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Research Article

Antimicrobial Characterization of Erythorbyl Laurate for Practical Applications in Food and Cosmetics

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In this study, antimicrobial spectrum of erythorbyl laurate (EL) against bacteria and fungi, leading to food-borne and infectious skin diseases, was evaluated for its practical applications in food and cosmetics. Furthermore, the influences of environmental factors including pH, oxidative stress, and dispersion medium on antimicrobial activity of EL were investigated. The three Gram-positive bacteria and the three molds were susceptible to 3.0 mM EL, while the yeast was susceptible to 6.0 mM EL. It was demonstrated EL retained antibacterial activity against *Staphylococcus aureus* after being oxidized while the antibacterial activity against the three Gram-positive bacteria including *S. aureus*, *Bacillus cereus*, and *Listeria monocytogenes* was significantly enhanced as decrease of pH from 7.0 to 5.0. Moreover, EL exhibited bactericidal effects against both Gram-positive and Gram-negative bacteria in an oil-in-water emulsion. Treatment of 5.0 mM EL for 4 h reduced 5.29 ± 0.24, 6.01 ± 0.18, 5.95 ± 0.13, and 6.24 ± 0.30 log CFU/mL against *S. aureus*, *L. monocytogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli*, respectively. In a multipassage resistance selection study, it was observed minimum inhibitory concentrations of EL against *S. aureus* were not increased over 20 passages, indicating EL might not develop drug resistance of bacteria. This study suggests EL has a potential to be applied as the multifunctional additive in food and cosmetics.

1. Introduction

Lipid oxidation of food and cosmetics has been considered as a major hazard for consumer health [1]. Moreover, microbial contamination influences the physical and chemical properties of food and cosmetics [2–4]. Therefore, preservatives have been employed to control the lipid oxidation and the microbial contamination in food and cosmetic industry [5]. Previously, erythorbyl laurate (EL) was suggested as a novel multifunctional emulsifier with antioxidative and antibacterial activity to control the lipid oxidation and microbial contamination with a single compound [6].

A previous study investigated EL had antibacterial activity against Gram-positive food-borne pathogens including *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of EL against these Gram-positive bacteria were determined, and the antibacterial mechanism was thought to be disruption of the bacterial cell membrane [7]. In addition, it was investigated EL exhibited more effective antioxidative activity than that of erythorbic acid in oil-in-water (O/W) emulsion system since its antioxidative moiety is located on the interface of droplets where lipid oxidation mostly occurs [8]. However, no study has examined the antibacterial properties of EL in an O/W emulsion, which is thought to be the best system for application of EL.

Food and cosmetics are made at various pH values, which affect the antimicrobial activity of preservatives, caused by structural changes [9]. In addition, structure of EL might be changed once it begins to act as an antioxidant. It was reported that ranalexin, an antimicrobial peptide, retained its antimicrobial activity after being oxidized, while

catechins with antioxidative activity exhibited antimicrobial effects when functioning as an antioxidant [10, 11].

Antimicrobial spectrum of an antimicrobial agent is one of the crucial considerations for practical applications since microbial contamination of food and cosmetics can be caused by bacteria, yeasts, and molds [12]. Moreover, increasing concerns about bacterial resistance to antibiotics have prompted the requirement to search for alternative antibacterial agents for clinical application, as well as use in food and cosmetics. Since there are no reports of bacteria developing resistance to fatty acids, EL may not induce bacterial resistance [13, 14].

Fundamental investigation on antibacterial properties of EL with regard to antibacterial activity and mode of action was carried out in the previous study [7], but no study has been performed to bring detailed knowledge of antimicrobial properties of EL for the practical applications. Therefore, the aim of this study is comprehensive evaluation of antimicrobial properties of EL for the practical applications in food and cosmetics. The antimicrobial spectrum of EL against bacteria, yeasts, and molds was investigated, and resistance study was performed to figure out bacterial resistance against EL. Furthermore, the effects of structural changes in EL due to protonation by pH variation and oxidation on its antibacterial activity were evaluated. Finally, the antibacterial activity of EL in an O/W emulsion system was investigated to assess its applicability to emulsion-based foods and cosmetics.

2. Materials and Methods

2.1. Materials. Immobilized lipase, from *Candida antarctica* (triacylglycerol hydrolase, EC 3.1.1.3; Novozym 435) with a catalytic activity of 7,000 PLU/g (the activity of PLU refers to the millimoles of propyl laurate synthesized per min at 60°C), was purchased from Novozymes (Bagsvaerd, Denmark). Erythorbic acid (≥99.0%), lauric acid (≥99.0%), amphotericin B, and ampicillin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Monolaurin (>98.0%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Tween-20 was purchased from Ilshinwells (Cheongju, Korea), and sunflower oil was purchased from Ottogi (Seoul, Korea). All other chemicals were of analytical grade.

2.2. Production of Erythorbyl Laurate. EL was produced in gas-solid-liquid multiphase system [15]. The esterification between erythorbic acid and lauric acid was catalyzed by the immobilized lipase (Novozym 435) for 95 h. The total volume was 450 mL, and the erythorbic acid (0.97 mol) to lauric acid (1.93 mol) molar ratio was 1 : 2. The enzyme amount was 840 PLU/mL (0.12 g/mL), and nitrogen gas was bubbled at a flow rate of 6.0 L/min. The lauric acid was preincubated at 60°C for 20 min on the reactor to be melted.

Quantitative analysis of the EL was conducted using high-performance liquid chromatography (HPLC; LC-2002; JASCO, Tokyo, Japan) with a silica-based column (5.0 μm, 4.6 × 150 mm; Luna C18; Phenomenex, Torrance, CA, USA) and an ultraviolet (UV) detector (UV-2075; JASCO). The mobile phase was acetonitrile/water/acetic acid (90 : 5:5, v/v/v) at a 1.0 mL/min flow rate. Peaks in the HPLC chromatograms were confirmed according to the retention time of an EL standard. The EL concentration was determined using the standard curve of EL, generated by integrating the peak area at 265 nm using Borwin (ver. 1.21; JASCO).

2.3. Antimicrobial Susceptibility Test

2.3.1. Bacteria, Fungi, and Media. Bacteria and fungi for susceptibility testing were selected from pathogens causing infectious skin diseases and food-borne diseases. These included three Gram-positive bacteria (*Propionibacterium acnes* ATCC 6919, *Streptococcus pyogenes* ATCC 19615, and *Clostridium perfringens* ATCC 13124), three molds (*Trichophyton mentagrophytes* ATCC 18748, *Rhizopus oryzae* ATCC 10404, and *Aspergillus nidulans* ATCC 10074), and one yeast (*Candida albicans* KCTC 7678).

S. pyogenes ATCC 19615 was cultured in tryptic soy agar (TSA) at 37°C for 24 h in an atmosphere of 5.0% CO_2. *C. perfringens* ATCC 13124 and *P. acnes* ATCC 6919 were cultured in TSA at 37°C in an anaerobic atmosphere for 1 and 5 days, respectively. All molds were cultured in potato dextrose agar (PDA) at 25°C for 5 days, and the yeast was cultured in yeast malt agar (YMA) at 25°C for 2 days.

2.3.2. Disk Diffusion Assay. The antimicrobial susceptibility to EL was assessed using disk diffusion assays with reference to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Inocula of Gram-positive bacteria and yeast were made by direct colony suspension in tryptic soy broth (TSB) and yeast mannitol broth (YMB), respectively, equivalent to 0.5 McFarland suspension, and molds were inoculated (5×10^4 spores/mL) in potato dextrose broth (PDB). The bacterial and fungal suspensions were inoculated on the surfaces of agar plates. Gram-positive bacteria, molds, and yeast were inoculated on the surfaces of TSA, PDA, and YMA, respectively. EL, a negative control (TSB, PDB, and YMB with 2.0% dimethyl sulfoxide), and antibiotics (10 ppm ampicillin and 250 ppm amphotericin B) were prepared. Paper disks (8.0 mm thickness) were laid on the surface of the agar, and 40.0 μL samples were dispensed on the disk; the plate was then dried for 5 min.

The plates inoculated with *S. pyogenes* ATCC 19615 and *C. perfringens* ATCC 13124 were incubated at 37°C, anaerobically for 1 day, and that with *P. acnes* ATCC 6919 was incubated for 5 days. The plates inoculated with molds were incubated at 25°C for 2 days and *T. mentagrophytes* ATCC 18748 was incubated for 7 days; the yeast was incubated at 25°C for 2 days, and the clear zones were then analyzed [16–18].

2.4. Structural Changes and Antimicrobial Properties of EL

2.4.1. Structural Changes in EL. The degree of protonation of EL according to the pH was predicted using Chemicalize.org. EL was dissolved in 2.0% dimethyl sulfoxide in 100 mM universal buffer at pH 5.0, 6.0, or 7.0 to protonate the EL.

At neutral and alkaline pH, ascorbic acid is highly unstable due to its much faster conversion to dehydroascorbic acid. Dehydroascorbic acid also degrades more rapidly at alkaline pH (7.0–8.0) than at acid pH (3.0–5.0) [19]. Similarly, EL might also be oxidized and degraded at alkaline pH. Therefore, EL was added to 5.0% dimethyl sulfoxide in 50 mM Tris-HCl buffer at pH 8.0 for oxidation. The degree of oxidation was presented as the mean of decrement in the reduced form of EL measured by HPLC in triplicate.

2.4.2. Evaluation of Antimicrobial Activity. To evaluate the antimicrobial activity of EL according to the degree of protonation, a 100 μL inoculum of *S. aureus* ATCC 12692, *L. monocytogenes* ATCC 19115, or *B. cereus* ATCC 10876, equivalent to 0.5 McFarland suspension, was added to 100 μL universal buffer containing EL. The colony-forming units (CFUs) in a 100 μL aliquot were enumerated by preparing serial dilutions in 100 mM universal buffer at pH 5.0, 6.0, and 7.0; plating was performed in triplicate for each dilution with TSA plates, for 1 h after incubation at 37°C for 1 h. Then, each plate was incubated at 37°C for 18 h.

The antimicrobial activity of EL according to the degree of oxidation was evaluated using the MIC test after storage for 0, 6, 15, 24, and 48 h at 40°C. The reduced form of EL was quantified by HPLC in triplicate. For the MIC test, Tris-HCl buffer containing EL was added to 1.25% dimethyl sulfoxide in TSB. *S. aureus* ATCC 12692 was tested to determine the MIC of the oxidized form of EL, and the bacterial inoculum was diluted in 2.00% dimethyl sulfoxide in TSB as 0.5 McFarland standard. The MIC was determined using the broth microdilution assay. Serial dilutions of each desired concentration of oxidized EL were prepared in sterile TSB to a final volume of 100 μL in 96-well microplates. Then, each well was inoculated with 100 μL of the test organisms in TSB. The MIC was obtained as the lowest concentration at which the test compound inhibited bacterial growth after incubation for 12 h at 37°C [20, 21].

2.5. Multipassage Resistance Selection Study. An inoculum was prepared as 0.5 McFarland suspension after incubating *S. aureus* ATCC 12692 in TSB at 37°C for 18 h. Serial passages were performed every 18 h in TSB (2.0% dimethyl sulfoxide). The strain was treated with a 2-fold dilution series of EL in 200 μL broth in 96-well plates. For each subsequent passage, a 2 μL aliquot was taken from the wells with concentrations below the MIC that matched the turbidity of the growth control and was used to inoculate the dilution series for the next passage. Every passage was performed until 20 consecutive passages were completed [22].

2.6. Antimicrobial Assay in O/W Emulsion System

2.6.1. Preparation of the Emulsion and Inoculum. An O/W emulsion containing EL was formulated using sunflower oil, Tween-20, EL, and sterilized water. The concentrations of sunflower oil (5.0%, w/w) and Tween-20 (0.5%, w/w) were fixed for all emulsion formulations. The emulsion was prepared by mixing the oil and Tween-20 with or without EL (5.6 mM) followed by the addition of sterilized water. The emulsion was then sonicated with Sonomasher (ULH-700S; Ulso High-Tech Co., Cheongwon, Korea) at 4°C for 20 min at 210 W using pulses of 10 s on followed by 30 s off [23].

Bacteria related to food-borne and infectious skin diseases were selected. The three Gram-positive bacteria tested were *S. aureus* ATCC 12692, *L. monocytogenes* ATCC 19115, and *B. cereus* ATCC 10876; the two Gram-negative bacteria tested were *E. coli* ATCC 35150 and *P. aeruginosa* ATCC 10145. These bacteria were incubated in TSB at 37°C for 18 h. Then, cultures of the bacteria were diluted in 10 mM phosphate-buffered saline (PBS) at pH 7.4 as a 0.5 McFarland standard.

2.6.2. Bactericidal Assay. The MBC was determined as the standard criterion for evaluating the antimicrobial effect with some modifications, to determine the effective concentration of EL in the O/W emulsion [24]. Serial dilutions of each desired concentration of emulsion containing EL were prepared in an emulsion without EL to a final volume of 180 μL in 96-well microplates. Then, each well was inoculated with 20 μL of the inoculum. After incubation at 37°C for 12 h, the MBC of the emulsion containing EL against each bacterium was determined as the lowest concentration producing a 99.9% reduction of the viable bacteria count in the subcultured wells. The serially diluted subcultured well contents were spread on TSA plates, and the colonies were counted after incubation at 37°C for 24 h.

2.6.3. Time-Killing Assay. The time-killing assay was performed to study the concentration and time-dependent killing effect [25]. For the O/W emulsion containing EL, a 200 μL inoculum was added to 1,800 μL of O/W emulsion containing EL. After inoculation, the solutions were incubated at 37°C with shaking (220 rpm). After incubation for 0, 1, 2, 3, or 4 h, a 100 μL aliquot was removed and serially diluted. The diluted samples were inoculated on TSA plates and incubated for 24 h at 37°C. The number of survivors (CFU/mL) was determined by counting the colonies, and time-killing curves were constructed by plotting the log CFU/mL versus time. The limit of detection in the assay was 10 CFU/mL (1 log CFU/mL). The experiments were conducted in triplicate.

2.7. Statistical Analysis. The statistical analysis was performed using SAS software (SAS Institute, Cary, NC, USA). Experiments were conducted in triplicate. Mean separations were evaluated using Duncan's multiple-range test. A p value <0.05 was taken to indicate a significant difference.

3. Results and Discussion

3.1. Antimicrobial Spectrum of EL. The three bacteria, the three molds, and the one yeast, causing food-borne diseases

and infectious skin diseases, were selected for the evaluation of the antimicrobial spectrum of EL (Table S1 in Supplementary data) [26–31].

In the disk diffusion assay, EL showed antimicrobial activity against the three Gram-positive bacteria, one yeast, and three molds. *P. acnes*, *S. pyogenes*, *C. perfringens*, *A. nidulans*, *R. oryzae*, and *T. mentagrophytes* were susceptible to 3 mM EL and *C. albicans* to 6 mM EL (Table 1). These results demonstrated that EL can be applied to food and cosmetics to control the molds, yeast, and Gram-positive bacteria that lead to food-borne diseases and infectious skin diseases.

3.2. Effects of Structural Change in EL on the Antimicrobial Activity

3.2.1. Effect of pH. Three Gram-positive bacteria were used to examine whether the degree of protonation of EL altered its antimicrobial activity (Table 2). When *S. aureus* ATCC 12692 was treated with 0.5 mM EL, no significant reduction in viable cells was seen at pH 7.0. By contrast, 4.11 ± 0.28 and more than 6.89 ± 0.20 log CFU reductions in viable cells were observed at pH 6.0 and 5.0, respectively. A similar result was obtained for *L. monocytogenes* ATCC 19115. There was no significant reduction in viable cells at pH 7.0, but reductions of 7.02 ± 0.18 and 7.04 ± 0.20 log CFU were seen at pH 6.0 and 5.0, respectively. For *B. cereus* ATCC 10876, there was no significant difference in log CFU reduction by pH variation. The results might be caused by surviving spores of *B. cereus*, spore-forming bacteria, germinated [32].

At acidic pH, the chemical structure of EL changes with the protonated form of EL increasing. The percentage of the protonated form of EL at pH 5.0, 6.0, and 7.0 was 22.03, 2.75, and 0.28%, respectively, because EL has a strongly acidic pK_a of 4.5 according to the prediction (Figure S1 in Supplementary Data).

Protonation of the head group of EL with decreasing pH should reduce the electrostatic repulsion between EL and the outer membrane of bacteria.

Other antimicrobial peptides with antibacterial mechanisms involving damage to the bacterial cell membrane also have greater antibacterial activity at acidic pH. Under acidic conditions, the anionicity of the peptides was decreased by the protonation of aspartic acid and glutamic acid residues of which pK_a values are 3.65 and 4.25, respectively. The reduced anionicity of the peptide enhances its ability to interact with negatively charged phospholipids on the cytoplasmic membranes of Gram-positive bacteria, increasing its antibacterial activity [9]. Therefore, the reduced anionicity of the head group of EL caused by protonation at acidic pH enhanced its antibacterial activity against Gram-positive bacteria.

It was reported that pH values of human skin and most of the foods are all in the pH 4.2–6.5 [33]. Therefore, it was demonstrated EL is adequate to be applied in food and cosmetics, since the antimicrobial activity of EL can be enhanced in acidic conditions than neutral conditions.

3.2.2. Effect of Oxidation of EL. The MIC of EL against *S. aureus* ATCC 12692 did not increase significantly with oxidation (Figure 1). During oxidation, 58.99% (mol/mol) of EL was oxidized, whereas its MIC did not increase significantly.

Ascorbic acid is unstable in alkaline solution, which leads to the formation of dehydro-1-ascorbic acid on exposure to active oxygen species or UV light [34]. During ascorbic acid oxidation, dehydroascorbic acid is formed by the removal of two hydrogen atoms and two electrons from ascorbic acid [35]. In addition, the lactone ring of dehydroascorbic acid induces its irreversible conversion to 2,3-diketo-1-gluconic acid by degradation in alkaline solution [36]. Therefore, in this study, the form of the EL head group was predicted to change during the oxidation of EL (Figure S2 in Supplementary Data).

Although the structure of EL changed with oxidation, degree of oxidation did not affect the antibacterial activity of EL. The reason why EL retained the antibacterial activity after being oxidized could be explained in terms of hydrophilicity. It was reported hydrophilic/hydrophobic balance between the head and tail group of fatty acid esters is one of the crucial factors determining their antibacterial activity [37]. In addition, it is necessary hydrophilicity of the head group of lauric acid esters is above a certain level to possess antibacterial activity.

Hence, hydrophilicity of EL and oxidized form of EL were assessed by calculation of octanol-water partition coefficient (log P) with the atom/fragment contribution method [38]. As a result, the log p values of EL and oxidized form of EL were calculated as -0.686 and -1.753, respectively. The values indicated that the hydrophilicity of EL was not decreased after being oxidized, and the hydrophilicity of the oxidized form of EL is above the certain level to possess antibacterial activity. Therefore, EL can retain the antibacterial activity after it functions as an antioxidant in food and cosmetics.

3.3. Antimicrobial Activity of EL in O/W Emulsion.

The MBCs of EL in the O/W emulsions against the Gram-positive bacteria *L. monocytogenes* and *S. aureus* were 2 mM and less than 0.50 mM, respectively. In comparison, the MBCs of EL in the O/W emulsions against the Gram-negative bacteria *E. coli and P. aeruginosa* were both 2.00 mM (Figure S3 in Supplementary Data). The previous study on the antibacterial activity of EL against bacteria found that Gram-negative bacteria were not susceptible to EL in the aqueous phase [7]. However, the result showed that EL in the O/W emulsion had antimicrobial activity against both Gram-positive and Gram-negative bacteria. Even though not fully understood, the difference in the antibacterial spectrum of EL between in the O/W emulsions and the aqueous phase could be related to negatively charged characteristics of EL as described in Section 3.2.1. The outer membrane of Gram-negative bacteria consists of lipopolysaccharide (LPS) with negatively charged phosphate groups

Table 1: Susceptibility of various pathogens to erythorbyl laurate.

Classification	Pathogen	3 mM EL	6 mM EL
Bacteria	*Propionibacterium acnes* ATCC 6919	+	+
	Streptococcus pyogenes ATCC 19615	+	+
	Clostridium perfringens ATCC 13124	+	+
Mold	*Aspergillus nidulans* ATCC 10074	+	+
	Rhizopus oryzae ATCC 10404	+	+
	Trichophyton mentagrophytes ATCC 18748	+	+
Yeast	*Candida albicans* ATCC 11006	NT*	+

*NT = not tested.

Table 2: Effect of pH on the antimicrobial activity of erythorbyl laurate.

Bacteria	pH	Log CFU/mL[a,b]		
		Control culture	0.5 mM EL-treated culture	1 mM EL-treated culture
S. aureus ATCC 12692	5	7.89 ± 0.20^{Aa}	$<1.00^{Ab}$	$<1.00^{Ab}$
	6	9.88 ± 0.03^{Ba}	5.77 ± 0.30^{Bb}	5.47 ± 0.10^{Bb}
	7	9.63 ± 0.12^{Ba}	7.78 ± 0.18^{Cb}	7.06 ± 0.43^{Cc}
L. monocytogenes ATCC 19115	5	8.04 ± 0.20^{Aa}	$<1.00^{Ab}$	$<1.00^{Ab}$
	6	8.02 ± 0.18^{Aa}	$<1.00^{Ab}$	$<1.00^{Ab}$
	7	8.04 ± 0.13^{Aa}	7.04 ± 0.06^{Bb}	4.84 ± 0.16^{Bc}
B. cereus ATCC 10876	5	6.99 ± 0.07^{Aa}	2.65 ± 0.02^{Ab}	2.63 ± 0.01^{Ab}
	6	7.05 ± 0.05^{Aa}	2.64 ± 0.08^{Ab}	2.64 ± 0.10^{Ab}
	7	6.85 ± 0.20^{Aa}	2.61 ± 0.05^{Ab}	2.56 ± 0.06^{Ab}

[a]Values in the same column followed by different uppercase letters are significantly different ($p < 0.05$). [b]Values in the same row followed by different lowercase letters are significantly different ($p < 0.05$).

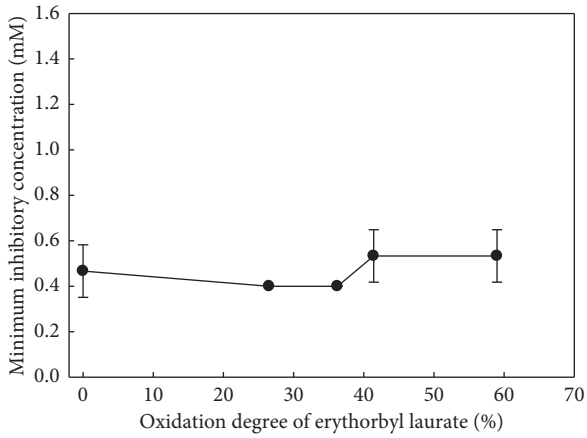

Figure 1: Effect of the oxidation of erythorbyl laurate on its minimum inhibitory concentration against *S. aureus* ATCC 12692.

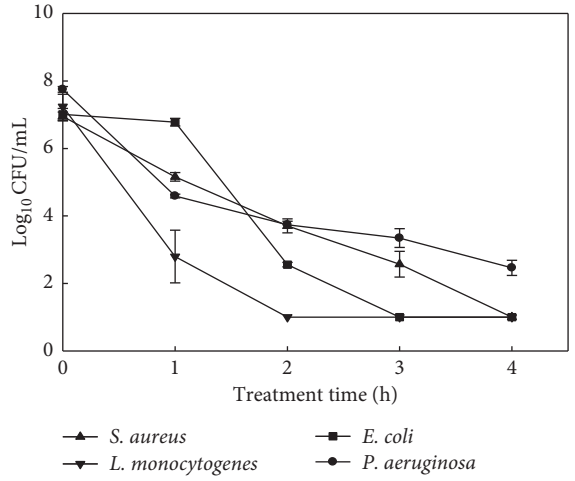

Figure 2: Time-killing curves of 5.0 mM erythorbyl laurate in oil-in-water emulsion against Gram-positive and Gram-negative bacteria.

[39]. In the aqueous phase, the electrostatic repulsion between EL and outer membrane (especially LPS) of the Gram-negative bacteria would harshly prevent EL interacting with the outer membrane. In contrast, the use of a nonionic surfactant (Tween-20) to stabilize the O/W emulsion might reduce the net negative charge of EL to overcome the electrostatic repulsion. As a result, in the O/W emulsions, EL can interact with the cell membranes of Gram-negative bacteria and consequently showed the antibacterial effect.

The time-killing assay quantified and confirmed the bactericidal activity of 5.0 mM EL in the O/W emulsion against two Gram-positive bacteria (*S. aureus* ATCC 12692 and *L. monocytogenes* ATCC 19115) and two Gram-negative bacteria (*E. coli* ATCC 35150 and *P. aeruginosa* ATCC 10145) (Figure 2). After treatment for 4 h, the numbers of *P. aeruginosa*, *E. coli*, *S. aureus*, and *L. monocytogenes* were reduced by 5.29 ± 0.24, 6.01 ± 0.18, 5.95 ± 0.13, and 6.24 ± 0.30 log CFU/mL, respectively.

3.4. Multipassage Resistance Selection Study. Resistance of microorganisms to EL can be selected for multipassage resistance selection, which enables strains to acquire greater resistance to antibiotics [40]. The initial MICs of EL and

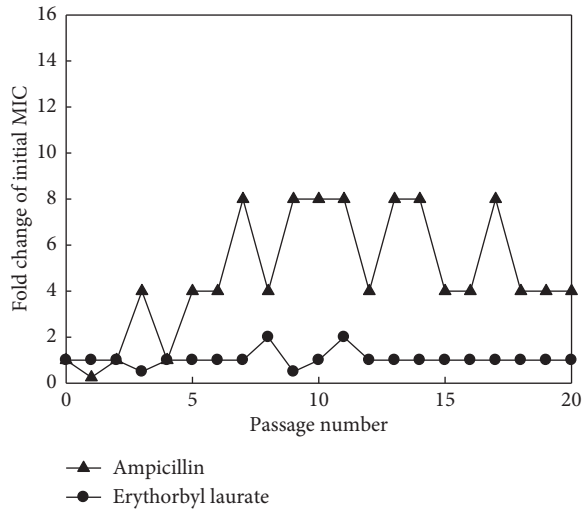

Figure 3: Changes in the minimum inhibitory concentrations of EL and ampicillin against *S. aureus* ATCC 12692 during 20 serial passages. Each line shows the result of one of three representative independent cultures passaged with each antimicrobial compound.

ampicillin for *S. aureus* ATCC 12692 were 286.76 and 0.125 μg/mL, respectively. A strain with reduced susceptibility to ampicillin was generated after 20 serial passages and was 4-fold less susceptible. Equal numbers of independent cultures were passaged with EL for 20 passages. No significant (>2-fold) increase in the MIC of EL was observed (Figure 3). Therefore, it was impossible to isolate bacteria that acquired resistance to EL.

As a β-lactam antibiotic, ampicillin is an irreversible inhibitor of the transpeptidase needed to make the bacterial cell wall. Generally, *S. aureus* acquires resistance to ampicillin by producing penicillinase and triggering the resistance pathway [41]. In comparison, evolution of bacteria acquiring resistance to free fatty acid was less problematic than conventional antibiotics [42]. Lauric acid and esters of fatty acids such as monocaprin kill microorganisms by disrupting bacterial cell membranes; hence, the emergence of resistance is unlikely [43]. Therefore, it was expected EL did not develop resistance since the antibacterial mechanism of EL is based on the damages to the bacterial cell membrane physically.

4. Conclusions

EL showed antimicrobial activity against Gram-positive bacteria, yeasts, and molds that cause food-borne diseases and infectious skin diseases. The protonation of EL in acidic conditions increased its antibacterial activity, while the oxidation of EL did not alter its antibacterial activity. EL exhibited bactericidal effects against both Gram-positive and Gram-negative bacteria in O/W emulsion system. A multipassage resistance study implied EL might not develop the drug resistance of bacteria. This study demonstrates EL is a promising additive to control microbial contamination in food and cosmetics, especially emulsion-based products.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Jin-Woo Kim, Hyunjong Yu, and Kyung-Min Park are co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jin-Woo Kim, Hyunjong Yu, and Kyung-Min Park contributed equally to this study.

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Supplementary Materials

Table S1: criteria to select microorganisms for the susceptibility tests based on potential diseases related to food and cosmetics. Figure S1: predicted chemical structures of erythorbyl laurate in various pH values. Figure S2: predicted chemical structures of erythorbyl laurate according to the oxidation. Figure S3: bacterial suspensions spread on TSA plates after the treatment with various concentrations of erythorbyl laurate, showing the bactericidal activity of erythorbyl laurate in the O/W emulsions. (*Supplementary Materials*)

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