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Erythorbyl laurate as a potential food additive with multi-functionalities: Interfacial characteristics and antioxidant activity

Kyung-Min Park [a,1], Min Joo Lee [a,1], Su-Kyung Jo [a], Seung Jun Choi [b], JaeHwan Lee [c], Pahn-Shick Chang [a,d,*]

[a] Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea
[b] Department of Food Science and Technology, Seoul National University of Science and Technology, Seoul 139-743, Republic of Korea
[c] Department of Food Science and Biotechnology, Sungkyunkwan University, Gyeonggi-do 440-746, Republic of Korea
[d] Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea

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ABSTRACT

The interfacial characteristics and antioxidant activities of erythorbyl laurate were investigated to provide information on practical applications as a multi-functional food additive. The critical micelle concentration (CMC) of erythorbyl laurate was 0.101 mM and its foam stability was three times (half-life 24.33 ± 0.94 h) higher than that of Tween 20 (8.00 ± 1.63 h). In free radical scavenging assay, the negligible decrease in EC_{50} of erythorbyl laurate compared to erythorbic acid manifested that C-5 selective esterification of erythorbic acid with an acyl group (lauric acid) did not reduce the inherent antioxidant activity of the donor (erythorbic acid). Erythorbyl laurate formed lipid peroxides slower (*i.e.* retarded oxidation) in an emulsion system than did erythorbic acid. The localization of erythorbyl laurate as an emulsifier allowed the antioxidant molecules to be concentrated at the oil-water interface where oxidation is prevalent, which led to more effective retardation of lipid oxidation.

1. Introduction

An emulsion is a heterogeneous dispersion of two immiscible liquids (*e.g.* water and lipid) wherein droplets of one phase (dispersed or internal phase) are encapsulated within another phase (continuous or external phase) in the presence of surface-active agents (*i.e.* emulsifiers) (Friberg, Larsson, & Sjoblom, 2003). For the emulsion-based products extensively used in food, cosmetic, and pharmaceutical industries, lipid oxidation and microbial contamination have been considered as the major hazards in terms of safety for human consumption (Luther et al., 2007). Under the strategy for simultaneously controlling the aforementioned hazards, our research group has performed lipase-catalyzed esterification between lauric acid and erythorbic acid to produce a novel multifunctional emulsifier with antibacterial and antioxidant activities (Park, Sung, Lee, & Chang, 2011).

Erythorbic acid is a stereoisomer of L-ascorbic acid that has been used widely as an antioxidant for various processed foods (Clark et al., 2009). On the other hand, lauric acid is a medium-chain fatty acid with a strong antimicrobial activities against a wide spectrum of food-borne pathogens (Nakatsuji et al., 2009). Therefore, erythorbyl laurate (6-*O*-lauroyl-erythorbic acid), which results from enzymatic esterification between erythorbic acid and lauric acid, was anticipated to be an amphiphilic material with multi-functionalities. And we have previously reported that subsequent studies related to the synthetic methodology for erythorbyl laurate including optimum conditions employing response surface methodology and continuous process through a packed bed enzyme reactor (Lee, Park, Choi, & Chang, 2012; Lee, Park, Choi, Shim, & Chang, 2013).

From the recent study on antibacterial susceptibility, it was confirmed that erythorbyl laurate had both bacteriostatic and bactericidal effects on Gram-positive pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. Furthermore, the antibacterial mechanism of erythorbyl laurate was revealed in results of the Live/Dead BacLight assay based on propidium iodide dye, which enters the cell and stains cellular DNA only if the membrane is damaged and abnormally permeable. Thus, exposure of *S. aureus* to erythorbyl laurate ruptures the cytoplasmic membrane, leading to altered membrane permeability.

The primary aim of this study was to investigate the emulsifying and antioxidant activities of erythorbyl laurate and to determine the effective concentration for practical applications. First, the emulsifying activity of erythorbyl laurate was assessed based on interfacial characteristics including surface tension and foaming

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea.
E-mail address: pschang@snu.ac (P.-S. Chang).
[1] Equal contribution as first authors.

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ability. We also evaluated the free radical scavenging activity of erythorbyl laurate and the retarding effect on lipid oxidation in an emulsion system.

2. Materials and methods

2.1. Materials

Erythorbic acid (⩾99.0%), dodecanoic acid (lauric acid ⩾ 99.0%), and 6-*O*-palmitoyl-L-ascorbic acid (ascorbyl palmitate ⩾ 99.0%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Novozym® 435 (*Candida antarctica* lipase immobilized on acrylic resin) and polyoxyethylene (20) sorbitan monolaurate (Tween 20) were kindly provided by Novozymes Korea, Ltd. (Seoul, Korea) and Ilshinwells Co. (Seoul, Korea), respectively. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Sigma-Aldrich to assess antioxidative activity. All other chemicals were of analytical grade and were used without further purification.

2.2. Preparation of erythorbyl laurate

Enzymatic synthesis, purification, and identification of erythorbyl laurate were performed according to our previous reports (Lee et al., 2012; Park et al., 2011). Briefly, erythorbic acid and lauric acid were added to a screw-capped glass vial with acetonitrile and pre-incubated in an orbital shaking water bath. The reaction was initiated by adding immobilized lipase (Novozym® 435; Novozymes) to the mixture. After terminating the enzymatic synthesis, erythorbyl laurate was purified using a solvent separation method, and quantitative analysis and identification were carried out with a high-performance liquid chromatography system (LC-2002; JASCO, Inc., Tokyo, Japan) equipped with a Spherisorb-ODS column (5 μm, 100 Å, I.D. 4.6 × 250 mm; Waters, Milford, MA, USA) and a matrix-assisted laser desorption/ionization time-of-flight system (Auto Flex II; Bruker Daltonics, Bremen, Germany).

2.3. Emulsifying ability of erythorbyl laurate

2.3.1. Surface tension and critical micelle concentration

Because of the low water solubility of erythorbyl laurate, sample solutions were prepared with distilled water containing 1% (v/v) dimethyl sulfoxide (DMSO). Critical micelle concentration (CMC) was determined by measuring the surface tension of a sample solution at different concentrations at 25 °C with a Wilhelmy plate tensiometer and a platinum plate (K100SF, Krüss, Hamburg, Germany).

2.3.2. Foaming ability and stability

Sample solutions were prepared to a concentration of 0.05% (w/v) with distilled water containing 1% (v/v) DMSO. The 0.05% concentrations of each surfactant referred to 0.41 mM of Tween 20, 1.21 mM of ascorbyl palmitate, and 1.40 mM of erythorbyl laurate, respectively. Then, each sample solution (50 mL) was poured into a 100-mL graduated cylinder. Foam was produced by homogenization at 10,000 rpm at 25 °C for 60 s using a high-speed blender (T-18 basic; IKA, Staufen, Germany). Foam volume was measured at 30 s after homogenization to determine foaming capacity. Foam stability was evaluated as the time until the foam collapsed by 50% of its original volume (half-life).

2.4. Antioxidant properties of erythorbyl laurate

2.4.1. Free radical scavenging activity

The free radical scavenging activity of erythorbyl laurate was determined based on the DPPH method (Chen, Bertin, & Froldi, 2013) with slight modifications. Briefly, 3.75 mL of 0.1 mM DPPH in methanol was mixed with 0.25 mL surfactant solution and vortex-mixed for 10 s. After the sample mixture stood in the dark for 30 min, the absorbance was measured at 517 nm using a UV/VIS-spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan). DPPH radical scavenging activity was expressed as [1 − (sample absorbance/blank absorbance)] × 100% (Miliauskas, Venskutonis, & Van Beek, 2004).

The ABTS assay was conducted according to Thaipong's method (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006) with slight modifications. A stock solution of 7.00 mM ABTS and 2.45 mM potassium persulfate was stored in the dark for 12 h to produce proton radicals. The solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. A surfactant solution (0.05 mL) dissolved in methanol was reacted with 1.90 mL ABTS solution in the dark, and absorbance was measured at 734 nm after 6 min. ABTS radical scavenging activity was expressed as [1 − (sample absorbance/blank absorbance)] × 100% (Khalil, Pepato, & Brunetti, 2008). Free radical scavenging activity was calculated from a linear regression as the 50% effective concentration (EC_{50}), which is the concentration required to reduce the initial absorbance 50%. Radical scavenging activities of ascorbyl palmitate and erythorbic acid were also determined for comparison.

2.4.2. Antioxidant activity of erythorbyl laurate in an emulsion system

Oil-in-water emulsions were prepared by mixing 5% (w/v) lipid phase (soybean oil) with 95% aqueous phase (0.2% [w/v] surfactant in distilled water). To prepare the surfactant solution, surfactant was dissolved in a 2% (v/v) ethanol solution, followed by subsequent stirring for 1 h at ambient temperature to remove the trace ethanol. A coarse emulsion premix was prepared by homogenizing the lipid and aqueous phases together using a high-speed blender (T-18 basic; IKA) at 16,000 rpm for 60 s at room temperature. Then, the droplet size in the premixed emulsions was reduced by sonication for 2 min at 210 W and a duty cycle of 0.5 s at 4 °C.

To elucidate the effect of erythorbyl laurate on thermal lipid oxidation, emulsion samples (5 mL) were stored at 60 °C in the dark for up to 11 days. Meanwhile, riboflavin (100 μM) was used as a photosensitizer for photo-oxidation. Emulsion samples (1 mL) were transferred to vials (10 mL), and the samples were oxidized at 25 °C for 12 h under fluorescence light (3420 lux).

Lipid hydroperoxides, which are the primary products of lipid oxidation, were measured by the ferric thiocyanate method (Kiokias & Varzakas, 2014). The emulsion sample (20 μL) was mixed with 3 mL methanol/1-butanol (2:1, v/v) and 30 μL thiocyanate/Fe^{2+} solution. The thiocyanate/Fe^{2+} solution was prepared immediately before use by combining 0.8 mL 3.940 M ammonium thiocyanate and 0.8 mL fresh Fe^{2+} solution. The fresh Fe^{2+} solution was obtained from the supernatant of 0.8 mL 144 mM $BaCl_2$ in 400 mM HCl and 0.8 mL 144 mM $FeSO_4$. After a 20-min reaction, the absorbance of each sample was detected at 510 nm. Lipid hydroperoxides were determined based on cumene hydroperoxide.

Thiobarbituric acid-reactive substances (TBARS) were used to measure lipid oxidation reaction products, particularly malondialdehyde (MDA), as an important auto-oxidation product (Cai, Cao, Aisikaer, & Ying, 2013). A solution of trichloroacetic acid (TCA)–TBA–HCl was prepared by mixing 15 g TCA, 375 mg TBA, 1.76 mL 12 N HCl, and 82.9 mL H_2O. Two milliliter of the