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A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

**Erythorbyl laurate as a multifunctional food additive:
interfacial characteristics and antioxidative activities
in O/W emulsion**

다기능성 유화제 erythorbyl laurate 의 계면특성과 유중수적형
에멀전에서의 항산화 특성

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석사학위논문

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이 논문을 석사학위 논문으로 제출함

2015 년 8 월

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Abstract

Interfacial properties and antioxidant activities of erythorbyl laurate in O/W emulsion were identified. Surface tension, contact angle, and foaming ability were measured to investigate interfacial properties. Surface tension was decreased with increasing concentrations up to critical micelle concentration (CMC, 0.12 mM) and surface tension at CMC was 34 mN/m. Contact angle showed similar pattern with surface tension. Foaming ability of erythorbyl laurate, Tween 20, and Triton X-100 solution was compared. Although no significant difference was observed in foaming capacity, foam produced by erythorbyl laurate had the highest half-life time among them. Based on the hydrophilic-lipophilic balance of erythorbyl laurate (9.8), soybean O/W emulsion (5:95, w/w) including 0.2%(w/w) of erythorbyl laurate was prepared. And the mean diameter ($d_{3,2}$) of erythorbyl laurate emulsion was 0.75 μm and spherical shape was observed by transmission electron microscopy. DPPH and ABTS assay were measured to observe its antioxidative activity and the EC_{50} of erythorbyl laurate was 331.43, and 425.95 μM , respectively. These results indicated that free-radical scavenging activity of erythorbyl laurate was retained after esterification. Antioxidative

activity of erythorbyl laurate in O/W emulsion was also investigated after thermal oxidation (60°C) and 100 µM of riboflavin sensitized photooxidation. Antioxidant activity of erythorbyl laurate was compared with various emulsions stabilized with SDS, Tween 20, non-treated emulsifier for thermal oxidation. Erythorbyl laurate emulsion effectively inhibited lipid oxidation than those of other emulsions during storage under 11 days. Also, erythorbyl laurate emulsion had the most effective antioxidative activity among other emulsions in photooxidation during storage under 3,340 lux fluorescent light for 12 h. Moreover, emulsion stabilized by erythorbyl laurate presence at oil-water interface had higher antioxidative activity than Tween 20 emulsion with erythorbic acid. These results indicated that erythorbyl laurate can be used as a promising multi-functional emulsifier having an antioxidative activity.

***Keywords:* erythorbyl laurate; interfacial characteristics; antioxidative activity; thermal oxidation; photooxidation; multifunctional food additive**

***Student Number:* 2013-21179**

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1. Introduction

Emulsion is defined as two immiscible liquids such as oil and water, with being small droplets in the dispersed liquid (Friberg, Larsson, & Sjoblom, 2003). There are many emulsifier-stabilized emulsion foods such as cream, dressing, mayonnaise, margarine, and butter (Mancuso, McClements, & Decker, 1999). Amphiphilic proteins (i.e., casein, soy and egg), phospholipids (i.e., egg or soy lecithin), and small-molecule surfactants (i.e., spans, tweens or fatty acids) are commonly used emulsifiers in food industry (Coupland & McClements, 1996).

Lipid oxidation (Shahidi & Zhong, 2005) and contamination of food pathogen (Maijala, Lyytikäinen, Johansson, Autio, Aalto, Haavisto, et al., 2001; Mason, Williams, Salmon, Lewis, Price, Johnston, et al., 2001) are major concerns that affect safety and quality of emulsion based foods. So, there are many processes to minimize deterioration of food. Storage temperature, water activity, and heat treatment are commonly used methods to decrease contamination of food pathogen. Using preservative is one of the popular preservation methods for reduction of microbial contamination

(Food & Administration, 2001). In addition, preventing lipid oxidation in emulsion based food is important because lipid oxidation causes not only producing rancid odors but also decreasing nutritional quality and shelf life (Frankel, 1993; Kellerby, Gu, McClements, & Decker, 2006). Interfacial area, droplet charge of emulsion, trace metals in oil, and types of antioxidants are factors that influence lipid oxidation (Waraho, McClements, & Decker, 2011). Among them, using antioxidant is one of the common ways to retard the rate of lipid oxidation (Ak & Gülçin, 2008)

Antioxidant, preservative, and emulsifier should be added in food emulsion such as salad dressing and mayonnaise to minimize deterioration of food (Peschel, Sánchez-Rabaneda, Diekmann, Plescher, Gartzía, Jiménez, et al., 2006). To control these factors at the same time, multi-functional food additive is required. So, erythorbyl laurate was synthesized by lipase-catalyzed esterification between erythorbic acid and lauric acid (Park, Sung, Lee, & Chang, 2011). Erythorbic acid, a stereoisomer of L-ascorbic acid, is a food antioxidant (Swern, Stirton, Turer, & Wells, 1943). Lauric acid is a fatty acid having a strong antimicrobial activity (Nakatsuji, Kao, Fang, Zouboulis,

Zhang, Gallo, et al., 2009). Erythorbyl laurate which is amphiphilic material expected to have antioxidative and antimicrobial activity. Molecular weight of erythorbyl laurate was 358.44 g/mol and continuous process was also optimized (Lee, Park, Choi, Shim, & Chang, 2013; Park, Choi, & Chang, 2012). Moreover, antimicrobial activity was investigated. From the recent study, erythorbyl laurate can control gram positive microorganisms as destroying cell membrane and cell wall.

Therefore, the objective of this study is to investigate interfacial properties and antioxidative activity of erythorbyl laurate as a multi-functional food additive. First, interfacial properties of erythorbyl laurate were investigated to estimate its possibility as an emulsifier. Free radical scavenging activity and antioxidative ability in O/W emulsion were also identified. Moreover, the effect of localization of antioxidant on lipid oxidation was observed in O/W emulsion between erythorbic acid and erythorbyl laurate.

2. Materials and methods

2.1. Materials

Ascorbic acid 6-palmitate, ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), barium chloride, iron(II) sulfate, potassium persulfate, cumene hydroperoxide, thiobarbituic acid, and 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich Co (St. Louix, MO, USA). Dimethyl sulfoxide (DMSO), methanol, 1-butanol, and hydrochloride were from Deajung (Gyeonggi-do, Korea). Triton X-100 and ammonium thiocyanate (Junsei, Tokyo, Japan) were used. Soy bean oil (Beksul, Seoul, Korea), trichloroacetic acid (Fisher scientific, Seoul, Korea) and Tween 20 (Ilshinwells, Seoul, Korea) were used. All chemicals were analytical grade.

2.2. Interfacial characteristics

Interfacial characteristics of erythorbyl laruate, ascorbyl palmitate, and Tween 20 were observed. Aqueous solution containing 1%(v/v) DMSO was

used because erythorbyl laurate was rarely dissolved in distilled water .

2.2.1. Surface tension and critical micelle concentration (CMC)

Surface tension of aqueous solutions was measured at 25°C with wilhelmy plate tensiometer using platinum plate (K100SF, Krüss, Germany). Critical micelle concentration (CMC) was determined from the surface tension with concentration plot (Weschayanwiwat, Scamehorn, & Reilly, 2005).

2.2.2. Contact angle

Contact angle was measured at 25°C with sessile drop method (DSA 100, Krüss, Germany). Average contact angle was determined from photographs by drawing tangent line between 10 µL of solution and hydrophobic solid surface, Teflon (LP23320, Tommyheco, Korea), for 30 s after dropping solution to solid surface.

2.3. Foaming ability

Foaming abilities of erythorbyl laurate, Triton X-100, ascorbyl palmitate

and Tween 20 were observed. All compounds were dissolved in 1%(v/v) DMSO solution. Fifty milliliter of aqueous solution (concentration of surfactant was 0.05% (w/v)) was poured into the 100 mL of graduated cylinder (Duran, Germany). Foam was produced by homogenization at 10,000 rpm, for 60 s (T-18 basic, IKA, Germany) at 25°C. Foam volume was recorded 30 s after homogenization (Foam capacity). Foam stability was evaluated by the time when the initial foam volume was collapsed by 50% of its original volume (half-life time).

2.4. Hydrophilic-lipophilic balance (HLB)

Emulsifier classification by HLB value had used since 1946. This method by Griffin is based on non-ionic surfactant molecule weight which combines hydrophilic and lipophilic group (Griffin, 1946). HLB is defined as

$$\text{HLB} = 20 \times \frac{M_h}{M}$$

and here, M_h is hydrophilic molecular mass and M is a whole molecular mass.

2.5. Preparation and characterization of emulsion

2.5.1. Determination of oil and water ratio and preparation of emulsion

Based on the HLB, O/W emulsions with various soybean oil concentrations (5.0%(w/w), 10.0%(w/w), and 15.0%(w/w)) were prepared with (0.2%, w/w) or without erythorbyl laurate in order to determine the most stable oil and water molar ratio. Erythorbyl laurate was dissolved in 2.0%(v/v) ethanol to the final concentration of 0.2%(w/w), followed by subsequent stirring for 1 h at ambient temperature to remove the trace alcohol (Trotta, Debernardi, & Caputo, 2003). Soy bean oil was added into aqueous solution. Coarse-emulsion was prepared by homogenization at 16,000 rpm for 60 s using Ultra Turrax (T-18 basic, IKA, Germany). The coarse-emulsion was then sonicated with Sonomasher (ULH-700S, Korea) at 4°C for 2 min at 210 W and 0.5 duty cycle.

Δ -Backscattering profile was measured at 25°C for 12 h using Turbiscan (Turbiscan LAB series, USA) and $\sum\Delta$ -backscattering was calculated with some modifications of the following study (Choi, Won, Park, & Chang, 2014). Briefly, $\sum\Delta$ -backscattering was measured at the sum of the Δ -backscattering up to 4 mm from the bottom of the sample after 12 h.

2.5.2. Size distribution of emulsion

Size distribution of the O/W emulsion (5% of soybean oil, w/w) with 0.2%(w/w) of erythorbyl laurate was measured using a static laser particle size analyzer (BT-9300ST, China). Sauter mean diameter was used which is defined as

$$d_{32} = \frac{\sum_i N_i d_i^3}{\sum_i N_i d_i^2}$$

and here, N_i is the number of droplet with the diameter d_i .

2.5.3. Transmission electron microscopy (TEM)

Morphology of emulsion stabilized with erythorbyl laurate was observed using TEM (80 kV, JEM1010, Japan). Emulsion sample (10 μ L) of emulsion was put onto a grid (Formvar coated carbon, 200 mesh) and 10 μ L of negative staining solution (2%(w/v) uranyl acetate) dropped onto the grid after 1 min. After washing with distilled water, the grid was dried completely at the ambient temperature before emulsion was observed by TEM.

2.6. Free radical scavenging activity

Free radical scavenging activity of erythorbyl laurate was determined by DPPH and ABTS assay. Ascorbic acid, ascorbyl palmitate, and erythorbic acid were used for comparing with erythorbyl laurate.

2.6.1. DPPH assay

Free radical scavenging activity of erythorbyl laurate was measured by DPPH method (Brand-Williams, Cuvelier, & Berset, 1995) with some modifications. To prepare stock solution, 0.1 mM of DPPH solution was dissolved in methanol. Sample solution (0.25 mL) was added in 3.75 mL of 0.1 mM DPPH solution and mixed for 10 s. After 30 min in the dark, absorbance was measured at 517 nm using spectrophotometer (Optizen pop, Mecasys, Korea). Half maximal effective concentration (EC_{50}) was calculated from the linear regression. DPPH radical scavenging activity was calculated by the following formula (Miliauskas, Venskutonis, & Van Beek,

2004):

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_{\text{sample (517nm)}}) / A_{\text{blank (517nm)}}] \times 100$$

2.6.2. ABTS assay

ABTS assay was followed Thaipong's method (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006) with some modifications. Stock solution, including 7.00 mM of ABTS solution and 2.45 mM of potassium persulfate solution, was stored in the dark condition for 12 h to produce proton radicals. This solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.05 at 734 nm. Sample solution (0.05 mL) dissolved in methanol was reacted with 1.90 mL of ABTS solution in a dark condition. After 6 min, absorbance was measured at 734 nm. EC_{50} was calculated from the linear regression. ABTS radical scavenging activity was calculated by the following formula (Khalil, Pepato, & Brunetti, 2008):

$$\text{ABTS radical scavenging activity (\%)} = [1 - (A_{\text{sample (734nm)}}) / A_{\text{blank (734nm)}}] \times 100$$

2.7. Lipid oxidation of emulsion

2.7.1. Lipid oxidation

Emulsions samples (5 mL) were stored in the dark condition at 60°C oven (DI-81, HYSC, Korea) for up to 11 days for thermal oxidation. To study the effect on the photosensitized oxidation, 100 µM of riboflavin was used. Emulsion samples (1 mL) were transferred into vial (10 mL). Samples were oxidized in at 25°C for 12 h under fluorescence light (3,420 lux).

2.7.2. Peroxide value

Lipid hydroperoxides were measured using ferric thiocyanate method (Mei, McClements, & Decker, 1999). Emulsion sample (20 µL) was mixed with 3 mL of methanol/1-butanol (2:1, v/v) and 30 µL of thiocyanate/Fe²⁺ solution. Fe²⁺/thiocyanate solution was prepared immediately before use by combining 0.8 mL of 3.940 M ammonium thiocyanate solution and 0.8 mL of fresh Fe²⁺ solution. Fresh Fe²⁺ solution was obtained from supernatant of 0.8 mL of 144 mM BaCl₂ in 400 mM HCl and 0.8 mL of 144 mM FeSO₄. After 20 min, absorbance of each sample was observed at 510 nm. Lipid

hydroperoxides were calibrated from cumene hydroperoxide.

2.7.3. Thiobarbituric acid-reactive substance (TBARs)

Malonaldehyde value was determined by thiobarbituric acid-reactive substance (TBARs) assay (Kiokias, Dimakou, & Oreopoulou, 2007; Mei, McClements, & Decker, 1999). Emulsion sample (20 μ L), 1.00 mL of distilled water, and 2.00 mL of TBA solution were mixed. TBA solution was prepared by mixing 15 g of trichloroacetic acid, 375 mg of thiobarbituric acid, 1.76 mL of 12 N HCl, and 82.90 mL of H₂O. Sample solution was boiled for 15 min, cooled room temperature for 10 min. Then samples were centrifuged at 2000xg (Micro17TR, Hanil, Korea) for 15 min. Absorbance was measured at 532 nm. Standard curve was prepared using 1,1,3,3-tetraethoxypropane.

2.8. Statistical analysis

All experiments were tested on triplicate. Mean values were analyzed

using Duncan's multiple range test. Significant difference level was determined at significant level of $p < 0.05$. All calculations were performed using the Statistical Analysis System (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Interfacial characterization of erythorbyl laurate

3.1.1. Surface characteristics of erythorbyl laurate compared to ascorbyl palmitate and Tween 20

Surface tension and contact angle (θ), important functions for surfactant, of erythorbyl laurate were investigated (Weschayanwiwat, Scamehorn, & Reilly, 2005). Surface tension was measured by Wilhelmy plate method and wettability was measured by contact angle of aqueous solution on hydrophobic surface, Teflon. Tween 20 and ascorbyl palmitate were determined as a comparative analysis. Ascorbyl palmitate was used because ascorbic acid is an isomer of erythorbic acid and has a similar molecular structure. Tween 20, emulsifier, was used due to same lipophilic tail (fatty acid) with erythorbyl laurate.

With increasing concentration of ascorbyl palmitate, Tween 20, and erythorbyl laurate, surface tension and contact angle decreased to a certain point called CMC (Fig. 1). CMC is defined as where its surface tension changes slightly with increasing concentration because surfactants are fully saturated air-solution interface (Reis, Moutinho, Matos, de Castro, Gameiro, & Lima, 2004). CMC of ascorbyl palmitate and Tween 20 from Fig. 1A was 1.10 and 0.06 mM, respectively, which was very similar with previous study (Graca, Bongaerts, Stokes, & Granick, 2007; Palma, Lo Nostro, Manzo, & Allemandi, 2002). Compared to surface tension of 1%(v/v) DMSO solution as a control (73 mN/m), surface tension of ascorbyl palmitate, Tween 20, and erythorbyl laurate decreased to 45 mN/m, 37 mN/m, and 34 mN/m until CMC. Ascorbyl palmitate had relatively less effective to reduce surface tension compared to erythorbyl laurate and Tween 20 which had similar surface activity. CMC of erythorbyl laurate was 0.12 mM which was in accordance with study that CMC range of non-ionic surfactant is from 10^{-3} to 10^{-5} mol/L (Lee, Kim, Lee, & Lim, 2011).

Contact angle against aqueous solutions of ascorbyl palmitate, Tween 20,

and erythorbyl laurate was shown in Fig. 1B. Contact angle sharply reduced near the CMC which was similar pattern in following research (Graca, Bongaerts, Stokes, & Granick, 2007). Contact angle of ascorbyl palmitate showed no drastic reduction regardless of concentration. Wettability of Tween 20 and erythorbyl laurate was relatively better than ascorbyl palmitate because these showed drastic reduction of contact angle with increasing concentration. Results of surface tension and contact angle indicated that erythorbyl laurate can be used as an emulsifier.

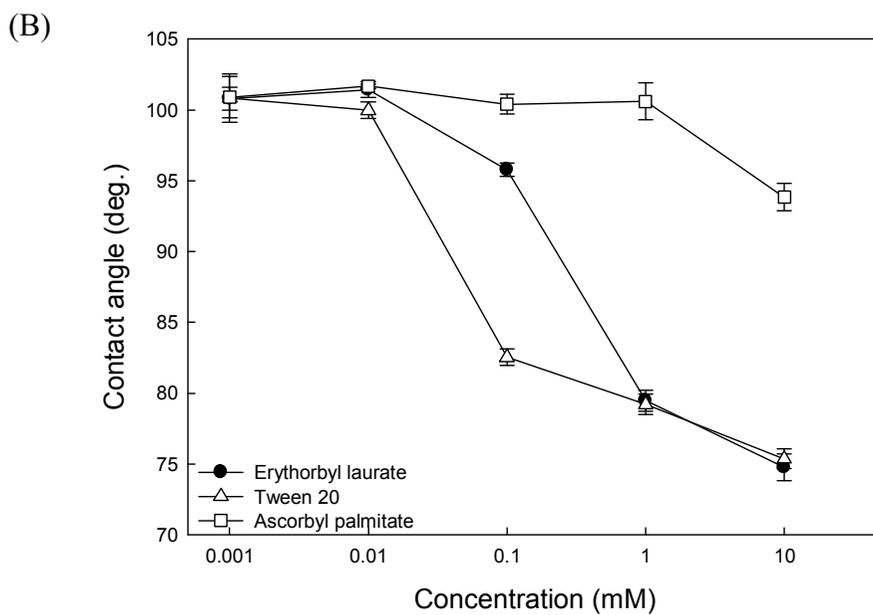
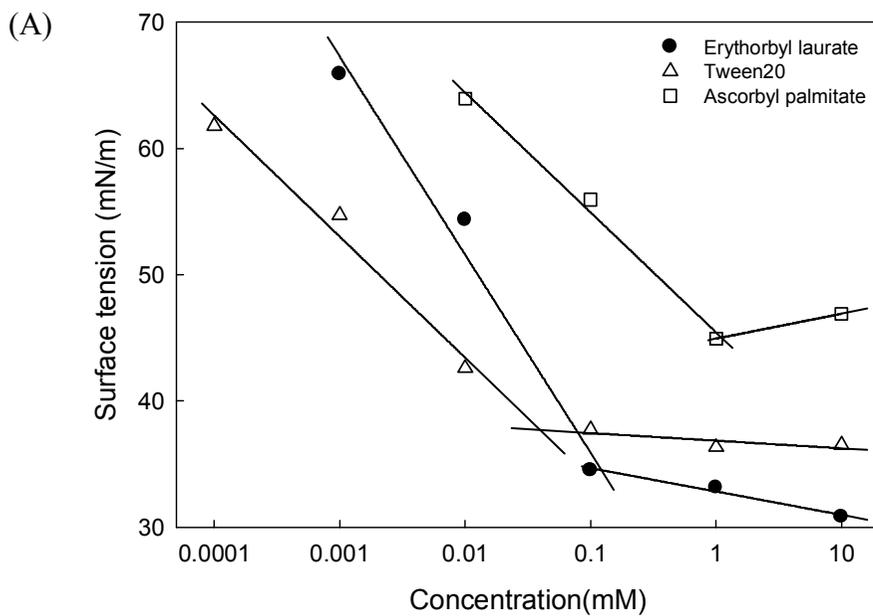


Fig. 1. Effect of concentration on (A) surface tension and (B) contact angle of erythorbyl laurate, Tween 20, and ascorbyl palmitate at 25°C.

3.1.2. Foaming ability of erythorbyl laurate compared with non-ionic surfactants

Foaming is an important properties for surfactant (Weschayanwiiwat, Scamehorn, & Reilly, 2005). Foaming capacity and foaming stability were determined by measurement of changes of the foam height after formation of foam. Foam stability was characterized by half-life time. Non-ionic surfactant, Triton X-100 and Tween 20 were used to compare with erythorbyl laurate which does not have ionic functional group. Ascorbyl palmitate was not shown because there was no foam. Concentration of materials (0.05%, w/v) was determined over range of CMC, because foam capacity is maximum at CMC and constant over concentration (Park & Kim, 1995). Figure 2 shows results of foaming capacity and foaming stability. The foam volume of Triton X-100, Tween 20, and erythorbyl laurate was 20.33^a, 18.67^a, and 18.00^a mL, respectively. This showed there was no significant difference

between erythorbyl laurate and other surfactants which are commonly used in industry.

The highest foam half-life time was observed for erythorbyl laurate, 24.33^a h. Triton X-100 and Tween 20 were 9.33^b and 8.00^b, respectively. Though these were similar foam capacity foam stability of erythorbyl laurate was better than other non-ionic surfactant. This result indicated that erythorbyl laurate can be used in foods such as ice cream as enhancing foam stability (Dickinson, 2010).

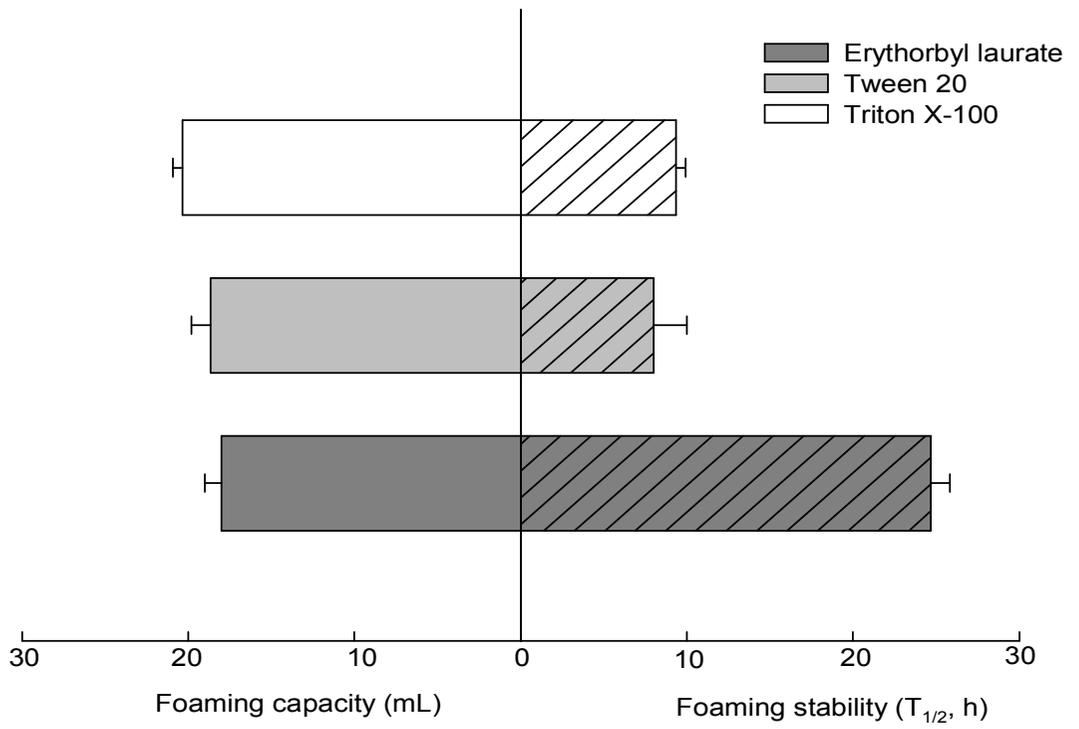


Fig. 2. Foaming ability of Triton X-100, Tween 20, and erythorbyl laurate. The concentration of each sample was 0.05%(w/v).

3.2. Erythorbyl laurate emulsion stability in various ratio of oil and water emulsion

Choosing a suitable emulsion system is required for using erythorbyl laurate as an emulsifier because it is an amphiphilic material having surface-active ability (Fig. 1). HLB is one of the most widely used indicators for emulsifier classification and selection (Pasquali, Taurozzi, & Bregni, 2008). Griffin's method was used based on non-ionic surfactant molecule weight combines hydrophilic and lipophilic group (Griffin, 1946). The molecular weight of erythorbyl laurate was 358.44 g/mol and HLB value was 9.8 which means appropriate to O/W emulsion (Griffin, 1946; Lee, Park, Choi, Shim, & Chang, 2013).

Various ratio of oil and water emulsion was prepared (5%, 10%, and 15%

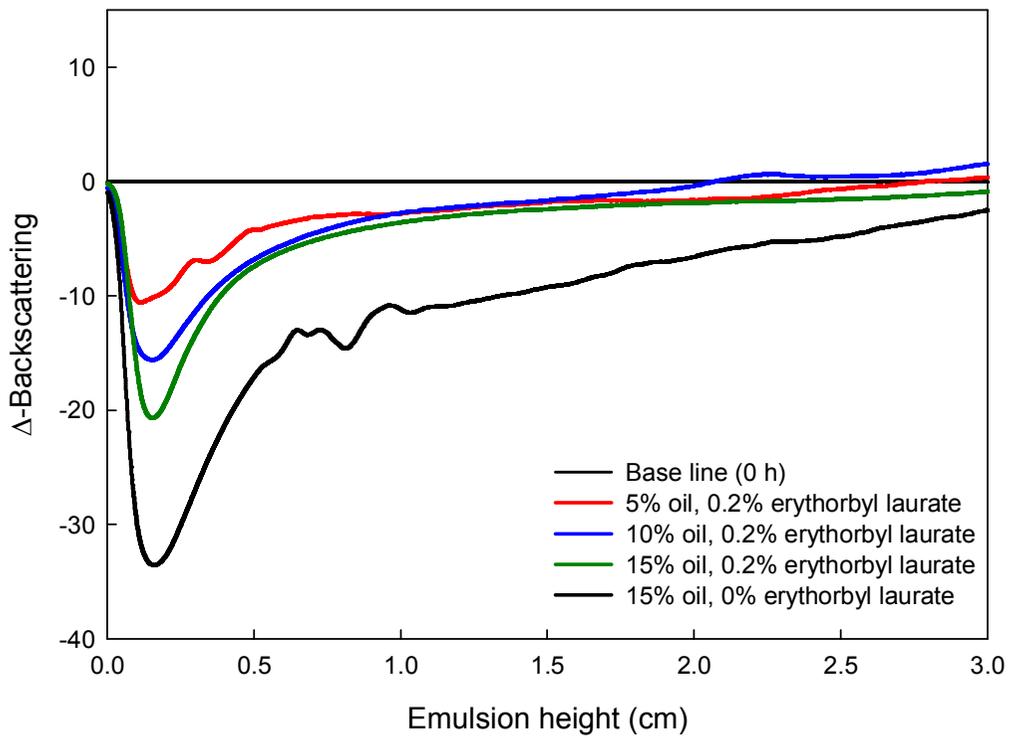
of oil, w/w) to estimate its stability using Δ -backscattering by Turbiscan. Monitoring the change of backscattering intensity is effective to detect destabilization phenomena in non-diluted emulsion (Lemarchand, Couvreur, Vauthier, Costantini, & Gref, 2003). The $\Sigma\Delta$ -backscattering at clarifier layer obtained from backscattering flux profile is a useful indicator to show emulsion stability (Choi, Won, Park, & Chang, 2014). Lower absolute $\Sigma\Delta$ -backscattering means more stable emulsion it is.

Figure 3A shows backscattering flux data profile in various oil and water ratio emulsion. At same ratio of oil and water emulsion, more rapid destabilization phenomenon were observed in emulsion without emulsifier than emulsion containing erythorbyl laurate. This indicated that erythorbyl laurate has emulsifying activity to emulsify oil and water. $\Sigma\Delta$ -backscattering at clarifier layer (bottom to 4 mm) of 5%, 10%, and 15%(w/w) of soybean oil water emulsion with erythorbyl laurate after 12 h, -807.55^a, -1139.15^b, and -1509.36^c, respectively. Stable emulsion model is required in thermal oxidation because emulsion should be incubated relatively high temperature and long time at 60°C for 11 days. Therefore, 5%(w/w) of

soybean oil in water emulsion with 0.2%(w/w) of erythorbyl laurate which was the most stable compared to other emulsion was determined as working concentration for antioxidant model emulsion.

Particle size distribution and TEM analysis of O/W emulsion (5:95, w/w) stabilized with 0.2%(w/w) of erythorbyl laurate were observed (Fig. 3B). Measuring particle size is important for optical property and rheology because droplet size has great impact on the emulsion stability (McClements, 2007; Pal, 1996). Monomodal distribution was presented and d_{32} value was determined as 0.75 ± 0.04 μm using sauter mean diameter. Spherical and monodispersed shape of emulsion was also shown in TEM analysis. The size distribution of the erythorbyl laurate emulsion is in the range of common food emulsion (Friberg, Larsson, & Sjoblom, 2003).

(A)



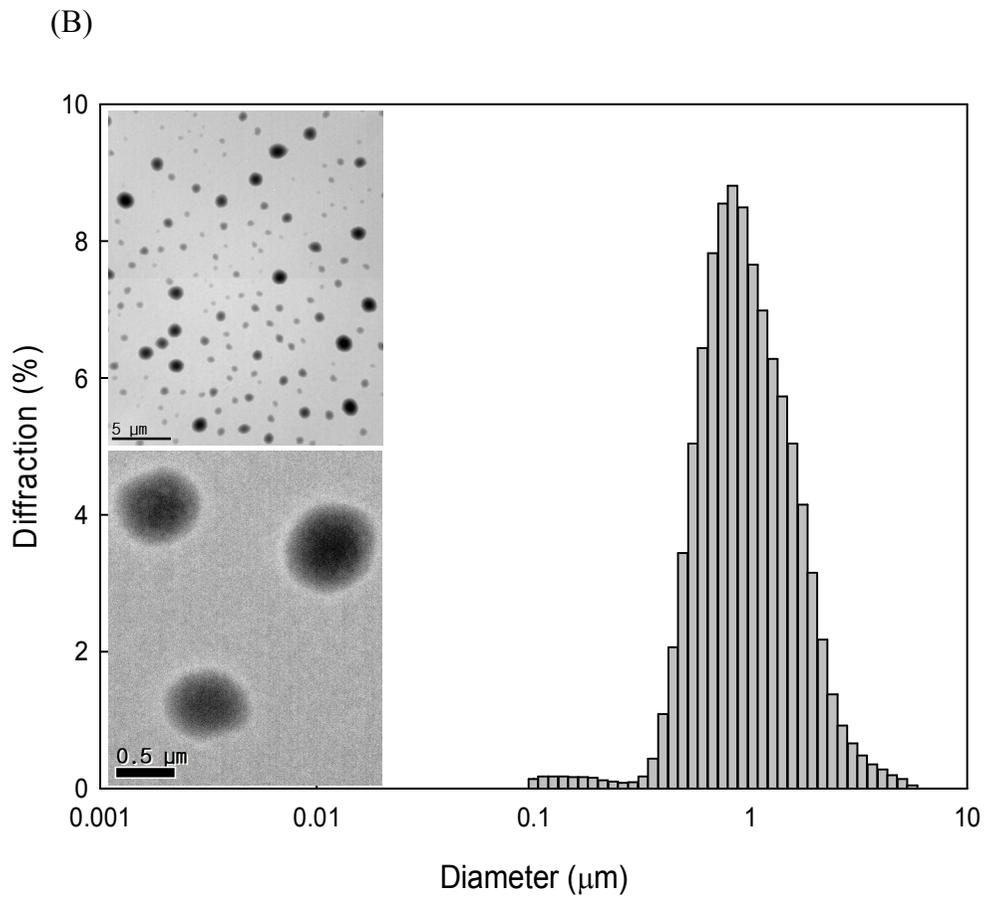


Fig. 3. Sum of Δ -backscattering data of various ratio of oil and water

emulsion (A) and particle size distribution (inserted TEM image) of O/W emulsion (5:95, w/w) stabilized with 0.2%(w/w) of erythorbyl laurate (B).

3.3. Antioxidant activity

3.3.1. Free radical scavenging activity (DPPH and ABTS assay)

Free radical scavenging activity of erythorbyl laurate was observed after esterification between erythorbic acid and lauric acid by immobilized enzyme (Novozyme 435). DPPH and ABTS assay are extensively used to measure antioxidative activity. DPPH assay is based on the reduction of DPPH solution of hydrogen donating ability of antioxidants because non-radical formed from DPPH-H by the reaction (Shon, Kim, & Sung, 2003). ABTS assay is the method that antioxidant reacts with radical cation and this assay is useful for lipid soluble antioxidant, and food compounds (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999). Erythorbic acid was used to compare its antioxidative activity with erythorbyl laurate.

Also, ascorbic acid and ascorbyl palmitate were used which were antioxidant in food industry. Figure 4 showed that DPPH and ABTS assay. Similar tendency was observed between ABTS and DPPH assay measurement that absorbance of samples reduced with increasing concentration of antioxidant.

The DPPH scavenging activity by various antioxidants was shown in Fig. 4A. The scavenging effect increased with increasing concentration of materials. DPPH radical scavenging activity of erythorbyl laurate was found to be 17.48, 36.34, and 74.83% at concentrations of 125, 250, and 500 μM , respectively. This result indicated erythorbyl laurate remained antioxidative activity after esterification. EC_{50} of ascorbic acid was 186.63 μM which has the highest free radical scavenger capacity compared to other antioxidants. The EC_{50} of ascorbyl palmitate, erythorbic acid, and erythorbyl laurate was 306.20, 288.13, and 331.42 μM , respectively.

Moreover, radical scavenging capacity was evaluated against ABTS (Fig. 4.B). Similar pattern was observed with DPPH assay. DPPH radical scavenging activity of erythorbyl laurate was found to be 18.84, 34.65, and 64.39% at concentrations of 125, 250, and 500 μM , respectively. EC_{50} of

ascorbic acid, ascorbyl palmitate, erythorbic acid, and erythorbyl laurate was 263.89, 378.83, 380.83, and 425.95 μM , respectively.

Hydrophilic compounds including ascorbic acid and erythorbic acid showed better radical scavenging activity than lipophilic compounds including ascorbyl palmitate and erythorbyl laurate. Although the EC_{50} value of erythorbyl laurate was higher than erythorbic acid, free radical scavenging activity of erythorbyl laurate was remained after esterification between erythorbic acid and lauric acid.

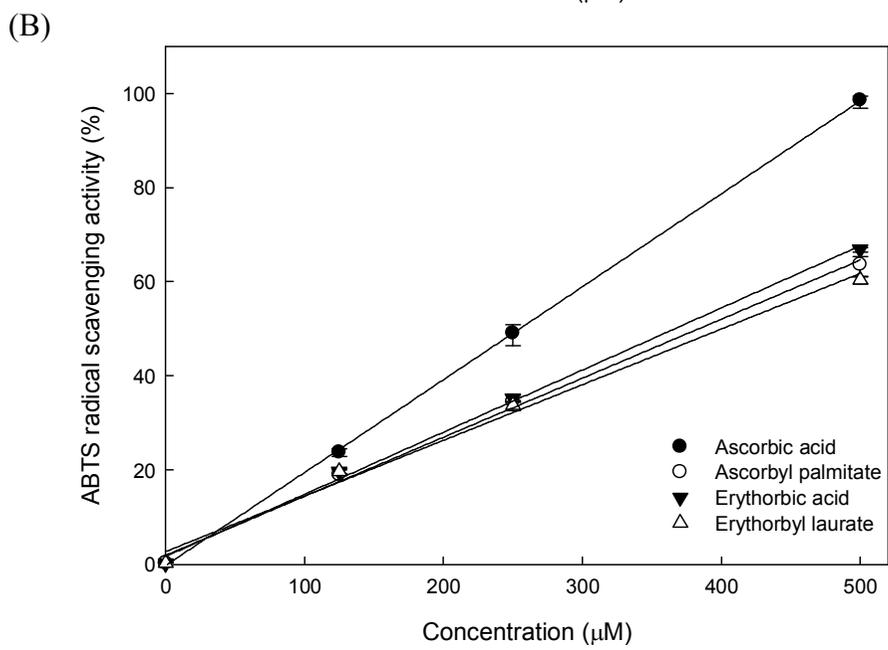
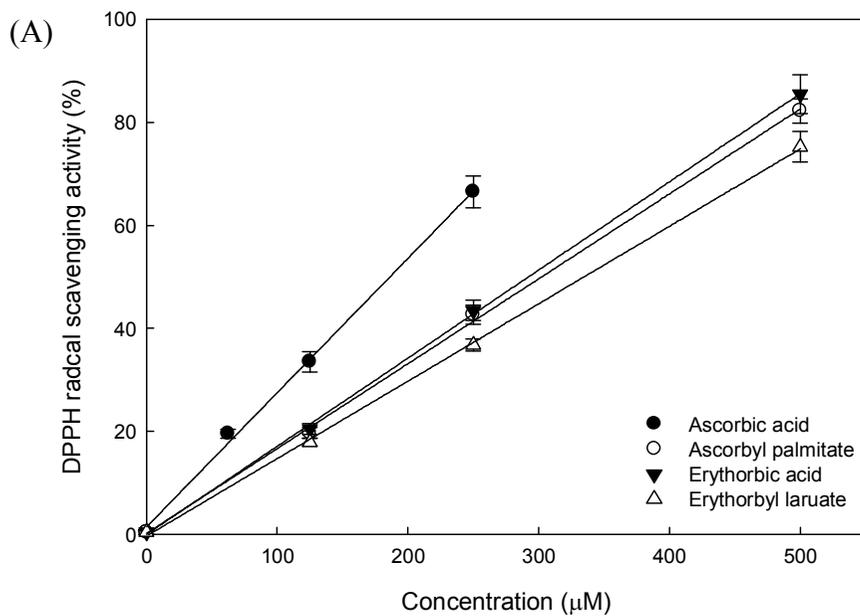


Fig. 4. DPPH radical scavenging activity (A) and ABTS radical scavenging activity (B) of ascorbic acid, ascorbyl palmitate, erythorbic acid, and erythorbyl laurate.

3.3.2. Effects of lipid oxidation on various emulsifiers in O/W emulsion

Oxidative stability of erythorbyl laurate was measured in O/W emulsion because it is expected as an emulsifier having antioxidative activity. Based on Fig. 3, O/W emulsion (5% of oil, w/w) with 0.2%(w/w) of emulsifier was prepared. Thermal oxidation and photooxidation were used to evaluate oxidative stability. For thermal oxidation, emulsion was incubated at 60°C for 11 days in order to accelerate oxidation rates and 60°C was chosen to minimize side reaction (Frankel, 1993). To accelerate photooxidation, riboflavin was chosen because this photosensitizer can generate free radicals by absorption of electron or hydrogen (Type 1 pathway) or form singlet oxygen from triplet oxygen transferring energy (Type 2 pathway) (Kim, Decker, & Lee, 2012; Min & Boff, 2002). Non-ionic surfactant, Tween 20 and anionic surfactant, SDS which are using in food industry were compared

with erythorbyl laurate. Peroxide value was measured by ferric thiocyanate method for primary products and malonaldehyde, one of the important secondary product of auto-oxidation was measured by TBARs (Shahidi & Zhong, 2005).

Figure 5 shows formation of hydroperoxide and TBARs in O/W emulsion stabilized by various emulsifiers for thermal and photooxidation. At first, thermal oxidation was observed (Fig. 5A). SDS-emulsion was the fastest on rate of lipid oxidation followed by Tween 20, without emulsifier. Lipid hydroperoxides of erythorbyl laurate emulsion remained at low level compared to emulsion with various emulsifier. Concentrations of lipid hydroperoxides in SDS, Tween 20, without emulsifier, and erythorbyl laurate were 26.84^d, 8.45^c, 2.19^b, and 0.66^a meq/kg oil, respectively, after 4 days of incubation when formation of hydroperoxides was maximum in SDS-emulsion. During 11 days, erythorbyl laurate emulsion was more effective to inhibit the formation of lipid hydroxide than other emulsion stabilized with various emulsifiers. TBARs value of various emulsifiers sharply increased when lipid hydroperoxide value decreased.

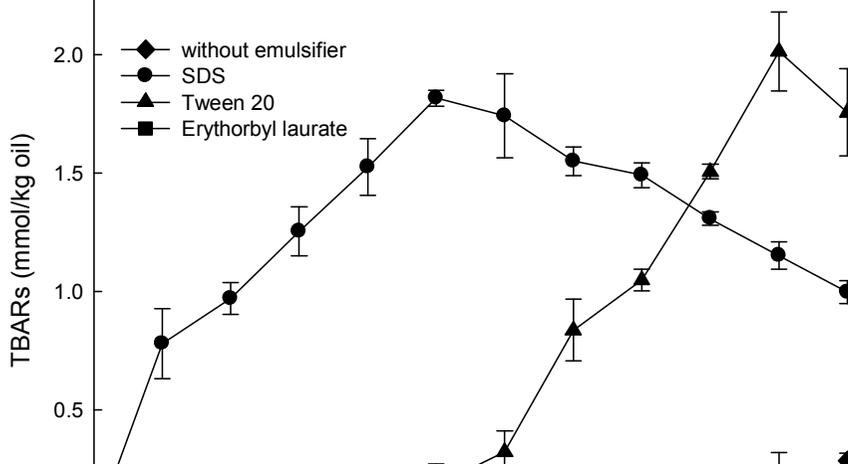
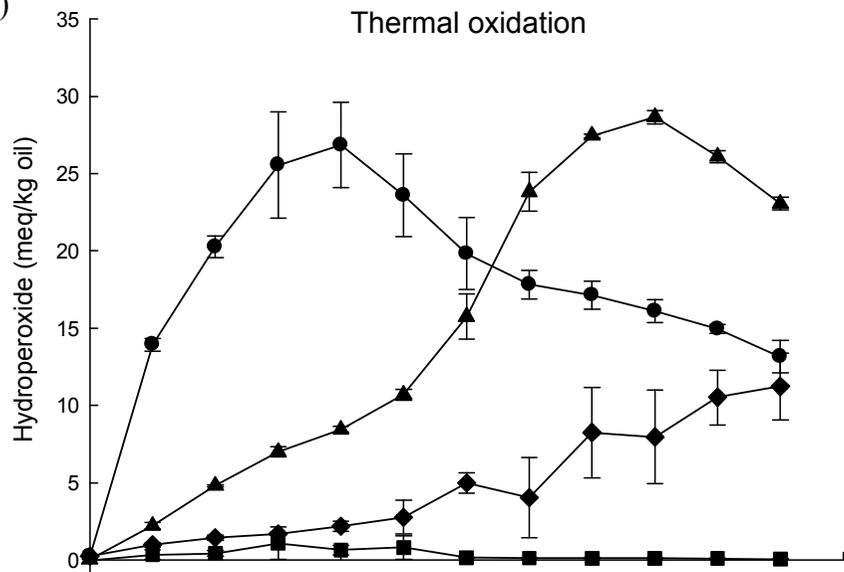
Previous works showed that anionic (SDS) emulsion droplets oxidized faster than non-ionic emulsion droplets (Brij) which was agreed with this experiment (Mei, McClements, & Decker, 1999). Cationic trace metals are active prooxidant that affects anionic surfactant than non-ionic surfactant (McClements & Decker, 2000; Mei, McClements, & Decker, 1999). Droplet size of emulsions with various emulsions was lower than 1.0 μm except without emulsifier (1.9 μm). Droplet size in O/W emulsion influences the oxidative stability since high surface area of oil droplets promotes lipid oxidation (Gohtani, Sirendi, Yamamoto, Kajikawa, & Yamono, 1999). This can explain that emulsions stabilized SDS or Tween 20 showed less oxidative stability than emulsion without emulsifier. Moreover, Conrnforth & West (2002) indicated that malonaldehyde, one of the important secondary products of auto-oxidation, can decompose and able to make volatile compound. This can be explained why TBARs was decreased.

The oxidative stability of emulsion with various emulsifiers to inhibit photooxidation was also tested (Fig. 5B). As fluorescence light was treated for 12 h at 25°C, lipid hydroperoxide increased in all emulsions. Similarly

with thermal oxidation, rate of oxidation in emulsions stabilized by Tween 20 and SDS were faster than erythorbyl laurate emulsion. During 12 h, 23.83^c, 8.43^b, and 1.75^a meq/kg oil of lipid hydroperoxides were formed by Tween 20, SDS, and erythorbyl laurate emulsion, respectively. There was no significant difference of lipid peroxidation between erythorbyl laurate and without light Tween 20 emulsion during 12 h. This showed erythorbyl laurate stabilized emulsion was more effective to inhibit photooxidation than emulsions with other emulsifiers. Lipid hydroperoxides formation of Tween 20 emulsion was faster than SDS emulsion unlike rate of lipid oxidation in thermal oxidation.

Although many emulsifiers such as SDS or Tween 20 can be used in emulsion model, only erythrobyl laurate presented great antioxidative activity in O/W emulsion model both thermal oxidation and photooxidation.

(A)



(B)

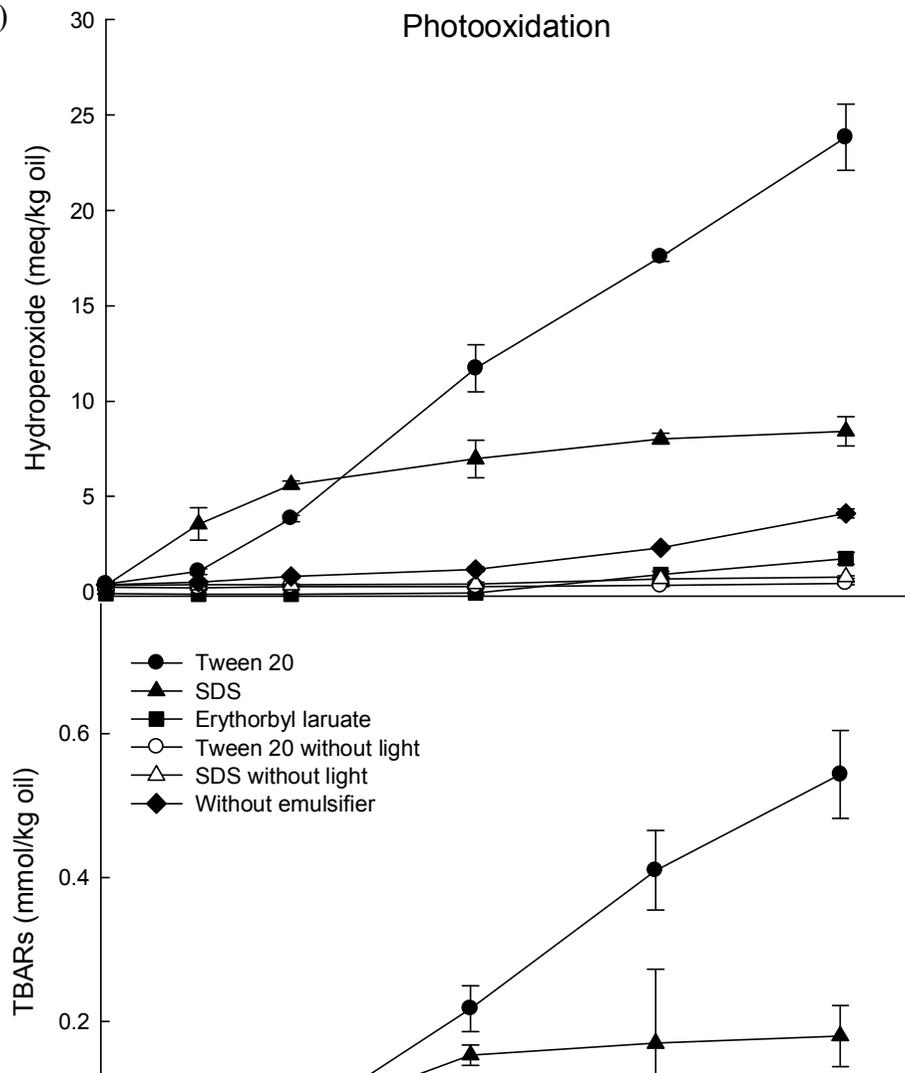


Fig. 5. Lipid oxidation of soybean O/W emulsion (5:95, w/w) stored at 60°C (A) and 100 µM riboflavin photosensitized at 25 °C (B).

3.3.3. Effect of localization on lipid oxidation

The effect of physical partition of antioxidants in lipid oxidation of O/W emulsions or bulk oil has been investigated since past decades. Polar paradox theory is one of the commonly explained theories about behavior of antioxidants (Frankel, Huang, Kanner, & German, 1994; Shahidi & Zhong, 2011). Accordingly, non-polar antioxidant is more effective in polar antioxidant in O/W emulsion because non-polar antioxidant would concentrate in oil in water interface where lipid oxidation might occur frequently. While, polar antioxidant is more effective in bulk oil (non-polar media) because it is easier to accumulate air-oil interface where oxidation is

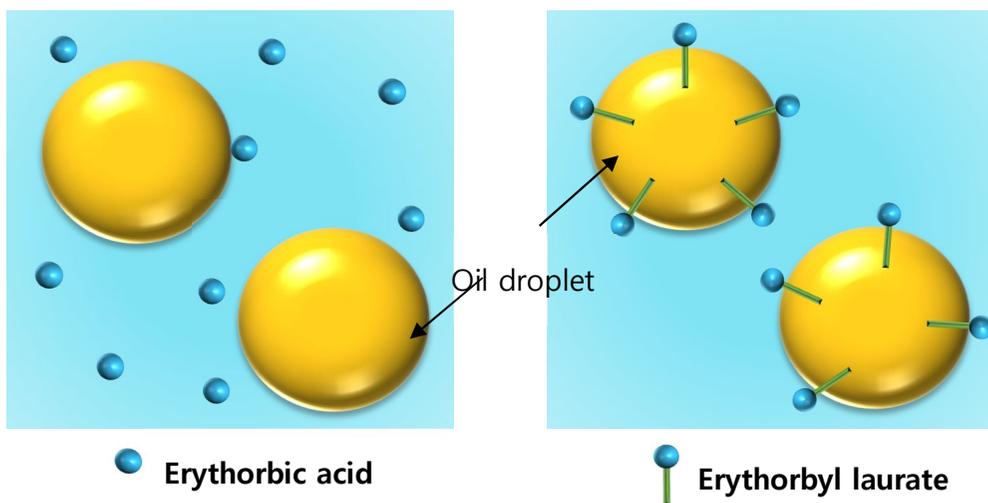
prevalent. Also, researchers postulated surface active non-polar antioxidant is more effective to inhibit lipid oxidation (Chaiyasit, Elias, McClements, & Decker, 2007). Based on this research, erythorbyl laurate emulsion expected to have strong antioxidant activity because erythorbyl laurate is presented in oil-water interface as an emulsifier (Fig 6A). To verify this assumption, erythorbyl laurate emulsion and erythorbic acid were used which has same antioxidative functional group. Emulsions (5% of oil, w/w) stabilized with erythorbyl laurate and Tween 20 (0.2%, w/w) were prepared. Tween 20 emulsion contained 5.57 mM of erythorbic acid which has same amount of erythorbyl laurate. Droplet size of Tween 20 emulsion was 0.9 μm which was similar with erythorbyl laurate emulsion.

Lipid oxidation rate of the Tween 20 emulsion without antioxidant is faster than the other emulsions in thermal oxidation (Fig. 6B). During 11 days, formation of hydroperoxide in Tween 20 emulsion with erythorbic acid was about 4.30 meq/ kg oil. However, the lipid oxidation of emulsion with erythorbyl laurate was not almost occurred (0.07 meq/kg oil). Hydroperoxide formation in riboflavin sensitized photooxidation in O/W emulsion was

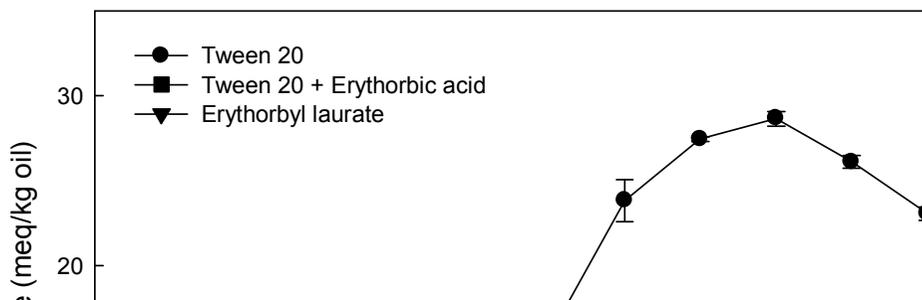
shown in Fig. 6C. After 12 h, hydroperoxides of Tween 20 emulsion, Tween 20 with erythorbic acid, and erythorbyl laurate were 23.82, 7.99, and 1.75 meq/kg oil, respectively. In accordance with thermal oxidation erythorbyl laurate emulsion was more effective to inhibit lipid oxidation than Tween 20 emulsion with erythorbic acid.

Though erythorbic acid and erythorbyl laurate showed similar free-radical scavenging activity, erythorbyl laurate emulsion had better oxidative stability of lipid oxidation in O/W emulsion than Tween 20 emulsion with erythorbic acid both thermal and photooxidation. This result can be explained localization of antioxidant affect oxidative stability in O/W emulsion. Because erythorbyl laurate is presented at oil-water interface, it is easier to scavenge free radical than erythorbic acid which is dissolved in water phase. This result supported that localization of antioxidant influences oxidative stability in O/W emulsion despite same functional group.

(A)



(B)



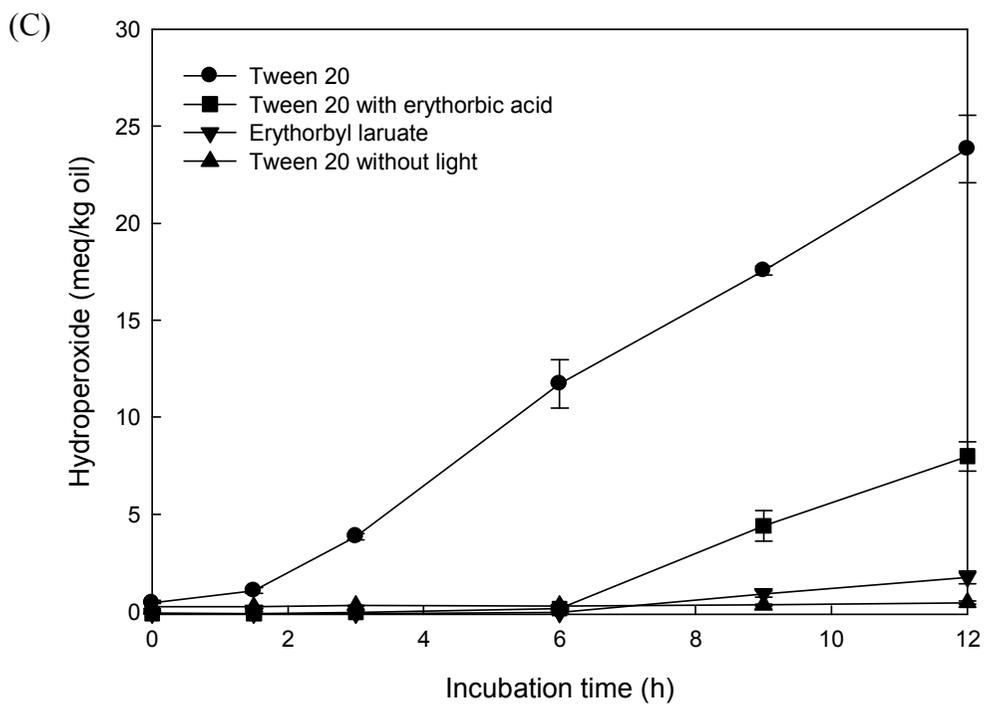


Fig. 6. Localization of erythorbic acid and erythorbyl laurate in O/W emulsion (A). Hydroperoxide value of lipid oxidation of soybean O/W emulsion (5:95, w/w) stored at 60°C (B) and 100 μ M riboflavin photosensitized at 25 °C (C).

4. Conclusions

Investigation of interfacial characteristics showed that erythorbyl laurate has surface-active ability and remarkable foaming stability. Free radical scavenging activity was remained after esterification. In thermal oxidation and photooxidation, erythorbyl laurate showed effective to inhibit lipid oxidation than SDS and Tween 20. Moreover, erythorbyl laurate emulsion was more effective to retard lipid oxidation than erythorbic acid contained emulsion. This shows that localization of antioxidative functionl group is important to inhibit lipid oxidation in O/W emulsion.

In food system, both emulsifiers and antioxidants are important food additives to form stable and retard lipid oxidation. Therefore, erythorbyl laurate can be used as a promising candidate for multi-functional emulsifier having remarkable antioxidative activity in O/W emulsion.

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국문초록

L-ascorbic acid의 이성질체인 erythorbic acid는 항산화능이 뛰어나고 가격이 저렴하나 수용성 물질이기 때문에 지질이 함유된 식품에서는 사용이 제한되어왔다. 그러므로 선행연구에서는 식품공정에 다양하게 활용하기 위해 소수성인 lauric acid와의 lipase 촉매 에스터화 반응을 통해 erythorbyl laurate를 합성하였으며, 이 물질이 그람 양성균의 세포막을 파괴하는 등 뛰어난 항균성을 띄고 있다는 것을 확인하였다. 본 연구에서는 erythorbyl laurate의 유화제로서의 특성과 수중유적형 에멀전에서의 항산화 특성을 확인하여 다기능성 식품 첨가물로서의 적용 가능성을 알아보고자 하였다.

Erythorbyl laurate의 표면장력과 접촉각은 농도가 증가함에 따라 감소하였고, 임계미셀농도(0.12 mM)에서의 표면장력은 34 mN/m였다. Erythorbyl laurate의 거품형성능은 다른 비이온 계면활성제(Tween 20, Triton X-100)와는 유의적인 차이가 없었으나, 거품의 반감기로 측정한 거품의 안정성이 다른 계면활성제에 비해 뛰어났다. Hydrophilic-lipophilic balance(HLB) 값이 9.8인 erythorbyl laurate를 0.2%, 대두유를 5% 함유한 수중유적형 에멀전의 입도분포와 TEM 분석에서 0.75 μm 의 균질한 기름입자가 생성되는 것을 확인하였다.

한편, DPPH와 ABTS 실험에서 각각의 EC_{50} 은 331.43, 425.95

μM 로, erythorbyl laurate의 라디칼소거능이 합성 후에도 유지된다는 것을 확인하였다. 열산화와 광산화실험에서 erythorbyl laurate는 다른 유화제(SDS, Tween 20, without emulsifier)를 넣어 만든 에멀전에 비해 산화속도가 현저히 지연되었다. 또한, erythorbic acid와의 비교실험을 통해서 같은 항산화기이며 비슷한 라디칼 소거능을 가지고 있더라도 erythorbyl laurate같이 계면에 존재하는 항산화제가 수중유적형 에멀전에 더 효과적임을 알 수 있었다.

본 연구는 erythorbyl laurate의 유화제로의 계면특성과, 수중유적형 에멀전에서의 항산화능을 연구함으로써, 식품 다기능성 첨가물로서의 가능성을 제시하는 것에 의의가 있다.