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Accepted Version

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Abstract

Over the past few decades, food scientists have investigated a wide range of emulsifiers to manufacture stable and safe emulsion-based food products. More recently, the development of emulsifiers with multi-functionality, which is the ability to have more than two functions, has been considered as a promising strategy for resolving rancidification and microbial contamination in emulsions. Erythorbyl fatty acid esters (EFEs) synthesized by enzymatic esterification of hydrophilic erythorbic acid and hydrophobic fatty acid have been proposed as multi-functional emulsifiers since they simultaneously exhibit amphiphilic, antioxidative, and antibacterial properties in both aqueous and emulsion systems. This review provides current knowledge about EFEs in terms of enzymatic synthesis and multi-functionality. All processes for synthesizing and identifying EFEs are discussed. Each functionality of EFEs and the proposed mechanism are described with analytical methodologies and experimental details. It would provide valuable insights into the development and application of a multi-functional emulsifier in food emulsion chemistry.

Keywords: erythorbyl fatty acid ester; multi-functional emulsifier; lipase-catalyzed esterification; amphiphilic property; antioxidative property; antibacterial property.

1. Introduction

Several natural and processed foods (*e.g*., milk, egg yolk, butter, margarine, mayonnaise, etc.) have a distinct three-dimensional colloidal network of food molecules called an emulsion (Owen, Srinivasan, & Kirk, 2017). These emulsion-based food products consist of two (or more) immiscible liquids, where spherical droplets of one liquid phase are uniformly dispersed in a large volume of another continuous liquid phase (McClements, 2015). The principle behind the fabrication of emulsions is the application of unique additives that contribute to reducing interfacial tension of the dispersed phase, stabilizing liquid-liquid phase separation, and preventing aggregation of emulsion droplets (flocculation and/or coalescence) (McClements & Jafari, 2018). These chemicals, which are typically small molecules with both hydrophilic and hydrophobic moieties (amphiphiles), are referred to as emulsifiers, stabilizers, or surface-active agents (surfactants) based on their modes of action. Considering that the colloidal and physicochemical characteristics of emulsions such as size, texture, appearance, stability, functionality, mouthfeel, and bioavailability depend on the variety and quantity of emulsifiers used (Lee, Niknafs, Hancocks, & Norton, 2013b), it is imperative to find a novel emulsifier with unique properties to manufacture emulsion-based products with specific functional attributes in the food industry. Over the past few decades, to fabricate desired emulsions, food scientists have discovered and developed a range of natural emulsifiers (*e.g*., lecithin, polysaccharides, proteins, gums, etc.) (McClements, Bai, & Chung, 2017; Ozturk & McClements, 2016) and synthetic emulsifiers (*e.g*., polysorbate esters, sorbitan esters, sugar esters, etc.) (Marhamati, Ranjbar, & Rezaie, 2021; Neta, Teixeira, & Rodrigues, 2015).

Unlike other food systems, emulsions provide many practical advantages as food matrix or delivery vehicles desirable for both hydrophilic and hydrophobic food ingredients (Lu, Kelly, & Miao, 2016; Mao, Roos, Biliaderis, & Miao, 2017). Unfortunately, however, two shortcomings−oxidative rancidification and microbial contamination−have become chronic issues that threaten the safety of emulsion-based food products (Villeneuve, Bourlieu-Lacanal, Durand, Lecomte, McClements, & Decker, 2021; Zhao, Zhang, Hao, & Li, 2015). Most food emulsions contain a relatively high amount of water and

lipids, and their colloidal structures with a large interfacial area between water and lipids make them highly susceptible to oxidative stress and radical formation. Unintentional chain reactions of oxidative degradation of lipids can be stimulated by heat, light, acid/alkali, mineral ion, or enzyme resulting in undesirable off-flavors and toxic compounds like hydroperoxides (Waraho, McClements, & Decker, 2011). Chemical intoxication by oxidized products can induce organ damage, inflammation, carcinogenesis, and advanced atherosclerosis in humans (Kanner, 2007). On the other hand, food emulsions provide a nutritionally abundant environment that is highly conducive to the bacterial growth, including food-borne pathogens, whereas it can be challenging to implement effective sterilization processes without affecting the colloidal stability of the emulsions (Kelleher, O'Mahony, Kelly, O'Callaghan, Kilcawley, & McCarthy, 2018; Liu, Sun, Xue, & Gao, 2016). These pathogens are infectious or produce endotoxins, which are responsible for food poisoning in emulsions (Jarvis & Highsmith, 1984). In order to avoid those deteriorations, most emulsion-based food products conventionally contain several food additives as antioxidants (*e.g.*, BHA, BHT, TBHQ, EDTA, etc.) and preservatives (*e.g.*, acetic acid, benzoic acid, sorbic acid, etc.). However, the complexity of the colloidal nature of food emulsions makes it difficult to predict or maintain their efficacies during processing, storage, and consumption (Decker et al., 2017; Weiss, Loeffler, & Terjung, 2015). Several conventional emulsifiers, especially sucrose fatty acid esters and ascorbyl fatty acid esters, exhibit other functions (Zieniuk, Białecka-Florjańczyk, Wierzchowska, & Fabiszewska, 2021), although most food emulsions contain above food additives to complement another deficiency. However, there is a trend toward trying to mitigate the use of food additives by consumers. For these reasons, more recently, food scientists have been interested in developing an emulsifier with multi-functionality, which is the ability to have more than two functions in a single molecule, called a multi-functional emulsifier.

Erythorbyl fatty acid ester (EFE) is one of the most successful cases of multi-functional emulsifiers having amphiphilic, antioxidative and antibacterial properties simultaneously. In most cases, researchers theoretically or practically screen several substances as candidates for the fatty acid derivatives to

find a promising amphiphilic compound with a desired function and then control their amphiphilicities by altering the hydrophobic moiety. Our research team, for example, found antioxidative erythorbic acid as a potent candidate for the multi-functional emulsifier by screening the antibacterial property of six kinds of lauric acid derivatives esterified with different hydrophilic moieties (Park et al., 2018b). The first EFE, erythorbyl laurate (6-*O*-lauroyl-erythorbic acid), was developed under the strategy for controlling both lipid rancidification and bacterial growth in food emulsions (Park, Lee, Sung, Lee, & Chang, 2011). This compound was synthesized via the lipase-catalyzed esterification between erythorbic acid (isoascorbic acid) and lauric acid ($C_{12:0}$) as substrates (Fig. 1a), and its multi-functionality was investigated in detail. Contrary to conventional or developed amphiphilic fatty acid esters, erythorbyl laurate exhibited emulsifying, antioxidative, and antibacterial properties in aqueous and emulsion systems (Park et al., 2018a; Park, Lee, Jo, Choi, Lee, & Chang, 2017). The results from erythorbyl laurate studies initiatively proposed that its fatty acid derivatives could be also potent multi-functional food emulsifiers. From this perspective, a solvent-free enzyme reaction system was constructed for the mass production of EFEs (Yu, Lee, Shin, Park, & Chang, 2019), and diversification of EFE derivatives via substituting the fatty acid moiety were successfully achieved (Fig. 1b), followed by investigating significant influence factors to determine the multi-functionality (Kim, Yu, Yang, Choi, & Chang, 2023). These recent studies finally validated the potential of EFEs as multi-functional emulsifiers.

This review article is mainly devoted to summarizing the current knowledge about EFEs as well as introducing key experimental results in terms of enzymatic synthesis and multi-functionality. The first section presents a detailed description of the overall process for the lipase-catalyzed synthesis of EFEs. Our focus is particularly on the use of an immobilized lipase and a solvent-free enzyme reaction system for the regioselective mass production of EFEs in eco-friendly circumstances. The multi-functionality of EFEs is then discussed in order of interfacial, antioxidative, and antibacterial properties, as well as their relationship with the amphiphilic property. Additionally, adopted analytical methods and experimental details are described in each section. This work will provide comprehensive insights into the

synthesis, identification, characterization, and practical application of a novel multi-functional emulsifier in the fields of food and emulsion chemistry.

2. Lipase-catalyzed Synthesis of EFEs

In the majority of recent cases to synthesize functional emulsifiers, chemical methods based on acid or base catalysts are no longer used because of low process yield, specificity/selectivity, and product stability. Ascorbyl fatty acid esters, which are stereoisomers of EFEs, for instance, were chemically synthesized before the 2000s; however, these non-regioselective and unstable reactions lead to a problem of the reduced yield of products, and inevitable degradation of ascorbic acid takes place under alkaline or elevated temperature conditions (Karmee, 2009). For this reason, EFEs have been synthesized through biocatalysts with a specific selectivity from the beginning (Park et al., 2011).

The main key to enzymatically synthesizing EFEs lies in the use of a commercial immobilized lipase Lipozyme® 435 or Novozym® 435 (from Novozymes A/S Co.) as a catalyst. Lipases (triacylglycerol hydrolase, EC 3.1.1.3), which belong to a group of esterases, can catalyze the cleavage and the formation of water-insoluble acyl compounds (R−C=O) such as mono-, di-, and triacylglycerols (Park & Park, 2022b). It is well-known that lipases catalyze the hydrolysis of long-chain acylglycerols in an aqueous medium, whereas in water-restricted conditions, other reactions including esterification, interesterification, transesterification, alcoholysis, aminolysis, acidolysis, and glycerolysis are also possible. Each lipase exhibits its unique regio- and stereoselectivity, resulting in desired products preferentially (Choi, Park, & Chang, 2021). On the other hand, lipases often exhibit somewhat promiscuous substrate specificity under certain reaction conditions, meaning they can react with substances of different structures compared to their major substrates (Kapoor & Gupta, 2012). These selective and versatile characteristics of lipases make them an overarching strategy for the bioconversion processing in organic chemistry, and a wide range of natural or immobilized lipases with different selectivities are currently available. In food chemistry, these enzymes are mainly used for manufacturing flavorings,

colorants, structured lipids (*e.g.*, cocoa butter equivalents, human milk fat substitutes, high omega-3 lipids, etc.), and other synthetic food additives (*e.g.*, antioxidants, antimicrobials, emulsifiers, etc.) or functional ingredients (Coelho & Orlandelli, 2021). For the synthesis of EFEs, the immobilized lipase derived from *Candida antarctica* lipase B, which is a non-specific lipase (Ortiz et al., 2019), can be employed because it exhibits chemoselectivity towards preferential esterification between the C-6 hydroxyl group (−OH) of erythorbic acid as an acyl acceptor and the carboxyl group (−COOH) of fatty acid as an acyl donor (Kim et al., 2023; Park et al., 2021; Park et al., 2011). Even though the exact mechanism remains unclear, it is probably due to the close proximity of the substituent groups within a steep funnel-shaped substrate binding pocket of the lipase (Arcens, Grau, Grelier, Cramail, & Peruch, 2020). This chemoselectivity has been observed in other studies on amphiphilic fatty acid esters with different hydrophilic moieties (Park et al., 2018b; Yu, Kim, & Chang, 2023; Yu, Park, & Chang, 2020).

2.1. Reaction systems for the lipase-catalyzed synthesis of EFEs

Lipase-catalyzed esterification of EFEs has been conducted in two different water-restricted reaction systems. One is a conventional organic solvent monophase system (OS-MPS) using acetonitrile or 2-methyl-2-butanol as reaction media (Fig. 2a), and the other is our original solvent-free gas-solid-liquid multiphase system (GSL-MPS) using fatty acid itself as the reaction medium (Fig. 2b). In the case of OS-MPS, it is essential to select an organic solvent with appropriate hydrophobicity that can dissolve both erythorbic acid and fatty acid, as well as synthesized EFEs. The hydrophobicity of solvent media generally determines the solubility of substrates, which is one of the significant factors affecting reaction efficiency. Additionally, the solvent compatibility of the immobilized lipase should be considered because it retains its native structure in relatively hydrophobic organic solvents (Kumar, Dhar, Kanwar, & Arora, 2016). The interaction of relatively hydrophilic solvents with enzymes might promote the denaturation of their protein structures by removing water from the surface and consequently perturbing hydrogen bonding (José et al., 2011). Considering these aspects comprehensively, organic solvents

showing intermediate hydrophobicity can be selected as reaction media for the lipase-catalyzed esterification of EFEs. Lee *et al*. achieved a maximum 77.81% conversion to erythorbyl laurate in a batch-type OS-MPS using acetonitrile solvent under the optimum reaction conditions (Lee, Park, Choi, & Chang, 2012). Sun *et al*. achieved a maximum 95.32% conversion to erythorbyl palmitate in a batch-type OS-MPS using 2-methyl-2-butanol (Sun et al., 2013), and their colleagues introduced ultrasonic treatment to effectively agitate the reaction medium, reducing reaction time by over 50% (Cui et al., 2013). Furthermore, a continuous-type packed-bed enzyme reactor (PBER) can be employed to resolve two deficiencies of the batch-type OS-MPS, which are the breakdown of immobilized lipase granules under the continuous shear stress of mechanical agitation (magnetic stirring or ultrasonication) and the accumulation of water during the process. Lee *et al*. designed a continuous-type OS-MPS based on PBER with ion exchange resins for removing free water and achieved a maximum 86.30% conversion to erythorbyl laurate under the optimum reaction conditions (Lee, Park, Choi, Shim, & Chang, 2013a).

The OS-MPS is simple to establish and suitable for the lab-scale lipase-catalyzed synthesis of EFEs. Unfortunately, however, there are several drawbacks to be considered on an industrial scale, including low solubility of substrates and irrecoverable lipases. Low concentration of substrates results in extremely low production yield versus reaction volume, and irreversible deformation of the immobilized lipase increases production costs. Furthermore, safety concerns regarding the use of dangerous organic solvents in the production of EFEs restrict their usage as food additives. For these reasons, Yu *et al*. constructed a solvent-free GSL-MPS, where erythorbic acids (solid) and immobilized lipases (solids) dispersed in fatty acids (liquid) are sufficiently mixed by nitrogen sparging (gas) (Yu et al., 2019). Inert gas bubbles can dramatically improve mixing efficiency without mechanical damage to the immobilized lipase and overcome the mass transfer limitations of a solid-liquid biphasic environment. Compared with the synthesis of erythorbyl laurate in OS-MPS, the maximum production yield obtained from the reaction in GSL-MPS (13.974 mg/mL) was 8.60-fold higher, and under thermal acceleration conditions, there was no significant reduction in the catalytic activity of the immobilized lipases. This

study suggested that the use of liquid phase fatty acids as reaction media protects lipases from thermal damage and induces structural changes in lipases into their active (open-form) conformations (Cabrera, Fernandez-Lorente, Fernandez-Lafuente, Palomo, & Guisan, 2009). The GSL-MPS was applied to the mass production of EFE derivatives with different fatty acid chains (Fig. 1b). On the other hand, it was proposed that the presence of solid phase (erythorbic acid) in GSL-MPS highly contributes to the relatively low conversion rate of EFEs than that of OS-MPS, resulting in large amounts of residual substrates (especially fatty acid) after the reaction. Hence, Yu *et al*. investigated a modified two-step lipase-catalyzed esterification using glycerol as a second acyl acceptor to increase the conversion rate of GSL-MPS dramatically (Yu, Byun, & Chang, 2022). The mixture of erythorbyl laurate and glyceryl laurate was finally obtained, and the conversion rate of these esters was approximately 100%.

2.2. Reaction variables affecting the lipase-catalyzed synthesis of EFEs

Several reaction variables such as reaction temperature, enzyme concentration, substrate molar ratio, and accumulation of free water can significantly affect the lipase-catalyzed synthesis of EFEs. In the case of OS-MPS, the effects of reaction variables on the synthesis of EFEs were systemically evaluated via a response surface methodology and a central composite experimental design (Cui et al., 2013; Lee et al., 2012; Lee et al., 2013a; Sun et al., 2013). The increase in both reaction temperature and enzyme concentration greatly elevates the conversion rate of EFEs, and it then levels off at certain levels. Notably, an immobilized lipase with high thermal stability allows a robust reaction at high temperatures where the solubility and molecular dynamics of substrates are improved. The initial substrate molar ratio (erythorbic acid:fatty acid) is the most critical variable for the lipase-catalyzed synthesis of EFEs. When the molar ratio of fatty acids to erythorbic acids is increased, the production of EFEs is increased regardless of the amount of enzyme used. As not fully understood, the initial formation of acyl-complexes at the active site is the rate-determining step as part of lipase-catalyzed esterification, which follows a Ping-Pong bi-bi mechanism (Choi & Chang, 2022). This implies that a relatively high ratio

of the acyl donor (fatty acid) might promote acyl-complex formation at the active site of lipase and the forward reaction (esterification). Of course, excessive treatment of these conditions can cause opposite results. Finally, it is recommended to use molecular sieves or PBERs to avoid water accumulation and mass transfer limitations in OS-MPS. The reaction rate of esterification (*i.e.*, dehydration reaction) is directly affected by the concentration of the water molecules in accordance with the law of mass action, implying that controlling water accumulation is one of the effective approaches to improving conversion rate of EFEs. Under the reaction in a batch-type sealed reactor, adding crystalline molecular sieves is the easiest way to remove free water without affecting lipase activity, although the breakdown under mechanical agitation cannot be avoided. Other methods using chemicals or distillation are challenging to implement the lipase-catalyzed esterification of erythorbic acid and fatty acid. For industrial practice, as aforementioned, the use of a continuous PBER equipped with ion exchange resins would be a suitable choice to overcome the drawbacks of a batch-type OS-MPS reactor.

On the other hand, in the case of GSL-MPS, there are a few other elements that need to be considered because of fundamental differences in reaction circumstances. First of all, there is an excessive amount of acyl donors because fatty acids are directly used as reaction media. This makes the availability of acyl acceptors determine the production yield, and consequently, an increase in the ratio of erythorbic acid can improve the lipase-catalyzed synthesis of EFEs (Ren & Lamsal, 2017; Yu et al., 2019). Nevertheless, excessive amounts of solid phases (erythorbic acid and immobilized lipase) hamper the ability of the gas phase to disperse reactants and decrease the production of EFEs. Second, the reaction is performed at a relatively high temperature (50−85°C) with inert gas sparging. The reaction temperature should be elevated to retain the liquid phase of fatty acids depending on melting point because highly viscous fatty acids can hinder the binding of substrates to the lipase and the release of products from the enzyme-substrate complex (Kim, Youn, & Shin, 2006; Yu et al., 2019). For example, the productivity of erythorbyl ricinoleate (4.9 mM/h) was 8.20 and 2.7-fold higher than that of erythorbyl laurate (0.6 mM/h) and erythorbyl myristate (1.8 mM/h) under the same bioconversion reactor, probably due

to the enhanced dispersibility of the reaction medium of ricinoleic acids (melting point: 5.5°C) (Park, Yu, & Chang, 2023). When the reaction temperature is within these ranges in a water-restricted condition, erythorbic acid and EFEs could be insignificantly oxidized and retained in stable forms. Of course, the differences in productivity among EFEs were also considered to be affected by the typoselectivity of *C. antarctica* lipase B, which showed a substrate preference for medium-chain fatty acid esters (Kim et al., 2023; Park et al., 2021). Controlling gas flux at proper levels to mix the reactants also promote an effective collision between substrates and lipases as well as removal of free water. Inert gases *(e.g.*, nitrogen, argon, and helium) should be used for GSL-MPS to avoid the unintended oxidation of substrates and EFEs, although nitrogen gas is the most suitable choice considering the production cost. In addition, it is occasionally possible for a long-term reaction at elevated reaction temperature to produce diesters (C-6 and C-5 hydroxyl groups of erythorbic acid), probably due to the relatively lower viscosity of the reaction mixture (Hilterhaus, Thum, & Liese, 2008). However, it is a negligible amount in most cases (data not reported).

2.3. Quantitative analysis and purification of synthesized EFEs

In the reactant after the lipase-catalyzed synthesis of EFEs, four kinds of substances with different hydrophobicities remain: two substrate remnants (erythorbic acids and fatty acids), synthesized EFEs, and residual immobilized lipases. The quantitative analysis requires simultaneous separation of those compounds, and typically, high-performance liquid chromatography (HPLC) equipped with a silica-based reverse-phase column has been used. The reactant from OS-MPS, in which the compounds are already dissolved in organic solvents, can be directly introduced to HPLC system after ultrafiltration to remove immobilized lipases and solid particles, whereas that of GSL-MPS needs to be dissolved in appropriate solvents (*e.g.*, acetonitrile and methanol) before further steps (Fig. 3a). Erythorbic acids and EFEs with the same dihydrofuran moiety can be easily detected by ultraviolet (UV) radiation near 260 nm wavelength, similar to its stereoisomer, ascorbic acids (Washko, Welch, Dhariwal, Wang, &

Levine, 1992). Erythorbic acid and its fatty acid derivatives absorb UV in the range of 220−300 nm wavelength, resulting in a peak value near 260 nm wavelength depending on the analytical conditions. Generally, a detection wavelength should be determined by analyzing the UV absorbance spectrum of substances and choosing the wavelength to give sufficient sensitivity and linearity against molar concentration. As shown in the HPLC-UV chromatogram (Fig. 3b), a putative peak of EFE is shown long after a peak of erythorbic acid because of the increased hydrophobicity caused by esterification with fatty acid. It is also observed that the peak height of EFE increases according to the reaction time. In addition, the other small peak just after erythorbic acid may be from soluble residues of immobilized lipase (peptides or polymethacrylate-divinylbenzene supporters) (Park et al., 2021). Although it is impossible to prevent their formation, they are practically eliminated through the purification process to be described after. Meanwhile, the other substrate, fatty acid, is undetectable in the same analytical conditions of HPLC-UV, considering the UV absorbance cutoff of organic solvents (acetonitrile: 195 nm, methanol: 205 nm) (Reichardt & Welton, 2010). Refractive index (RI) detector, which is useful to analyze compounds with limited or no UV absorption, such as alcohols, lipids, carbohydrates, and polymers (Guillén & Cabo, 1997), easily detects fatty acids in the reactant as shown in the HPLC-RI chromatogram (Fig. 3c). An evaporative light scattering detector (ELSD) based on nebulization and desolvation can be also adopted to quantify the change of fatty acids precisely because HPLC-RI relatively lacks analytical sensitivity and suitability for temperature dependent or gradient elution HPLC conditions (Hopia & Ollilainen, 1993). Using a reverse-phase column like C_{18} column, the detection time of fatty acids is prolonged as their hydrophobicity increases (Bravi, Perretti, & Montanari, 2006), while the peak of EFEs is detected before fatty acids. These results strongly indicate that the synthesized compounds have intermediate hydrophobicity between their precursors.

Liquid-solid extraction methodology, which is analytical technique for sample preparation of solids by partition of analytes between the two involved phases (Priego-Capote, 2021), is the most practical approach to purify EFEs from the reactant for laboratory scale. Considering that the solubility of EFEs

is very low in both hydrophilic and hydrophobic solvents, the remnants of erythorbic acid and fatty acid are exclusively removed from the reactant using hydrophilic and hydrophobic solvents, respectively (Fig. 3d). First of all, a slurry of the reactant is obtained after ultrafiltration and/or evaporation to remove immobilized lipases and/or solvents. Then, *n*-hexane (or other food grade solvents) is added to the slurry to solubilize fatty acids into the extractant, and consequently insoluble materials as the solid matrix can be separated either through gravitational force (centrifugation) or vacuum filtration. Since this process traditionally exhibits low quantitative efficiency, sufficient repetitions (over 6 times) are necessary to eliminate the target compounds. Notably, performance can be enhanced with additional treatments such as high temperature, high pressure, and moderate auxiliary energies (*e.g.*, vortexing, homogenization, ultrasonication, etc.) (Meullemiestre, Petitcolas, Maache-Rezzoug, Chemat, & Rezzoug, 2016). Water is subsequently added to the slurry without fatty acids to solubilize erythorbic acids and immobilized lipase residues, and finally, insoluble materials containing only EFEs can be obtained in the same manner. In order to preserve the purified sample in a more stable state, lyophilization (freeze drying) is recommended. The purity (%) of EFEs in the final sample can be expressed in the absence of a standard curve for EFEs as follows (Kim et al., 2023):

$$Purity\ (\%) = \left(1 - \frac{M_e + M_f}{M_t}\right) \times 100\ (\%)$$

where M_e and M_f are the calculated mass of erythorbic acid and fatty acid from the quantitative analysis of the final sample, and M_t is the measured mass of the final sample. However, liquid-solid extraction is also inapplicable to purifying EFEs in some cases. Erythorbyl ricinoleate, which is synthesized using ricinoleic acid ($C_{18:1}$, *cis*-Δ^9, $-OH^{12}$) as a fatty acid substrate, was purified via preparatory HPLC since there is no solvent to selectively extract ricinoleic acid or erythorbyl ricinoleate (Park et al., 2023). Hence, there was an attempt to make the purification process unnecessary. Under modified two-step lipase-catalyzed esterification in GSL-MPS, approximately 100% lauric acid was converted into mixed lauric acid esters, requiring no further purification of fatty acids (Yu et al., 2022). This mixed lauric

acid esters containing erythorbyl laurate retains the multi-functionality of erythorbyl laurate. Therefore, researchers need to decide the purification strategy according to the solubility of substrates and products, desired compositions and aspects of products, and the target yield and purity values.

2.4. Chemical identification of synthesized EFEs

Immobilized lipase Lipozyme® 435 or Novozym® 435 can produce one type of erythorbyl monoester from the esterification of erythorbic acid and fatty acid (Kim et al., 2023; Park et al., 2023; Park et al., 2021; Park et al., 2011). Esterification between the C-6 hydroxyl group of erythorbic acid and the carboxyl group of fatty acid is selectively catalyzed depending on the chemoselectivity of the lipase having a steep funnel-shaped substrate binding pocket. What methodology can be employed to ascertain this? Although HPLC analysis can observe the production of a novel compound during the reaction, it is impossible to verify not only whether erythorbic acid and fatty acid are esterified into one molecule but also which of the hydroxyl groups of erythorbic acid is esterified. Therefore, three different analytical techniques, Fourier-transform infrared spectroscopy (FTIR), mass spectrometry (MS) combined with a liquid chromatography system, and nuclear magnetic resonance (NMR) spectroscopy have been applied to investigate the esterification of EFE.

First of all, FTIR is used to obtain an infrared absorption/emission spectrum of the target compound. Covalent bonds in one molecule selectively absorb infrared radiation of specific wavelengths, which induces the change of vibrational energy (stretching or bending) in the chemical bond (Griffiths, 1983). The transmittance (the flipside of absorbance) spectrum is different for each molecule depending on its chemical structure. After the lipase-catalyzed synthesis of EFEs, a relatively strong band around 1,700 cm^{-1} can be detected, which is the absorption of C=O stretching vibration in a synthesized ester bond (O=C−O). Stretch vibrations of the hydroxyl groups in erythorbic acid moiety (3000−3500 cm^{-1}) and the C−H bonds in fatty acid moiety (3000−2800 cm^{-1}) remain after esterification of EFEs. These patterns were commonly observed in the FTIR spectra of highly purified erythorbyl laurate (1,739

cm^{-1}) (Lee et al., 2012) and erythorbyl palmitate (1,711 cm^{-1}) (Sun et al., 2013). The FTIR methodology is useful to verify the production of EFEs more easily and rapidly than other techniques without sample damage; however, it explains neither molecular weight nor specific chemical structure of EFEs.

Second, MS is an analytical technique to measure the mass-to-charge (m/z) of one or more ionized molecules and calculate their exact molecular weights (Hoffmann & Stroobant, 2007). Liquid chromatography-mass spectrometry (LC-MS), which combines the physical separation capability of LC and the mass analysis capability of MS, is usually used to analyze mixtures of multiple compounds (Zhan, Thakur, Feng, Zhu, Zhang, & Wei, 2023). While HPLC or ultra-performance liquid chromatography (UPLC) separates unidentified compounds in a sample, MS provides spectral information to identify each separated compound after the ionization process (negatively or positively). In most cases of EFEs, each highly purified sample was analyzed by an LC-MS with an electrospray ionization interface to investigate their molecular weights and confirm the formation of ester bonds. Several specific patterns of the MS spectrum of EFEs can be obtained depending on the type of ionization and its operating condition. When target analytes are highly concentrated, multiple isotopes of $[2M-H]^-$ or $[2M+H]^+$ can be dominant as much as base peak ions of $[M-H]^-$ or $[M+H]^+$ in the MS spectrum (Fig. 3e). This phenomenon was observed in the majority of MS analyses of EFEs (Kim et al., 2023; Park et al., 2023). On the other hand, a peculiar ion of $[M-18+H]^+$ is often detected together with a base peak ion of $[M+H]^+$ in positive ion mode (Fig. 3f). During the ionization, protonated ions of ascorbic acid can incidentally be converted into $[M-(H_2O)+H]^+$ and $[M-2(H_2O)+H]^+$ ions by non-oxidative degradation (Cimino et al., 2018). Likewise, erythorbic acid moiety of EFEs can undergo this phenomenon theoretically, and $[M-(H_2O)+H]^+$ or $[M-2(H_2O)+H]^+$ ions of EFEs can be observed in their MS spectra. Notably, $[M-2(H_2O)+H]^+$ ions are generated in the presence of the C-6 hydroxyl group of erythorbic acid, indicating that no detection of $[M-2(H_2O)+H]^+$ ions involve the formation of ester bonds in the C-6 hydroxyl group of erythorbic acid during the lipase-catalyzed bioconversion (Cimino et al., 2018; Park et al., 2021). This explanation, of course, is incomplete and needs to be validated by

other clear-cut methodologies.

Finally, NMR spectroscopy is an advanced methodology for clearly identifying chemical structures of monomolecular organic compounds. Nuclei in a strong constant magnetic field are perturbed by an oscillating magnetic field and produce specific electromagnetic signals (resonance) with a frequency characteristic of the magnetic field at the nucleus (Claridge, 2016). Based on the measurement of absorption of the electromagnetic signals around each atomic nuclei, it provides information about the structure, dynamics, and chemical environment of the target molecule (Elyashberg, 2015; Fan & Lane, 2016). In most cases of EFEs, both ^{1}H NMR and ^{13}C NMR spectroscopies were applied in order to reveal the chemical structures of EFEs. In the ^{13}C NMR spectrum of highly purified EFEs, all chemical shifts corresponding to carbons of erythorbic acid (60−175 ppm) and fatty acid (10−40, 170−180 ppm) are detected (Fig. 3g), indicating that these substrates are merged into one molecule by lipase-catalyzed bioconversion. Compared to the ^{13}C NMR spectra of the precursors, a chemical shift of carbonyl carbon in the carboxyl group of fatty acid (170−180 ppm) retains or exhibits an up-field shift after the reaction, due to the formation of ester bonds. Chemical shifts of aliphatic C-5 and C-6 carbons with the hydroxyl groups in erythorbic acid (60−70 ppm) exhibit up-field and down-field shifts, respectively, although others remain intact after esterification. These NMR results commonly obtained from previous studies suggest the presence of an ester bond at the C-6 of the erythorbic acid moiety in EFEs (Kim et al., 2023; Park et al., 2023; Park et al., 2021). Moreover, this hypothesis was confirmed using two-dimensional [^{1}H–^{1}H] NMR correlation spectroscopy, which provides information about the structure of a complicated molecule. Detected correlations (proton couplings) between protons of C-6 (2H, –CH_2) and C-5 (1H, –OH) in the erythorbic acid moiety demonstrate that the hydroxyl group at C-5 of the erythorbic acid moiety in EFEs remains, in other words, the site of esterification is to be C-6 (Kim et al., 2023; Park et al., 2021). The esterification of EFEs is theoretically possible (and often actually occurs) at both hydroxyl groups (C-6 and C-5) of erythorbic acid; however, previous results validated that the immobilized lipase regioselectively catalyzes the esterification at the C-6 hydroxyl group of

erythorbic acid in practical experiments.

3. Amphiphilic Property of EFEs

A primary function of emulsifiers in food emulsions is to induce emulsification and improve colloidal stability (McClements, 2015). Conventional emulsifiers vary considerably in emulsifying characteristics and according to their chemical structures and fabricate a variety of colloidal structures such as aggregates, polymers, micelles, droplets, particles, and crystals (Kralova & Sjöblom, 2009). Small molecule emulsifiers typically have both hydrophilic and hydrophobic groups (head and tail) in a single molecule, and the nature of these moieties, called an amphiphilic property, determines their practical interfacial and emulsifying characteristics (Cox, Sandall, Smith, Rossi, & Whelan, 2021). Hence, describing these amphiphilic properties of emulsifiers is essential to find the most appropriate single emulsifier or combination (co-stabilization) for desired emulsions (McClements et al., 2018).

3.1. Amphiphilic structure of EFEs

Like other small molecule surfactants, EFEs have both hydrophilic and hydrophobic moieties, exhibiting an amphiphilic property. The hydrophilic moiety of EFEs is originated from erythorbic acid, which is a stereoisomer of ascorbic acid (vitamin C) and a food antioxidant (E315). The hydrophilic moiety of emulsifiers is differentiated by a degree of ionization (*e.g.*, non-ionic, anionic, cationic, and zwitterionic) (McClements, 2015). The majority of EFEs exists in the anionic form of the erythorbyl moiety at neutral and basic pH, while the non-ionic form increases at acidic pH (22.03% protonated erythorbyl laurate at pH 5.0) (Kim, Yu, Park, & Chang, 2020). The alterations in the ionic strength of EFEs might affect the surface properties of micelles or emulsions, as well as their multi-functionality. The hydrophobic moiety of EFEs consists of a hydrocarbon fatty acid like other conventional small molecule emulsifiers. This moiety increases the hydrophobicity of EFEs, which mainly influences the interfacial and emulsifying properties of EFEs. To make EFE-based emulsifiers individually suitable

for each desired emulsifying condition, the fatty acid derivatization has been attempted.

Before investigating the actual interfacial and emulsifying properties of emulsifiers, the amphiphilic property can be theoretically predicted based on their molecular structures. The hydrophile-lipophile balance (HLB) is a widely used concept for indicating their relative affinity for each type of emulsion (Pasquali, Taurozzi, & Bregni, 2008; Yamashita & Sakamoto, 2016). Food emulsions, which are typically composed of water and lipids, can be divided into three different types according to the oil-water phase configuration: (1) oil-in-water (O/W) emulsion; (2) water-in-oil (W/O) emulsion; (3) multiple emulsion (Sundarraj & Ranganathan, 2019). An emulsifier with a high HLB value (8−16) preferentially forms stable micelles in the aqueous phase and stabilizes O/W emulsions. In most cases, the HLB values of EFEs were calculated based on the Davies method as follows (Davies, 2003):

$$HLB\ value\ =\ 7 + \sum(h_g m_h) + \sum(l_g n_l)$$

where h_g is the hydrophilic group number, l_g is the lipophilic group number, m_h is the frequency of the hydrophilic group, and n_l is the frequency of the lipophilic group. The HLB value of EFEs (9.60−13.40) indicates that they might be suitable for forming an O/W emulsion (Table 1). Indeed, previous studies have produced O/W emulsions using EFEs as emulsifiers or co-stabilizers. Notably, the HLB value of EFEs gradually decreases as the chain length of fatty acid moiety elongates, meaning the increase of hydrophobicity. The hydrophobicity of emulsifiers can be estimated by the *n*-octanol/water partition coefficient (log *P*) based on the atom/fragment contribution method of Meylan and Howard as follows (Meylan & Howard, 1995):

$$Log\ P\ =\ 0.229\ +\ \sum(f_i m_i) + \sum(c_j n_j)$$

where f_i is the coefficient of the i^{th} fragment, c_j is the coefficient of the j^{th} correction factor, and m_i and n_j are the frequency of each coefficient. The log *P* value reflects the relationship between hydrophilicity and hydrophobicity of emulsifiers, and it demonstrates the hydrophobicity of EFEs (Table 1). Unfortunately, however, one exception, erythorbyl oleate with an unsaturated fatty acid (oleic acid, $C_{18:0}$),

is unable to stabilize the O/W emulsion (Park et al., 2023), although its theoretical amphiphilicity is the same as that of erythorbyl stearate. This study highlights the importance of experimental indicators of amphiphilicity to understand the actual interfacial and emulsifying properties of emulsifiers.

3.2. Interfacial and emulsifying properties of EFEs

In an aqueous phase, small amphiphilic molecules showing interfacial activity (called surfactants) can spontaneously form micelles, which are spherical colloidal structures with a hydrophobic core and a hydrophilic shell, when their concentration exceeds a certain level called critical micelle concentration (CMC) in which the surface tension becomes independent of the concentration (Israelachvili, 2011; McClements, 2015). The shape and behavior of micelles depends on the arrangement of amphiphilic molecules at the interface, and it is mainly determined by the amphiphilicity of emulsifiers. Hence, the CMC of each emulsifier provides more practical and quantitative information about its interfacial and emulsifying properties. The CMC can be determined by several methodologies (Mabrouk, Hamed, & Mansour, 2023), but typically calculated from the measurement of changes in interfacial tension depending on the concentration of emulsifiers (Israelachvili, 2011; McClements, 2015). The CMC of erythorbyl laurate was measured as 0.101 mM at 25°C using a Wilhelmy plate tensiometer (Park et al., 2017). This is the simplest and most accurate method for surface tension measurement, though a considerable amount of emulsifier is required for a single measurement. The CMC of erythorbyl myristate was also measured as 0.36 mM at 25°C using an isothermal titration calorimetry (ITC) (Loh, Brinatti, & Tam, 2016; Park et al., 2021). Below the CMC of emulsifiers, micelles spontaneously disintegrate and release the heat of demicellization. The exothermic ITC trace of enthalpy change during the demicellization can describe the micellar thermodynamic behavior and consequently calculate the CMC of emulsifiers (Hildebrand, Garidel, Neubert, & Blume, 2004).

In a more recent study, comparative analyses of the interfacial characteristics of EFEs via the pendant drop method have been conducted to investigate the effects of the fatty acid chain length on the

amphiphilicity of EFEs (Berry, Neeson, Dagastine, Chan, & Tabor, 2015; Kim et al., 2023; Park et al., 2023; Tamm, Sauer, Scampicchio, & Drusch, 2012). The interfacial tension of the oil-water interface under each EFE concentration was measured from the shape of a pendant drop (the curvature of the interface). The CMC of EFEs dramatically decreased as their hydrophobicity increased (Table 1), indicating that the elongation of the fatty acid chain promotes the formation of micelles or O/W emulsions. It is because EFEs might stabilize the colloidal systems through weak hydrophobic interactions among their fatty acid moieties (Czajka, Hazell, & Eastoe, 2015; Kim et al., 2023; Nowicki, Łuczak, & Stańczyk, 2016). In contrast, erythorbyl oleate reduced no interfacial tension despite its amphiphilicity because the skewed geometry of the *cis* double bond inhibits hydrophobic interaction and accumulation at the interface phase (Park et al., 2023). Hence, the balance between hydrophobicity and chemical structure is also crucial for EFEs to exhibit interfacial characteristics. For example, polysorbate 80 (containing oleic acid) or erythorbyl ricinoleate exhibited strong interfacial characteristics (Park et al., 2023), probably due to intermolecular hydrogen bonding induced by their strong hydrophilic groups.

Generally, emulsion droplets can be assembled under conditions of proper concentrations of water and lipids together with sufficient energy inputs such as homogenization or ultrasonification. However, these common emulsions without emulsifiers for the colloidal stabilization are inherently unstable and rapidly separate into two phases due to thermodynamically unfavorable contact between water and lipid molecules. Using EFEs, except erythorbyl oleate, as emulsifiers or co-stabilizers, the O/W emulsions mimicking the beverage type of emulsion-based food can be fabricated in a highly stable form. Park *et al*. prepared O/W emulsions of 5%(w/v) soybean oil and 0.2%(w/v) erythorbyl laurate by homogenization for the first time (Park et al., 2017). Under a wide range of colloidal conditions such as droplet size (200−700) and co-stabilizer (Tween 20−80), the colloidal stability of 5%(w/w) O/W emulsions treated with various concentrations of erythorbyl laurate was maintained during experiment (maximum 60°C, 12 days) (Park, Choi, Yu, Choi, Park, & Chang, 2022a). Several recent studies also have manufactured 5%(w/w) O/W emulsions containing each EFE as a co-stabilizer of polysorbates

(Tween) or a single emulsifier (Kim et al., 2023; Park et al., 2023). Notably, O/W emulsions stabilized either by erythorbyl laurate or erythorbyl myristate showed slight destabilization relatively earlier than erythorbyl ricinoleate under the same storage conditions (25°C, 15 days) (Park et al., 2023), implying that the hydroxyl group (−OH) of erythorbyl ricinoleate might promote the formation of stable O/W emulsions, as aforementioned. On the other hand, O/W emulsions stabilized by erythorbyl oleate maintained their colloidal stabilities for only a short time.

These summarized results provide critical information about the amphiphilic properties of EFEs and support their practical applicability as promising emulsifiers in the food industry.

4. Antioxidative Property of EFEs

Oxidation-reduction is a chemical reaction that involves the transfer of electrons, oxygen atoms, or hydrogen atoms between substances. These reactions commonly occur in foods, and some are beneficial although others can lead to detrimental effects, including degradation of lipids, vitamins, pigments, and other food compounds with loss of nutrition and development of undesirable off-flavors (Jacobsen & Sørensen, 2015; Owen et al., 2017). In particular, lipids in emulsion-based food products are highly susceptible to oxidation under stimulating conditions. Decomposed triacylglycerols and phospholipids can produce several detrimental volatile organic compounds through auto-, photo-, thermal-, and enzymatic oxidations (Berton-Carabin, Ropers, & Genot, 2014), and this process is referred to as oxidative rancidification. Control of oxidative rancidification have been achieved by employing processing and packaging techniques or adding proper antioxidants, and of course, in practice, the most effective means is to use a combination of different antioxidant strategies. However, the efficacy of conventional hydrophilic antioxidants is often unpredictable or even worse totally neutralized in emulsion-based food products due to their high levels of lipids and colloidal complexity. For that reason, research has focused on the synthesis of non-polar antioxidants or amphiphilic derivatives of antioxidants. For example, ascorbic acid, which is a natural water-soluble food antioxidant (E300) as a reducing or oxygen-

scavenging agent, can be made less polar by esterification with fatty acids to form fatty acid derivatives, such as ascorbyl palmitate (E304) (Karmee, 2009; Stamatis, Sereti, & Kolisis, 1999).

4.1. Antioxidative mechanism of EFEs

In a similar manner to ascorbyl fatty acid esters, the amphiphilic EFEs with an erythorbic acid moiety can also act as antioxidants themselves. The free hydroxyl groups at C-2 and C-3 carbons of ascorbic acid as well as its stereoisomeric erythorbic acid, can form radicals by transferring hydrogens and electrons as reducing agents (Fig. 4a) or react readily with oxygen as oxygen scavengers. Fortunately, the C-2 and C-3 hydroxyl groups of erythorbic acid are totally conserved even after esterification with fatty acids because the immobilized lipase Lipozyme® 435 or Novozym® 435 regioselectively reacts with the C-6 hydroxyl group of erythorbic acid. Upon removing two hydrogen atoms and two electrons from an erythorbic acid moiety of EFEs, it can be converted into dehydroerythorbic acid, which has no antioxidative properties (Nimse & Pal, 2015). Notably, a lactone ring of dehydroerythorbic acid induces irreversible degradation in strong alkaline conditions, resulting in decrement of UV absorption (Kim et al., 2020). In most cases of free radical scavenging assays on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, EFEs with a saturated fatty acid moiety exhibited effective radical scavenging activities on both DPPH and ABTS radicals, similar to that of erythorbic acid at the same molar concentration. These results explained that the antioxidative properties of EFEs in the aqueous phase are attributed to their erythorbic acid moieties (more specifically, the free hydroxyl groups at C-2 and C-3 carbons), regardless of alkyl chain length (Kim et al., 2023). It was reported that the radical scavenging activity of erythorbyl laurate was almost identical to that of ascorbyl palmitate (Park et al., 2017), providing another evidence to support the proposed mode of action. On the other hand, erythorbyl ricinoleate exhibited less radical scavenging activity than erythorbic acid (Park et al., 2023). Compared to other EFEs with saturated alkyl chains, the fatty acid moiety of erythorbyl ricinoleate has a skewed geometry of a *cis*-type double bond. Due

to this structure, erythorbyl ricinoleate might be relatively inaccessible to free radicals, and in other words, effective collisions are insufficient for antioxidative reactions.

4.2. Antioxidative property of EFEs in the O/W emulsion

Amphiphilic EFEs can act more effectively and efficiently in emulsion-based food products as antioxidative emulsifiers. Lipids in common oil-water two-phase systems or O/W emulsion systems, in which water is still much more than lipids, undergo rancidification at the oil-water interface (Fig. 4b). Oxidation products such as lipid peroxides, volatile aldehydes, ketones, and dicarboxylic acids, which are relatively polar compounds, tend to migrate away from the nonpolar lipid phase to the polar aqueous phase. The use of polar antioxidants in emulsion-based food products almost totally neutralizes their antioxidative properties because polar antioxidants are drawn to the vast aqueous phase and far away from the lipid phase (oil droplet or emulsion droplet) (Laguerre et al., 2015). How about nonpolar antioxidants, which are retained in oil and emulsion droplets, such as α-tocopherol (E307), butylated hydroxyanisole (E320), and butylated hydroxytoluene (E321)? These lipophilic food antioxidants are conventionally used for emulsion-based food products because of their proximity to where lipid oxidation is prevalent. However, depending on the amounts of lipids and their colloidal complexity, it can be considerably difficult to predict, maintain, and control the efficacy of antioxidants (Decker et al., 2017). It is therefore suitable to select amphiphilic antioxidants that can be localized at the oil-water interface in O/W emulsions. Hydrophilic erythorbic acid moieties of EFEs are highly enriched at the oil-water interface thereby preventing the neutralization of the antioxidative property and retarding lipid oxidation more effectively than one-sided antioxidants like erythorbic acid.

In the previous studies, two conventional methodologies have been used to detect rancidification of lipids in emulsion-based food products. One is ferric thiocyanate (FeSCN) colorimetry to measure the primary products of lipid oxidation (called peroxide value). Lipid peroxides are generally unstable and easily react with ferrous ions (Fe^{2+}) to produce ferric ions (Fe^{3+}), which can be detected by thiocyanate

ions (SCN^-) as follows (Gasparovic, Jaganjac, Mihaljevic, Sunjic, & Zarkovic, 2013):

$$ROOH + Fe^{2+} \rightarrow RO^{\bullet} + Fe^{3+}$$

$$RO^{\bullet} + Fe^{2+} + H^{+} \rightarrow ROH + Fe^{3+}$$

$$Fe^{3+} + SCN^{-} \rightarrow FeSCN^{2+}\,(red)$$

The other method is thiobarbituric acid reactive substances (TBARS) test, also known as thiobarbituric acid (TBA), to measure the amounts of secondary products from lipid oxidation, especially malonaldehyde. Malonaldehyde is a split product of an endoperoxide of unsaturated fatty acids and reacts with TBA, resulting in the malonaldehyde-TBA complex (pink) (O'Keefe & Pike, 2010). Under both thermal- and photo-oxidation conditions, compared to O/W emulsions stabilized with Tween 20, the production of lipid peroxides and TBARS was greatly mitigated in O/W emulsions stabilized with erythorbyl laurate in a concentration-dependent manner (Park et al., 2017). Notably, the addition of erythorbic acid (the same moles as erythorbyl laurate) into the Tween 20-stabilized O/W emulsion also alleviated the production of lipid peroxides, although the rate of production was still faster than that of erythorbyl laurate-stabilized O/W emulsion.

Additionally, unlike in aqueous phase, the antioxidative properties of EFEs in O/W emulsions can be affected by their chemical structures (the length of the fatty acid moiety) and several colloidal conditions (droplet size and co-stabilizer). It was reported that the antioxidative properties of EFEs as co-stabilizers in Tween 20-stabilized O/W emulsions increase as the chain length of fatty acid moiety elongates (erythorbyl palmitate ≥ erythorbyl stearate > erythorbyl myristate > erythorbyl laurate > erythorbyl caprate) under thermal acceleration conditions (40°C, 18 days) (Kim et al., 2023). The solubility of alkyl ester homologs in emulsion phase (water, oil, and oil-water interface) affects their partitioning behaviors at the oil-water interface (Costa, Losada-Barreiro, Paiva-Martins, & Bravo-Díaz, 2017), suggesting that the difference in the antioxidative properties of EFEs is because they might be partitioned at the oil-water interface as their solubilities in the oil phase decrease. Colloidal conditions

of O/W emulsions, such as co-stabilizer and droplet size, also affect the retardation of lipid oxidation by EFEs. When emulsifier molecules associate with each other, they tend to form a monolayer at the oil-water interface, which has an optimum curvature to pack the molecules (McClements, 2015), indicating that structural similarity between EFEs and co-stabilizers influences their arrangement and effective concentration at the oil-water interface. The rate of lipid oxidation in Tween 20-stabilized O/W emulsions treated with erythorbyl laurate (notably, they have the same fatty acid moiety) was the slowest among Tween 20−80 co-stabilizers (Park et al., 2022a). The droplet size of O/W emulsions is generally known to affect the rate of lipid oxidation based on the change in surface area. Although the rate of lipid oxidation decreased as the droplet size of O/W emulsions increased, erythorbyl laurate exhibited its antioxidative property irrespective of droplet size (Park et al., 2022a), due to the localization of a fixed amount of EFEs at the oil-water interface.

These summarized results provide critical information about the antioxidative properties of EFEs and support their practical applicability as promising antioxidative emulsifiers in the food industry.

5. Antibacterial Property of EFEs

The final major functionality of EFEs is the antibacterial property (in a large sense, the antimicrobial property), and this is why EFEs are proposed as multi-functional food emulsifiers. Emulsion-based food products are highly susceptible to being contaminated by food-borne pathogens because of their nutritionally-abundant environment and difficulty to implement sterilization processes. Hence, the use of antibacterial agents is an indispensable portion of ensuring the safety of emulsion-based food products. Over the past few decades, numerous food preservatives with lipophilicity or amphiphilicity, such as lipophilic acids (Freese, Sheu, & Galliers, 1973), medium-chain fatty acids (Yoon, Jackman, Valle-González, & Cho, 2018), sugar fatty acid esters (Neta et al., 2015), and lipopeptides (Raaijmakers, De Bruijn, Nybroe, & Ongena, 2010), have been investigated and used in practical applications. In particular, natural fatty acids and their derivatives are highly suitable antibacterial additives for emulsion-

based food products because these compounds are generally recognized as safe (non-toxic to humans) and free of bacterial resistance, despite the relatively low antibacterial efficacy and narrow spectrum. Indeed, lauric acid and its derivatives synthesized by the immobilized-lipase esterification have been proposed as antibacterial agents against Gram-positive food-borne pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes* (Dayrit, 2015; Kabara, 1984; Lieberman, Enig, & Preuss, 2006). The main target of these amphiphilic antibacterials is to reduce or disrupt the integrity of bacterial cytoplasmic membranes based on their amphiphilic property (Desbois & Smith, 2010; Park et al., 2018a). This leads to significant bacterial reduction via the collapse of cytoplasmic membranes and subsequent leakage of intracellular components.

5.1. Antibacterial spectrum of EFEs

Amphiphilic EFEs with a non-ionic hydrophilic moiety (erythorbic acid) can also act as antibacterial agents themselves. All developed EFEs, except erythorbyl oleate (not evaluated), exhibit bacteriostatic activities against Gram-positive food-borne pathogens in the aqueous phase (Kim et al., 2023; Park et al., 2023; Park et al., 2021; Park et al., 2018a). Under the treatment of EFEs, the lag time of bacterial growth dramatically increased as well as the maximum specific growth rate decreased, although there are discrepancies depending on the bacterial strain and the amphiphilic structure of EFEs. Furthermore, several studies on erythorbyl laurate and erythorbyl myristate showed that they have bactericidal activities against Gram-positive bacteria in the aqueous phase (Park et al., 2021; Park et al., 2018a). No viable vegetative cells were observed under the treatment of EFEs above each minimum bactericidal concentration, indicating that their mode of action is directly related to the killing of microorganisms. Meanwhile, EFEs are totally inactive against Gram-negative food-borne pathogens, including *Escherichia coli* and *Salmonella* Typhimurium, in the aqueous phase (Kim et al., 2023; Park et al., 2023; Park et al., 2021; Park et al., 2018a), due to the structural differences in the cytoplasmic membranes of Gram-positive and Gram-negative bacteria (Fig. 5). Notably, it was reported that EFEs can control

the microbial contamination of both Gram-positive and Gram-negative bacteria in the O/W emulsion. This will be discussed further below along with the proposed mechanism. In addition, EFEs can control thermophilic spore-forming bacteria based on the same antibacterial mechanism. Erythorbyl laurate not only showed antibacterial properties on the vegetative cells of *Geobacillus stearothermophilus*, which is a spore-forming bacteria isolated from dairy products, but also inhibited its spore germination (both sporostatic and sporicidal) in both aqueous and O/W emulsion systems (Shin, Kwon, Lee, Yu, & Chang, 2022). This result proposes that EFEs can be used as alternatives to conventional spore-controlling agents such as sucrose fatty acid esters (E473), which prevent the flat-sour spoilage caused by spore germination in emulsion-based food products stored in heat cabinets. Finally, it is proposed that the antimicrobial spectrum of EFEs extends beyond bacteria. Several molds (*e.g.*, *Aspergillus*, *Rhizopus*, and *Trichophyton*) and yeasts (*e.g.*, *Candida*) were significantly susceptible to the treatment of erythorbyl laurate in a preliminary disk diffusion assay (Kim et al., 2020). The antimicrobial spectrum of EFEs, of course, needs to be further validated, although the previous results sufficiently showed the possibility of applying EFEs as more inclusive antimicrobial agents in the food industry.

5.2. Antibacterial mechanism of EFEs

The amphiphilic properties of EFEs have been proposed to be fundamentally responsible for their antibacterial properties, similar to other amphiphilic antibacterial compounds. Several studies on the antibacterial mechanism elucidate that EFEs can interact with the bacterial cytoplasmic membranes. Using crystal violet uptake and bacterial viability assays, it was confirmed that erythorbyl laurate can alter membrane permeability up to a minimum inhibitory concentration without cell death (Park et al., 2018a). This phenomenon was similar to that of nisin (E234), which targets dissipating potential and pH gradient of the bacterial cytoplasmic membrane (Breukink, Van Kraaij, Demel, Siezen, Kuipers, & De Kruijff, 1997), in contrast to antibiotics like ampicillin, which permanently inhibits transpeptidases (Rafailidis, Ioannidou, & Falagas, 2007). Morphological analyses using microscopic techniques

visualized the membrane disintegrations induced by EFEs. Membrane damages and release of intracellular components were observed in the microscopic images of both vegetative cells (cytoplasmic membrane) and spores (inner membrane) of Gram-positive bacteria treated with sublethal concentrations of EFEs (Park et al., 2021; Park et al., 2018a; Shin et al., 2022). In addition to this physiological evidence, transcriptomics based on RNA sequencing (RNA-Seq) provided genetic insights to support the antibacterial modes of action of EFEs. It was found that the treatment of erythorbyl laurate significantly up-regulated gene expression of the *vra*SR signal transduction and other related genes in Gram-positive *S. aureus* Newman (Park et al., 2019). The genes of *vra*SR encode a two-component regulatory system composed of VraS (sensor kinase) and VraR (response regulator), which accomplishes the expression of genes involved in peptidoglycan biosynthesis in response to cell wall or membrane deficiencies (McCallum, Stutzmann Meier, Heusser, & Berger-Bächi, 2011). This transcript profile suggests that EFEs can work as cell wall-active agents targeting the membrane, consistent with other evidence.

Finally, similar to other antimicrobials, EFEs should be treated above a certain concentration (called MIC or MBC) to control microbial growth through sufficient membrane depolarization and disruption. Nevertheless, it was reported that the fatty acid chain length of EFEs critically affected their antibacterial properties. Erythorbyl myristate with a medium-chain alkyl moiety (myristic acid, $C_{14:0}$) showed the strongest bacteriostatic activity among EFE derivatives of saturated fatty acids, and the antibacterial activity decreased with longer alkyl chain lengths, exhibiting an inverted U-shaped profile (Kim et al., 2023). These results proposed that appropriate solubility (or hydrophobicity) of EFEs and their affinities to bacterial membrane are critical to determine their antibacterial properties. Indeed, another comparative study on the antibacterial properties of lauric acid esters derivatized with different non-fatty acid moieties also suggested that a relatively low lipophilicity of amphiphilic compounds was favorable for interacting with bacterial cytoplasmic membranes (Park et al., 2018b). Taken together, here is the proposed mechanism for antibacterial behavior of EFEs (Fig. 5): (1) Hydrophilic erythorbyl

moieties of EFEs (non-ionic or anionic) adhere to the hydrophilic surface of the bacterial cytoplasmic membranes, (2) Hydrophobic alkyl moieties of EFEs are inserted into the membranes by hydrophobic interaction with phosphatidylglycerols, (3) Inserted EFEs depolarize the surface of membranes, perturb their permeability, and disrupt them (by forming pores and releasing intracellular components).

Unfortunately, however, Gram-negative bacteria are non-susceptible to EFEs in the aqueous phase because their inner membranes are protected by outer membranes with negatively-charged lipopolysaccharide (LPS) molecules (Fig. 5). The EFE molecules are sufficiently small to penetrate the peptidoglycan layer, whereas they are inaccessible to the LPS layer due to electrostatic repulsion and steric hindrance. Therefore, food preservatives targeting Gram-negative bacteria are generally coupled with other treatments such as heat shock and chelator EDTA to dismantle the outer membrane (Alirezalu et al., 2020; Martin-Visscher, Yoganathan, Sit, Lohans, & Vederas, 2011). This is called hurdle technology. Several recent studies showed the synergistic effects of UV irradiation (Chang, Bai, Yu, Chang, & Nitin, 2021) or mild heat shock (Chang, Bai, Yu, Yang, Chang, & Nitin, 2022) on the treatment of EFEs to control Gram-negative bacteria (*Escherichia coli*) in the aqueous phase. On the other hand, EFEs can reduce the bacterial proliferation of both Gram-positive and Gram-negative food-borne pathogens in the O/W emulsion system. It has been reported that erythorbyl laurate in the O/W emulsions co-stabilized by polysorbate emulsifiers exhibited both bacteriostatic and bactericidal activities against Gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa*, while the emulsions without erythorbyl laurate showed no antibacterial property (Kim et al., 2020; Park et al., 2022a). Despite being poorly understood, the use of non-ionic emulsifiers to co-stabilize emulsions might reduce the interfacial negative charge to overcome electrostatic repulsion of LPS layers (Kim et al., 2020). The colloidal structure of O/W emulsion is also considered to play a crucial role in penetrating LPS layers and outer membranes because the surface of the droplet is more hydrophilic than EFEs themselves (Park et al., 2023; Teng, Stewart, Hai, Li, Banwell, & Lan, 2021). Indeed, colloidal conditions of emulsions affect the antibacterial property of EFEs. Like the antioxidative property, structural similarity between EFEs

and co-stabilizers determines the antibacterial properties of EFEs depending on their arrangement at the oil-water interface. It was observed that, under 0.1%(w/w) erythorbyl laurate, the decimal reduction time (D-value) of *E. coli* was the shortest in Tween 20-stabilized O/W emulsions and the longest in Tween 80-stabilized O/W emulsions (Park et al., 2022a). In the same study, the antibacterial properties of EFEs in O/W emulsions were slightly stronger as the droplet size increased, unlike the antioxidative properties. This is because the emulsion droplets might be more likely to come in contact with bacteria when they are relatively enlarged. Finally, a multistep resistance selection study confirmed that EFEs can develop no resistance to bacteria for 20 passages, corresponding to their proposed mode of action.

These summarized results provide critical information about the antibacterial properties of EFEs and support their practical applicability as promising antibacterial emulsifiers in the food industry.

6. Conclusions and Future Perspectives

Emulsion science and technology are commonly used in the food industry to create a wide variety of emulsion-based food products. There have been numerous emulsifiers currently available to manufacture stable food emulsions. More recently, food scientists have focused on developing multi-functional emulsifiers to simultaneously control lipid oxidation and bacterial growth in food emulsions. In this review article, the current scientific knowledge of EFEs as promising multi-functional food emulsifiers was discussed, especially the practical information underlying the lipase-catalyzed synthesis of EFEs as well as the proposed mechanisms of their multi-functionality. It is noteworthy to introduce a solvent-free GSL-MPS for the eco-friendly synthesis of EFEs, considering recent safety and sustainability concerns surrounding the food industry. All the topics covered here would provide researchers with comprehensive insights into the development and application of a multi-functional emulsifier.

Finally, it is firstly important to ascertain the chemical properties of each emulsifier, including functional characteristics and toxicities, as food ingredients. However, considering practical applications in industry, it has become more imperative to employ hurdle technology combined with non-chemical

processes to overcome the complexity of emulsion-based food products in the future. The combination of erythorbyl laurate and other processes showed synergistic effects on bacterial reduction (Chang et al., 2021; Chang et al., 2022). Moreover, a newly-developed compound may exhibit unintended functionality! Our EFEs showed an anti-inflammatory effect in the early stage of atherosclerosis by suppressing TNF-α-induced monocyte adhesion of the vascular endothelium and expression of inflammatory cytokines (Ha et al., 2021). These further multidisciplinary approaches will spur the development of multi-functional emulsifiers or food additives in the fields of food and emulsion chemistry.

CRediT Authorship Contribution Statement

Jun-Young Park: Conceptualization, Data curation, Writing - Original draft, Writing - Review & Editing, Visualization. **Hyunjong Yu**: Conceptualization, Writing - Original draft, Writing - Review & Editing. **Dimitris Charalampopoulos**: Writing - Review & Editing. **Kyung-Min Park**: Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition. **Pahn-Shick Chang**: Conceptualization, Writing - Review & Editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was carried out with the supports of the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020R1C1C1009678) and “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01488802)” provided by Rural Development Administration, Republic of Korea.

Data Availability

No data was used for the research described in the article.

References

Alirezalu, K., Munekata, P. E. S., Parniakov, O., Barba, F. J., Witt, J., Toepfl, S., . . . Lorenzo, J. M. (2020). Pulsed electric field and mild heating for milk processing: A review on recent advances. *Journal of the Science of Food and Agriculture, 100*(1), 16-24.

Arcens, D., Grau, E., Grelier, S., Cramail, H., & Peruch, F. (2020). Impact of fatty acid structure on CALB-catalyzed esterification of glucose. *European Journal of Lipid Science and Technology, 122*(4), 1900294.

Berry, J. D., Neeson, M. J., Dagastine, R. R., Chan, D. Y. C., & Tabor, R. F. (2015). Measurement of surface and interfacial tension using pendant drop tensiometry. *Journal of Colloid and Interface Science, 454*, 226-237.

Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 945-977.

Bravi, E., Perretti, G., & Montanari, L. (2006). Fatty acids by high-performance liquid chromatography and evaporative light-scattering detector. *Journal of Chromatography A, 1134*(1), 210-214.

Breukink, E., Van Kraaij, C., Demel, R. A., Siezen, R. J., Kuipers, O. P., & De Kruijff, B. (1997). The C-terminal region of nisin is responsible for the initial interaction of nisin with the target membrane. *Biochemistry, 36*(23), 6968-6976.

Cabrera, Z., Fernandez-Lorente, G., Fernandez-Lafuente, R., Palomo, J. M., & Guisan, J. M. (2009). Novozym 435 displays very different selectivity compared to lipase from *Candida antarctica* B adsorbed on other hydrophobic supports. *Journal of Molecular Catalysis B: Enzymatic, 57*(1), 171-176.

Chang, Y., Bai, J., Yu, H., Chang, P.-S., & Nitin, N. (2021). Synergistic inactivation of bacteria using a combination of erythorbyl laurate and UV type-A light treatment. *Frontiers in Microbiology, 12*.

Chang, Y., Bai, J., Yu, H., Yang, X., Chang, P.-S., & Nitin, N. (2022). Synergistic inactivation of *Listeria* and *E. coli* using a combination of erythorbyl laurate and mild heating and its application in decontamination of peas as a model fresh produce. *Food Microbiology, 102*, 103869.

Choi, Y., & Chang, P.-S. (2022). Kinetic modeling of lipase-catalysed hydrolysis of triacylglycerol in a reverse micelle system for the determination of integral stereoselectivity. *Catalysis Science & Technology, 12*(9), 2819-2828.

Choi, Y., Park, J.-Y., & Chang, P.-S. (2021). Integral stereoselectivity of lipase based on the chromatographic resolution of enantiomeric/regioisomeric diacylglycerols. *Journal of Agricultural and Food Chemistry, 69*(1), 325-331.

Cimino, P., Troiani, A., Pepi, F., Garzoli, S., Salvitti, C., Di Rienzo, B., . . . Ricci, A. (2018). From ascorbic acid to furan derivatives: The gas phase acid catalyzed degradation of vitamin C. *Physical Chemistry Chemical Physics, 20*(25), 17132-17140.

Claridge, T. D. W. (2016). *High-resolution NMR techniques in organic chemistry*. (3rd ed.): Elsevier.

Coelho, A. L. S., & Orlandelli, R. C. (2021). Immobilized microbial lipases in the food industry: A systematic literature review. *Critical Reviews in Food Science and Nutrition, 61*(10), 1689-1703.

Costa, M., Losada-Barreiro, S., Paiva-Martins, F., & Bravo-Díaz, C. (2017). Physical evidence that the variations in the efficiency of homologous series of antioxidants in emulsions are a result of differences in their distribution. *Journal of the Science of Food and Agriculture, 97*(2), 564-571.

Cox, S., Sandall, A., Smith, L., Rossi, M., & Whelan, K. (2021). Food additive emulsifiers: A review of their role in foods, legislation and classifications, presence in food supply, dietary exposure, and safety assessment. *Nutrition Reviews, 79*(6), 726-741.

Cui, F.-J., Zhao, H.-X., Sun, W.-J., Wei, Z., Yu, S.-L., Zhou, Q., & Dong, Y. (2013). Ultrasound-assisted

lipase-catalyzed synthesis of D-isoascorbyl palmitate: Process optimization and kinetic evaluation. *Chemistry Central Journal, 7*(1), 180.

Czajka, A., Hazell, G., & Eastoe, J. (2015). Surfactants at the design limit. *Langmuir, 31*(30), 8205-8217.

Davies, J. (2003). A quatitative kinetic theory of emulsion type. I. Physical chemistry of the emulsifying. *Proceedings of 2nd International Congress Surface Activity* (pp. 426–438).

Dayrit, F. M. (2015). The properties of lauric acid and their significance in coconut oil. *Journal of the American Oil Chemists' Society, 92*(1), 1-15.

Decker, E. A., McClements, D. J., Bourlieu-Lacanal, C., Durand, E., Figueroa-Espinoza, M. C., Lecomte, J., & Villeneuve, P. (2017). Hurdles in predicting antioxidant efficacy in oil-in-water emulsions. *Trends in Food Science & Technology, 67*, 183-194.

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.

Elyashberg, M. (2015). Identification and structure elucidation by NMR spectroscopy. *TrAC Trends in Analytical Chemistry, 69*, 88-97.

Fan, T. W. M., & Lane, A. N. (2016). Applications of NMR spectroscopy to systems biochemistry. *Progress in Nuclear Magnetic Resonance Spectroscopy, 92-93*, 18-53.

Freese, E., Sheu, C. W., & Galliers, E. (1973). Function of lipophilic acids as antimicrobial food additives. *Nature, 241*(5388), 321-325.

Gasparovic, A. C., Jaganjac, M., Mihaljevic, B., Sunjic, S. B., & Zarkovic, N. (2013). Assays for the measurement of lipid peroxidation. In L. Galluzzi, I. Vitale, O. Kepp & G. Kroemer (Eds.), *Cell senescence: Methods and protocols* (pp. 283-296). Totowa, NJ. Humana Press.

Griffiths, P. R. (1983). Fourier transform infrared spectrometry. *Science, 222*(4621), 297-302.

Guillén, M. D., & Cabo, N. (1997). Infrared spectroscopy in the study of edible oils and fats. *Journal of the Science of Food and Agriculture, 75*(1), 1-11.

Ha, S. J., Kim, M. J., Park, J., Choi, H.-W., Yu, H., Chang, P.-S., & Jung, S. K. (2021). Erythorbyl laurate suppresses TNF-α-induced adhesion of monocytes to the vascular endothelium. *Journal of Functional Foods, 80*, 104428.

Hildebrand, A., Garidel, P., Neubert, R., & Blume, A. (2004). Thermodynamics of demicellization of mixed micelles composed of sodium oleate and bile salts. *Langmuir, 20*(2), 320-328.

Hilterhaus, L., Thum, O., & Liese, A. (2008). Reactor concept for lipase-catalyzed solvent-free conversion of highly viscous reactants forming two-phase systems. *Organic Process Research & Development, 12*(4), 618-625.

Hoffmann, E. d., & Stroobant, V. (2007). *Mass spectrometry: Principles and applications*. (3rd ed.): WILEY.

Hopia, A. I., & Ollilainen, V. M. (1993). Comparison of the evaporative light scattering detector (ELSD) and refractive index detector (RID) in lipid analysis. *Journal of Liquid Chromatography, 16*(12), 2469-2482.

Israelachvili, J. N. (2011). *Intermolecular and surface forces*. (3rd ed.): Academic Press.

Jacobsen, C., & Sørensen, A.-D. M. (2015). The use of antioxidants in the preservation of food emulsion systems. In *Handbook of antioxidants for food preservation* (pp. 389-412). Elsevier.

Jarvis, W. R., & Highsmith, A. K. (1984). Bacterial growth and endotoxin production in lipid emulsion. *Journal of Clinical Microbiology, 19*(1), 17-20.

José, C., Bonetto, R. D., Gambaro, L. A., Torres, M. d. P. G., Foresti, M. L., Ferreira, M. L., & Briand, L. E. (2011). Investigation of the causes of deactivation–degradation of the commercial biocatalyst Novozym® 435 in ethanol and ethanol–aqueous media. *Journal of Molecular Catalysis B: Enzymatic, 71*(3), 95-107.

Kabara, J. J. (1984). Antimicrobial agents derived from fatty acids. *Journal of the American Oil Chemists' Society, 61*(2), 397-403.

Kanner, J. (2007). Dietary advanced lipid oxidation endproducts are risk factors to human health.

Molecular Nutrition & Food Research, 51(9), 1094-1101.

Kapoor, M., & Gupta, M. N. (2012). Lipase promiscuity and its biochemical applications. *Process Biochemistry, 47*(4), 555-569.

Karmee, S. K. (2009). Biocatalytic synthesis of ascorbyl esters and their biotechnological applications. *Applied Microbiology and Biotechnology, 81*(6), 1013-1022.

Kelleher, C. M., O'Mahony, J. A., Kelly, A. L., O'Callaghan, D. J., Kilcawley, K. N., & McCarthy, N. A. (2018). The effect of direct and indirect heat treatment on the attributes of whey protein beverages. *International Dairy Journal, 85*, 144-152.

Kim, H. J., Youn, S. H., & Shin, C. S. (2006). Lipase-catalyzed synthesis of sorbitol-fatty acid esters at extremely high substrate concentrations. *Journal of Biotechnology, 123*(2), 174-184.

Kim, J.-W., Yu, H., Park, K.-M., & Chang, P.-S. (2020). Antimicrobial characterization of erythorbyl laurate for practical applications in food and cosmetics. *Journal of Chemistry, 2020*, 5073508.

Kim, J., Yu, H., Yang, E., Choi, Y., & Chang, P.-S. (2023). Effects of alkyl chain length on the interfacial, antibacterial, and antioxidative properties of erythorbyl fatty acid esters. *LWT, 174*, 114421.

Kralova, I., & Sjöblom, J. (2009). Surfactants used in food industry: A review. *Journal of Dispersion Science and Technology, 30*(9), 1363-1383.

Kumar, A., Dhar, K., Kanwar, S. S., & Arora, P. K. (2016). Lipase catalysis in organic solvents: advantages and applications. *Biological Procedures Online, 18*(1), 2.

Laguerre, M., Bayrasy, C., Panya, A., Weiss, J., McClements, D. J., Lecomte, J., . . . Villeneuve, P. (2015). What makes good antioxidants in lipid-based systems? The next theories beyond the polar paradox. *Critical Reviews in Food Science and Nutrition, 55*(2), 183-201.

Lee, D. E., Park, K. M., Choi, S. J., & Chang, P.-S. (2012). Optimal production and structural characterization of erythorbyl laurate obtained through lipase-catalyzed esterification. *Food Science and Biotechnology, 21*(4), 1209-1215.

Lee, D. E., Park, K. M., Choi, S. J., Shim, J.-H., & Chang, P.-S. (2013a). Enhancing operational stability and exhibition of enzyme activity by removing water in the immobilized lipase-catalyzed production of erythorbyl laurate. *Biotechnology Progress, 29*(4), 882-889.

Lee, L. L., Niknafs, N., Hancocks, R. D., & Norton, I. T. (2013b). Emulsification: Mechanistic understanding. *Trends in Food Science & Technology, 31*(1), 72-78.

Lieberman, S., Enig, M. G., & Preuss, H. G. (2006). A review of monolaurin and lauric acid: Natural virucidal and bactericidal agents. *Alternative and Complementary Therapies, 12*(6), 310-314.

Liu, S., Sun, C., Xue, Y., & Gao, Y. (2016). Impact of pH, freeze–thaw and thermal sterilization on physicochemical stability of walnut beverage emulsion. *Food Chemistry, 196*, 475-485.

Loh, W., Brinatti, C., & Tam, K. C. (2016). Use of isothermal titration calorimetry to study surfactant aggregation in colloidal systems. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1860*(5), 999-1016.

Lu, W., Kelly, A. L., & Miao, S. (2016). Emulsion-based encapsulation and delivery systems for polyphenols. *Trends in Food Science & Technology, 47*, 1-9.

Mabrouk, M. M., Hamed, N. A., & Mansour, F. R. (2023). Spectroscopic methods for determination of critical micelle concentrations of surfactants: A comprehensive review. *Applied Spectroscopy Reviews, 58*(3), 206-234.

Mao, L., Roos, Y. H., Biliaderis, C. G., & Miao, S. (2017). Food emulsions as delivery systems for flavor compounds: A review. *Critical Reviews in Food Science and Nutrition, 57*(15), 3173-3187.

Marhamati, M., Ranjbar, G., & Rezaie, M. (2021). Effects of emulsifiers on the physicochemical stability of oil-in-water nanoemulsions: A critical review. *Journal of Molecular Liquids, 340*, 117218.

Martin-Visscher, L. A., Yoganathan, S., Sit, C. S., Lohans, C. T., & Vederas, J. C. (2011). The activity of bacteriocins from *Carnobacterium maltaromaticum* UAL307 against Gram-negative

bacteria in combination with EDTA treatment. *FEMS Microbiology Letters, 317*(2), 152-159.

McCallum, N., Stutzmann Meier, P., Heusser, R., & Berger-Bächi, B. (2011). Mutational analyses of open reading frames within the *vra*SR operon and their roles in the cell wall stress response of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 55*(4), 1391-1402.

McClements, D. J. (2015). *Food emulsions: Principles, practices, and techniques*. (3rd ed.): CRC Press.

McClements, D. J., Bai, L., & Chung, C. (2017). Recent advances in the utilization of natural emulsifiers to form and stabilize emulsions. *Annual Review of Food Science and Technology, 8*(1), 205-236.

McClements, D. J., & Jafari, S. M. (2018). Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Advances in Colloid and Interface Science, 251*, 55-79.

Meullemiestre, A., Petitcolas, E., Maache-Rezzoug, Z., Chemat, F., & Rezzoug, S. A. (2016). Impact of ultrasound on solid–liquid extraction of phenolic compounds from maritime pine sawdust waste. Kinetics, optimization and large scale experiments. *Ultrasonics Sonochemistry, 28*, 230-239.

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.

Neta, N. S., Teixeira, J. A., & Rodrigues, L. R. (2015). Sugar ester surfactants: Enzymatic synthesis and applications in food industry. *Critical Reviews in Food Science and Nutrition, 55*(5), 595-610.

Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances, 5*(35), 27986-28006.

Nowicki, J., Łuczak, J., & Stańczyk, D. (2016). Dual functionality of amphiphilic 1-alkyl-3-methylimidazolium hydrogen sulfate ionic liquids: Surfactants with catalytic function. *RSC Advances, 6*(14), 11591-11601.

O'Keefe, S. F., & Pike, O. A. (2010). Fat characterization. In S. S. Nielsen (Ed.), *Food analysis* (pp. 239-260). Boston, MA. Springer US.

Ortiz, C., Ferreira, M. L., Barbosa, O., dos Santos, J. C. S., Rodrigues, R. C., Berenguer-Murcia, Á., . . . Fernandez-Lafuente, R. (2019). Novozym 435: The "perfect" lipase immobilized biocatalyst? *Catalysis Science & Technology, 9*(10), 2380-2420.

Owen, R. F., Srinivasan, D., & Kirk, L. P. (2017). *Fennema's food chemistry*. (5th ed.): CRC Press.

Ozturk, B., & McClements, D. J. (2016). Progress in natural emulsifiers for utilization in food emulsions. *Current Opinion in Food Science, 7*, 1-6.

Park, I., Yu, H., & Chang, P.-S. (2023). Lipase-catalyzed synthesis of antibacterial and antioxidative erythorbyl ricinoleate with high emulsifying activity. *Food Chemistry, 404*, 134697.

Park, J.-Y., Choi, M.-J., Yu, H., Choi, Y., Park, K.-M., & Chang, P.-S. (2022a). Multi-functional behavior of food emulsifier erythorbyl laurate in different colloidal conditions of homogeneous oil-in-water emulsion system. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 636*, 128127.

Park, J.-Y., Jo, S.-K., Park, K.-M., Yu, H., Bai, J., Ryu, S., & Chang, P.-S. (2019). Transcriptomic analysis of *Staphylococcus aureus* under the stress condition of antibacterial erythorbyl laurate by RNA sequencing. *Food Control, 96*, 1-8.

Park, J.-Y., Myeong, J., Choi, Y., Yu, H., Kwon, C. W., Park, K.-M., & Chang, P.-S. (2021). Erythorbyl fatty acid ester as a multi-functional food emulsifier: Enzymatic synthesis, chemical identification, and functional characterization of erythorbyl myristate. *Food Chemistry, 353*, 129459.

Park, J.-Y., & Park, K.-M. (2022b). Lipase and its unique selectivity: A mini-review. *Journal of Chemistry, 2022*, 7609019.

Park, K.-M., Jo, S.-K., Yu, H., Park, J.-Y., Choi, S. J., Lee, C. J., & Chang, P.-S. (2018a). Erythorbyl laurate as a potential food additive with multi-functionalities: Antibacterial activity and mode

of action. *Food Control, 86*, 138-145.

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.

Park, K.-M., Lee, M. J., Jo, S.-K., Choi, S. J., Lee, J., & Chang, P.-S. (2017). Erythorbyl laurate as a potential food additive with multi-functionalities: Interfacial characteristics and antioxidant activity. *Food Chemistry, 215*, 101-107.

Park, K.-M., Lee, S. J., Yu, H., Park, J.-Y., Jung, H.-S., Kim, K., . . . Chang, P.-S. (2018b). Hydrophilic and lipophilic characteristics of non-fatty acid moieties: Significant factors affecting antibacterial activity of lauric acid esters. *Food Science and Biotechnology, 27*(2), 401-409.

Pasquali, R. C., Taurozzi, M. P., & Bregni, C. (2008). Some considerations about the hydrophilic–lipophilic balance system. *International Journal of Pharmaceutics, 356*(1), 44-51.

Priego-Capote, F. (2021). Solid–liquid extraction techniques. In R. Lucena & S. Cárdenas (Eds.), *Analytical sample preparation with nano- and other high-performance materials* (pp. 111-130). Elsevier.

Raaijmakers, J. M., De Bruijn, I., Nybroe, O., & Ongena, M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiology Reviews, 34*(6), 1037-1062.

Rafailidis, P. I., Ioannidou, E. N., & Falagas, M. E. (2007). Ampicillin/sulbactam. *Drugs, 67*(13), 1829-1849.

Reichardt, C., & Welton, T. (2010). *Solvents and Solvent Effects in Organic Chemistry*. (4th ed.): WILEY-VCH.

Ren, K., & Lamsal, B. P. (2017). Synthesis of some glucose-fatty acid esters by lipase from *Candida antarctica* and their emulsion functions. *Food Chemistry, 214*, 556-563.

Shin, H., Kwon, C. W., Lee, M.-W., Yu, H., & Chang, P.-S. (2022). Antibacterial characterization of erythorbyl laurate against *Geobacillus stearothermophilus* spores. *LWT, 155*, 112824.

Stamatis, H., Sereti, V., & Kolisis, F. N. (1999). Studies on the enzymatic synthesis of lipophilic derivatives of natural antioxidants. *Journal of the American Oil Chemists' Society, 76*(12), 1505-1510.

Sun, W.-J., Zhao, H.-X., Cui, F.-J., Li, Y.-H., Yu, S.-L., Zhou, Q., . . . Dong, Y. (2013). D-isoascorbyl palmitate: Lipase-catalyzed synthesis, structural characterization and process optimization using response surface methodology. *Chemistry Central Journal, 7*(1), 114.

Sundarraj, A. A., & Ranganathan, T. V. (2019). Classification and uses of emulsions in food and agro applications. In T. J. Gutiérrez (Ed.), *Polymers for agri-food applications* (pp. 143-158). Cham. Springer International Publishing.

Tamm, F., Sauer, G., Scampicchio, M., & Drusch, S. (2012). Pendant drop tensiometry for the evaluation of the foaming properties of milk-derived proteins. *Food Hydrocolloids, 27*(2), 371-377.

Teng, Y., Stewart, S. G., Hai, Y.-W., Li, X., Banwell, M. G., & Lan, P. (2021). Sucrose fatty acid esters: Synthesis, emulsifying capacities, biological activities and structure-property profiles. *Critical Reviews in Food Science and Nutrition, 61*(19), 3297-3317.

Villeneuve, P., Bourlieu-Lacanal, C., Durand, E., Lecomte, J., McClements, D. J., & Decker, E. A. (2021). Lipid oxidation in emulsions and bulk oils: A review of the importance of micelles. *Critical Reviews in Food Science and Nutrition*, 1-41.

Waraho, T., McClements, D. J., & Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends in Food Science & Technology, 22*(1), 3-13.

Washko, P. W., Welch, R. W., Dhariwal, K. R., Wang, Y., & Levine, M. (1992). Ascorbic acid and dehydroascorbic acid analyses in biological samples. *Analytical Biochemistry, 204*(1), 1-14.

Weiss, J., Loeffler, M., & Terjung, N. (2015). The antimicrobial paradox: Why preservatives lose activity in foods. *Current Opinion in Food Science, 4*, 69-75.

Yamashita, Y., & Sakamoto, K. (2016). Hydrophilic–lipophilic balance (HLB): Classical indexation

and novel indexation of surfactant. In *Encyclopedia of biocolloid and biointerface science 2V set* (pp. 570-574).

Yoon, B. K., Jackman, J. A., Valle-González, E. R., & Cho, N.-J. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. *International Journal of Molecular Sciences* (Vol. 19).

Yu, H., Byun, Y., & Chang, P.-S. (2022). Lipase-catalyzed two-step esterification for solvent-free production of mixed lauric acid esters with antibacterial and antioxidative activities. *Food Chemistry, 366*, 130650.

Yu, H., Kim, S., & Chang, P.-S. (2023). Lipase-catalyzed production of pyridoxine monolaurate in solvent-free bioreactor system. *Food Chemistry, 399*, 133949.

Yu, H., Lee, M.-W., Shin, H., Park, K.-M., & Chang, P.-S. (2019). Lipase-catalyzed solvent-free synthesis of erythorbyl laurate in a gas-solid-liquid multiphase system. *Food Chemistry, 271*, 445-449.

Yu, H., Park, K.-M., & Chang, P.-S. (2020). Lipase-catalyzed synthesis of lauroyl tripeptide-KHA with multi-functionalities: Its surface-active, antibacterial, and antioxidant properties. *Food Chemistry, 319*, 126533.

Zhan, Q., Thakur, K., Feng, J.-Y., Zhu, Y.-Y., Zhang, J.-G., & Wei, Z.-J. (2023). LC–MS based metabolomics analysis of okara fermented by *Bacillus subtilis* DC-15: Insights into nutritional and functional profile. *Food Chemistry, 413*, 135656.

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.

Zieniuk, B., Białecka-Florjańczyk, E., Wierzchowska, K., & Fabiszewska, A. (2021). Recent advances in the enzymatic synthesis of lipophilic antioxidant and antimicrobial compounds. *World Journal of Microbiology and Biotechnology, 38*(1), 11.

Figure captions

Fig. 1. Overview of the derivatization of erythorbyl fatty acid ester (EFE). (a) Schematic diagram of the immobilized lipase-catalyzed production of EFE using erythorbic acid and fatty acid as substrates. This diagram depicts the esterification process of erythorbyl laurate, the first EFE developed. An ester bond (−COO−) between the hydroxyl group (−OH) of erythorbic acid and the carboxyl group (−COOH) of fatty acid is selectively formed by immobilized lipase under specific reaction conditions. These EFE compounds possessing both hydrophilic and hydrophobic moieties exhibited amphiphilic property and other functionalities simultaneously. (b) Chemical structures for all previously synthesized fatty acid derivatives of EFE. The synthesis of EFE derivatives was successfully achieved by using seven different fatty acids, and their functionalities were also characterized.

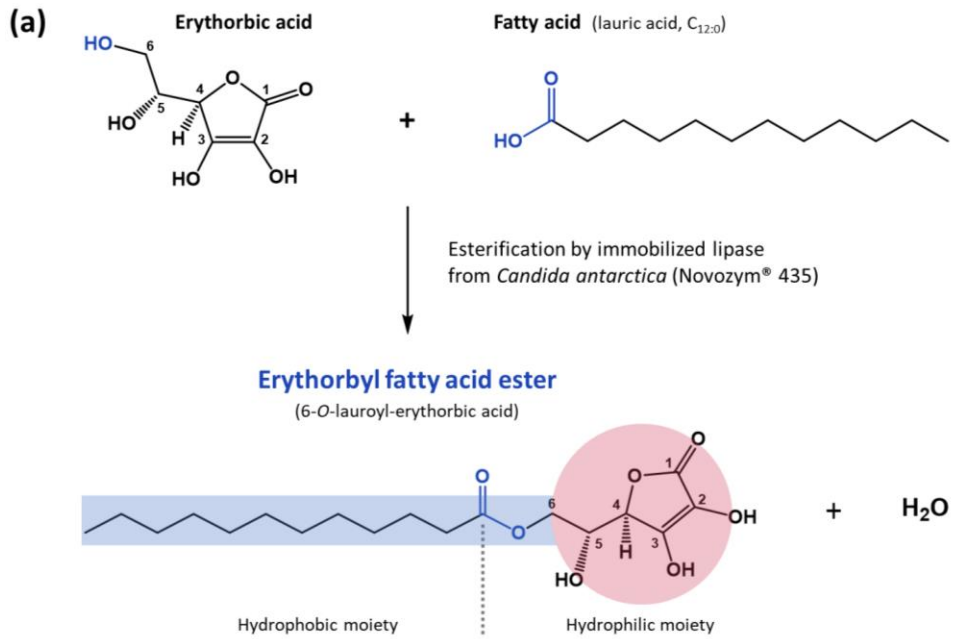
Fig. 2. Two different reactors for the immobilized lipase-catalyzed synthesis of erythorbyl fatty acid ester (EFE). (a) Organic solvent monophase system (OS-MPS) with mechanical agitation method. The organic solvent medium should be selected based on its hydrophobicity to dissolve both substrates and synthesized EFE molecules, as well as its compatibility with immobilized lipase. (b) Solvent-free gas-solid-liquid multiphase system (GSL-MPS) with inert gas sparging method. It is critical to increase the rheological properties of the fatty acid medium and maximize the catalysis efficiency during EFE production by controlling the reaction temperature and gas flux.

Fig. 3. Detailed procedures and general interpretations for detecting, purifying, and chemically identifying of erythorbyl fatty acid ester (EFE). (a) Quantitative analysis of the reactant from organic solvent monophase system (OS-MPS) or gas-solid-liquid multiphase system (GSL-MPS). In the reactant, there could be a presence of both synthesized EFEs and leftover substrates such as erythorbic acid (EA)

and fatty acid (FA). R, immobilized lipase residue. (b, c) Common examples of HPLC-UV and HPLC-RI chromatograms of the reactant without further purification. Most EFE molecules have an intermediate extent of hydrophobicity between their precursors. (d) Lab-scale purification of synthesized EFEs from the reactant based on a liquid-solid extraction methodology. (e, f) Common examples of ESI/MS spectrum of the purified EFE. Multiple isotopes (2M) or dehydrated isotopes (M−18) can be observed according to the analysis conditions. (g) Common example of ^{13}C-NMR spectrum of the purified EFE. All chemical shifts (δ, ppm) detected in the ^{13}C-NMR spectra of precursors (EA and FA) are merged into a single ^{13}C-NMR spectrum for EFE.

Fig. 4. Mechanism of antioxidative activity of erythorbyl fatty acid ester (EFE). (a) Schematic diagram of the radical formation of erythorbic acid (EH_2) as an electron donor. Erythorbate anions (EH^- or E^{2-}) can donate their electrons to adjacent lipid radicals and form erythorbyl radicals ($^{\bullet}EH$ or $^{\bullet}E^-$), resulting in postponing the lipid peroxidation. The erythorbyl moiety of EFE is structurally conserved even after esterification and behaves similarly to erythorbic acid. E, dehydroerythorbic acid. (b) Localization of three types of antioxidants in oil-water two-phase and oil-in-water emulsion systems. In comparison to simple hydrophilic or hydrophobic antioxidants, amphiphilic EFEs can localize at the oil-water interface effectively, where the lipid peroxidation occurs.

Fig. 5. Mechanism of antibacterial activity of erythorbyl fatty acid ester (EFE). In the case of Gram-positive bacteria, the hydrophilic moiety of EFE can interact with a positively charged bacterial membrane after penetrating through a peptidoglycan (PG) layer and disrupt the membrane by three-phases (membrane insertion, surface charge depolarization, and permeability alteration). In the case of Gram-negative bacteria, EFE is inaccessible to an outer membrane due to the electrostatic repulsion against a negatively charged lipopolysaccharide (LPS) layer.



(b)

Fatty acid chain	Chemical structure	Synthesized EFE derivative
Capric acid	$C_{10:0}$ (saturated)	
Lauric acid	$C_{12:0}$ (saturated)	
Myristic acid	$C_{14:0}$ (saturated)	
Palmitic acid	$C_{16:0}$ (saturated)	
Stearic acid	$C_{18:0}$ (saturated)	
Oleic acid	$C_{18:1}$ (*cis*-Δ^9)	
Ricinoleic acid	$C_{18:1}$ (*cis*-Δ^9, $-OH^{12}$)	

Fig. 1.

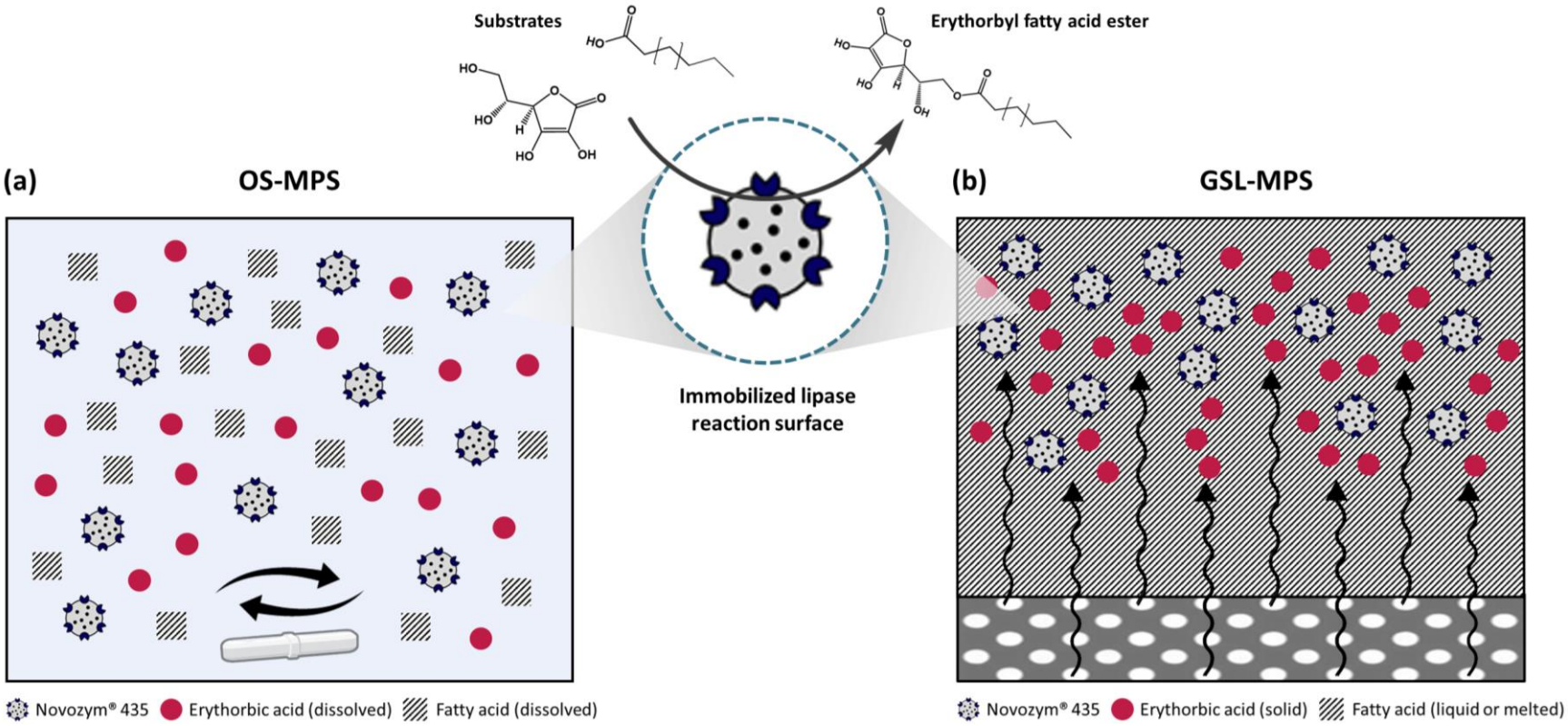

Substrates
Erythorbyl fatty acid ester
(a)
OS-MPS
(b)
GSL-MPS
Immobilized lipase
reaction surface
Novozym® 435
Erythorbic acid (dissolved)
Fatty acid (dissolved)
Novozym® 435
Erythorbic acid (solid)
Fatty acid (liquid or melted)

Fig. 2.

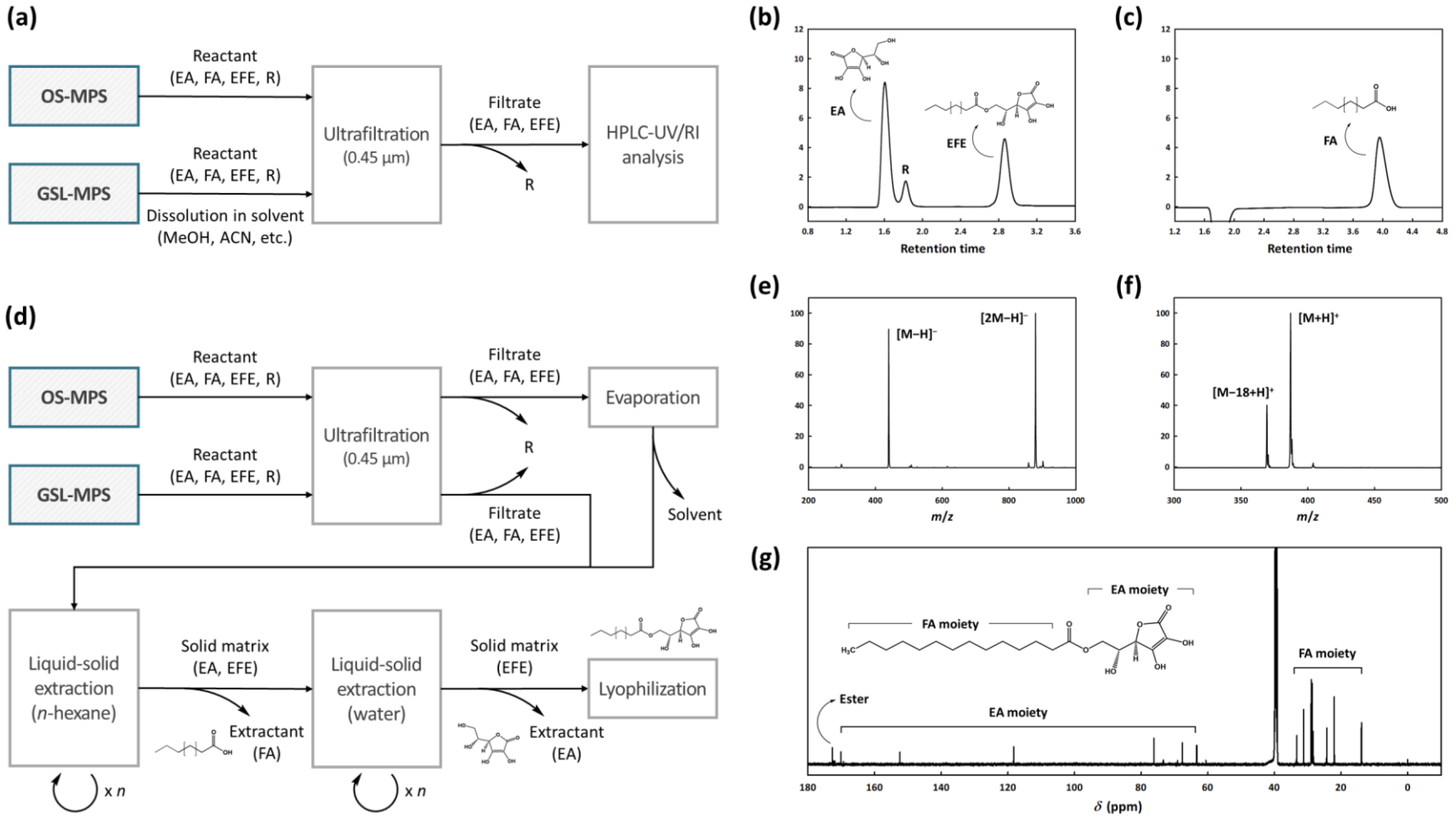

(a)
OS-MPS
GSL-MPS
Reactant
(EA, FA, EFE, R)
Reactant
(EA, FA, EFE, R)
Dissolution in solvent
(MeOH, ACN, etc.)
Ultrafiltration
(0.45 μm)
Filtrate
(EA, FA, EFE)
R
HPLC-UV/RI
analysis
(b)
EA
EFE
R
Retention time
(c)
FA
Retention time
(d)
OS-MPS
GSL-MPS
Reactant
(EA, FA, EFE, R)
Reactant
(EA, FA, EFE, R)
Ultrafiltration
(0.45 μm)
Filtrate
(EA, FA, EFE)
R
Filtrate
(EA, FA, EFE)
Evaporation
Solvent
Liquid-solid
extraction
(n-hexane)
Solid matrix
(EA, EFE)
Extractant
(FA)
x n
Liquid-solid
extraction
(water)
Solid matrix
(EFE)
Extractant
(EA)
Lyophilization
x n
(e)
[M−H]⁻
[2M−H]⁻
m/z
(f)
[M+H]⁺
[M−18+H]⁺
m/z
(g)
EA moiety
FA moiety
Ester
EA moiety
FA moiety
δ (ppm)

Fig. 3.

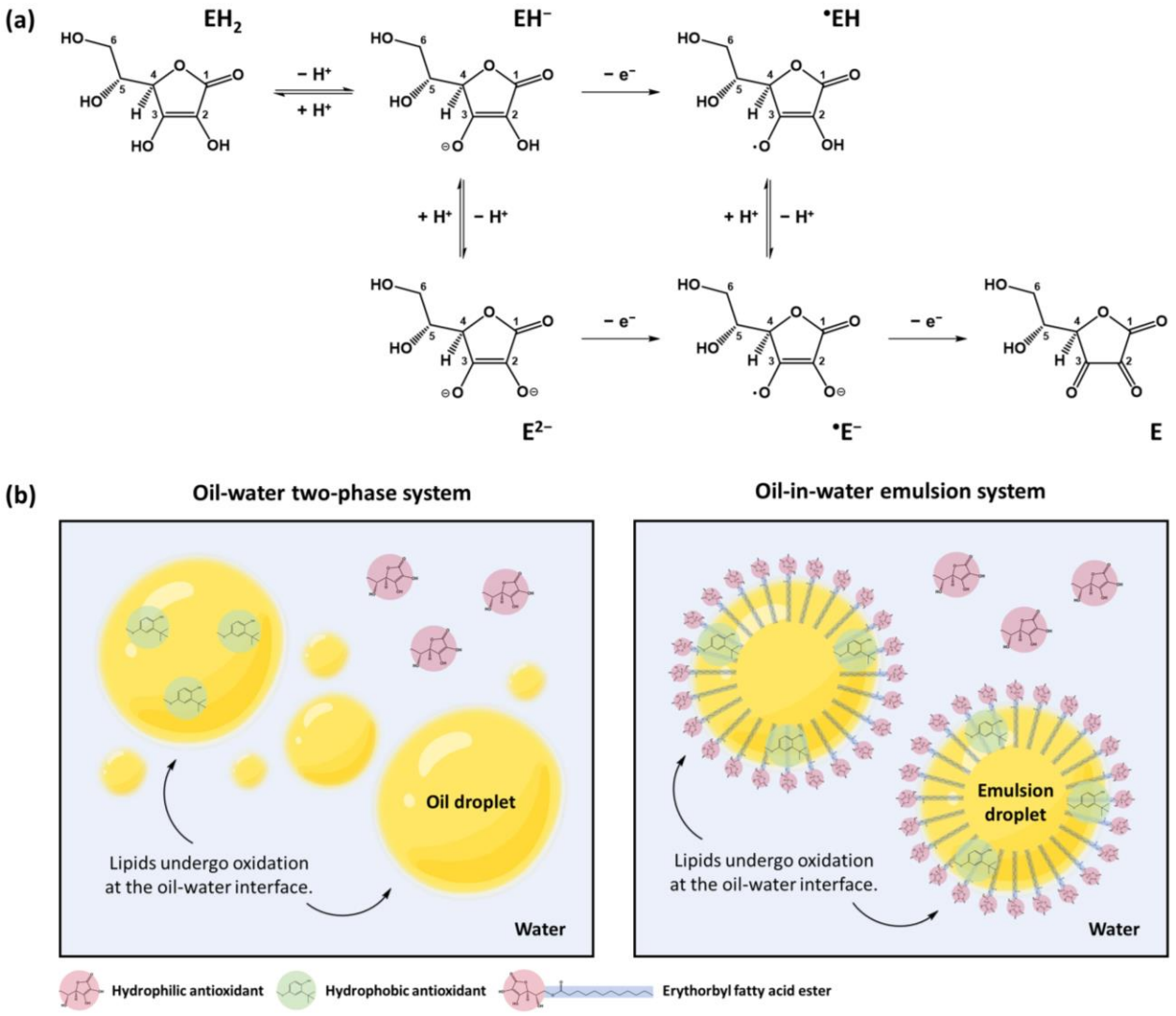

(a)
EH2
EH−
•EH
− H+
+ H+
− e−
+ H+
− H+
+ H+
− H+
− e−
− e−
E2−
•E−
E
(b)
Oil-water two-phase system
Oil-in-water emulsion system
Oil droplet
Lipids undergo oxidation at the oil-water interface.
Water
Emulsion droplet
Lipids undergo oxidation at the oil-water interface.
Water
Hydrophilic antioxidant
Hydrophobic antioxidant
Erythorbyl fatty acid ester

Fig. 4.

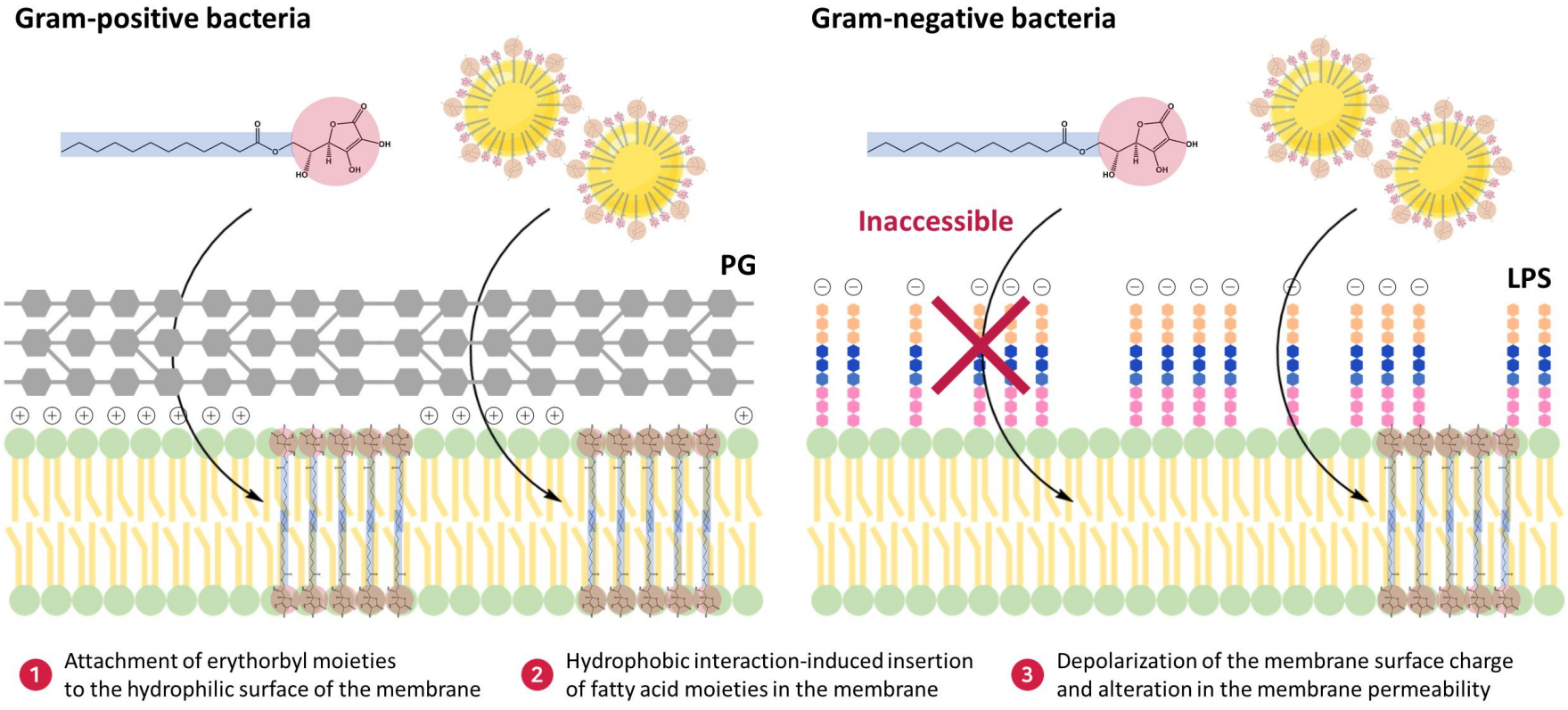

Gram-positive bacteria
Gram-negative bacteria
PG
Inaccessible
LPS
1 Attachment of erythorbyl moieties to the hydrophilic surface of the membrane
2 Hydrophobic interaction-induced insertion of fatty acid moieties in the membrane
3 Depolarization of the membrane surface charge and alteration in the membrane permeability

Fig. 5.

Table 1. Summary of theoretical and experimental amphiphilic properties of erythorbyl fatty acid ester (EFE) derivatives

EFE derivative	Chemical structure	Molecular weight (g/mol)	CMC_{IFT}[1] (mM)	HLB[2]	Log *P*[3]
Erythorbyl caprate	$C_{10:0}$ (saturated)	330.38	5.52[a]	13.40	3.06
Erythorbyl laurate	$C_{12:0}$ (saturated)	358.43	0.74[b] / 1.56[a]	12.45	4.04
Erythorbyl myristate	$C_{14:0}$ (saturated)	386.48	0.62[b] / 1.19[a]	11.50	5.02
Erythorbyl palmitate	$C_{16:0}$ (saturated)	414.54	0.80[a]	10.55	6.00
Erythorbyl stearate	$C_{18:0}$ (saturated)	442.59	0.36[a]	9.60	6.98
Erythorbyl oleate	$C_{18:1}$ (*cis*-Δ^9)	440.58	N.D.[4,b]	9.60	6.80
Erythorbyl ricinoleate	$C_{18:1}$ (*cis*-Δ^9, $-OH^{12}$)	456.58	0.73[b]	11.50	4.90

[1]Critical micelle concentration based on the interfacial tension measurement; [2]Hydrophilic-lipophilic balance based on the data from Davies (1957);
[3]*n*-Octanol-water partition coefficient based on the data from Meylan and Howard (1994); [4]Not determined.
[a]Data from Kim *et al*. (2023); [b]Data from Park *et al*. (2023).