.59^{A}	13.03±1.20^{AB}
2×MIC thymol+20 kHz US	26.52±0.27^{A}	4.56±0.20^{A}	13.40±0.53^{AB}
3% LA+2×MIC thymol+20 kHz US	29.94±1.41^{AB}	2.99±0.54^{A}	13.07±0.19^{AB}

$^{A-C}$Means in the same row with different lowercase letters denote significant (P<0.05) differences.

The principles of US are known to cause a chain of chemical and physical damages to bacterial cells in terms of its specific acoustic streaming, cavitation bursting, and mechanical vibration, thereby causing neither structural alterations nor significant damages to overall food anatomy and quality[40]. In this study, however, the US operation did not result in significant (P>0.05) reductions against *E. coli* O157:H7 and *L. monocytogenes* in comparison with the control for all samples treated, but provided an additive effect when combined with LA and/or thymol dipping.

Prevention of food-borne diseases and illnesses arising from the consumption of edible mushrooms is a new challenge to the current food industries. Mushroom should be decontaminated before its consumption primarily to reduce the risks of outbreaks caused by food-borne pathogenic bacteria that may be present. In a spite of the routine preservation and/or sanitization measures adopted in the food industry, very few methods are practically effective in controlling pathogenic microorganisms present in edible mushrooms. There is a growing interest in the development of novel combinations of natural antimicrobials and other food processing technologies, giving rise to a stable and safe product without the loss of nutritional and sensorial properties. In this study, the usage of a mixture of 3% LA and 2×MIC thymol plus 20 kHz US appeared to be the most effective in inactivating both *E. coli* O157:H7 and *L. monocytogenes* inoculated on enoki mushrooms; the simultaneous utilization of 3% LA+2×MIC thymol+20 kHz US at 25°C for 15 min gave more than 3.0-log reductions in the viable count of *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms. Such an addictive effect observed for the reduction of *E. coli* O157:H7 and *L. monocytogenes* inoculated in enoki mushrooms by the combination of 3% LA+2×MIC thymol+20 kHz US was greater than that for all other treatments tested. A possible contributor to the underlying mechanisms of US would be closely linked to the sonoporation (i.e. the formation of temporary pores in the cell membrane), allowing the direct cytoplasmic entry of cell-impermeable molecules with different sizes[41]. US may contribute to the weakening of cell walls, boosting the transfer of antimicrobial agents within the inaccessible inner site of fresh fruits and vegetables to be treated by enhancing the permeability of the outer membranes[23, 25, 42] which indicates the potential of US as an alternative decontamination step in the food industry to guarantee the preservation of edible mushrooms when applied in combination with LA and/or thymol. We also found that the combination of 3% LA+2×MIC thymol+20 kHz US may compromise the integrity of bacterial cells, leading to the noticeable alteration in the cell morphology. Obviously, there are other unknown mechanisms underlying interactions between LA, thymol, and US, which may result in the additive or synergistic inactivation activities against *E. coli* O157:H7 and *L. monocytogenes*. Further, a better understanding of the combined operation used in this study should involve in molecular analysis tools in order to aid in identifying its accurate functionality and mechanism against food-borne pathogenic bacteria inoculate in enoki mushrooms. Additionally, a more in-depth investigation should be needed not only to optimize the process conditions, but also to evaluate its impacts on the organoleptic properties of edible mushrooms.

국문요약

식용버섯은 1-3일의 제한된 유효 섭취기간을 가진 가장 부패하기 쉬운 재료 중 하나로서 바실러스 세레우스(*Bacillus cereus*), 장(관)출혈성 대장균(enterohemorrhagic *Escherichia coli*), 리스테리아 모노사이토제넨스(*Listeria monocytogenes*) 및 황색포도상구균(*Staphylococcus aureus*) 등의 병원성 세균에 오염될 수 있으며, 특히 팽이버섯은 포장 및 소비 전에 추가적인 세척이나 가공 단계를 거치지 않기 때문에 이를 그대로 섭취 시 소비자의 식품안전에 문제를 미칠 수 있다. 무엇보다 팽이버섯은 소비자들이 자연스럽고 영양가 있는 식단을 선호하는 이유로 종종 날 것으로, 즉 샐러드와 샌드위치 등의 형태로 섭취된다는 점에 유의할 필요가 있다. 따라서 본 연구는 팽이버섯(*Flammulina velutipes*)에 접종한 병원성 대장균 O157:H7(pathogenic *Escherichia coli* O157:H7) 및 리스테리아 모노사이토제넨스를 제어하기 위하여 초음파(ultrasound, US)와 유기산(organic acids, OAAs) 및/또는 식물성 에센셜(essential) 오일의 병행 처리에 따른 저감 효과를 조사하고자 수행되었다.

팽이버섯에 10^{5-6} CFU/g의 수준에 상응하는 각 병원성 세균을 접종하고 다양한 농도의 유기산, 최소저해농도(minimal inhibitory concentrations, MICs)의 티몰(thymol) 및/또는 20 kHz 초음파를 상온에서 15분 동안 개별적으로 또는 병행하여 처리하였다. 그 결과로써, 상온에서 15분 동안 3% 유기산, 2×MIC 티몰 또는 20 kHz 초음파를 개별적으로 처리한 후 병원성 대장균 O157:H7과 리스테리아 모노사이토제넨스의 균 밀도가 약 1.7 로그 이하로 감소한 반면, 3% 젖산과 2×MIC 티몰의 혼합액에 20 kHz 초음파를 상온에서 15분간 병행처리한 경우 팽이버섯에

접종한 병원성 대장균 O157:H7과 리스테리아 모노사이토제네스의 균 밀도가 대략 3.0 로그 이상으로 감소함에 따라 additive 효과가 관찰되었다. 특히 3% 젖산(lactic acid, LA)+2×MIC 티몰+20 kHz 초음파의 병행처리를 하기 전·후에 팽이버섯의 색상 변화에 유의미한(P<0.05) 차이가 관찰되지 않았다.

결론적으로 팽이버섯에 접종한 병원성 대장균 O157:H7 및 리스테리아 모노사이토제네스의 제어에 단일 처리로서 3% 젖산이 가장 효과적이었던 반면, 초음파의 단일 처리로서는 이러한 병원성 세균에 대한 저감 효과를 관찰할 수 없었다. 특히 유기산(즉 젖산) 및 초음파를 단일 처리하였을 때보다 젖산과 티몰의 혼합액(mixture)에 20 kHz 초음파를 동시에 병행 처리하였을 때 유의적으로(P<0.05) 가장 높은 저감 효과를 관찰할 수 있었다. 이에 따라 초음파는 유기산과 티몰의 혼합용액이 병원성 대장균 O157:H7의 세포에 더 수월하게 접근을 가능케 하여 결국 세포막 파괴를 수반하는 것으로 확인되었다.

Conflict of interests

The authors declare no potential conflict of interests.

ORCID

Sa-Rang Park	https://orcid.org/0009-0009-3656-395X
Sang-Woo Lee	https://orcid.org/0009-0000-8680-9721
Se-Gyeong Yoon	https://orcid.org/0009-0005-5147-9343
Hyun-Dong Cho	https://orcid.org/0000-0001-8756-3217
In-Yong Kim	https://orcid.org/0000-0002-0565-8341
Jae-Hyun Yoon	https://orcid.org/0000-0002-8144-7458

References

1. Samadpour, M., Barbour, M.W., Nguyen, T., Cao, T.M., Buck, F., Depavia, G.A., Mazengia, E., Yang, P., Alfi, D., Lopes, M., Stopforth, J.D., Incidence of enterohemorrhagic *Escherichia coli*, *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* in retail fresh ground beef, sprouts, and mushrooms. *J. Food Prot.*, **69**, 441-443 (2006).
2. Pereira, E., Conrad, A., Tesfai, A., Palacios, A., Kandar, R., Kearney, A., Locas, A., Jamieson, F., Elliot, E., Otto, M., Kurdilla, K., Tijerina, M., Son, I., Pettengill, J.B., Chen, Y., Fox, T., Lane, C., Aguillon, R., Huffman, J., Sheau Fong Low, M., Wise, M., Edwards, L., Bidol, S., Blankenship, H.M., Rosen, H.E., Leclercq, A., Lecuit, M., Tourdjman, M., Herber, H., Singleton, L.S., Viazis, S., Bazaco, M.C., Multinational outbreak of *Listeria monocytogenes* infections linked to enoki mushrooms imported from the Republic of Korea 2016-2020. *J. Food Prot.* , **86**, 100101 (2023).
3. Ok, Y.J., Kwon, Y.H., Hwang, H.S., Byun, Y.J., Park, J.Y., Kim, B.J., A study of the distribution of *Listeria* spp. in fresh agricultural products distributed in the Busan area, the Republic of Korea. *J. Food Hyg. Saf.*, **39**, 9-15 (2024).
4. Meng, B., Jang, A.R., Song, H., Lee, S.Y., Microbiological quality and safety of fresh mushroom products at retail level in Korea. *Food Sci. Biotechnol.*, **33**, 1261-1268 (2024).
5. Lee, S.W., Park, S.R., Yoon, S.G., Cho, H.D., Lee, M.K., Lee, S.Y., Yoon, J.H., Prevalence and characterization of *Listeria monocytogenes* isolated from online market-purchased enoki mushrooms (*Flammulina velutipes*) in the Republic of Korea. *LWT*, **215**, 117235 (2025).
6. Singha, S., Thomas, R., Viswakarma, J.N., Gupta, V.K., Foodborne illnesses of *Escherichia coli* O157 origin and its control measures. *J. Food Sci. Technol.*, **60**, 1274-1283 (2023).
7. Zhu, Q., Gooneratne, R., Hussain, M.A., *Listeria monocytogenes* in fresh produce: Outbreaks, prevalence, and contamination levels. *Foods*, **6**, 21 (2017).
8. Zhang, X., Wang, S., Chen, X., Qu, C., Review: controlling *Listeria monocytogenes* in ready-to-eat meat and poultry products: An overview of outbreaks, current legislations, challenges, and future prospects. *Trends Food Sci. Technol.*, **116**, 24-35 (2021).
9. Duze, S.T., Marimani, M., Patel, M., Tolerance of *Listeria monocytogenes* to biocides used in food processing environments. *Food Microbiolol.*, **97**, 103758 (2021).
10. Quereda, J.J., Morón-García, A., Palacios-Gorba, C., Dessaux, C., García-del Portillo, F., Pucciarelli, M.G., Ortega, A.D., Pathogenicity and virulence of *Listeria monocytogenes*: A trip from environmental to medical microbiology. *Virulence*, **12**, 2509-2545. (2021).
11. Khan, I., Khan, J., Miskeen, S., Tango, C.N., Park, Y.S., Oh, D.H., Prevalence and control of *Listeria monocytogenes* in the food industry: a review. *Czech J. Food Sci.*, **34**, 469-487 (2016).
12. Jeon, E.B., Kim, J.Y., Choi, M.S., Choi, S., Bang, H.J., Park, S.Y., Microbial contamination levels in the raw materials of home meal replacement shabu-shabu meal kit distributed in markets. *J. Food Hyg. Saf.*, **35**, 375-381 (2020).
13. Kim, N.H., Kim, H.W., Moon, H., Rhee, M.S., Sodium chloride significantly enhances the bactericidal actions of carvacrol and thymol against the halotolerant species *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*. *LWT–Food Sci. Technol.*, **122**, 109015 (2020).
14. Lee, S.W., Park, S.R., Yoon, S.G., Cho, H.D., Lee, M.K., Lee, S.Y., Yoon, J.H., Prevalence and characterization of *Listeria monocytogenes* isolated from online market-purchased enoki mushrooms (*Flammulina velutipes*) in the Republic of Korea. *LWT–Food Sci. Technol.*, **215**, 117235 (2025).
15. US Mushroom Council, (2025, May, 23). Versatility in varieties. Retrieved from https://www.mushroomcouncil.com/wp-content/uploads/2017/12/MC-Versatility_FactSheet_020323.pdf
16. Pennone, V., Dygico, K.L., Coffey, A., Gahan, C.G.M., Grogan, H., McAuliffe, O., Burgess, C.M., Jordan, K., Effectiveness of current hygiene practices on minimization of *Listeria monocytogenes* in different mushroom production-related

environments. *Food Sci. Nutr.*, **8**, 3456-3468 (2020).
17. Zhang, K., Pu, Y.Y., Sun, D.W., Recent advances in quality preservation of postharvest mushrooms (*Agaricus bisporus*): a review. *Trends Food Sci. Technol.*, **78**, 72-82 (2018).
18. Marçal, S., Sousa, A.S., Taofiq, O, Antunes, F., Morais, A.M.M.B., Freitas, A.C., Barros, L., Ferreira, I.C.F.R., Pintado, M., Impact of postharvest preservation methods on nutritional value and bioactive properties of mushrooms. *Trends Food Sci. Technol.*, **110**, 418-431 (2021).
19. Özcan, G., Zorba, N.N.D., Combined effect of ultrasound and essential oils to reduce *Listeria monocytogenes* on fresh produce. *Food Sci. Technol. Int.*, **22**, 353-362 (2015).
20. Sagong, H.G., Lee, S.Y., Chang, P.S., Heu, S., Ryu, S., Choi, Y.J., Kang, D.H., Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. *Int. J. Food Microbiol.*, **145**, 287-292 (2011).
21. Turhan, E.U., Polat, S., Erginkaya, Z., Konuray, G., Investigation of synergistic antibacterial effect of organic acids and ultrasound against pathogenic biofilms on lettuce. *Food Biosci.*, **47**, 101643 (2022).
22. Yoon, J.H., Lee, S.Y., Comparison of the effectiveness of decontaminating strategies for fresh fruits and vegetables and related limitations. *Crit. Rev. Food Sci. Nutr.*, **58**, 3189-3208 (2019).
23. Yoon, J.H., Jeong, D.Y., Lee, S.B., Choi, S., Jeong, M.I., Lee, S.Y., Kim, S.R., Decontamination of *Listeria monocytogenes* in king oyster mushrooms (*Pleurotus eryngii*) by combined treatments with organic acids, nisin, and ultrasound. *LWT-Food Sci. Technol.*, **144**, 111207 (2021).
24. Akata, I., Torlak, E., Erci, F., Efficacy of gaseous ozone for reducing microflora and foodborne pathogens on button mushroom. *Postharvest Biol. Technol.*, **109**, 40-44. (2015).
25. Yoon, J.H., Jeong, D.Y., Lee, S.B., Kim, S.R., Control of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in enoki mushrooms (*Flammulina velutipes*) by combined treatments with organic acids, nisin, and ultrasound. *Food Control*, **129**, 108204 (2021).
26. Guan, W., Fan, X., Yan, R., Effects of UV-C treatment on inactivation of Escherichia coli O157:H7, microbial loads, and quality of button mushrooms. *Postharvest Biol. Technol*, **64**, 119-125 (2012).
27. Guan, W., Fan, X., Yan, R., Effect of a combination of ultraviolet light and hydrogen peroxide on inactivation of *Escherichia coli* O157:H7, native microbial loads, and quality of button mushrooms. *Food Control*, **34**, 554-559 (2013).
28. Coban, H.B., Organic acids as antimicrobial food agents: applications and microbial productions. *Bioprocess Biosyst. Eng.,* **43**, 569-591 (2020).
29. Memar, M.Y., Raei, P., Alizadeh, N., Aghdam, M.A., Kafil, H.S., Carvacrol and thymol: strong antimicrobial agents against resistant isolates. *Rev. Med. Microbiol.*, **28**, 63-68 (2017).
30. Lauteri, C., Ferri, G., Piccinini, A., Pennisi, L., Vergara, A., Ultrasound technology as inactivation method for foodborne pathogens: a review. *Foods*, **12**, 1212 (2023).
31. Taylor, T.M., Doores, S.X., 2020. Organic acids. In: Davidson, P.M., Taylor, T.M., David, J.R.D. (Eds), Antimicrobials in food, CRC Press, Boca Raton, FL, USA, pp. 133-190.
32. Yoon, J.H., Oh, M.S., Lee, S.Y., Effectiveness of organic acids for inactivating pathogenic bacteria inoculated in laboratory media and foods: an updated minireview. *Food Sci. Biotechnol.*, **33**, 2715-2728 (2024).
33. Marchese, A., Orhan, I.E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S.F., Gortzi, O., Izadi, M., Nabavi, S.M., Antibacterial and antifungal activities of thymol: a brief review of the literature. *Food Chem.*, **210**, 402-414 (2016).
34. He, Q., Zhang, L., Yang, Z., Ding, T., Ye, X., Liu, D., Guo, M., Antibacterial mechanisms of thyme essential oil nanoemulsions against *Escherichia coli* O157:H7 and *Staphylococcus aureus*: Alterations in membrane compositions and characteristics. *Innov. Food Sci. Emerg. Technol.*, **75**, 102902 (2022).
35. Tian, L., Wang, X., Liu, R., Zhang, D., Wang, X., Sun, R., Guo, W., Yang, S., Li, H., Gong, G., Antibacterial mechanism of thymol against *Enterobacter sakazakii*. *Food Control,* **123**, 107716 (2021).
36. Gu, Y., Lan, W., Xie, J., Antibacterial mechanism of ultrasound combined with thymol against *Shewanella putrefaciens*: Implications for cell integrity and biofilm control. *Int. J. Food Sci. Technol.*, **59**, 3045-3055 (2024).
37. Wang, X., Tian, L., Fu, J., Liao, S., Yang, S., Jia, X., Gong, G., Evaluation of the membrane damage mechanism of thymol against *Bacillus cereus* and its application in the preservation of skim milk. *Food Control*, **131**, 108435 (2022).
38. Zhang, Y., Liu, X., Wang, Y., Jiang, P., Quek, S.Y., Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. *Food Control*, **59**, 282-289 (2016).
39. Posgay, M., Greff, B., Kapcsándi, V., Lakatos, E., Effect of *Thymus vulgaris* L. essential oil and thymol on the microbiological properties of meat and meat products: a review. *Heliyon*, **8**, e10812 (2022).
40. Dai, J., Bai, M., Li, C., Cui, H., Lin, L., Advances in the mechanism of different antibacterial strategies based on ultrasound technique for controlling bacterial contamination in food industry. *Trends Food Sci. Technol.*, **105**, 211-222 (2020).
41. He, Q., Guo, M., Jin, T.Z., Arabi, S.A., Liu, D., Ultrasound improves the decontamination effect of thyme essential oil nanoemulsions against *Escherichia coli* O157:H7 on cherry tomatoes. *Int. J. Food Microbiol.*, **337**, 108936 (2021).
42. Yoon, J.H., Kim, J.Y., Bae, Y.M., Lee, S.Y., Control of *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* on lettuce and radish sprouts by combined treatments with thymol, acetic acid, and ultrasound. *Food Control*, **147**, 109588 (2023).