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Lipase-catalyzed solvent-free synthesis of erythorbyl laurate in a gas-solid-liquid multiphase system

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ABSTRACT

Erythorbyl laurate is a potential food additive as a multi-functional emulsifier having antioxidant and antimicrobial activities. In this study, a gas-solid-liquid multiphase system (GSL-MPS) was established to enhance the production yield of erythorbyl laurate in a lipase-catalyzed solvent-free synthesis. The significant reaction variables were optimized as follows: substrate molar ratio of 2:1 (lauric acid:erythorbic acid) and enzyme concentration of 120 mg/mL (840 PLU/mL). Under these conditions, the maximum production yield in GSL-MPS was 13.974 mg/mL, which is 8.60- and 4.26-fold higher than the yields obtained in an organic solvent monophase system (OS-MPS) and a solid-liquid biphase system (SL-BPS), respectively. Moreover, the operational stability of the immobilized lipase was significantly improved in GSL-MPS compared with OS-MPS. These results indicate that GSL-MPS can be an enzymatic reaction system facilitating efficient production of ester compounds as a means of increasing production yields and the reusability of the immobilized lipase.

1. Introduction

Lipid oxidation and microbial contamination are major factors leading to deterioration of emulsion-based foods (Luther et al., 2007; McClements & Decker, 2000). Under the strategy to control the aforementioned factors with a single food additive, our research group has developed erythorbyl laurate (6-*O*-lauroyl-erythorbic acid) by lipase-catalyzed esterification between erythorbic acid and lauric acid (Park, Sung, Lee, & Chang, 2011). Erythorbyl laurate can act as an emulsifier and also exhibits antioxidant activity in oil-in-water emulsions (Park et al., 2017). Recently, an antibacterial characterization of erythorbyl laurate suggested that erythorbyl laurate could be used to control microbial contamination in food (Park et al., 2018). These results indicated that erythorbyl laurate is a promising multi-functional food additive that could be used as an effective alternative to conventional antioxidants and antimicrobial agents for emulsion-based food.

Several synthetic methodologies to improve conversion yield of erythorbyl laurate have been reported by our research group (Lee, Park, Choi, Shim, & Chang, 2013; Park, Choi, & Chang, 2012). Under the optimum reaction conditions, maximum degrees of esterification of 77.81 and 86.30% were obtained in batch- and continuous-type reactions, respectively, in organic solvent monophase system (OS-MPS) by using acetonitrile. Unfortunately, despite the high degree of esterification, the enzymatic production of erythorbyl laurate in OS-MPS was not applicable at the industrial scale. Both substrates (erythorbic acid and lauric acid) exhibit limited solubility in organic solvents, hence must be used at low concentrations. This results in extremely low production yields in terms of reaction volume and consequently increases production costs. Moreover, safety concerns limit the use of organic solvents in the production of food ingredients (Foresti & Ferreira, 2005; Santos, Bueno, Molgero, Rós, & de Castro, 2007).

Solvent-free synthesis, in which a simple mixture of reactants is used as a reaction medium, eliminates organic solvents and allows much higher substrate concentrations (Ghamgui, Karra-Chaâbouni, & Gargouri, 2004). Thus, it is possible to obtain more product with the same reaction volume, rendering the process more amenable to industrial scale production (Dossat, Combes, & Marty, 2002; Soumanou & Bornscheuer, 2003; Sun, Yu, Curran, & Liu, 2012). However, due to differences in their relative hydrophilicity, erythorbic acid and lauric acid are immiscible. The lipase-catalyzed esterification is

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predominantly performed in the temperature range of 50 to 80 °C; lauric acid with a melting point of 43.2 °C exists as a liquid throughout the range. Whereas erythorbic acid having a melting point of 172.0 °C remains solid at these temperatures. Therefore, the solvent-free synthesis of erythorbyl laurate in a solid-liquid biphase system (SL-BPS) ought to be restricted due to low production yields caused by mass transfer limitations between the two phases (Erbeldinger, Ni, & Halling, 1998; Kim, Youn, & Shin, 2006; Romero, Calvo, Alba, & Daneshfar, 2007).

Multiphase reaction systems or three-phase (gas-solid-liquid) reaction systems have been used in bioreactors, namely bubble column reactor, to produce bioproducts such as enzymes, proteins, and antibiotics from microorganisms (bacteria or yeast). The reactor is basically equipped with gas distributor to form bubbles improving mixing efficiency to overcome mass transfer limitations between a solid phase, specified as suspensions of microorganisms, and a liquid phase (Kantarci, Borak, & Ulgen, 2005). Several studies have suggested reaction systems incorporating gaseous phase to produce chemicals by enzymatic synthesis (Kashid & Kiwi-Minsker, 2009). In the reaction systems, immobilized enzymes were utilized as solid phases and gaseous phases were incorporated to mix liquid phase substrates with reduced mechanical damage to the immobilized enzymes. Lipase-catalyzed solvent-free synthesis in a multiphase reaction system improved esterification degree of polyglycerol-3, a high viscous substrate, with fatty acid by overcoming mass transfer limitations between substrates of liquid-liquid phase (Hilterhaus, Thum, & Liese, 2008). Development of a gas-solid-liquid multiphase system (GSL-MPS), employing a gaseous phase to the SL-BPS, would overcome the mass transfer limitations by improving the dispersibility of the solid phase and consequently enhance the production yield (Zhang et al., 2016).

The overall aim of the present study was to establish an enzymatic reaction system to enhance the production yield of erythorbyl laurate in the lipase-catalyzed solvent-free synthesis. GSL-MPS was established by incorporating a gaseous phase into the solid-liquid biphase. Production yields were compared among three reaction systems (OS-MPS, SL-BPS, and GSL-MPS) and the operational stability of the immobilized lipase in GSL-MPS was assessed. In addition, the effects of substrate molar ratio, enzyme concentration, and reaction time on the production yield in GSL-MPS were investigated.

2. Materials and methods

2.1. Materials

Erythorbic acid (≥99.0%) and dodecanoic acid (lauric acid ≥99.0%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Daejung Chemicals and Metals (Siheung, Korea), respectively. Immobilized lipase from *Candida antarctica* (triacylglycerol, hydrolase, EC 3.1.1.3; Novozym 435) was kindly provided by Novozymes Korea, Ltd. (Seoul, Korea) with a reported catalytic activity of 7000 PLU/g (the activity of PLU refers to the millimoles of propyl laurate synthesized per min at 60 °C). Acetonitrile, water, and acetic acid (J.T. Baker Co., Phillipsburg, NJ, USA) were of high-performance liquid chromatography (HPLC) grade. All other chemicals and solvents were of analytical grade.

2.2. Enzymatic production in a gas-solid-liquid multiphase system

The enzymatic production of erythorbyl laurate in GSL-MPS is illustrated in Fig. 1. Nitrogen gas was used due to its inertness. The reaction vessel was equipped with a porous glass filter (0.5 cm thickness and 27.5 μm pore size) on the bottom and the gas was sparged through the filter. The reaction temperature was controlled by a water circulator. The dimensions of the reaction vessel were 4.7 × 6.0 and 10.0 × 12.0 cm for the 20 and 500 mL reactors, respectively.

2.3. Lipase-catalyzed synthesis of erythorbyl laurate

For the production of erythorbyl laurate in GSL-MPS, the reaction temperature was fixed at 60 °C to melt the lauric acid. The gas flow rate was 2.0 L/min and the purity of the gas was higher than 99.9%. After pre-incubation for 10 min to melt the lauric acid, the reaction was initiated by adding erythorbic acid and the immobilized lipase. Unless otherwise noted, the quantity of lauric acid was fixed at 85.86 mmol (17.201 g) for reaction volume of 20 mL. For the production of erythorbyl laurate in OS-MPS, the reaction was performed in acetonitrile under optimum reaction conditions determined by our previous study (Park et al., 2011). Lauric acid and erythorbic acid were pre-dissolved in acetonitrile and the reaction was initiated by adding the immobilized lipase. In this study, the production yield is defined as the mass of product (erythorbyl laurate) divided by the volume of the reactants.

2.4. Quantitative analysis of erythorbyl laurate

Reactants (50 μL) were withdrawn, dissolved in 950 μL of acetonitrile, and filtered through a 0.45-μm membrane filter. After filtration, aliquots (20 μL) were injected into HPLC system for quantitative analysis. HPLC analysis was conducted on LC-2002 system (Jasco, Tokyo, Japan) with a C18 reverse-phase column (5 μm, 4.6 × 150 mm; Phenomenex, Torrance, CA, USA) and an ultraviolet detector (UV-2075; Jasco). The mobile phase was acetonitrile/water/acetic acid (90:5:5, v/v/v) and the flow rate was 1.0 mL/min. All of the peaks in the chromatograms were identified by its retention times and the quantity of the substances were determined by the peak areas at 265 nm according to previous report (Park et al., 2011).

2.5. Evaluation of operational stability of the immobilized lipase

The operational stabilities of the lipase in GSL-MPS and OS-MPS were assessed under simulated conditions with thermal acceleration over 7 days. Stability was assessed by analyzing the residual hydrolytic activity of the lipase. For GSL-MPS, the lipase was treated with lauric acid and a gas flow of 2.0 L/min at 80 °C. Lipase stability in OS-MPS was evaluated during the treatment in acetonitrile with magnetic stirring (450 rpm) at 80 °C. The residual hydrolytic activity of the lipase was determined using a spectrophotometric method with *p*-nitrophenyl palmitate by quantitative analysis of the *p*-nitrophenol liberated from *p*-nitrophenyl palmitate (Wrolstad et al., 2005). The residual activity was expressed as the percent *p*-nitrophenol produced by the reaction for 60 min relative to that of lipase without treatments. To obtain the lipase for the enzyme assay, acetonitrile was removed by evaporation for 45 s and lauric acid was washed with *n*-hexane for 30 s. The *n*-hexane was removed by evaporation for 45 s.

2.6. Statistical analysis

All experiments were performed in triplicate and the results are presented as means and standard deviation. Analysis of variance was performed and the significance of differences was determined by Tukey's test at *P*-value of 0.05.

3. Results and discussion

3.1. Effects of reaction variables on production yield in gas-solid-liquid multiphasic system

The effect of substrate molar ratio (lauric acid:erythorbic acid) on the production yield of erythorbyl laurate in GSL-MPS was investigated in the range of 5:1-1:1 (Fig. 2A) while holding the concentration of lauric acid constant. The production yield significantly increased with increasing molar ratio, *i.e.*, increase of erythorbic acid between 5:1 and 2:1. In general, the degree of esterification improves as the molar ratio

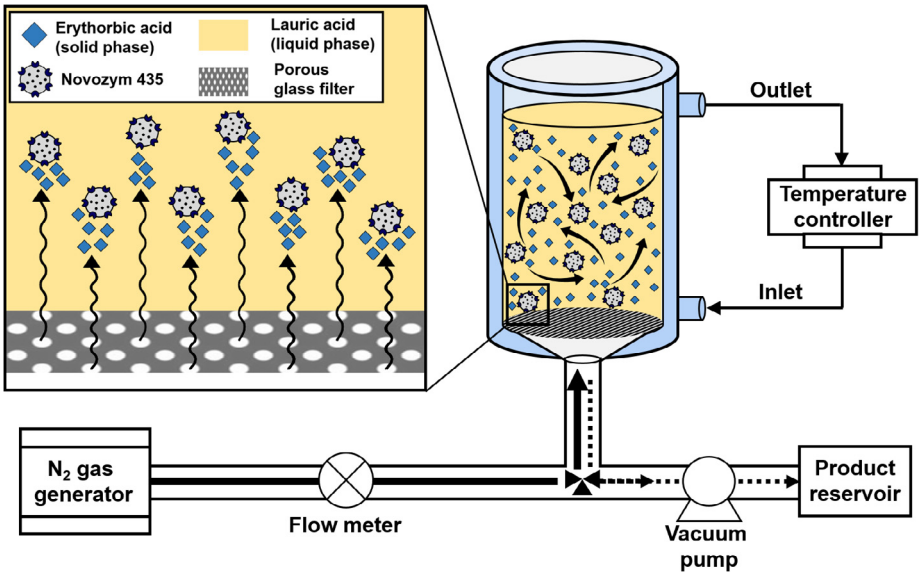

Fig. 1. Illustration of the enzymatic process in gas-solid-liquid multiphase system for the lipase-catalyzed solvent-free synthesis of erythorbyl laurate.

of acyl donor to acyl acceptor increases (Kim & Park, 2017; Ren & Lamsal, 2017; Soultani, Engasser, & Ghoul, 2001). In the presence of excess acyl donor, production yield is limited by the availability of the acyl acceptor since the acyl acceptor is inadequate to maximize a production of an ester. Conversely, the production yield decreased with increasing molar ratio from 2:1 to 1:1. Also, when the concentration of erythorbic acid was higher than that of lauric acid, *e.g.*, at a molar ratio of 1:2, it was observed that the reactant was not agitated (data not shown). The decrease of production yield in the range of 2:1–1:1 may be due to increasing viscosity, in accordance with increasing levels of solid erythorbic acid. High viscosity can limit the mass transfer of both substrates to the active site of the lipase and product release from the enzyme-substrate complex (Kim et al., 2006; Sun et al., 2012). Therefore, the molar ratio of 2:1 was determined as the optimum substrate molar ratio and used in subsequent experiments.

In general, high enzyme concentration accelerates reaction rates, resulting in improved production yields. However, excessive enzyme concentration can lead to a decrease in catalytic efficiency, especially in a reaction involving solid phase substrates and immobilized enzymes (Kuperkar, Lade, Prakash, & Rathod, 2014; Sun, Zhu, & Bi, 2014). The effects of enzyme concentration on the production yield in GSL-MPS were evaluated in terms of catalytic efficiency and production costs (Fig. 2B). The higher enzyme concentrations increased the production yield in the range of 60–120 mg/mL. However, additional increase of enzyme concentration over 120 mg/mL resulted in lower production yields. Excess immobilized lipase may hinder the ability of the gaseous phase to disperse the solid erythorbic acid, and the accessibility of the substrates to the enzyme. Therefore, 120 mg/mL was determined as optimum enzyme concentration and used in the subsequent reactions.

3.2. Time course of the esterification

Under the optimum reaction conditions, the production yield of erythorbyl laurate in GSL-MPS was evaluated over reaction time (Fig. 3). Scale-up of a reactor can affect catalytic performance due to changes in transport characteristics, especially in multiphase systems containing solid-phase. Therefore, the scale-up should be considered when assessing the amenability of a process to industrial applications (Kantarci et al., 2005). The production yield was measured over reaction time in 20 and 500 mL reactor. In the 20 mL reactor, the production yield increased linearly until 24 h, then the reaction rate was gradually decreased between 24 and 72 h. The production yield of erythorbyl laurate reached a maximum (13.974 mg/mL) at 72 h, and did not fluctuate thereafter due to reaction equilibrium. Production yields were higher in 500 mL reactor than in the 20 mL reactor at all reaction times. Reaction equilibrium was achieved at 72 h with a maximum production yield of 15.457 mg/mL, which is 10.6% higher than that obtained in the 20 mL reactor. These results suggest that increasing reaction volume may enhance catalytic performance in large-scale reactors for the production of erythorbyl laurate in GSL-MPS.

3.3. Comparison of production yields among different reaction systems

Erythorbyl laurate was synthesized in OS-MPS, SL-BPS, and GSL-MPS to evaluate the effects of reaction systems on the production yields (Table 1). The reaction in OS-MPS where the substrates were dissolved in organic solvent (acetonitrile) reached equilibrium at 12 h, indicating the highest reaction rate compared to the other reaction systems (Supplementary Data S1). However, the maximum production yield in the OS-MPS (1.625 mg/mL) was the lowest of all the reaction systems, despite the highest degree of esterification (75.61%). The production yield in SL-BPS (1.176 mg/mL) did not significantly differ from that in OS-MPS at the same reaction time (8 h) although substrate concentration was much higher than that in OS-MPS. The low production yield indicates that mass transfer was limited in SL-BPS by the presence of solid phase erythorbic acid. In contrast, GSL-MPS exhibited a 2.60-fold higher production yield than SL-BPS after reaction for 8 h. The increased production yield in GSL-MPS can be attributed to the incorporated gaseous phase into the solid-liquid biphase. The gaseous phase helps overcome the mass transfer limitations between the solid and liquid phase through the formation of large phase interfaces (Kantarci et al., 2005). Furthermore, the gaseous phase also improves the dispersion of solid phase erythorbic acid in the liquid phase lauric acid, which facilitates access of substrate to the lipase (Hilterhaus et al., 2008). Differences in the production yield depending on the reaction systems were conspicuous when the reactions attained the equilibrium. The maximum production yield obtained from GSL-MPS (13.974 mg/mL) was 8.60- and 4.25-fold higher than those from OS-MPS (1.625 mg/mL) and SL-BPS (3.284 mg/mL), respectively. GSL-MPS improving dispersion of solid phase substrates could be used for solvent-free synthesis of ester compounds, especially emulsifiers, where one substrate with high melting temperature and high hydrophilicity exists as a solid phase in a reaction medium (liquid phase) consisting of the other hydrophobic substrate.

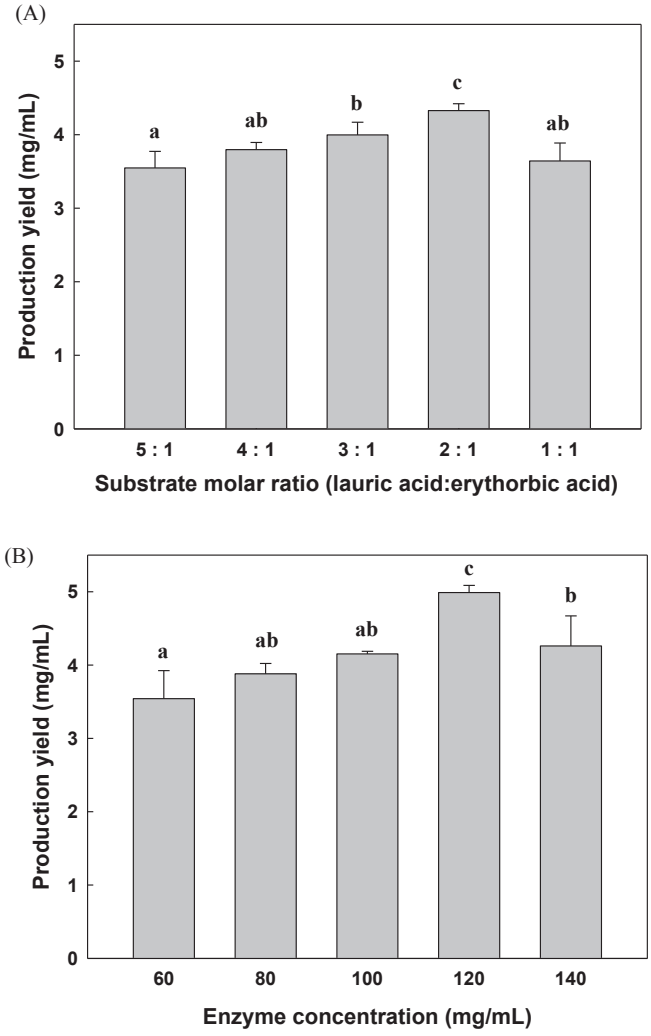

Fig. 2. Effects of the reaction variables on the production yield of erythorbyl laurate: (A) substrate molar ratio (lauric acid:erythorbic acid), and (B) enzyme concentration. Reaction conditions: (A) lauric acid, 85.86 mmol; erythorbic acid, 17.17–85.86 mmol; N_2 gas flow, 2.0 L/min; reaction temperature, 60 °C; enzyme concentration, 120 mg/mL; reaction time, 14 h, (B) lauric acid, 85.86 mmol; erythorbic acid, 42.93 mmol; N_2 gas flow, 2.0 L/min; reaction temperature, 60 °C; enzyme concentration, 60–140 mg/mL; reaction time, 14 h. Different letters indicate significant differences.

3.4. Operational stability of immobilized lipase in GSL-MPS

The operational stability of immobilized enzymes is the most important characteristic in terms of production costs since stability determines reusability. Stability varied across reaction systems due to the effects of reaction media on enzyme deactivation. The stability of the immobilized lipase (Novozym 435) in GSL-MPS was compared with that in OS-MPS (Fig. 4). The immobilized lipase exhibited extremely poor stability under the OS-MPS conditions, retaining only 11.68% of its original activity following the treatment (Supplementary Data S2). This deactivation may be caused by the high temperature (80 °C) and use of an organic solvent (acetonitrile). It was reported that lipase showed less than 50% of its original activity after being held at 80 °C for 60 min, which indicates that lipase underwent severe thermal deactivation (Pirozzi & Greco, 2004). In addition, it has been suggested that acetonitrile, a hydrophilic organic solvent, led to dissolution of the supporting material of the bead immobilizing enzyme. Then, acetonitrile could result in deactivation by unfolding of free lipase liberated from the immobilized medium (José et al., 2011).

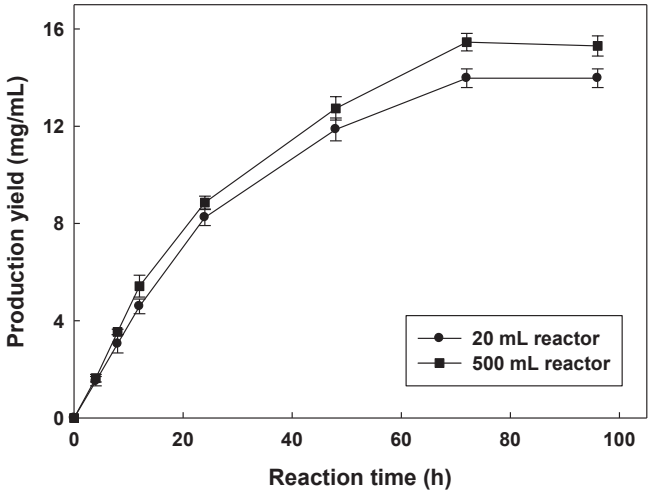

Fig. 3. Time course of lipase-catalyzed solvent-free synthesis of erythorbyl laurate in 20 and 500 mL reactor under the optimum reaction conditions in a gas-solid-liquid multiphase system. Reaction conditions: substrate molar ratio (lauric acid:erythorbic acid), 2:1; reaction temperature, 60 °C; enzyme concentration, 120 mg/mL; N_2 gas flow, 2.0 and 8.0 L/min in 20 and 500 mL reactor, respectively.

Table 1
Comparison of production yields of erythorbyl laurate among different reaction systems.

Reaction system	Production yield (mg/mL)	
	8 h [d]	Maximum [e]
Organic solvent monophase [a]	1.369 ± 0.147	1.625 ± 0.073
Solid-liquid biphase [b]	1.176 ± 0.259	3.284 ± 0.119
Gas-solid–liquid multiphase [c]	3.052 ± 0.378	13.974 ± 0.382

[a] Reaction conditions: 0.12 mmol erythorbic acid, 0.60 mmol lauric acid, 10 mg/mL immobilized lipase, magnetic stirring at 400 rpm.
[b] Reaction conditions: 42.93 mmol erythorbic acid, 85.86 mmol lauric acid, 120 mg/mL immobilized lipase, magnetic stirring at 400 rpm.
[c] Reaction conditions were the same as those in the solid-liquid biphase system except for the use of 2.0 L/min nitrogen gas in place of magnetic stirring.
[d] Production yields were evaluated at the same reaction time.
[e] Maximum production yield was calculated when the reactions were reached the equilibrium, indicated by no further fluctuations in production yields. Equilibrium was obtained after 12 h in the organic solvent monophase system, after 60 h in the solid-liquid biphase system, and after 72 h in the gas-solid-liquid multiphase system.

In contrast, the activity of lipase treated under the GSL-MPS conditions increased during the treatment with residual activity of 107.37%. These results could be inferred from effects of liquid phase lauric acid on the prevention of the thermal deactivation of the lipase and enhancement of its activity. It has been reported lauric acid is a phase change material exhibiting latent heat storage properties when undergoing a phase change (Sarı & Kaygusuz, 2002). Although these properties are generally observed around its melting point (43.2 °C), in the presence of mesoporous materials such as beads, the properties could be performed at high temperature due to changes of physical characteristic of lauric acid (Mitran, Berger, Munteanu, & Matei, 2015). Thus, under the GSL-MPS conditions, lauric acid may protect the lipase against thermal damage by absorbing heat that would otherwise deactivates the lipase. Furthermore, the hydrophobicity of lauric acid may enhance the lipase activity, leading to the structural changes of the lipase. Interactions with a hydrophobic surface such as oils have been shown to induce the open-form conformation of the lipase, which is considered as its active form (Cabrera, Fernandez-Lorente, Fernandez-Lafuente, Palomo, & Guisan, 2009). Hence, the reusability of the lipase

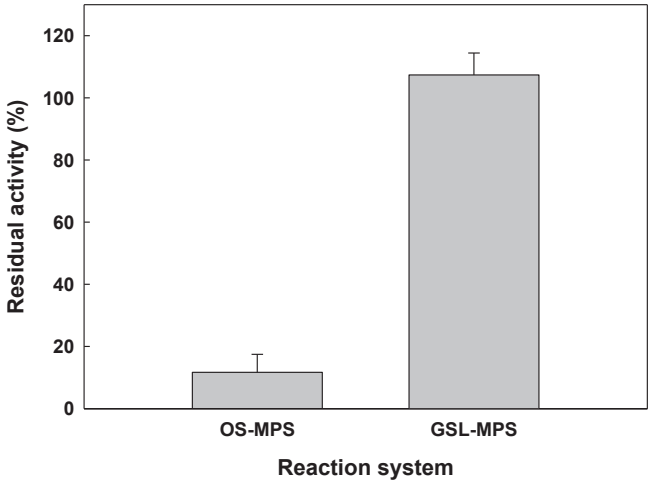

Fig. 4. The operational stability of the immobilized lipase under simulated conditions with thermal acceleration in gas-solid-liquid multiphase system (treatment conditions: 17.2 g (20.0 mL) lauric acid, 2.0 L/min N_2 gas flow, 80 °C) and organic solvent monophase system (treatment conditions: 20.0 mL acetonitrile, 450 rpm magnetic stirring, 80 °C). OS-MPS: organic solvent monophase system; GSL-MPS: gas-solid-liquid multiphase system.

can be significantly higher in GSL-MPS than that in OS-MPS.

4. Conclusions

In this study, GSL-MPS was established as a means of enhancing the production yield of erythorbyl laurate. Under the optimum conditions, the production yield of erythorbyl laurate in GSL-MPS was 8.60- and 4.25-fold higher than those in OS-MPS and SL-BPS, respectively. The operational stability of the lipase in GSL-MPS was also significantly higher than in OS-MPS. These results indicate that GSL-MPS enhances the production efficiency of erythorbyl laurate with respect to production yield and reusability of the lipase. Furthermore, GSL-MPS can potentially be an efficient enzymatic reaction system for solvent-free production of ester compounds.

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Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.07.134.

References

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.

Dossat, V., Combes, D., & Marty, A. (2002). Lipase-catalysed transesterification of high oleic sunflower oil. *Enzyme and Microbial Technology, 30*(1), 90–94.

Erbeldinger, M., Ni, X., & Halling, P. J. (1998). Enzymatic synthesis with mainly undissolved substrates at very high concentrations. *Enzyme and Microbial Technology, 23*(1), 141–148.

Foresti, M., & Ferreira, M. (2005). Frequent analytical/experimental problems in lipase-mediated synthesis in solvent-free systems and how to avoid them. *Analytical and Bioanalytical Chemistry, 381*(7), 1408–1425.

Ghamgui, H., Karra-Chaâbouni, M., & Gargouri, Y. (2004). 1-Butyl oleate synthesis by immobilized lipase from Rhizopus oryzae: A comparative study between n-hexane and solvent-free system. *Enzyme and Microbial Technology, 35*(4), 355–363.

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.

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.

Kantarci, N., Borak, F., & Ulgen, K. O. (2005). Bubble column reactors. *Process Biochemistry, 40*(7), 2263–2283.

Kashid, M. N., & Kiwi-Minsker, L. (2009). Microstructured reactors for multiphase reactions: State of the art. *Industrial & Engineering Chemistry Research, 48*(14), 6465–6485.

Kim, H., & Park, C. (2017). Enzymatic synthesis of phenethyl ester from phenethyl alcohol with acyl donors. *Enzyme and Microbial Technology, 100*, 37–44.

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.

Kuperkar, V. V., Lade, V. G., Prakash, A., & Rathod, V. K. (2014). Synthesis of isobutyl propionate using immobilized lipase in a solvent free system: Optimization and kinetic studies. *Journal of Molecular Catalysis B: Enzymatic, 99*(Supplement C), 143–149.

Lee, D. E., Park, K. M., Choi, S. J., Shim, J. H., & Chang, P. S. (2013). 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.

Luther, M., Parry, J., Moore, J., Meng, J., Zhang, Y., Cheng, Z., & Yu, L. (2007). Inhibitory effect of Chardonnay and black raspberry seed extracts on lipid oxidation in fish oil and their radical scavenging and antimicrobial properties. *Food Chemistry, 104*(3), 1065–1073.

McClements, D., & Decker, E. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science, 65*(8), 1270–1282.

Mitran, R. A., Berger, D., Munteanu, C., & Matei, C. (2015). Evaluation of different mesoporous silica supports for energy storage in shape-stabilized phase change materials with dual thermal responses. *The Journal of Physical Chemistry C, 119*(27), 15177–15184.

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.

Park, K.-M., Jo, S.-K., Yu, H., Park, J.-Y., Choi, S. J., Lee, C. J., & Chang, P. S. (2018). 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, 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., Sung, H., Lee, J., & Chang, P. S. (2011). Lipase-catalysed synthesis of erythorbyl laurate in acetonitrile. *Food Chemistry, 129*(1), 59–63.

Pirozzi, D., & Greco, G. (2004). Activity and stability of lipases in the synthesis of butyl lactate. *Enzyme and Microbial Technology, 34*(2), 94–100.

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.

Romero, M. D., Calvo, L., Alba, C., & Daneshfar, A. (2007). A kinetic study of isoamyl acetate synthesis by immobilized lipase-catalyzed acetylation in *n*-hexane. *Journal of Biotechnology, 127*(2), 269–277.

Santos, J. C., Bueno, T., Molgero, P. C., Rós, D., & de Castro, H. F. (2007). Lipase-catalyzed synthesis of butyl esters by direct esterification in solvent-free system. *Journal of Chemical Technology and Biotechnology, 82*(10), 956–961.

Sarı, A., & Kaygusuz, K. (2002). Thermal and heat transfer characteristics in a latent heat storage system using lauric acid. *Energy Conversion and Management, 43*(18), 2493–2507.

Soultani, S., Engasser, J.-M., & Ghoul, M. (2001). Effect of acyl donor chain length and sugar/acyl donor molar ratio on enzymatic synthesis of fatty acid fructose esters. *Journal of Molecular Catalysis B: Enzymatic, 11*(4), 725–731.

Soumanou, M. M., & Bornscheuer, U. T. (2003). Improvement in lipase-catalyzed synthesis of fatty acid methyl esters from sunflower oil. *Enzyme and Microbial Technology, 33*(1), 97–103.

Sun, J., Yu, B., Curran, P., & Liu, S.-Q. (2012). Lipase-catalysed transesterification of coconut oil with fusel alcohols in a solvent-free system. *Food Chemistry, 134*(1), 89–94.

Sun, S., Zhu, S., & Bi, Y. (2014). Solvent-free enzymatic synthesis of feruloylated structured lipids by the transesterification of ethyl ferulate with castor oil. *Food Chemistry, 158*, 292–295.

Wrolstad, R. E., Acree, T. E., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., ... Sporns, P. (2005). *Handbook of food analytical chemistry*. New Jersey: John Wiley & Sons (Chapter 8).

Zhang, N., Yang, X., Fu, J., Chen, Q., Song, Z., & Wang, Y. (2016). Production of diacylglycerol-enriched oil by glycerolysis of soybean oil using a bubble column reactor in a solvent-free system. *Journal of Oleo Science, 65*(3), 207–216.