有機溶媒中でのリパーゼ触媒によるエリソルビン酸と脂肪酸の縮合における至適条件

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Optimal Conditions for Lipase-catalyzed Condensation of Erythorbic Acid with Fatty Acids in Organic Solvents

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The optimal conditions for the synthesis of lauroyl erythorbate through the condensation of erythorbic acid with lauric acid using immobilized lipase in organic solvents were determined, which are as follows: 0.5 mmol of erythorbic acid, 3.75 mmol of lauric acid, 5 mL of acetonitrile, 50 mg of Chirazyme® L-2 C2, 60℃. Octanoic, decanoic, myristic, and palmitic acid were also used for the synthesis of acyl erythorbate; however, the effect of the acyl chain length of the fatty acid on the reaction conversion was not observed. The DPPH radical scavenging activities of erythorbic acid and acyl erythorbates were measured and it was indicated there was no difference in the activity between erythorbic acid and acyl erythorbates in ethanol solution. Additionally, the suppressive ability of acyl erythorbate against lipid oxidation was investigated. Palmitoyl erythorbate significantly improved the oxidative stability of methyl linoleate to the same extent as that of palmitoyl ascorbate. Based on these results, acyl erythorbate could be considered to be a useful food additive as an amphiphilic antioxidant in a food system such as lipid microcapsules.

Keywords: Acyl erythorbate, amphiphilic antioxidant, erythorbic acid, immobilized lipase, organic solvent

1. Introduction

L-Ascorbic acid, commonly known as vitamin C, is a water-soluble vitamin and is widely used in foods as an antioxidant because of its strong reducing ability. Ascorbic acid in some vegetables such as cucumber, carrot, and pumpkin is easily oxidized through the catalysis of ascorbate oxidase, and the oxidation leads to a decrease in the antioxidative ability and bioavailability of ascorbic acid. D-Erythorbic acid is a stereoisomer of ascorbic acid and is a by-product in the synthesis of ascorbic acid from glucose using both chemical and microbial processes [1]. Erythorbic acid has only ca. 5% of the vitamin activity of ascorbic acid [2], although it is approved as a food antioxidant owing to its reducing properties. Obata et al. estimated the first-order rate parameters for the oxidation of erythorbic and ascorbic acids with ascorbate oxidase. They found that the Michaelis constant, K_m, of the enzymatic reaction for erythorbic acid was 1.3 times that of ascorbic acid and that the maximum rate, V_{max}, for erythorbic acid was about 21% of that of ascorbic acid [3]. These data indicate that erythorbic acid has a weaker affinity for ascorbate oxidase than ascorbic acid, and is far less prone to enzymatic oxidation. Erythorbic acid would serve as a more effective antioxidant than ascorbic acid in vegetables containing ascorbate oxidase.

The synthesis of 6-*O*-acyl ascorbate through the lipase-catalyzed condensation of ascorbic acid and a fatty acid in an organic solvent has been previously reported [4, 5]. The enzymatic synthesis is advantageous compared to a chemical procedure owing to the simplicity of its reaction process and its high regioselectivity. Acyl ascorbate is an amphiphilic antioxidant; it consists of a hydrophilic antioxidative moiety and a lipophilic group. It has also been reported that ascorbates exhibit antitumor and metastasis-inhibitory effects [6, 7]. We have synthesized acyl ascorbates using an immobilized lipase and have applied them to the microencapsulation of lipids by spray-drying [8, 9]. Lipid oxidative stability was significantly improved following microencapsulation with acyl ascorbate. The enzymatic condensation of erythorbic acid and lauric acid has also been reported [10]. However, the effects of various reaction conditions on the productivity of the reaction and the antioxidative

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properties of the product, acyl erythorbate, for lipid oxidation have not been investigated.

It was hypothesized that both acyl ascorbate and acyl erythorbate might be effective for lipid microencapsulation as amphiphilic antioxidants. Additionally, erythorbate efficiently exerts antioxidative activity in a food system despite the presence of ascorbate oxidase. In this study, the optimal conditions for the synthesis of acyl erythorbate using an immobilized lipase were determined, and the suppressive ability against lipid oxidation was evaluated.

2. Materials and Methods

2.1 Materials

D-Erythorbic acid (purity: 98%) and L-ascorbic acid (purity: 99.5%) were purchased from Kishida Chemical (Osaka) and Nacalai Tesque (Kyoto), respectively. Immobilized lipases from *Candida antarctica*-Chirazyme® L-2 c.-f. C2 (lipase type B with Carrier 2), Chirazyme® L-2 c.-f. C3 (lipase type B with Carrier 3), and Chirazyme® L-5 c.-f., (lipase type A with Carrier 1)-and that from *Mucor miehei*-Chirazyme® L-9 c.-f. C2 (lipase with Carrier 2)-were obtained from Roche Molecular Biochemicals (Mannheim). Chirazyme® L-2 C2 was used for the condensation of erythorbic acid with the fatty acid in this study, because the activity of Chirazyme® L-2 C2 for the reaction was the highest among these four immobilized lipases. Palmitoyl ascorbate (purity: 95%) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals were purchased from Tokyo Chemical Industry (Tokyo). All other chemicals of analytical grade were obtained from Wako and Yoneyama Chemicals (Osaka).

2.2 Synthesis and purification of acyl erythorbate

Erythorbic acid (0.0625-1.0 mmol) and the fatty acid (0.47-7.5 mmol) were weighed into an amber glass vial with a screw-cap, and immobilized lipase (5-200 mg) and 5 mL of organic solvent as a reaction medium were added to the vial. Octanoic, decanoic, lauric, myristic and palmitic acid were individually used as lipophilic substrates for the synthesis of acyl erythorbate. Chirazyme® L-2 C2, Chirazyme® L-2 C3, Chirazyme® L-5, and Chirazyme® L-9 C2 were tested as catalysts for the condensation. Dimethyl sulfoxide, acetonitrile, acetone, 2-methyl-2-propanol, 2-methyl-2-butanol, ethyl acetate, chloroform, and hexane were selected for investigating the effect of organic solvents on the conversion to the product in the reaction. The headspace of the vial was filled with nitrogen gas to prevent substrate and product oxidation during the reaction, and the vial was tightly sealed over the blowing gas. The vial was then immersed in a water-bath at 30-70℃ with vigorous shaking. At appropriate intervals, 20 μL of the reaction mixture was sampled and diluted with an eluent, methanol/water/phosphoric acid (75 : 25 : 0.1 by vol.), for HPLC analysis. After the sampling, the headspace was filled with nitrogen gas again. The analysis was carried out using an HPLC (LC-10AT, Shimadzu, Kyoto) with an ODS column (4.6 mmϕx250 mm, Chemcosorb 300-5C18, Chemco Scientific, Osaka) and a UV detector (245 nm, SPD-10A, Shimadzu). The mixture (20 μL) was applied to the column and eluted with the eluent at 0.8 mL/min. The conversion of acyl erythorbate was calculated on the initial molar basis of erythorbic acid, which was the limiting reactant, and was measured in triplicate.

Acyl erythorbates were synthesized on a large scale and purified to determine the molecular structure by NMR, to prepare the calibration curve in HPLC analysis and to evaluate the antioxidative abilities by measuring DPPH radical scavenging activity and the transient change in the peroxide value of methyl linoleate with erythorbate. The condensation reaction was carried out at 60℃ for 24 h, after 7.5 mmol of erythorbic acid, 22.5 mmol of the fatty acid, 0.5 g of Chirazyme® L-2 C2, and 150 mL of acetonitrile were mixed. The used amount of the fatty acid was saved, as a large amount of unreacted fatty acid resulted from the large scale synthesis is inconvenient for purification of the product. Then, acyl erythorbates were purified according to previous methods [11].

The ^{1}H-NMR (400 MHz, CD_3OD, TMS, 297 K) and ^{13}C-NMR (100 MHz, CD_3OD, TMS, 297 K) analyses for lauroyl erythorbate were carried out on a spectrometer (JNM-EX400WB FT, JEOL. Ltd., Tokyo).

2.3 Solubility of erythorbic acid in organic solvents

The solubility of substrate in a solvent affects a reaction. The measurement of the solubility is effective for understanding the reactivity in that solvent and for selection of a suitable organic solvent for the reaction. The solubility of erythorbic acid in the above mentioned organic solvents was measured as follows: Two hundred milligrams of erythorbic acid was added to 5 mL of each organic solvent except for dimethyl sulfoxide, in an

amber glass vial. In the case of dimethyl sulfoxide, 1 g of erythorbic acid was used. The vial was immersed in a water-bath at 60℃ with shaking. After 24 h, 20 μL of the mixture was sampled and adequately diluted with the eluent for the HPLC analysis. The HPLC analysis was carried out under the same conditions as mentioned above. Solubility measurements were done in duplicates.

2.4 DPPH radical scavenging activity

Based on a reported method [12], the DPPH radical scavenging activities of erythorbic acid and acyl erythorbates were measured. Four milliliters of 0.125 mmol/L erythorbic acid or acyl erythorbate in 50% ethanol solution and 1 mL of 0.5 mmol/L DPPH radical in ethanol solution were added to an amber vial. After filling the headspace with nitrogen gas and sealing the vial tightly, it was vigorously shaken and incubated for 20 min at 25℃. The radical scavenging activity of each erythorbate was measured by the decolorization of the DPPH radical at 516 nm using a UV-Vis spectrophotometer (V-520, JASCO Corporation, Tokyo). The measurements were done in duplicates, and the mean value was calculated.

2.5 Measurement of the oxidation process of methyl linoleate with acyl erythorbate

The peroxide value of methyl linoleate with acyl erythorbate was measured as follows: a plastic container was charged with a Petri dish filled with a saturated lithium chloride solution to maintain relative humidity at 12%. The container was stored in the dark at 65℃ for 1 d. Next, 200 mg of methyl linolate was dissolved in 5 ml of hexane, followed by erythorbic acid or palmitoyl erythorbate dissolved in ethanol. The erythorbic acid or palmitoyl erythorbate was added to methyl linolate at a molar ratio of 0.01. Next, 250 μL of the mixture was placed in flat-bottomed glass cups (15 mm i.d. and 30 mm height), and hexane and ethanol were evaporated under reduced pressure in a desiccator. The cups were placed in the container and stored at 65℃. Each cup was periodically removed, and 2.7 mL of a mixture of chloroform and methanol (1/2 (v/v)) was added to the sample in the cup. Moreover, 120 μL of a 25 mmol/L hydrochloric acid/methanol solution and the same volume of a 12.5 mmol/L ammonium/iron(III) sulfate solution were added, and the mixture was fully agitated by using the test tube mixer. Then, 80 μL of a saturated potassium iodide aqueous solution was added, and the sample was centrifuged at 3,000 rpm for 3 min. The absorbance of the supernatant at 15 min after the addition of the saturated potassium iodide solution was measured at 363 nm using the above mentioned spectrophotometer. Each peroxide value was measured in duplicate, and the mean was evaluated.

3. Results and Discussion

3.1 Effect of the concentration of substrates on the condensation

The NMR analysis for lauroyl erythorbate synthesized through the lipase-catalyzed condensation consistently revealed that the primary hydroxyl group at the C6 position in erythorbic acid was esterified with lauric acid. The NMR parameters are as follows: ^{1}H-NMR (400 MHz, CD_3OD, TMS, 297 K) δ; 0.89 (3H, t, J=7.1 Hz, $-CH_3$), 1.27 (16H, m, J=7.9 Hz, $-CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3-$), 1.60 (2H, quint, J=7.1 Hz, $-CH_2-CH_2-COO-$), 2.33 (2H, t, J=7.3 Hz, $-CH_2-COO-$), 4.16 (1H, dt, J=4.2 Hz, $-CH-CH_2-OCO-$), 4.17 (1H, dd, J=3.0 Hz, $-CH_2OCO-$), 4.87 (1H, d, J=2.0 Hz, -CHO-); ^{13}C-NMR (100 MHz, CD_3OD, TMS, 297 K) δ; 14.48 (CH_3), 23.75 (CH_2), 25.94 (CH_2), 30.20 (CH_2), 30.41 (CH_2), 30.46 (CH_2), 30.61 (CH_2), 30.74 (CH_2), 30.74 (CH_2), 33.07 (CH_2), 34.87 (CH_2), 64.67 (CH_2), 69.87 (COH), 78.01 (CH), 119.98 (COH), 154.12 (CH), 172.73 (COO), 175.05 (COO).

Syntheses of lauroyl erythorbate were executed in acetonitrile at 60℃ using four immobilized lipases, Chirazyme® L-2 C2, Chirazyme® L-2 C3, Chirazyme® L-5 and Chirazyme® L-9 C2, with different lipase types and carriers. The activity of Chirazyme® L-2 C2 was the highest in these lipases and the conversion at 48 h was 85.3%. The conversion by Chirazyme® L-2 C3, which had same lipase type as Chirazyme® L-2 C2, was 62.8%, while the reactions hardly progress in the cases of Chirazyme® L-5 and L-9 C2. The types of these lipases are different from that of Chirazyme® L-2 C2 and C3, suggesting that lipase type is more important for the productivity than carrier type.

Figure 1 shows the conversions for the synthesis of lauroyl erythorbate at various initial concentrations of substrates in acetonitrile at 60℃ using immobilized lipase, Chirazyme® L-2 C2. The initial molar ratio of lauric acid to erythorbic acid was fixed at 7.5, because the ratio was appropriate for the condensation between ascorbic acid and the fatty acid in acetonitrile using the lipase [5]. The maximum reaction conversion increased as the initial amounts of substrate increased. When the initial amount of erythorbic acid was 1.0 mmol, the maxi-

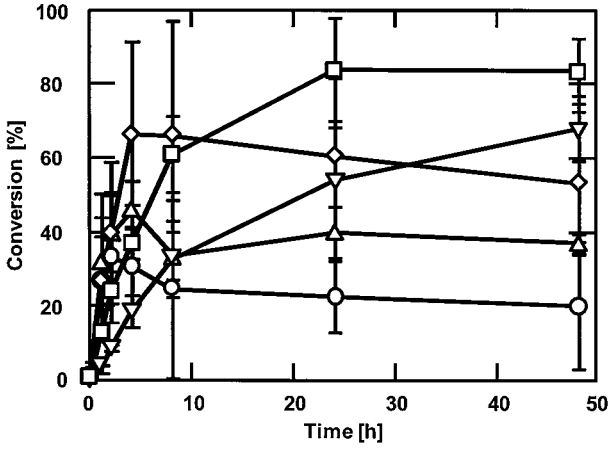

Fig. 1 Transient changes in the conversions for the synthesis of lauroyl erythorbate at the initial amount of erythorbic acid of (○) 0.0625, (△) 0.125, (◇) 0.25, (□) 0.50, and (▽) 1.0 mmol in 5 mL of acetonitrile at 60°C. The initial molar ratio of lauric acid to erythorbic acid was 7.5, and 50 mg of Chirazyme® L-2 C2 was used as a catalyst for the condensation. The bars represent 95% confidence intervals.

mum conversion was not achieved during the experimental period of 2 d. Such a low reaction rate at high initial concentrations of substrates could be attributed to substrate inhibition of the lipase-catalyzed reaction. The initial amount of erythorbic acid at 0.5 mmol was adopted thereafter for all syntheses of acyl erythorbate. The gradual decrease in conversions would indicate the oxidative degradation of erythorbic acid and/or erythorbate during the reaction.

The effect of the molar ratio between the substrates on the conversion for the synthesis of lauroyl erythorbate at 60℃ was examined (Fig. 2). The maximum conversion increased with the increase of the ratio; however, the conversion did not increase beyond the maximum ratio of 7.5. The first step in the lipase-catalyzed condensation is acylation on substrate binding sites in lipase and formation of an enzyme-substrate complex. Next, transesterification between the donor compound containing the hydroxyl group and the complex occurs. Possibly, the immobilized lipase becomes saturated with lauric acid at ratios above 7.5. Octanoic, decanoic, myristic, and palmitic acid were used for the synthesis of the corresponding acyl erythorbate under the same conditions as for lauric acid. The time-courses of the conversions for these syntheses were similar and there was no difference among the reaction rates (data not shown). It was shown that the acyl chain length of the fatty acid did not influence on the lipase-catalyzed reaction.

3.2 Synthesis of acyl erythorbate in various solvents and at various temperatures

Lauroyl erythorbate was synthesized in various organic solvents as shown in Fig. 3. The maximum conversion was in the following order: acetonitrile> acetone> 2-methyl-2-propanol> 2-methyl-2-butanol> ethyl acetate> chloroform. The product was not detected in either dimethyl sulfoxide or hexane. The solubilities of erythorbic acid in dimethyl sulfoxide, acetonitrile, acetone,

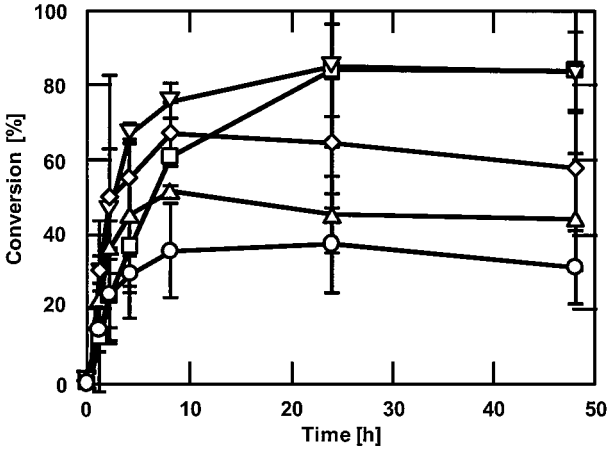

Fig. 2 Effect of the molar ratio of substrates on the conversion for lauroyl erythorbate synthesis in acetonitrile at 60°C. The initial amount of erythorbic acid was 0.5 mmol, and the molar ratios (lauric acid / erythorbic acid) were (○) 1, (△) 2, (◇) 5, (□) 7.5, and (▽) 10. The amounts of Chirazyme® L-2 C2 and acetonitrile were the same as in Fig. 1. The bars represent 95% confidence intervals.

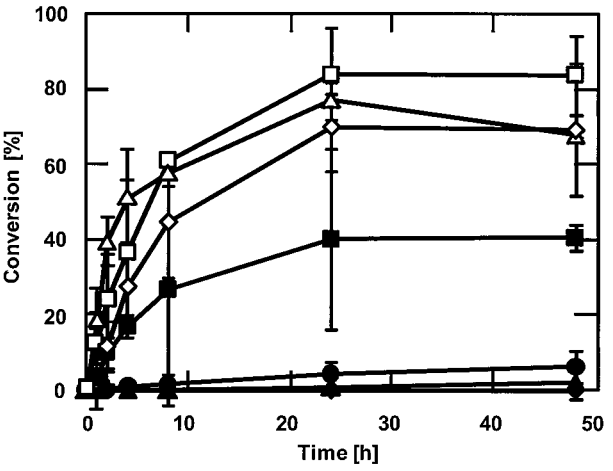

Fig. 3 Lipase-catalyzed condensation of erythorbic acid and lauric acid in (□) acetonitrile, (△) acetone, (◇) 2-methyl-2-propanol, (■) 2-methyl-2-butanol, (●) ethyl acetate, (▲) chloroform, and (◆) hexane at 60℃. The initial amount of erythorbic acid was 0.5 mmol. The initial molar ratio of substrates and the amounts of organic solvents and Chirazyme® L-2 C2 were the same as in Fig. 1. The bars represent 95% confidence intervals.

2-methyl-2-propanol, 2-methyl-2-butanol, ethyl acetate, chloroform, and hexane at 60℃ were 2180, 50.8, 48.4, 38.6, 23.9, 4.8, 4.5, and 1.2 mmol/L, respectively. The conversion seemed to be in proportion to the polarity of the organic solvent. We estimated the equilibrium constant based on the concentrations of substrates and products, K_C, for the lipase-catalyzed condensation of lauric acid and mannose in water-soluble solvents [13]. It was found that the K_C value correlated with the relative dielectric constant of the solvent, because the solubility of a hydrophilic substrate was higher in the solvent with a higher relative dielectric constant. However, the solubility in dimethyl sulfoxide would be too high for erythorbic acid molecules to diffuse to the active site in the immobilized lipase.

Figure 4 shows the conversions for the synthesis of lauroyl erythorbate at various temperatures. The reaction rate was faster at higher temperature. The low conversions at 70℃ could be ascribed to the denaturation of the immobilized lipase at that temperature. Therefore, the reaction at 60℃ should be adopted for the effective synthesis of the product.

3.3 Relationship between the amount of the immobilized lipase and the reaction rate

The condensation of erythorbic acid with lauric acid was carried out with various amounts of Chirazyme® L-2 C2 in acetonitrile. The effect of the amount of immobilized lipase on the reaction rate is shown in Fig. 5. The initial reaction rate increased as the amount of the lipase

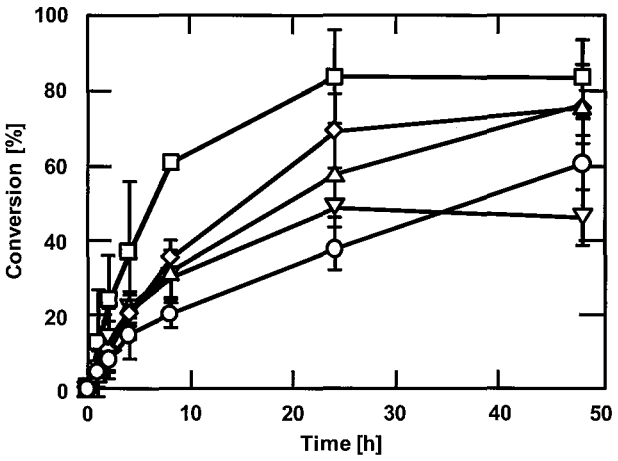

Fig. 4 Temperature dependencies of the conversions for the synthesis of lauroyl erythorbate. The reaction temperatures were (○) 30℃, (△) 40℃, (◇) 50℃, (□) 60℃, and (▽) 70℃. The amounts of erythorbic acid, lauric acid, acetonitrile, and Chirazyme® L-2 C2 were 0.5 mmol, 3.75 mmol, 5 mL, and 50 mg, respectively. The bars represent 95% confidence intervals.

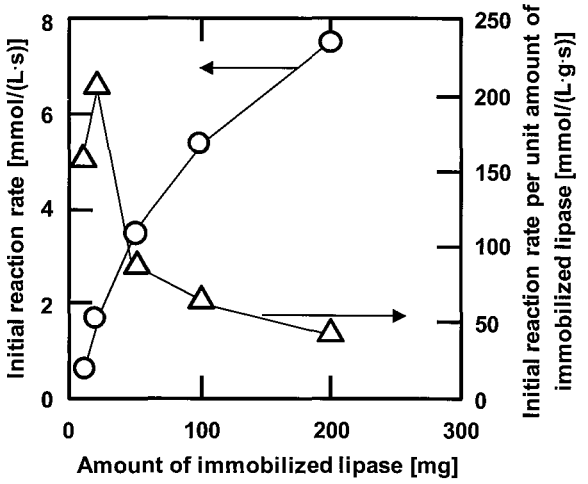

Fig. 5 Relationship between the amount of the immobilized lipase, Chirazyme® L-2, and (○) the initial reaction rate, and (△) the initial reaction rate per unit amount of the immobilized lipase for lauroyl erythorbate synthesis in 5 mL of acetonitrile at 60℃. The amounts of erythorbic acid and lauric acid were 0.5 mmol and 3.75 mmol, respectively.

increased. The reaction rate per unit amount of the lipase reached a peak at 10 mg of the lipase and then decreased at amounts higher than 10 mg. The percent conversions at 48 h in the cases of 5, 10, 50, 100, and 200 mg of the lipase were, however, 15.8, 58.2, 83.7, 85.3, and 78.5%, respectively. Thus, more than 50 mg of lipase was required to obtain high conversion.

3.4 Suppressive ability of acyl erythorbate against lipid oxidation

DPPH radical scavenging activities of erythorbic acid and acyl erythorbates were measured, and the 50% scavenging concentrations (SC_{50}) were estimated. The radical scavenging activities of ascorbic acid and palmitoyl ascorbate were also evaluated. The SC_{50} values of erythorbic acid, octanoyl, decanoyl, lauroyl, myristoyl and palmitoyl erythorbate, ascorbic acid, and palmitoyl ascorbate were 5.41, 5.33, 5.39, 5.06, 5.19, 5.68, 5.28, and 4.72, μmol/L, respectively. These data indicated that there was no difference in radical scavenging activity between erythorbic acid and acyl erythorbates and between erythorbate and ascorbate in ethanol solution. Figure 6 shows the transient changes in the peroxide value of methyl linoleate with erythorbates at 65℃ and under 12% relative humidity. Palmitoyl erythorbate was chosen from various acyl erythorbates with different acyl chain length for this experiment, because the antioxidative ability of the erythorbate against a lipid oxidation could be compared with that of palmitoyl ascorbate, which had the same acyl chain length as the erythorbate and could be

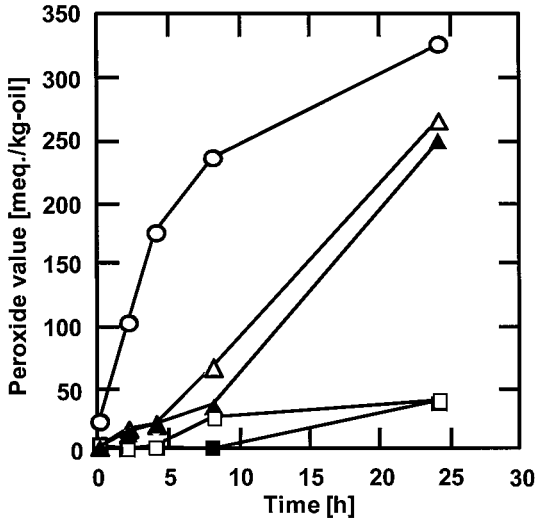

Fig. 6 Transient changes in the peroxide value for the oxidation of 10 mg of methyl linoleate with (○) no additive, (△) erythorbic acid, (□) palmitoyl erythorbate, (▲) ascorbic acid, and (■) palmitoyl ascorbate at 0.01 molar ratio of each additive to methyl linoleate. The oxidation processes were carried out at 65℃ and under 12% relative humidity.

obtained from manufacturers. The suppressive effect of erythorbic acid on the oxidation of methyl linoleate was very similar to that of ascorbic acid. Palmitoyl erythorbate and ascorbate are more effective at preventing oxidation than erythorbic and ascorbic acids. The suppressive abilities of the palmitoyl erythorbate and ascorbate against oxidation were similar, and it was consistent with the radical scavenging activity results. The enhancement of the solubilities of erythorbate and ascorbate in a bulky lipid by the introduction of the acyl group to erythorbic and ascorbic acids would contribute to the improvement of the suppressive ability against lipid oxidation.

4. Conclusion

Acyl erythorbate was synthesized through the lipase-catalyzed condensation of erythorbic acid with saturated fatty acids, and the optimal conditions for the reaction were determined. The maximum conversion of more than 85% for 48 h was attained under the standard conditions in a batch reaction. The suppressive ability of erythorbic acid against lipid oxidation was improved by acylation, and acyl erythorbate exhibited almost the same antioxidative activity as acyl ascorbate. Therefore, acyl erythorbate could be effectively used for microencapsulation of lipids owing to its abilities to act as an antioxidant and an emulsifier.

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◇◇◇ 和文要約 ◇◇◇

有機溶媒中でのリパーゼ触媒によるエリソルビン酸と脂肪酸の縮合における至適条件

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野菜中のL-アスコルビン酸は，アスコルビン酸酸化酵素の触媒作用により容易に酸化され，その機能性の低下が生じる．一方，D-エリソルビン酸はアスコルビン酸の立体異性体であり，その還元能により食品の酸化防止剤としての使用が認められている．エリソルビン酸はアスコルビン酸よりもアスコルビン酸酸化酵素に対してより弱い親和性を有し，酸化反応を受けにくい傾向があることが報告されている．有機溶媒中でのリパーゼ触媒下のアスコルビン酸と脂肪酸の縮合による両親媒性抗酸化剤6-*O*-アシルアスコルビン酸の合成が報告されている．噴霧乾燥法による脂質の粉末化技術にアシルアスコルビン酸を利用した結果，粉末化脂質の酸化安定性が大きく改善された．アシルエリソルビン酸も両親媒性抗酸化剤として粉末化脂質の利用に有効である可能性があり，さらにアシルエリソルビン酸はアスコルビン酸酸化酵素の存在する食品においても効果的に抗酸化活性を発現することが期待される．本研究では，固定化リパーゼを用いたアシルエリソルビン酸合成のための最適条件が決定され，脂質酸化に対する抑制効果が評価された．

所定量のエリソルビン酸，脂肪酸および固定化リパーゼを褐色バイアル瓶入れ，5 mLの各種有機溶媒が加えられた．炭素数8から16の飽和脂肪酸が使用された．固定化リパーゼChirazyme® L-2 C2，L-2 C3，L-5およびL-9 C2が用いられた．反応溶媒には，ジメチルスルホキシド，アセトニトリル，アセトン，2-メチル-2-プロパノール，2-メチル-2-ブタノール，酢酸エチル，クロロホルムおよびヘキサンが選択された．バイアル瓶は激しく振とうしながら30～70℃の水浴中に浸漬され，適当な間隔で反応液が採取された後，HPLC分析により生成物量の経時変化が測定された．脂質酸化に対する抑制効果はリノール酸メチルの過酸化物価の経時変化にて評価され，さらにアシルエリソルビン酸のDPPHラジカル消去活性が測定された．

エリソルビン酸とラウリン酸との縮合反応における生成物のNMR構造解析結果から，生成物はエリソルビン酸のC6位の一級水酸基がラウリン酸によりエステル化された構造を有することが明らかとなった．4種の固定化リパーゼではChirazyme® L-2 C2の活性が最も高かった．0.0625から1.0 mmolの初期エリソルビン酸量にて60℃のアセトニトリル中で縮合反応を行ったところ，基質の初期濃度が高いほど最大反応率が高くなる傾向が示された．基質モル比を1:1から1:10の範囲で同反応を実施したところ，モル比の増加とともに最大反応率は増大したが1:7.5以上では差異が認められなかった．各種飽和脂肪酸での反応率の経時変化はいずれもほぼ同じ挙動を示し，アシル鎖長は反応に影響しないことが示された．種々の有機溶媒中で反応が行われた結果，反応率は溶媒極性に起因するエリソルビン酸の溶解度に依存する傾向が示された．30℃から70℃で反応が行われたところ反応は高い温度でより迅速に進んだが，70℃ではリパーゼの熱変性が示唆された．固定化リパーゼ量を5から200 mgにて反応を実施したところ，反応速度は固定化リパーゼ量に顕著に依存した．DPPHラジカル消去活性の測定から，エタノール溶液中でのラジカル消去能について，エリソルビン酸とアシルエリソルビン酸との間に差異がないことが示された．また，65℃，相対湿度12％下でのリノール酸メチルの酸化に対する抑制効果は，パルミトイルエリソルビン酸およびパルミトイルアスコルビン酸の方が，エリソルビン酸およびアスコルビン酸よりも大きかった．エリソルビン酸やアスコルビン酸へのアシル基の導入は脂質へのそれらの溶解度を向上させ，その結果脂質酸化に対する抑制能が改善されたものと考えられる．アシルエリソルビン酸はその抗酸化能および乳化能により，脂質の粉末化に対して効果的に使用することができるものと考えられる．

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