A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Hydrophilicity of non-fatty acid moiety: significant determinant affecting antibacterial activity of lauric acid esters

라우르산 에스터의 항균성에 영향을 미치는 기질 특성 규명

February, 2016

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석사학위논문

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이 논문을 석사학위 논문으로 제출함

2016년 2월

서울대학교 대학원

농 생 명 공 학 부

이 선 주

이선주의 석사 학위논문을 인준함

2016년 2월

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Abstract

What affects to the antibacterial activities of lauric acid esters was investigated. Based on the results of antibacterial activity test evaluating minimum inhibitory concentration and minimum bactericidal concentration, sesamol laurate was found to have no effect on either Gram positive bacteria or Gram negative bacteria. On the other hand, erythorbyl laurate had antibacterial activity to Gram positive bacteria. To investigate why the antibacterial activities are shown differently between sesamol laurate and erythorbyl laurate, monolaurin, sucrose monolaurate, isoamyl laurate, and methyl laurate, erythorbyl laurate, and sesamol laurate were chosen based on hydrophilicity of non-fatty acid moiety.

Minimum inhibitory concentration was assessed by broth micro-dilution method against *Staphylococcus aureus*, *Listeria monocytogenes, Escherichia coli,* and *Salmonella* Typhimurium. Monolaurin, erythorbyl laurate, and sucrose monolaurate showed antibacterial activities against Gram positive bacteria. On the other hand, isoamyl laurate, methyl laurate, and sesamol laurate had no inhibitory effect on both Gram positive and Gram negative bacteria even treated up to 1.0 mM.

The mechanism of monolaurin, erythorbyl laurate, and sucrose monolaurate was investigated by measuring the released cell constituents at 260 nm using spectrophotometer and the lipid compositional changes using gas chromatography (GC). 260 nm absorbing materials of *Staphylococcus aureus* treated with monolaurin, erythorbyl laurate, and sucrose monolaurate were increased for 2 h, and membrane lipid composition was also changed.

Octanol/water partition coefficient was calculated by atom/fragment contribution method. The partition coefficients indicating lipophilicity were 7.175, 5.284, and 5.717 for isoamyl laurate, methyl laurate, and sesamol laurate, respectively, whereas monolaurin, erythorbyl laurate, and sucrose monolaurate showed 3.670, -0.6858, and -4.122, respectively. The hydrophilic-lipophilic balance values of isoamyl laurate, methyl laurate, and sesamol laurate were 1.800, 3.700, and 4.835, respectively, while monolaurin, erythorbyl laurate, and sucrose monolaurate had the higher HLB value of 7.025, 15.25, and 16.09, respectively.

These results suggested that lauric acid esters should retain proper hydrophilicity based on the log *P* value of lower than 4, and the HLB value of higher than 7 to incorporate into bacterial cell membrane as antibacterial agents.

Keywords: lauric acid esters; antibacterial activity; non-fatty acid moiety; hydrophilicity; octanol-water partition coefficient; hydrophilic-lipophilic balance

Student Number: 2014-20693

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aureus ATCC 49444, (b) *L. monocytogenes* ATCC 7644, (c) *E. coli* ATCC 43889, and (d) *Sal.* Typhimurium ATCC 43971.

Fig. 6. Effects of monolaurin, sucrose monolaurate, and erythorbyl laurate on membrane integrity in *S. aureus* ATCC 49444 measured by UV absorbing components at 260 nm.

Fig. 7. SYTO 9 and propidium iodide fluorescence images of *S. aureus* ATCC 49444 without treatment of lauric acid esters (A), or with treatment of 2xMIC monolaurin (B), sucrose monolaurate (C), and erythorbyl laurate (D). SYTO 9 stained both intact and damaged cells, and propidium iodide stained only cells with compromised membranes (red).

1. Introduction

An emulsion is traditionally defined as a dispersion of droplets of one liquid in another, the two being immiscible (Coupland & McClements, 1996). In foods, emulsions have a broad meaning encompassing systems where there may also be solids, gases and/or liquid crystals present (cake batter, ice cream, mayonnaise, etc.) (Rousseau, 2000).

Contamination of food pathogen (Maijala, Lyytikäinen, Johansson, Autio, Aalto, Haavisto, et al., 2001; Mason, Williams, Salmon, Lewis, Price, Johnston, et al., 2001) is one of the major concerns in emulsion foods due to the problem of safety and quality. Therefore, using food preservative, one of the popular methods to reduce microbial contamination, has been developed (Food & Administration, 2001).

Lauric acid is known to have the most inhibitory activity against Gram positive bacteria among the saturated fatty acid (J. J. Kabara, Swieczkowski, Conley, & Truant, 1972). Therefore, the applications of lauric acid to food industry have been studied, for example, in milk and ground beef as antibacterial agents (Mansour & Millière, 2001; McLay, Kennedy, O'Rourke, Elliot, & Simmonds, 2002).

Preliminary study was performed by lipase-catalyzed esterification between

lauric acid and erythorbic acid derived natural materials to introduce antioxidative and antimicrobial activity to an O/W emulsifier (Park, Lee, Sung, Lee, & Chang, 2011). The synthesized erythorbyl laurate could be used as an antibacterial agent that suppresses the cell growth of Gram positive bacteria, *Staphylococcus aureus*, and *Listeria monocytogenes*, causative organism of food borne illness. The growth inhibitory effects of erythorbyl laurate were considered to be derived from lauric acid, hydrophobic moiety. Similarly, various lauric acid esters, such as fructose laurate, galactose laurate (T. Watanabe, Katayama, Matsubara, Honda, & Kuwahara, 2000), monolaurin (Fu, Feng, & Huang, 2006), and so on, were synthesized and revealed as antimicrobial agents.

Therefore, lipase-catalyzed synthesis of lauric acid esters is potential for developing antimicrobial agents applicable to food additives and other promising lauric acid esters are still necessary for enlarging its application fields. To provide fundamental information for synthesizing lauric acid esters which have antibacterial effects, the effective concentrations and physiological characteristics were investigated depending on the various hydrophilic moieties.

2. Materials and methods

2.1. Materials

2.1.1. Sample preparation

Immobilized lipase, from *Candida antarctica* (triacylglycerol hydrolase, EC 3.1.1.3; Novozym®435) was purchased from Novozymes (Bagsvaerd, Denmark) with a catalytic activity of 7000 PLU/g (the activity of PLU refers to the millimoles of propyl laurate synthesized per minute at 60℃). Sesamol (98%) and lauric acid (≥99%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade acetonitrile (J.T. Baker Co., Phillipsburg, NJ, USA) was dehydrated by molecular sieves 4 Å (Sigma-Aldrich Co.) and filtered through a membrane filter (0.45 μm) prior to use as a reaction medium. Erythorbyl laurate was synthesized by lipase-catalyzed esterification between erythorbic acid and lauric acid (Park, Lee, Sung, Lee, & Chang, 2011).

2.1.2. Chemicals

Monolaurin (>98%) was purchased from Tokyo Chemical Industry Co., LTD., Tokyo, Japan). Sucrose monolaurate (≥97%), isoamyl laurate (≥97%), methyl laurate (99.5%), nisin (2.5% w/w), and ampicillin were purchased from Sigma-Aldrich Co. All other chemicals were of extra pure grade.

2.2. Procedure for lipase-catalyzed esterification between sesamol and lauric acid

Sesamol (0.6 mmol) and lauric acid (3.0 mmol) were placed in a crimp top glass vial with 15 mL of acetonitrile and pre-incubated at 70℃ for 15 min using a water circulator with a stirrer (200 rpm). The reaction was initiated by adding 150 mg of immobilized lipase to the mixture. The temperature was kept constant at 70±1℃ during the reaction.

2.2.1. Quantitative analysis of esterification product

Esterification products were periodically analyzed using a HPLC instrument (LC-2002, Jasco Inc., Tokyo, Japan) equipped with a silica-based column (5 μm, I.D. 4.6 mm x 150 mm: Luna C_{18}, Phenomenex, Torrance, CA, USA), a refractive index (RI) detector (RI-2031, Jasco Inc.), and a ultraviolet (UV) detector (UV-2075, Jasco Inc.). The mobile phase was acetonitrile/water/acetic acid (90:5:5, v/v/v) at 1.0 mL/min flow rate. The reaction mixture was sampled at appropriate intervals and filtered through a membrane filter (0.45 μm); then, each aliquot of 20 μL was injected into the HPLC. Peaks in the HPLC chromatograms were identified using retention times of sesamol and lauric acid standards. Lauric acid was detected using a RI detector, and sesamol and sesamol laurate were detected using a UV

detector at 290 nm.

2.2.2. Purification and identification of sesamol laurate

After enzymatic esterification, sesamol laurate was isolated from the reaction mixture according to the reported method with a slight modification (Karmee, 2008; Y. Watanabe, Ishido, Fang, Adachi, & Matsuno, 2005; Yan, Bornscheuer, & Schmid, 1999). Briefly, the reaction mixture was filtered through a membrane (0.45 μm) to separate the immobilized lipase and molecular sieves, and then lyophilized in a FD8512 freeze-dryer (Ilshin Lab Co., Ltd., Seoul, Korea) at -76℃. The concentrate was washed three times with 10 mL of water and the retentate was discarded to remove the residual lauric acid after filtration through a membrane (0.45 μm) using vacuum. Then, filtrated water containing sesamol and sesamol laurate was frozen at -80℃ and lyophilized. The dried sample was dissolved in acetonitrile and 3 mL was injected into preparative HPLC (LC-918, Japan Analytical Industry Co., Ltd., Tokyo, Japan) equipped with poly(vinyl alcohol) gel columns (500 × 20 mm, JAIGEL GS-510, Japan Analytical Industry Co., Ltd.), and a UV detector (UV detector 3702, Japan Analytical Industry Co., Ltd.). The mobile phase was acetonitrile/water (90:5, v/v) at 5.0 mL/min flow rate. The retention times of sesamol laurate and sesamol detected using UV detector at 290 nm were

28.38±0.01 and 33.70±0.2 min, respectively. Sesamol laurate was recycled and collected through fraction nozzle.

The obtained sesamol laurate was identified by LC-ESI-MS (Thermo Finnigan, San Jose, CA, USA) equipped with autosampler and PDA-UV detector.

2.3. Cell culture

The Gram positive strains were *Staphylococcus aureus* ATCC 49444 and *Listeria monocytogenes* ATCC 7644. Gram negative strains were *Escherichia coli* ATCC 43889 and *Salmonella* Typhimurium ATCC 43971. Stock cultures were maintained in tryptic soy broth (30 g/L of Difco Bacto Tryptic Soy Broth, Becton Dickinson Co., Sparks, MD, USA) supplemented with 50% glycerol at -80℃. All microorganisms were cultured for 18-24 h at 37℃ in tryptic soy broth.

2.4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Staphylococcus aureus ATCC 49444, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 43889, and *Salmonella* Typhimurium ATCC 43971 were tested for determination of effective concentration of sesamol laurate, methyl laurate, isoamyl laurate, monolaurin, erythorbyl laurate, and sucrose

monolaurate. MIC and MBC, which are standard criteria for evaluation of antimicrobial effect, were determined by broth micro-dilution assay. Briefly, serial dilutions of each desired concentrations of samples were prepared in sterile tryptic soy broth to final volume of 100 μL in 96-well microplate (Corning Incorporated, Corning, NY, USA). Then, each well was inoculated with 100 μL of test microorganisms in tryptic soy broth to final concentration of $5.0x10^5$ CFU/mL (Bechert, Steinrücke, & Guggenbichler, 2000; Magalhães & Nitschke, 2013; Patricia Nobmann, Bourke, Dunne, & Henehan, 2010; Wiegand, Hilpert, & Hancock, 2008). The MIC was defined as the lowest concentration of test compound at which bacterial growth was inhibited after 12 h of incubation at 37℃. And the MBC was defined as the lowest concentration producing a 99.9% reduction in bacterial viable count in the sub-cultured well contents (Pridmore, Burch, & Lees, 2011).

2.5. Release of cellular constituents

The cell integrity was examined by determining the release of cellular constituents into supernatant (Diao, Hu, Zhang, & Xu, 2014; Lv, Liang, Yuan, & Li, 2011). Cells from the working culture of tested bacteria were harvested by centrifugation for 10 min at 10,000 xg at 4℃. The supernatant was discarded and the cells were washed twice with 0.1 M phosphate buffer (pH 7.4, PBS),

and resuspended in the same buffer. The suspensions were adjusted to achieve a bacterial concentration of $1.0x10^9$ CFU/mL. Monolaurin, erythorbyl laurate, and sucrose monolaurate were added to the bacterial suspensions at 4xMIC, and then, incubated at 37℃ under agitation for 2 h. Then, each sample of 0.4 mL was collected and centrifuged at 10,000 xg for 10 min at 4℃ at the predetermined intervals. Supernatant of 2 μL for each treatment was added to a 16-well plate. The total amount of DNA and RNA released from the cytoplasm was estimated by detecting the absorbance at 260 nm.

A bacterial suspension in sterile PBS without antimicrobial agents as the negative control and nisin as a positive control were included. Nisin was suspended in 0.02 M HCl, and the supernatant following centrifugation at 10,000 xg for 10 min was used (Brumfitt, 2002). Sucrose monolaurate stock solution was prepared by dissolving sucrose monolaurate of 2.6 mg into 150 μL of ethanol. And the other lauric acid ester stock solutions were prepared by dissolving in dimethyl sulfoxide (DMSO). To correct the absorbance of lauric acid ester itself, the PBS in the presence of lauric acid esters only were taken. The bacterial suspension in sterile PBS with DMSO, ethanol and 0.02 M HCl were also taken to prove that solvent did not affect the cell death (P. Nobmann, Smith, Dunne, Henehan, & Bourke, 2009).

2.6. Fluorescence microscopy

The Live/Dead BacLight viability kit (Molecular Probes, Inc., Eugene, OR, USA) was used for assessment of cell rupture according to the manufacturer instructions. In this assay, the SYTO 9 and propidium iodide stains compete for binding to the bacterial nucleic acid. SYTO 9 labels cells with both damaged and intact membranes, whereas propidium iodide penetrates only cells with damaged membranes. A culture of *S. aureus* ATCC 49444 as a model organism was grown for 18-24 h in tryptic soy broth. The bacterial culture was harvested by centrifugation at 10,000 xg for 10 min at 4℃. The supernatant was removed and the pellet was washed once with PBS (pH 7.4) and resuspended in 0.85% NaCl solution. Bacterial suspension adjusted to $1.0x10^5$ CFU/mL were treated with monolaurin, sucrose monolaurate, and erythorbyl laurate and then, all the suspensions were incubated at 37℃ for 30 min. At the end of the incubation period, the dye mixture of 3 μL was added to 1 mL of bacterial suspensions including untreated bacterial suspensions. After another incubation in the dark at 25℃ for 15 min, stained bacterial suspension of 5 μL was trapped between a slide and coverslip, and observed using a DE/Axio imager A1 microscope (Carl Zeiss, Oberkochen, Germany) equipped with fluorescence filters for SYTO 9 (filter set 38 HE, Carl Zeiss) and propidium iodide (filter set 43 HE, Carl Zeiss).

2.7. Membrane lipid composition

Monolaurin, erythorbyl laurate, and sucrose monolaurate were assessed for their impact on changes in *S. aureus* membrane fatty acid profiles. The concentration of the antimicrobial compound allowing the microorganisms to survive half compared to the status of the end of the stationary phase grown at the optimal temperature without antimicrobials. The antimicrobial concentration was determined based on the result of micro-dilution MIC test. Treatments (4.5 mL sterile TSB in test tube) contained 0.1 mM monolaurin, 0.2 mM of sucrose monolaurate, and 0.05 mM of erythorbyl laurate. Each treatment tube was inoculated with 0.5 mL working culture yielding 1.0×10^6 CFU/mL. All tubes were incubated in an environmental shaker under aerobic conditions at 37℃ and 220 rpm for 24 h.

After appropriate incubation, an aliquot of 5 mL from each tube was centrifuged at 4,000 xg and 25℃ for 10 min to obtain cell pellets. Each pellet was subjected to fatty acid extraction and identification as described by Garcés and Mancha (1993). Each pellet was lyophilized and then placed in tubes with Teflon lined caps. The methylation mixtures containing methanol : benzene : 2,2-dimethoxypropane : H_2SO_4 (39:20:5:2, by vol) were used. The mixtures of 340 μL and *n*-heptane of 200 μL were added to the sample. After flushing with N_2, the tube was placed in a water bath at 80℃ for 2 h to assure complete lipid

extraction and methylation. After heating and shaking to mix the components into a single phase, the tube was cooled at room temperature and shaken again. Fatty acid methyl esters (FAMEs) in the upper phase were analyzed on an Agilent gas chromatograph (model 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a split-capillary injector and a flame ionization detector. Separations were obtained using a DB-23 column (60 mm x 0.25 mm I.d., 0.25 μm, Agilent Technologies). The injector temperature was set at 250℃, the column oven at 50℃ for 1 min, followed by an increase at a rate of 15℃/min to 130℃, 8℃/min to 170℃, and 2℃/min to 215℃, which was held for 10 min. Hydrogen, air, and helium were used as the carrier gas, and the flow rate was set to 35 mL/min, 350 mL/min, and 35 mL/min, respectively. Supelco 37 component FAME mix (Supelco, Inc., PA, USA) was used for analyzing fatty acid profiles.

2.8. Octanol-water partition coefficient (log *P*)

Atom/fragment contribution method was used to estimate the log octanol-water partition coefficient (log *P)* of lauric acid esters. Meylan and Howard (1995) determined atom/fragment contribution values (Table 1) and correction factors (Table 2) by a multiple linear regression of 1,120 compounds with measured log *P* values and 1,231 compounds, respectively. Log *P* was

estimated by following equation

$$\log P = \Sigma(f_i n_i) + \Sigma(c_j n_j) + 0.229$$

(n = 2351, r^2 = 0.982, SD = 0.216, ME = 0.161).

Table 1. Lists of a representative selection of the 130 atom/fragments for estimating octanol-water partition coefficients

	Coeff[a]		Coeff[a]
Aromatic atoms		**Aliphatic nitrogen**	
Carbon	0.2940	$-NO_2$ (aliphatic attachment)	-0.8132
Oxygen	-0.0423	$-NO_2$ (aromatic attachment)	-0.1823
Sulfur	0.4082	>N< (5+ valance, single bonds)	-6.6000
Aromatic nitrogen		-N=C=S (aliphatic attachment)	0.5236
Nitrogen [N=O, oxide type]	-2.4729	-N=C=S (aromatic attachment)	1.3369
Nitrogen [+5 valence type]	-6.6500	-NP (phosphorus attachment)	-0.4367
Nitrogen at a fused ring location	-0.0001	-N (two aromatic attachment)	-0.4657
Nitrogen in a five-member ring	-0.5262	-N (one aromatic attachment)	-0.9170
Nitrogen in a six-member ring	-0.7324	-N(O) (nitroso, +5 valence)	-1.0000
Aliphatic carbon		-N=C (aliphatic attachment)	-0.0010
$-CH_3$ (methyl)	0.5473	$-NH_2$ (aliphatic attachment)	-1.4148
$-CH_2-$	0.4911	-NH- (aliphatic attachment)	-1.4962
-CH	0.3614	-N< (aliphatic attachment)	-1.8323
>C< (no hydrogens, single bonds, three or more carbons attached)	0.2676	-N(O) (nitroso)	-0.1299
C (no hydrogens)	0.9723	-N=N- (azo, includes both N)	0.3541
Olefinic and acetylenic carbon		**Aliphatic oxygen**	
=C< (two aromatic attachments)	-0.4186	-OH (nitrogen attachment)	-0.0427
$=CH_2$	0.5184	-OH (phosphorus attachment)	0.4750
=CH- or =C<	0.3836	-OH (olefinic attachment)	-0.8855
Carbonyls and thiocarbonyls		-OH (carbonyl attachment)	0.0
CHO- (aldehyde, aliphatic attachment)	-0.9422	-OH (aliphatic attachment)	-1.4086
CHO- (aldehyde, aliphatic attachment)	-0.2828	-OH (aromatic attachment)	-0.4802
-C(=O)OH (acid, aliphatic attachment)	-0.6895	-O- (two aromatic attachments)	0.2923
-C(=O)OH (acid, aromatic attachment)	-0.1186	-OP (aromatic attachment)	0.5345
-C(=O)O (ester, aliphatic attachment)	-0.9505	-OP (aliphatic attachment)	-0.0162
-C(=O)O (ester, aromatic attachment)	-0.7121	-ON- (nitrogen attachment)	0.2352
-C(=O)- (noncyclic, 2 aromatic attachments)	-0.6099	-O- (carbonyl attachment)	0.0
-C(=O)- (cyclic, 2 aromatic attachment)	-0.2063	-O- (one aromatic attachment)	-0.4664
-C(=O)- (cyclic, aromatic, olefinic attachment)	-0.5497	-O- (aliphatic attachment)	-1.2566
-C(=O)- (olefinic attachment)	-1.2700	=O	0.0
-C(=O)- (aliphatic attachment)	-1.5586		
-C(=O)- (one aromatic attachment)	-0.8666		

[a]Coefficient determined by regression.

Adopted from (Meylan & Howard, 1995)

Table 2. Correction factors involving aromatic ring substituent positions for more complex substructures than atoms

	Coeff[a]		Coeff[a]
Ortho interaction		**Can be either ortho or non-ortho**	
-COOH/-OH	1.1930	$-NO_2$ with –OH, -N<, or –N=N-	0.5770
-OH/ester	1.2556	$-NO_2$/-NC(=O) (cyclic type)	0.3994
Amino (at 2-position) on pyridine	0.6421	$-NO_2$/-NC(=O) (non-cyclic-type)	0.7181
Alkyloxy ortho to 1 aromatic nitrogen	0.4549	**Non-ortho reactions**	
Alkyloxy ortho to two aromatic nitrogens	0.8955	-N</-OH	-0.3510
Alkylthio ortho to two aromatic nitrogen	0.5415	-N</ester	0.3953
Carboxamide (-C(=O)N) ortho to an aromatic nitrogen	0.6427	-OH/ester	0.6487
any[b]/-NHC(=O)C	-0.5634	**Others**	
any[b] two/-NHC(=O)C	-1.1239	Amino-type (at 2-position) on triazine, pyrimidine	0.8566
any[b]/-C(=O)NH	-0.7352	NC(=O)NS on triazine	-0.7500
any[b] two/-C(=O)NH	-1.1284	1,2,3-trialkyloxy	-0.7317
Amino-type[c]	0.6194		

[a]Coefficient determined by regression. [b]Any refers to any ortho substituent other than hydrogen with the exception of –OH or an amino-type). [c]Can be a primary, secondary, or tertiary amine, including –N-C(=O) types.

Adopted from (Meylan & Howard, 1995)

Table 3. Correction factors involving non-aromatic ring substituent positions for more complex substructures than atoms

	Coeff[a]		Coeff[a]
Various carbonyl factors		**Various ring factors**	
More than one aliphatic –C(=O)OH	-0.5865	1,2,3-Triazole ring	0.7525
HO-CC(=O)CO-	1.7838	Pyridine ring (nonfused)	-0.1621
-C(=O)-C-C(=O)N	0.9739	*sym*-Triazine ring	0.8856
-C(=O)NC(=O)NC(=O)-	1.0254	Fused aliphatic ring	-0.3421
-NC(=O)NC(=O)-	0.6074	**Various alcohol, ether, and nitrogen factors**	
Cyclic ester (non-olefin type)	-1.0577	More than one aliphatic -OH	0.4064
Cyclic ester (olefin type)	-0.2969	-NC(C-OH)C-OH	0.6365
Amino acid (α-carbon type)	-2.0238	-NCOC	0.5494
C(C(=O)OH) aromatic	-0.3662	HO-CHCOCH-OH	1.0649
CC(=O)NCC(=O)OH	0.4193	HO-CHC(OH)CH-OH	0.5944
CC(=O)NC(C(=O)OH)S-	1.5505	-NH-NH- structure	1.1330
>C=NOC(=O)	-1.0000	>N-N< structure	0.7306

[a]Coefficient determined by regression.

Adopted from (Meylan & Howard, 1995)

2.9. Hydrophilic-lipophilic balance (HLB)

There have been large numbers of works to determine HLB value experimentally or computationally. Among all those methods, Davies method has been most widely used. Davies assumed that the HLB value was an additive and constitutive indicator and the group numbers were assigned to various structural elements (Table 4) (Guo, Rong, & Ying, 2006). HLB is defined as

$$\text{HLB} = 7 + \Sigma(\text{hydrophilic group numbers}) + \Sigma(\text{lipophilic group numbers})$$

Table 4. Values of the hydrophilic and lipophilic groups obtained by the Davies method

Hydrophilic group	Numbers	Lipophilic group	Numbers
$-SO_4Na$	38.7	-CH-	-0.475
-COOK	21.1	$-CH_2-$	-0.475
-COONa	19.1	$-CH_3$	-0.475
$-SO_3Na$	11	=CH-	-0.475
-N (tertiary amine)	9.4	$-CF_2-$	-0.87
Ester (free)	2.4	$-CF_3$	-0.87
-COOH	2.1	Phenyl	-1.662
-OH (free)	1.9	$-CH_2CH_2CH_2O-$	-0.15
$-CH_2OH$	-	$-CH(CH_3)CH_2O-$	-0.15
$-CH_2CH_2OH$	-	$-CH_2CH(CH_3)O-$	-0.15
$-CH_2CH_2CH_2OH$	-	Sorbitan ring	-
-O-	1.3		
$-CH_2CH_2O-$	0.33		
$-CH_2CH_2OOC-$	-		
-OH (sorbitan ring)	0.5		
Ester (sorbitan ring)	6.8		

Adopted from (Guo, Rong, & Ying, 2006)

2.10. Statistical analysis

Statistical analysis was performed using SPSS software (Version 23.0, IBM Corp., Armonk, NY, USA). Experiments were conducted in triplicate. Mean separations were determined using Duncan's one way analysis of variance ($p < 0.05$).

3. Results and discussion

3.1. Progression of enzymatic synthesis of sesamol laurate in acetonitrile

To monitor the lipase-catalyzed esterification between sesamol and lauric acid over time (Fig. 1), the products from the esterification were analyzed at predetermined time intervals by HPLC. Typical HPLC chromatograms of each component of the lipase-catalyzed esterification are represented in Fig. 2. As shown in Fig. 2, the retention times of sesamol, sesamol laurate, and lauric acid were determined to be 1.76±0.01, 5.76±0.1, and 3.75±0.02 min, respectively.

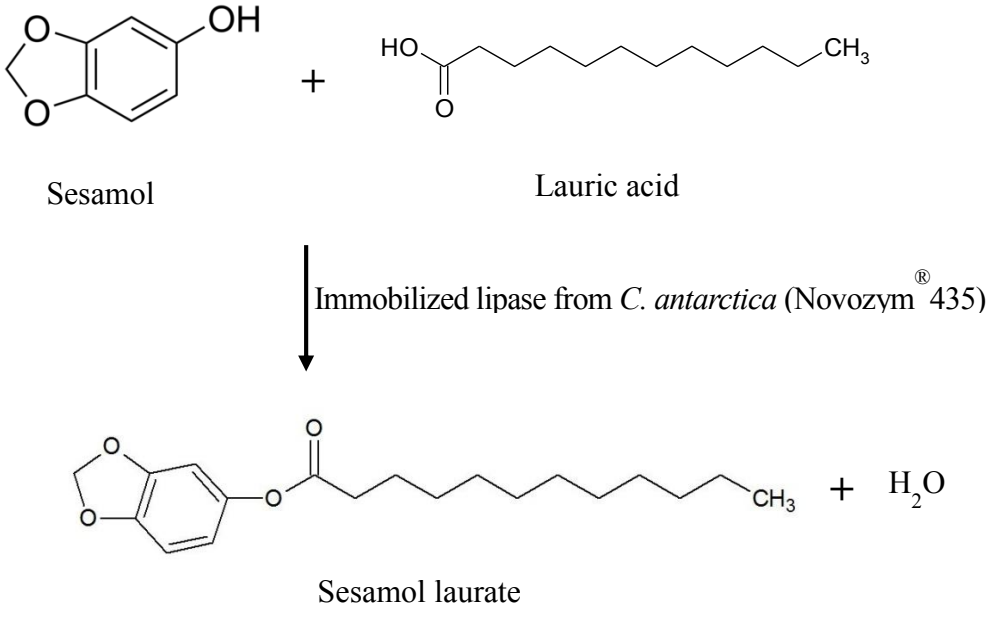


Fig. 1. Scheme of lipase-catalyzed synthesis of sesamol laurate in acetonitrile.

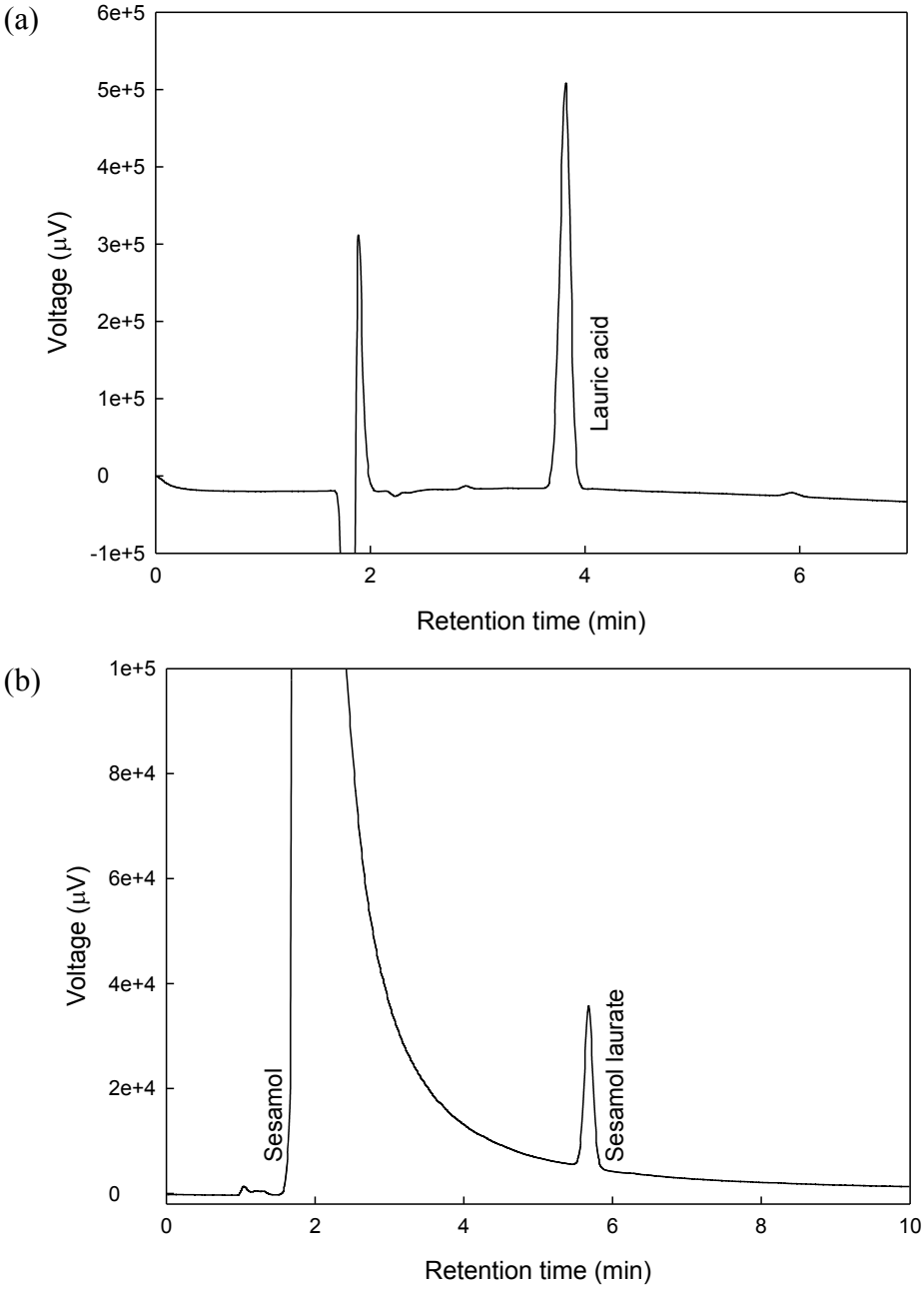


Fig. 2. HPLC chromatograms for the components obtained from the immobilized lipase-catalyzed esterification between sesamol and lauric acid in acetonitrile (a, RI-; b, UV-detector).

3.2. Structural analysis of sesamol laurate produced

After the purification procedure, the purity was confirmed by no peaks except one, indicating sesamol laurate in HPLC chromatograms of the purified product (data not shown). The sesamol laurate, obtained from the final product as a brown fluid, was identified by LC–ESI-MS. Mass spectrometry in full mode revealed the presence of sesamol laurate. The spectra gave a molecular ion at m/z = 321.1 $[M+H]^+$, corresponding exactly to the molecular mass of sesamol laurate (Fig. 3).

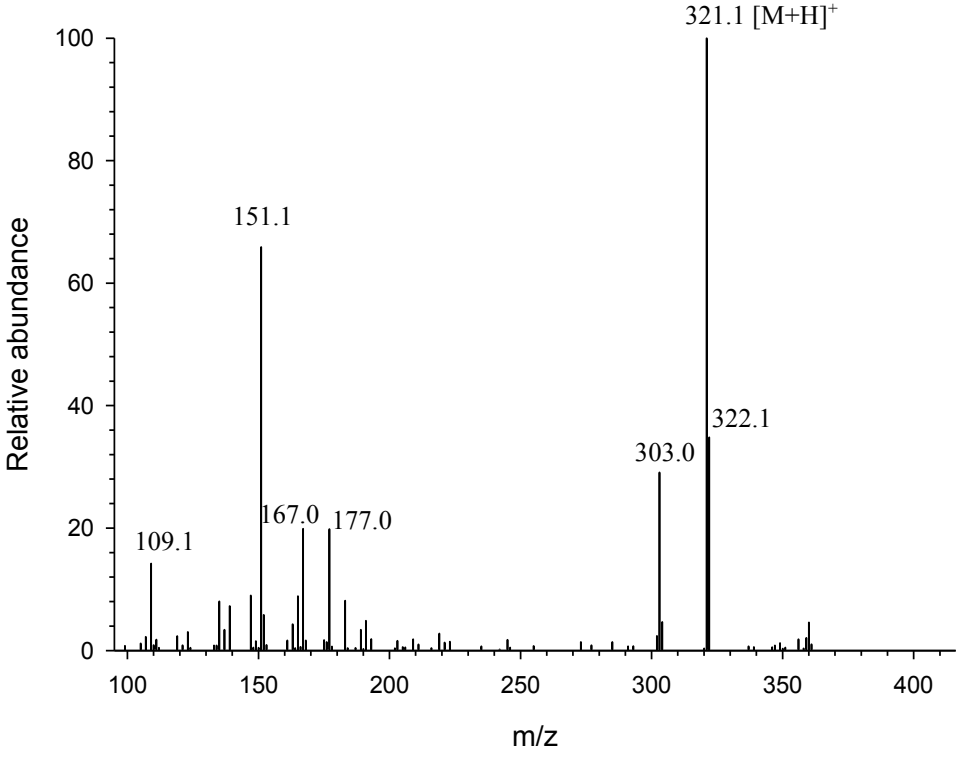


Fig. 3. LC-ESI-MS spectra of sesamol laurate synthesized by lipase in this study (full scan mode).

3.3. Susceptibility screening of sesamol laurate

Initial screening of the antibacterial activity of sesamol laurate against five tested food-related bacteria was conducted using MIC. Sesamol laurate displayed no antibacterial activity against different tested strains, even treated as much as the concentration of 30 times of lauric acid MIC against *Staphylococcus aureus* ATCC 49444 (data not shown).

3.4. Selection of lauric acid esters

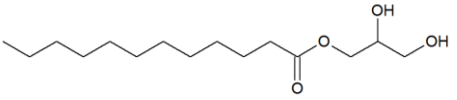
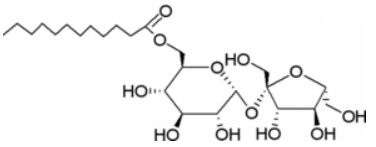
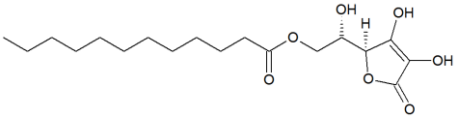
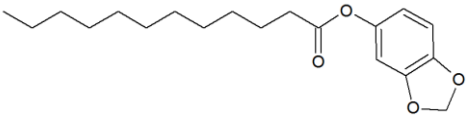
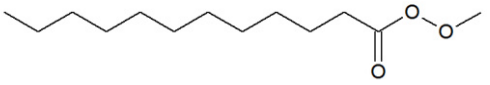
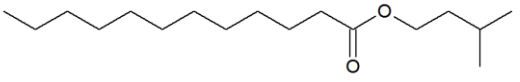
As the sesamol laurate did not show the antibacterial activity, it was assumed that the hydrophilicity of non-fatty acid moieties in lauric acid esters affects to their antibacterial activities due to the ability incorporating into the bacterial cell membrane. This hypothesis was based on the other reports. J. Kabara, Vrable, and Jie (1977) suggested that the mechanism of bactericidal action of long chain fatty acids and derivatives is due to a balance between hydrophilic and hydrophobic parts of the molecule. But their discussion that the hydrophobic portion of the molecule is more important to antimicrobial action did not correspond to my result, because lauric acid esters with different hydrophilic moiety did not show antibacterial activity. Zhao, Zhang, Hao, and Li (2015) evaluated the antibacterial activities of sugar fatty acid esters with different saccharide moieties. The result suggested that disaccharide monoesters of capric acid exhibited better antibacterial activity than monosaccharide monoesters, which was in accordance with the results of Ferrer, Soliveri, Plou, López-Cortés, Reyes-Duarte, Christensen, et al. (2005). The hydroxyl group in the carboxyl group seems to be important for the antibacterial activity of free fatty acids, as methylated free fatty acids often have reduced or no activity (Kodicek & Worden, 1945; Zheng, Yoo, Lee, Cho, Kim, & Kim, 2005). Also, lauric acid esterified with the monohydric alcohols, cholesterol showed no antibacterial activity (J. J. Kabara, Swieczkowski,

Conley, & Truant, 1972). From the above results, the hydrophilicity of lauric acid esters due to the non-fatty acid moiety was assumed to be important in antibacterial action.

Also, the prime target of free fatty acids is the cell membrane, where free fatty acids disrupt the electron transport chain and oxidative phosphorylation (Desbois & Smith, 2010). And the prior research investigated the antibacterial mechanism of erythorbyl laurate which acted on the bacterial cell membrane. Therefore, it could be proposed that the target of lauric acid esters would be the cell membrane, and only the esters which have certain hydrophilicity enable themselves to insert into bacterial cell membrane would have inhibitory or bactericidal effect.

To verify this suggestion, six lauric acid esters with different non-fatty acid moieties were selected; sesamol laurate, monolaurin, sucrose monolaurate, methyl laurate, and isoamyl laurate (Table 5), and the effect of hydrophilicity to the antibacterial activities was investigated.

Table 5. The structure of lauric acid esters with different hydrophilicity from their non-fatty acid moieties

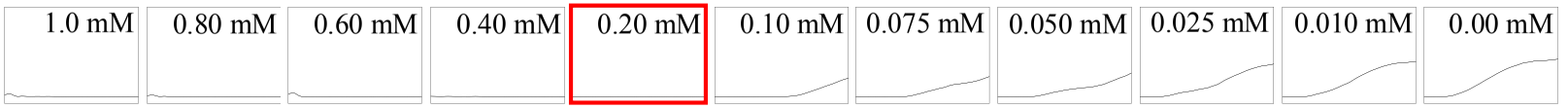
Compounds	Structures
Monolaurin	
Sucrose monolaurate	
Erythorbyl laurate	
Sesamol laurate	
Methyl laurate	
Isoamyl laurate	

3.5. Antibacterial activities of lauric acid esters

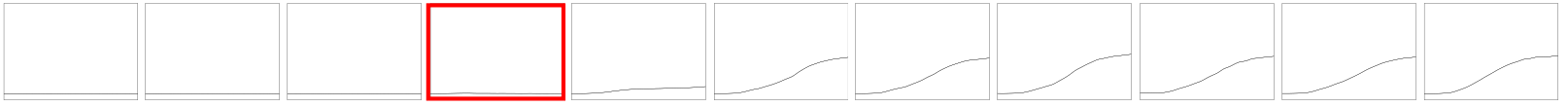
3.5.1. Minimum inhibitory concentration (MIC)

MIC of six lauric acid esters were determined for the two Gram positive bacterial strains and two Gram negative bacterial strains which is shown in Fig. 4. Monolaurin, sucrose monolaurate, and erythorbyl laurate showed inhibitory effect against Gram positive bacteria; *S. aureus* ATCC 49444 and *L. monocytogenes* ATCC 7644, while sesamol laurate, methyl laurate, and isoamyl laurate did not show any bacteriostatic or bactericidal effect. Meanwhile, all the tested lauric acid esters did not affect the growth of Gram negative bacteria.

(A) Monolaurin

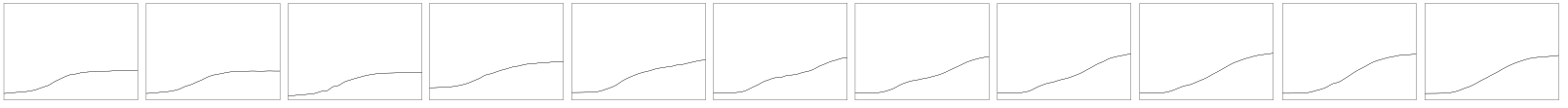


(A) Sucrose monolaurate

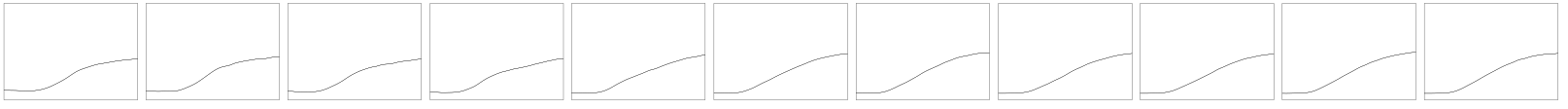


(A) Erythorbyl laurate

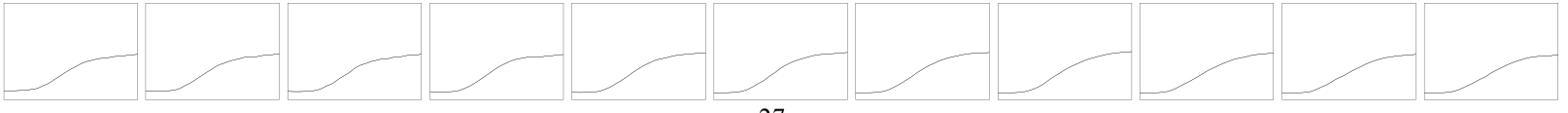
(A) Sesamol laurate



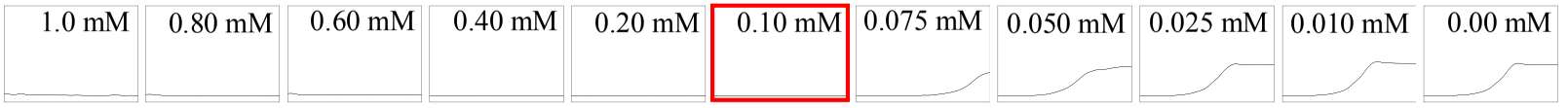
(A) Methyl laurate



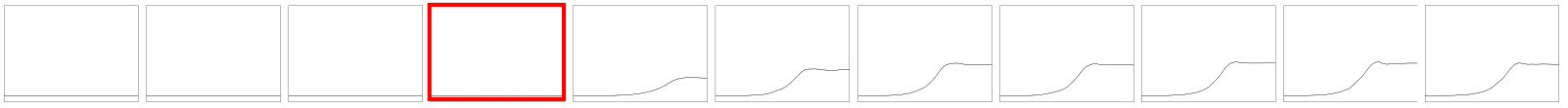
(A) Isoamyl laurate



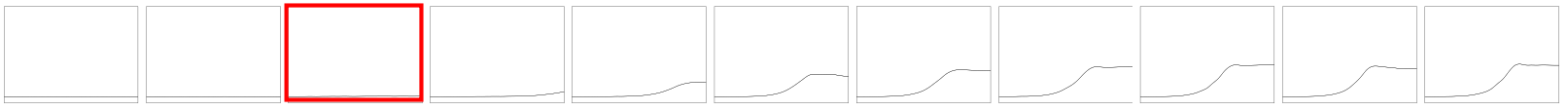
(B) Monolaurin



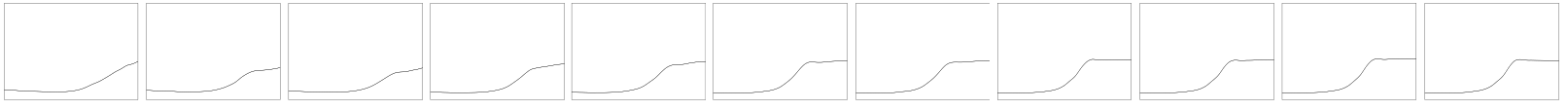
(B) Sucrose monolaurate



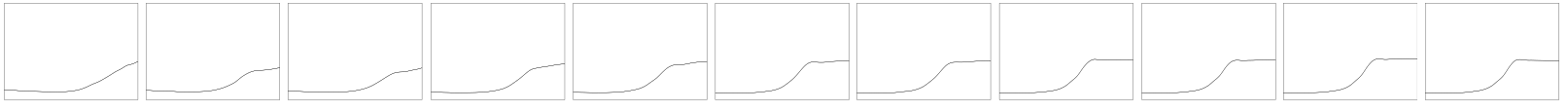
(B) Erythorbyl laurate



(B) Sesamol laurate

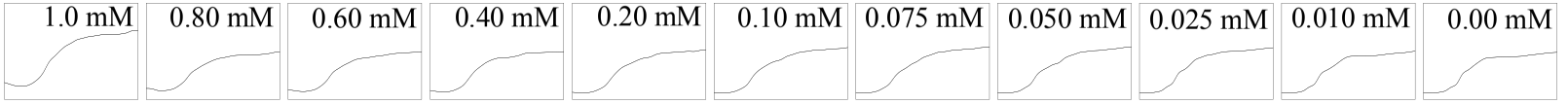


(B) Methyl laurate



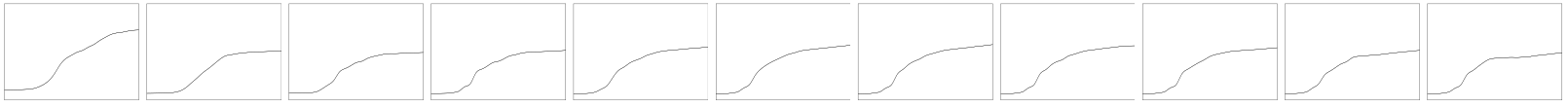
(B) Isoamyl laurate

(C) Monolaurin

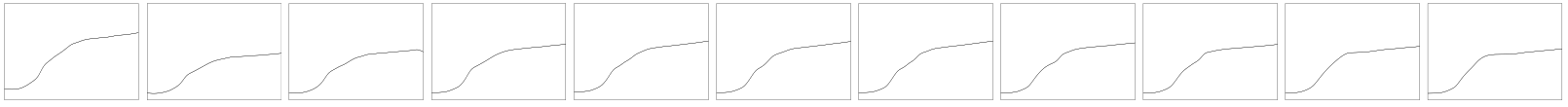


(C) Sucrose monolaurate

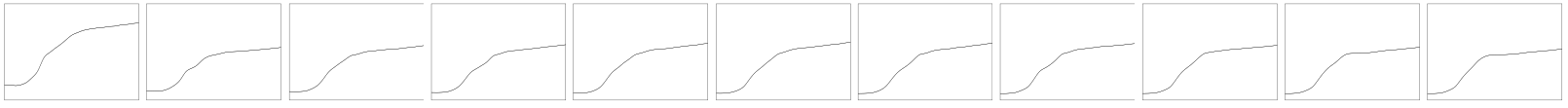
(C) Erythorbyl laurate



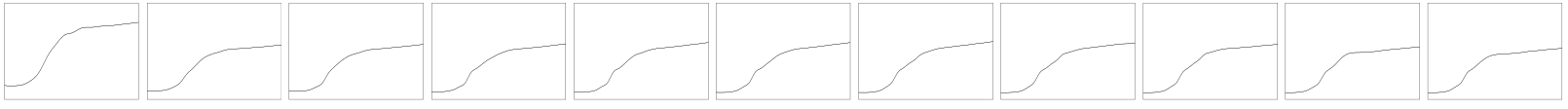
(C) Sesamol laurate



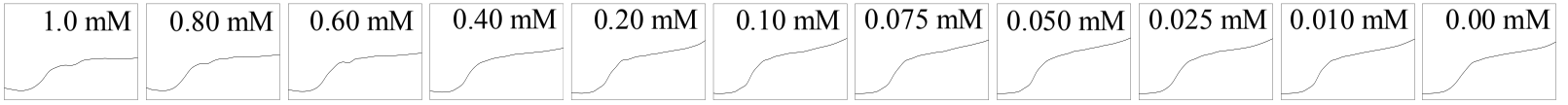
(C) Methyl laurate



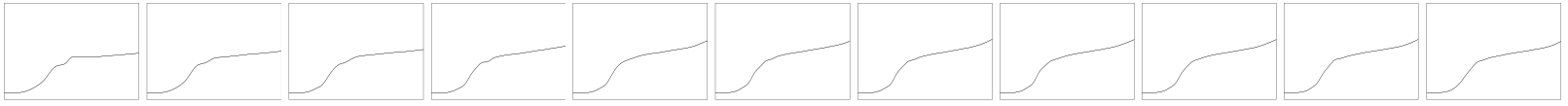
(C) Isoamyl laurate



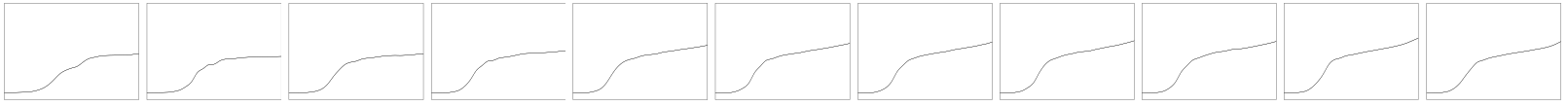
(D) Monolaurin



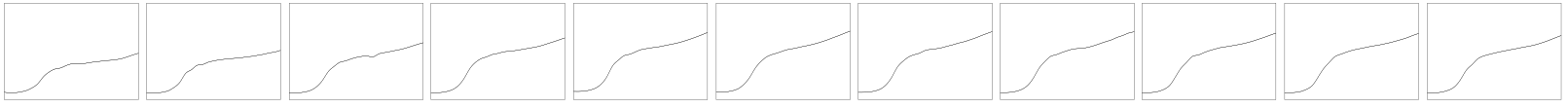
(D) Sucrose monolaurate



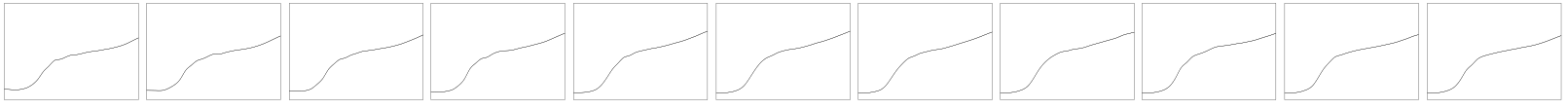
(D) Erythorbyl laurate



(D) Sesamol laurate



(D) Methyl laurate



(D) Isoamyl laurate

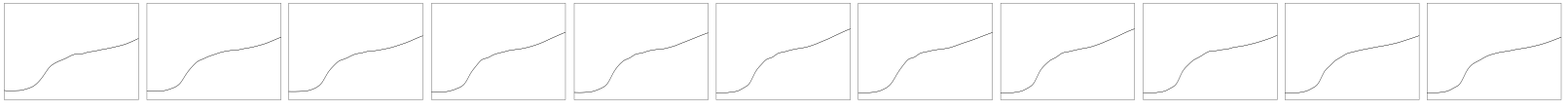


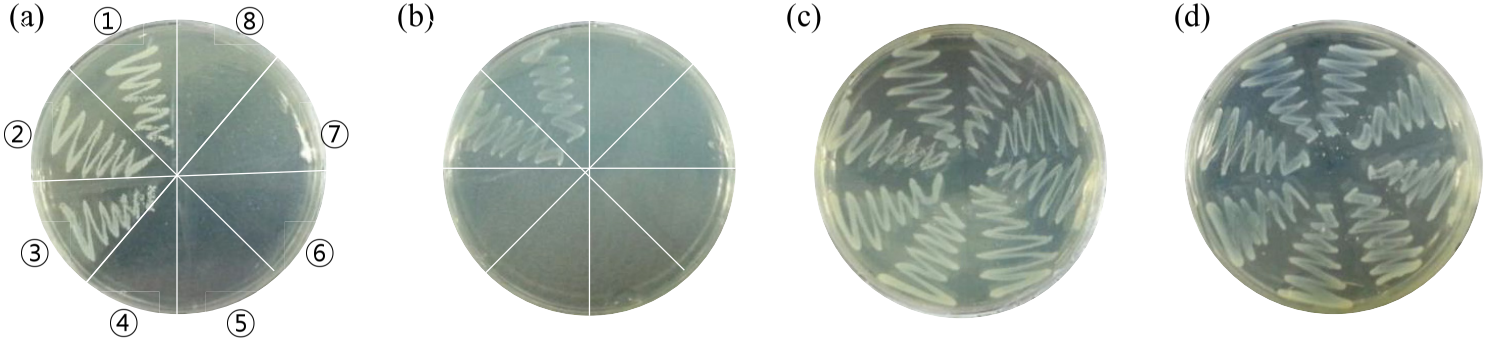
Fig. 4. Growth curves of *S. aureus* ATCC 49444 (A), *L. monocytogenes* ATCC 7644 (B), *E. coli* ATCC 43889 (C), and *Sal.* Typhimurium ATCC 43971 (D) treated with lauric acid esters at various concentrations, 1.0 mM, 0.80 mM, 0.60 mM, 0.40 mM, 0.20 mM, 0.10 mM, 0.075 mM, 0.050 mM, 0.025 mM, 0.010 mM, 0.00 mM from the left to the right graphs. MIC (□) of lauric acid esters were determined.

3.5.2. Minimum bactericidal concentration (MBC)

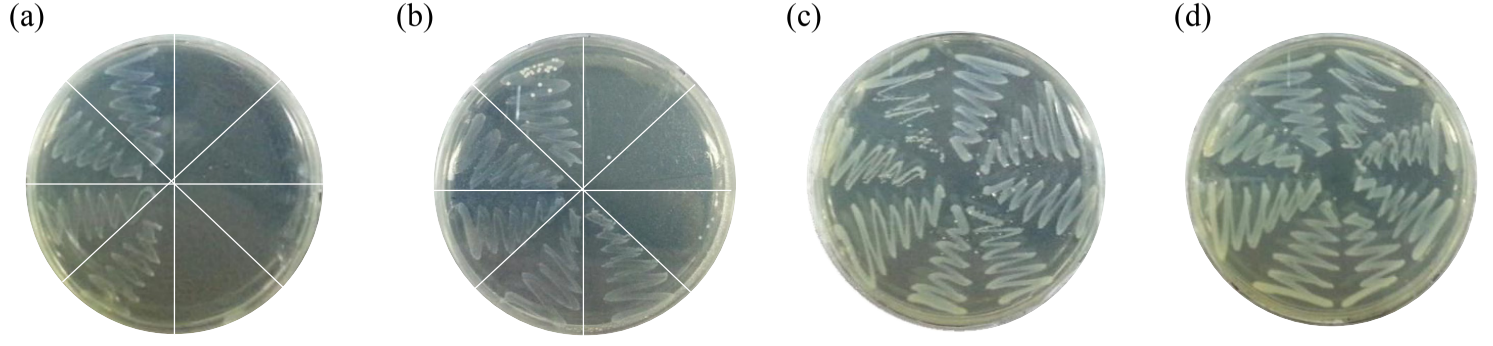
MBCs of six lauric acid esters were determined (Fig. 5). The MBC of monolaurin against *S. aureus* ATCC 49444 was 0.20 mM, while the MBCs of sucrose monolaurate and erythorbyl laurate were the same of 0.40 mM. Against *L. monocytogenes* ATCC 7644, monolaurin and sucrose monolaurate indicated bactericidal effect at 0.10 mM, and 0.80 mM, respectively, while erythorbyl laurate showed the MBC value of higher than 1.0 mM.

For sesamol laurate, methyl laurate, and isoamyl laurate, there was no bactericidal effect as shown in MIC test.

(A)

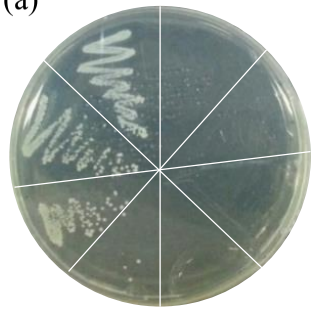


(B)

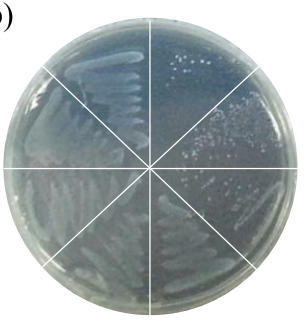


(C)

(a)



(b)

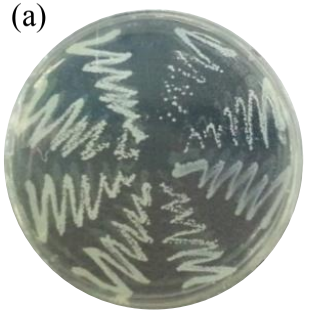


(c)

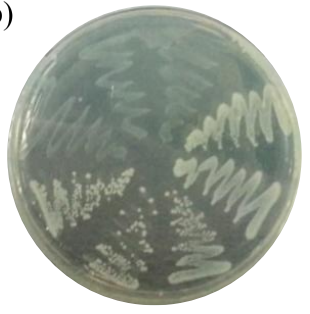
(d)

(D)

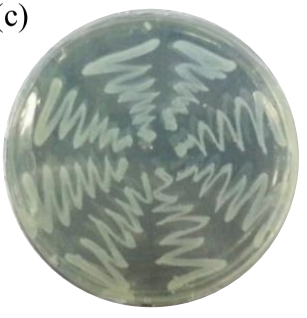
(a)



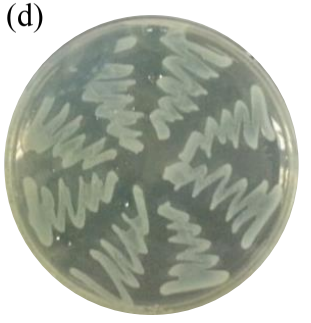
(b)



(c)



(d)



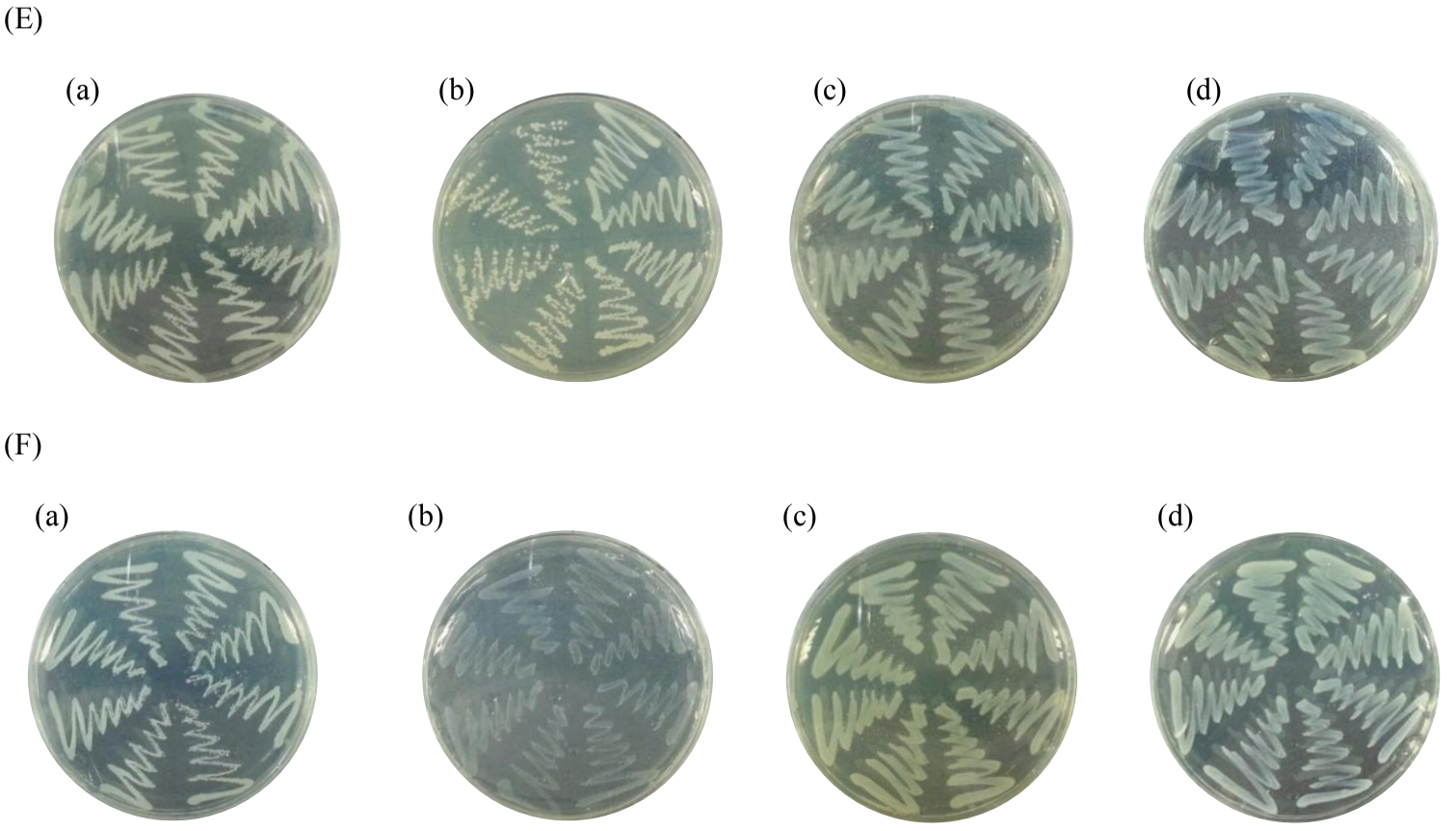


Fig. 5. (A) Monolaurin, (B) sucrose monolaurate, (C) erythorbyl laurate, (D) sesamol laurate, (E) methyl laurate, and

(F) isoamyl laurate were treated at various concentrations; ①:0.050 mM, ②:0.075 mM, ③:0.10 mM, ④:0.20 mM, ⑤:0.40 mM, ⑥:0.60 mM, ⑦:0.80 mM, and ⑧:1.0 mM against (a) *S. aureus* ATCC 49444, (b) *L. monocytogenes* ATCC 7644, (c) *E. coli* ATCC 43889, and (d) *Sal.* Typhimurium ATCC 43971.

3.6. Release of cellular constituents

After incubation with pre-determined concentrations of lauric acid esters, nucleic acid and its related compounds, such as pyrimidines and purines were measured at a wavelength of 260 nm. The presence of these materials in a suspension may be used as an indicator of damage to the cell membrane. Leakage was determined using nisin of 5 mg/mL as a control, a compound that is known to cause cellular membrane damage. Tested bacteria, *S. aureus* ATCC 49444 showed the increase of OD_{260} after 120 min of exposure (Fig. 6). The amount of UV absorbing substances increased as the time of exposure, when *S. aureus* suspensions were treated with nisin, monolaurin, and erythorbyl laurate at 4xMIC, and treated with sucrose monolaurate at 1xMIC. And the effect of solvent, DMSO, HCl, ethanol was mimic, proving that the damage of cell membrane was caused by lauric acid esters.

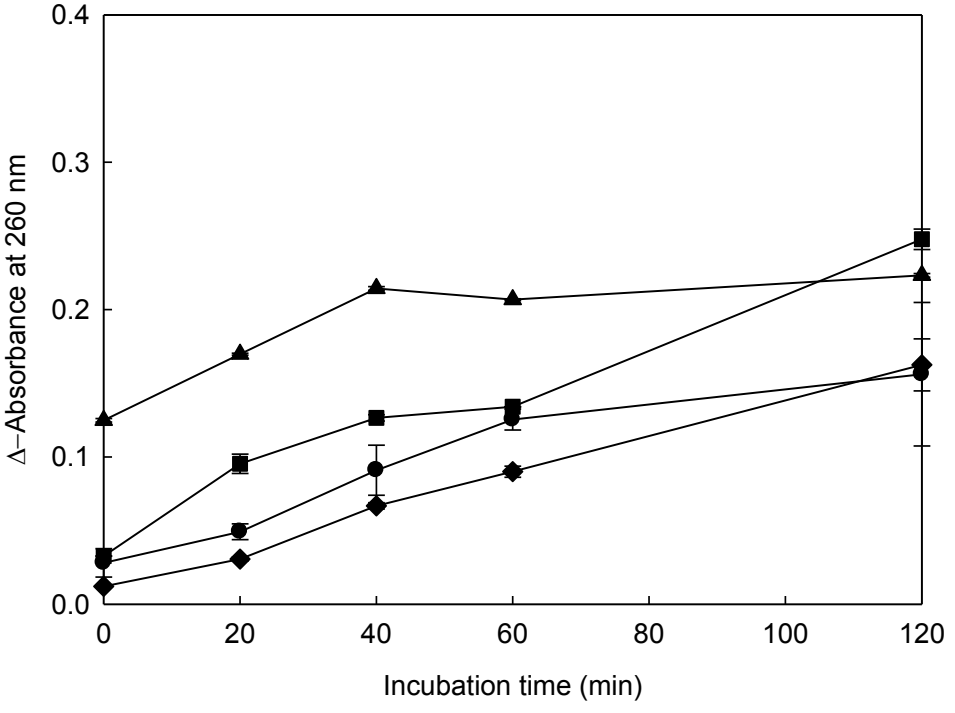


Fig. 6. Effects of monolaurin, sucrose monolaurate, and erythorbyl laurate on membrane integrity in *S. aureus* ATCC 49444 measured by UV absorbing components at 260 nm; ●: monolaurin of 4xMIC, ▲: sucrose monolaurate of 1xMIC, ■: erythorbyl laurate of 4xMIC, ◆: nisin of 5 mg/mL.

3.7. Fluorescence microscopy

To further verify the cellular membrane rupture, fluorescence microscopy was performed with Live/Dead viability kit. In fluorescence micrographs of stained bacteria suspension without lauric acid esters treatment, most cells showed only green light stained with SYTO 9 (Fig. 7A). However, samples treated with 2xMIC of monolaurin, sucrose monolaurate, and erythorbyl laurate fluoresced both green and red (Fig. 7B, 7C, and 7D). Ant it indicated that tested lauric acid esters caused the cellular membrane rupture.

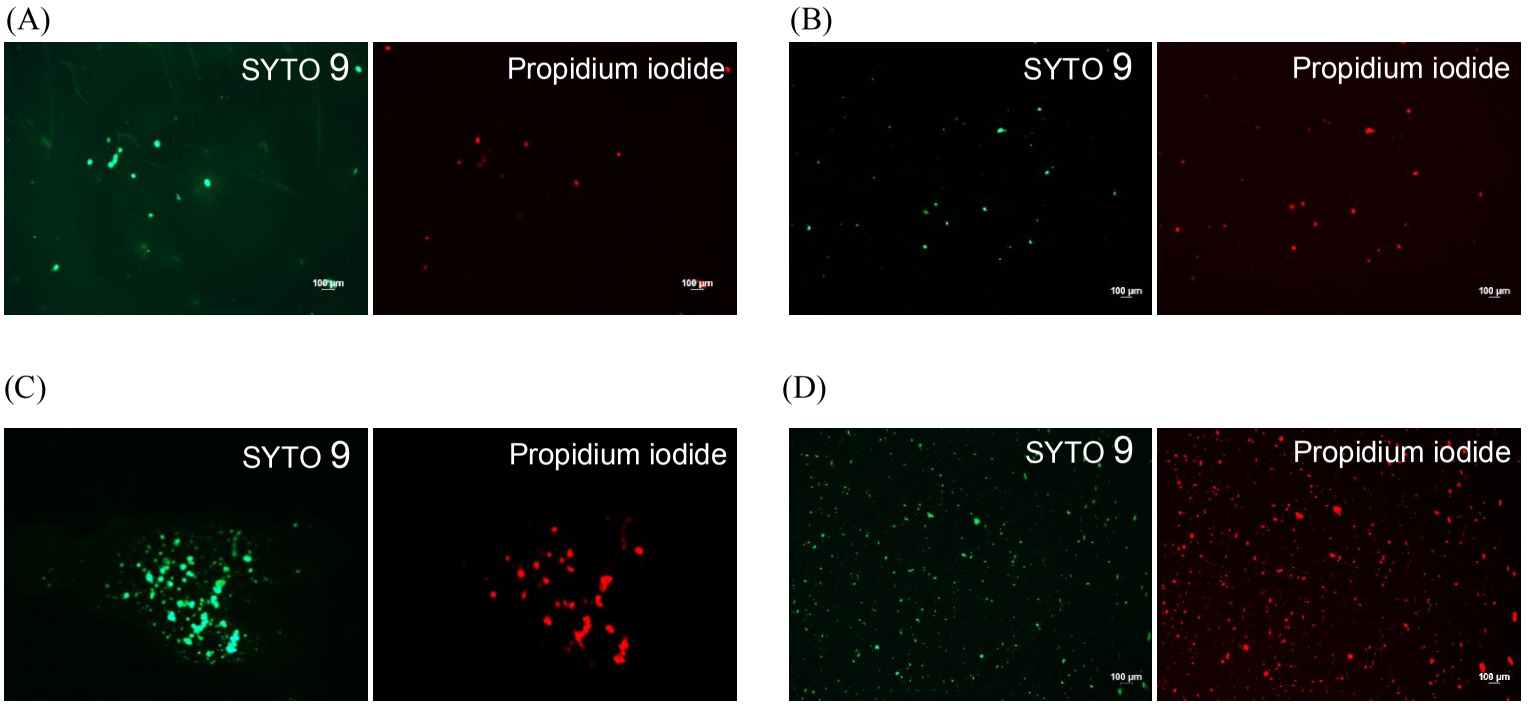


Fig. 7. SYTO 9 and propidium iodide fluorescence images of *S. aureus* ATCC 49444 without treatment of lauric acid esters (A), or with treatment of 2xMIC monolaurin (B), sucrose monolaurate (C), and erythorbyl laurate (D). SYTO 9 stained both intact and damaged cells, and propidium iodide stained only cells with compromised membranes (red).

3.8. Membrane lipid composition

Membrane fatty acid composition were investigated by using GC analysis. Table 5 shows the profiles of cells treated with monolaurin, sucrose monolaurate, and erythorbyl laurate. For all tested esters, there were changes in the composition of fatty acids in membrane.

S. aureus grown in the presence of monolaurin resulted in decrease in the proportion of 16:0 fatty acid with increase in 22:0 and 24:0 fatty acids. This result is correspond to a study describing the effects of monolaurin which is known to produce highly ordered membrane, and disrupt membrane function by affecting signal transduction due to blockage of promoters, uncoupling of energy systems, altered respiration, and altered amino acid uptake (Tokarskyy & Marshall, 2008). In contrast, sucrose monolaurate caused an increase in the proportion of 14:0 and 16:0 fatty acids with a decrease in 18:0, 20:0, 22:0, and 24:0 fatty acids. Similar result was indicated by Iwami, Schachtele, and Yamada (1995) that sugar esters reorganize the cellular membrane altering its permeability, which causes a loss of important metabolites. Erythorbyl laurate increased fatty acids of short chain length. The increase of short chain fatty acids and decrease of long chain fatty acids, was also reported by Ingram, Ley, and Hoffmann (1978) who indicated that a drug, pentobarbital caused shift of long chain fatty acids to the short chain fatty acids resulting increase of membrane fluidity.

When growth temperature alters, bacterial membrane preserve its fluidity by changing the ratio of unsaturated- to saturated-fatty acids, acyl chain length, cis/trans-unsaturation ratio as adaptation mechanism, because membrane fluidity is critical to live and growth (Russell, 2002). Decrease of long acyl chains and increase of short acyl chains weaken the van der Waals forces between fatty acids followed by more fluidic membrane. The increased membrane fluidity also occurs when unsaturated free fatty acids insert into membrane followed by the leakage of cellular constituents and membrane instability causing cell lysis (Shin, Bajpai, Kim, & Kang, 2007). Conversely, saturated free fatty acids reduce membrane fluidity and restrict movement of electron transport carriers, which can cause growth inhibition or even death (Sheu & Freese, 1972).

Table 6. Changes in fatty acid profiles of *S. aureus* membranes treated with lauric acid esters

FAME	Control	Monolaurin	Sucrose monolaurate	Erythorbyl laurate
C12:0	1.28[a ab]	0.88 [a]	16.47 [c]	1.85 [b]
C14:0	1.45 [a]	1.52 [a]	5.77 [b]	2.57 [c]
C16:0	13.45 [a]	8.94 [b]	14.76 [c]	13.37 [a]
C17:0	0.31 [a]	ND[b b]	ND [b]	0.25 [c]
C18:0	27.02 [a]	14.94 [b]	20.23 [c]	28.46 [a]
C20:0	53.52 [a]	65.95 [b]	40.45 [c]	50.15 [a]
C20:1cis	0.27 [a]	0.28 [a]	0.15 [b]	0.39 [c]
C20:3cis	0.38 [a]	0.35 [ab]	0.28 [c]	0.32 [b]
C22:0	2.17 [a]	6.67 [b]	1.79 [c]	2.08 [ac]
C22:1cis	0.15 [a]	0.14 [a]	0.10 [b]	0.11 [b]
C24:0	ND [a]	0.33 [b]	ND [a]	0.44 [c]
Total	100	100	100	100

[a] Percentage values, means of triplicate determinations. [b] Not detected.

3.9. Octanol-water partition coefficient (log *P*)

To identify the hydrophilicity which enable the lauric acid esters to incorporate into the membrane, octanol-water partition coefficient was estimated by atom/fragment contribution method of Meylan and Howard (Meylan & Howard, 1995).

The octanol-water partition coefficient is a physical property used extensively to describe a chemical's lipophilic or hydrophobic properties. It is the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two phase system at equilibrium (Meylan & Howard, 1995).

Many studies have shown that log *P* is useful for correlating a drug chemical's transport processes, its interactions with receptor molecules, and its observed changes with structure with various biological, biochemical, or toxic effects (Sabljic, Guesten, Hermens, & Opperhuizen, 1993). Since experimental measurement can be difficult, time-consuming, and expensive, it is common to estimate log *P*.

Calculated log *P* by the above method, monolaurin, sucrose laurate, and erythorbyl laurate which indicated antibacterial effect on Gram positive bacteria, showed lower log *P* values than the others (Table 7). This result suggested that lauric acid esters with antibacterial activity are more hydrophilic than the esters without antibacterial effect.

3.10. Hydrophilic-lipophilic balance (HLB) value

Davies method was used based on the hydrophilic, lipophilic group numbers assigned to various structural elements. Following the method, HLB value of monolaurin, sucrose monolaurate, and erythorbyl laurate was 7.025, 16.09, and 15.25, respectively. On the other hand, the HLB value of sesamol laurate, methyl laurate, and isoamyl laurate was 4.835, 3.700, and 1.800, respectively (Table 7). This result indicated that antibacterial active lauric acid esters have higher HLB values which applicable to detergent or solubilizer. Monolaurin, sucrose monolaurate, and erythorbyl laurate could solubilize the membrane by the detergent effect, hence account for the loss of vital components of membrane, leakage of internal contents, finally causing the growth inhibition or cell lysis. And, also the higher HLB values indicate more hydrophilic properties of those esters, which correspond to the result of log *P* calculations.

Table 7. Estimation of log *P* by the atom/fragment contribution method and HLB value by Davies method

Compound	Log *P*	HLB
Monolaurin	3.670	7.025
Sucrose monolaurate	-4.122	16.09
Erythorbyl laurate	-0.6858	15.25
Sesamol laurate	5.717	4.835
Methyl laurate	5.284	3.700
Isoamyl laurate	7.175	1.800

4. Conclusion

Investigation of membrane disruption ability and hydrophilicity of monolaurin, sucrose monolaurate, erythorbyl laurate, sesamol laurate, methyl laurate, and isoamyl laurate was conducted. Among the lauric acid estsers, monolaurin, sucrose monolaurate, and erythorbyl laurate which are more hydrophilic than the others, based on the log *P* and HLB value, had antibacterial activities against Gram positive bacteria by acting on the cell membrane, followed by the leakage of cell contents, cell rupture or changing the lipid composition.

From the results of this study, the esters of antibacterial activity possessing log *P* lower than 4 and HLB value higher than 7 were revealed. This fundamental information provides which non-fatty acid moiety is potent to retain the antibacterial activity of its esters during the synthesis of functional emulsifiers.

5. References

Bechert, T., Steinrücke, P., & Guggenbichler, J.-P. (2000). A new method for screening anti-infective biomaterials. *Nature Medicine, 6*(9), 1053-1056.

Brumfitt, W. (2002). Nisin, alone and combined with peptidoglycan-modulating antibiotics: activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. *Journal of Antimicrobial Chemotherapy, 50*(5), 731-734.

Coupland, J. N., & McClements, D. J. (1996). Lipid oxidation in food emulsions. *Trends in Food Science & Technology, 7*(3), 83-91.

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology, 85*(6), 1629-1642.

Diao, W.-R., Hu, Q.-P., Zhang, H., & Xu, J.-G. (2014). Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (*Foeniculum vulgare* Mill.). *Food Control, 35*(1), 109-116.

Ferrer, M., Soliveri, J., Plou, F. J., López-Cortés, N., Reyes-Duarte, D., Christensen, M., Copa-Patiño, J. L., & Ballesteros, A. (2005). Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their

antimicrobial properties. *Enzyme and Microbial Technology, 36*(4), 391-398.

Food, & Administration, D. (2001). Evaluation and definition of potentially hazardous foods. *Center for Food Safety and Applied Nutrition.*< *http://www. cfsan. fda. gov/~ comm/ift4-toc. html*.

Fu, X., Feng, F., & Huang, B. (2006). Physicochemical characterization and evaluation of a microemulsion system for antimicrobial activity of glycerol monolaurate. *International Journal of Pharmaceutics, 321*(1), 171-175.

Garcés, R., & Mancha, M. (1993). One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Analytical Biochemistry, 211*(1), 139-143.

Guo, X., Rong, Z., & Ying, X. (2006). Calculation of hydrophile-lipophile balance for polyethoxylated surfactants by group contribution method. *Journal of Colloid and Interface Science, 298*(1), 441-450.

Ingram, L., Ley, K., & Hoffmann, E. (1978). Drug-induced changes in lipid composition of *E. coli* and of mammalian cells in culture: Ethanol, pentobarbital, and chlorpromazine. *Life Sciences, 22*(6), 489-494.

Iwami, Y., Schachtele, C., & Yamada, T. (1995). Effect of sucrose monolaurate on acid production, levels of glycolytic intermediates, and enzyme activities of *Streptococcus mutans* NCTC 10449. *Journal of Dental*

Research, 74(9), 1613-1617.

Kabara, J., Vrable, R., & Jie, M. L. K. (1977). Antimicrobial lipids: natural and synthetic fatty acids and monoglycerides. *Lipids, 12*(9), 753-759.

Kabara, J. J., Swieczkowski, D. M., Conley, A. J., & Truant, J. P. (1972). Fatty acids and derivatives as antimicrobial agents. *Antimicrobial Agents and Chemotherapy, 2*(1), 23-28.

Karmee, S. K. (2008). Lipase catalyzed synthesis of ester-based surfactants from biomass derivatives. *Biofuels, Bioproducts and Biorefining, 2*(2), 144-154.

Kodicek, E., & Worden, A. (1945). The effect of unsaturated fatty acids on *Lactobacillus helveticus* and other Gram-positive micro-organisms. *Biochemical Journal, 39*(1), 78.

Lv, F., Liang, H., Yuan, Q., & Li, C. (2011). In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International, 44*(9), 3057-3064.

Magalhães, L., & Nitschke, M. (2013). Antimicrobial activity of rhamnolipids against *Listeria monocytogenes* and their synergistic interaction with nisin. *Food Control, 29*(1), 138-142.

Maijala, R., Lyytikäinen, O., Johansson, T., Autio, T., Aalto, T., Haavisto, L., & Honkanen-Buzalski, T. (2001). Exposure of *Listeria monocytogenes*

within an epidemic caused by butter in Finland. *International Journal of Food Microbiology, 70*(1), 97-109.

Mansour, M., & Millière, J.-B. (2001). An inhibitory synergistic effect of a nisin–monolaurin combination on *Bacillus* sp. vegetative cells in milk. *Food Microbiology, 18*(1), 87-94.

Mason, B., Williams, N., Salmon, R., Lewis, A., Price, J., Johnston, K., & Trott, R. (2001). Outbreak of *Salmonella indiana* associated with egg mayonnaise sandwiches at an acute NHS hospital. *Communicable Disease and Public Health/PHLS, 4*(4), 300-304.

McLay, J., Kennedy, M., O'Rourke, A.-L., Elliot, R., & Simmonds, R. (2002). Inhibition of bacterial foodborne pathogens by the lactoperoxidase system in combination with monolaurin. *International Journal of Food Microbiology, 73*(1), 1-9.

Meylan, W. M., & Howard, P. H. (1995). Atom/fragment contribution method for estimating octanol–water partition coefficients. *Journal of Pharmaceutical Sciences, 84*(1), 83-92.

Nobmann, P., Bourke, P., Dunne, J., & Henehan, G. (2010). In vitro antimicrobial activity and mechanism of action of novel carbohydrate fatty acid derivatives against *Staphylococcus aureus* and MRSA. *Journal of Applied Microbiology, 108*(6), 2152-2161.

Nobmann, P., Smith, A., Dunne, J., Henehan, G., & Bourke, P. (2009). The

antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against *Listeria* spp. and food spoilage microorganisms. *International Journal of Food Microbiology, 128*(3), 440-445.

Park, K.-M., Lee, D. E., Sung, H., Lee, J., & Chang, P.-S. (2011). Lipase-catalysed synthesis of erythorbyl laurate in acetonitrile. *Food Chemistry, 129*(1), 59-63.

Pridmore, A., Burch, D., & Lees, P. (2011). Determination of minimum inhibitory and minimum bactericidal concentrations of tiamulin against field isolates of *Actinobacillus pleuropneumoniae*. *Veterinary Microbiology, 151*(3-4), 409-412.

Rousseau, D. (2000). Fat crystals and emulsion stability—a review. *Food Research International, 33*(1), 3-14.

Russell, N. J. (2002). Bacterial membranes: the effects of chill storage and food processing. An overview. *International Journal of Food Microbiology, 79*(1), 27-34.

Sabljic, A., Guesten, H., Hermens, J., & Opperhuizen, A. (1993). Modeling octanol/water partition coefficients by molecular topology: chlorinated benzenes and biphenyls. *Environmental Science and Technology, 27*(7), 1394-1402.

Sheu, C. W., & Freese, E. (1972). Effects of fatty acids on growth and envelope

proteins of *Bacillus subtilis*. *Journal of Bacteriology, 111*(2), 516-524.

Shin, S., Bajpai, V., Kim, H., & Kang, S. (2007). Antibacterial activity of eicosapentaenoic acid (EPA) against foodborne and food spoilage microorganisms. *LWT-Food Science and Technology, 40*(9), 1515-1519.

Tokarskyy, O., & Marshall, D. L. (2008). Mechanism of synergistic inhibition of *Listeria monocytogenes* growth by lactic acid, monolaurin, and nisin. *Applied and Environmental Microbiology, 74*(23), 7126-7129.

Watanabe, T., Katayama, S., Matsubara, M., Honda, Y., & Kuwahara, M. (2000). Antibacterial carbohydrate monoesters suppressing cell growth of *Streptococcus mutans* in the presence of sucrose. *Current Microbiology, 41*(3), 210-213.

Watanabe, Y., Ishido, E., Fang, X., Adachi, S., & Matsuno, R. (2005). Oxidation kinetics of linoleic acid in the presence of saturated acyl l-ascorbate. *Journal of the American Oil Chemists' Society, 82*(5), 389-392.

Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols, 3*(2), 163-175.

Yan, Y., Bornscheuer, U. T., & Schmid, R. D. (1999). Lipase-catalyzed synthesis of vitamin C fatty acid esters. *Biotechnology Letters, 21*(12),

1051-1054.

Zhao, L., Zhang, H., Hao, T., & Li, S. (2015). In vitro antibacterial activities and mechanism of sugar fatty acid esters against five food-related bacteria. *Food Chemistry, 187*, 370-377.

Zheng, C. J., Yoo, J. S., Lee, T. G., Cho, H. Y., Kim, Y. H., & Kim, W. G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters, 579*(23), 5157-5162.

국문초록

Lauric acid ester는 유화능과 항균력 등이 뛰어나 다기능성 식품 첨가제로서의 활용 가능성이 높게 평가되어, 이를 합성하는 연구가 활발히 진행되어왔다. 특히 선행연구에서 합성한 erythorbyl laurate는 항산화력과 유화능뿐만 아니라 그람 양성균에 대한 항균력이 뛰어남이 밝혀졌다. 그러나 lauric acid와 sesame oil의 성분 중 상대적으로 높은 항산화능을 가진 sesamol을 합성한 sesamol laurate의 경우에는 그람 양성균과 그람 음성균에 대한 항균력이 나타나지 않았다. 따라서 lauric acid ester의 항균력에는 비지방산 잔기의 친수성 정도가 영향을 미칠 것이라고 가정하였고 이를 검증하고자 하였다.

서로 다른 비지방산 잔기를 가진 lauric acid ester 중, monolaurin과 sucrose monolaurate, erythorbyl laurate는 그람 양성균에 항균효과가 있었으나, sesamol laurate와 methyl laurate, isoamyl laurate는 그람 양성균의 생장에 저해효과가 없었다. 항균효과를 나타낸 물질들을 처리했을 때 그람 양성균의 내부 물질이 유출되었으며, 형광현미경을 통해 세포막의 파괴를 확인하였다.

또한 GC 분석을 통해 세포막을 구성하는 지방산 조성이 변화하였음을 확인하였다. 이로부터 항균성을 나타낸 lauric acid ester가 세포막에 끼어들어 지방산 조성을 변화시켰고 세포막을 파괴하였음을 알 수 있었다. 한편, 옥탄올-물 분배계수 (log *P*)와 친수성-친유성 밸런스 (HLB) 수치를 통해 볼 때, 항균성을 나타내는 물질들과 항균성이 없는 물질들 간의 친수성 정도에 차이가 있었으며 항균성을 나타내는 lauric acid ester의 친수성이 더 컸다. 따라서 세포막에 끼어들기 위한 적절한 정도의 친수성을 가지고 있는 lauric acid ester만이 항균성을 나타낸다는 것을 알 수 있었다.

본 연구는 항균력을 가지는 lauric acid ester를 합성하기 위한 비지방산 잔기의 특성을 제시함으로써, 효과적인 다기능성 식품첨가물의 합성에 지표가 될 수 있다는 것에 의의가 있다.