

Enzymatic Synthesis: Acyl Ascorbate and Its Industrial Use as a Food Additive

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ABSTRACT

Acyl ascorbates were synthesized through the condensation of various fatty acids with L-ascorbic acid using immobilized lipase in a water-soluble organic solvent, and their properties as food additive were examined. The optimal conditions, which were the type of organic solvent, reaction temperature, the initial concentrations of substrates and the molar ratio of fatty acid to ascorbic acid, for the enzymatic synthesis in a batch reaction were determined. The continuous production of acyl ascorbate was carried out using a continuous stirred tank reactor (CSTR) and plug flow reactor (PFR) at 50°C, and each productivity was ca. 6.0×10 for CSTR and 1.9×10^3 g/(L-reactor·d) for PFR for at least 11 days, respectively. The temperature dependences of the solubility of acyl ascorbate in both soybean oil and water could be expressed by the van't Hoff equation, and the dissolution enthalpy, ΔH , values for the soybean oil and water were ca. 20 and 90 kJ/mol, respectively, irrespective of the acyl chain length. The decomposition kinetics of saturated acyl ascorbate in an aqueous solution and air was empirically expressed by the Weibull equation, and the rate constant, k , was estimated. The activation energy, E , for the rate constant for the decomposition in both systems depended on the acyl chain length. The surface tensions of acyl ascorbates in an aqueous solution were measured by the Wilhelmy method, and the critical micelle concentration (CMC) and the residual area per molecule were calculated. The CMC values were independent of temperature but dependent on the pH. The effect of pH of aqueous phase on the stability of O/W emulsion prepared using acyl ascorbate as an emulsifier was examined, and the high stability at pHs 5 and 6 was ascribed to the largely negative surface-charge of droplets in the emulsion. The addition of saturated acyl ascorbate, whose acyl chain length was from 8 to 16, lengthened the induction period for the oxidation of linoleic acid in a bulk and microcapsule with maltodextrin as a wall material. The oxidative stability in bulk system increased with increasing

the acyl chain length, whereas that in the microcapsule was the highest at the acyl chain length of 10. The esterification of various polyunsaturated fatty acids, such as linoleic, α - and γ -linolenic, dihomo- γ -linolenic, arachidonic, eicosapentaenoic, docosahexaenoic and conjugated linoleic acids with ascorbic acid and subsequent microencapsulation significantly improved their oxidative stability.

Keywords: *Acyl ascorbate; Immobilized lipase; Continuous production; Amphiphilic food additive; Antioxidative emulsifier*

1 INTRODUCTION

Lipid oxidation is a complicated process. Antioxidants prolong the oxidation such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), propyl gallate, vitamin C and tocopherols (vitamin E), inhibit free radical chain reactions. Derivatives of ascorbic acid acylated with a long-chain fatty acid such as palmitic acid are common additives in foods rich in lipid. 6-O-palmytoyl ascorbate shows antitumor activity and metastasis-inhibitory effects. Polyunsaturated fatty acid (n-6 PUFA) have advantage as a substrate for the synthesis of acyl ascorbate along with other physiological functions such as antithrombotic, cholesterol depressant and antiallergenic properties, as a precursor of prostaglandins, leukotrienes and related compounds in inflammation and immunity. But they suffer as 6-O-palmitoyl and stearyl ascorbates are insoluble in water than the shorter acyl chains ascorbates. Enzymatic vitamin C esters is other advantage than a chemical method because of the simplicity of its reaction process and its high regioselectivity. Examples are synthesis sugar and fatty acids esterification by lipase, 6-O-Palmitoyl ascorbate using lipase shown in Figure 1. Enzymatic synthesis of 6-O-acyl ascorbates using immobilized lipase in a batch reaction system a CSTR or PFR, solubility in water - oil conditions (Section 3), emulsifier property and antioxidative ability of acyl ascorbate in a lipid microencapsulation are evaluated (Sections 4, 5).

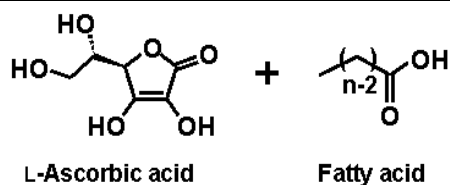


Figure 1. Scheme of condensation reaction of ascorbic acid with fatty acid using lipases from various origins in a microaqueous organic solvent with low water content.

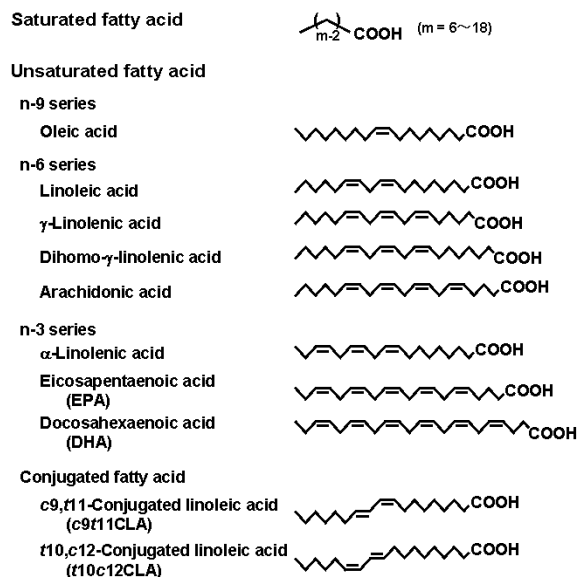


Figure 2. Molecular structures of fatty acids used for the synthesis of acyl ascorbate.

2 CONDITIONS FOR ENZYMATIC ACYL ASCORBATE SYNTHESIS : BATCH REACTION

The condensation of L-ascorbic acid and lauric acid in dehydrated acetonitrile by lipases (Chirazyme®L-2, Novozym®435, lipase types PS,QL,PL,A,F,M,MY,OF immobilized on macroporous acrylic resin can catalyze the condensation in the medium. Optimal conditions are 60°C. Examples: eicosapentaenoic acid and ascorbic acid condensation is shown in Figure 3(a). Major factors: water content of reaction medium; molar ratio of eicosapentaenoic acid : ascorbic acid = 0.129 mmol:0.125 mmol; transient changes in the conversion (see Figure 4a and 4b). The equilibrium constant, K_C , is defined by

$$K_C = \frac{C_{P_e} C_{W_e}}{C_{A_e} C_{F_e}}$$

where C is the concentration and the subscripts A, F, P, and W represent ascorbic acid, lauric acid, lauroyl ascorbate, and water, respectively. The subscript e indicates the equilibrium. The values of C_{P_e} and C_{W_e} were experimentally determined.

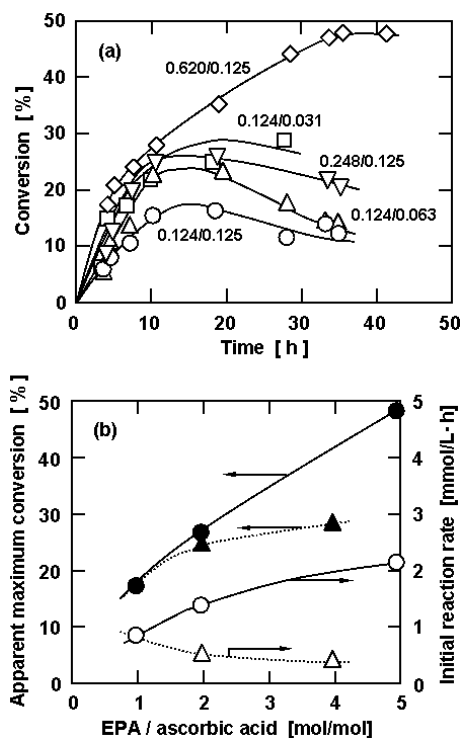


Figure 3. Lipase-catalyzed condensation of eicosapentaenoic acid (EPA) and ascorbic acid at their various molar ratios in acetone at 55°C.

Figure 3(a) and 4(a), show the formation rate, conversion (equilibrium conversion).

3 CONTINUOUS PRODUCTION OF ACYL ASCORBATE USING A CSTR OR PFR

As illustration, continuous stirred tank reactor (CSTR) system is shown in Figure 5(a). An immobilized lipase (3.0 g by dry weight), Chirazyme®L-2 C2, is shown packed into the stainless steel basket. The volume of the solvent in the reactor, mass of ascorbic acid, fatty acid (decanoic, lauric or myristic acid) added with right solvent concn, reactor size, flow rate, water-bath temperature and magnetic stirrer speed are very crucial factors in this plug flow reactor system (PFR) is shown in Figure 5(b) for Chirazyme®L-2 C2 particles. The dependence mean residence times in the reactor, τ , and the concentrations of the decanoyl, lauroyl and myristoyl ascorbates in the effluent shown in Figure 6 with productivity

of lauroyl ascorbate, decanoyl and myristoyl ascorbate in Figure 7.

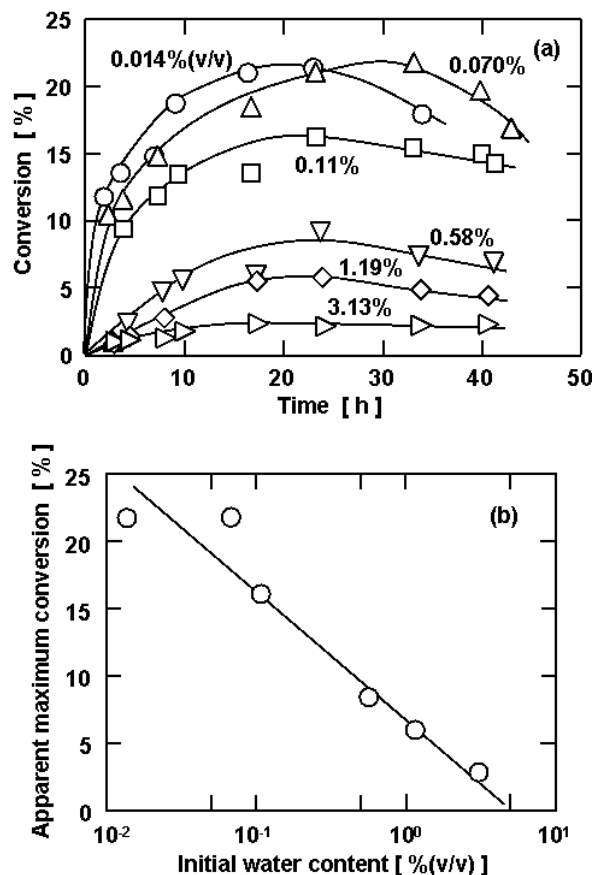


Figure 4. Effect of initial acetone water content on the synthesis of 6-O-eicosapentaenoyl ascorbate through lipase-catalyzed condensation.

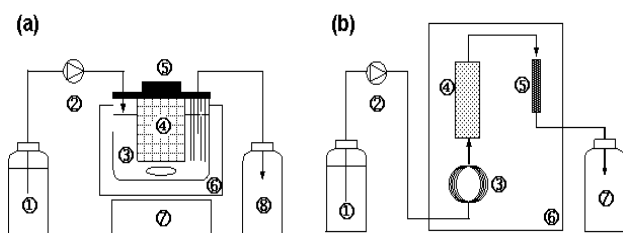


Figure 5 (a) Scheme of CSTR for the continuous synthesis of saturated acyl ascorbates. 1: feed reservoir, 2: pump, 3: reactor, 4: basket packed with immobilized lipase, 5: lid, 6: water-bath, 7: magnetic stirrer, 8: effluent reservoir; (b) Scheme of a PFR; 1: feed reservoir, 2: pump, 3: pre-heating coil, 4: column packed with ascorbic acid, 5: column packed with immobilized lipase, 6: thermo-regulated chamber, 7: effluent reservoir.

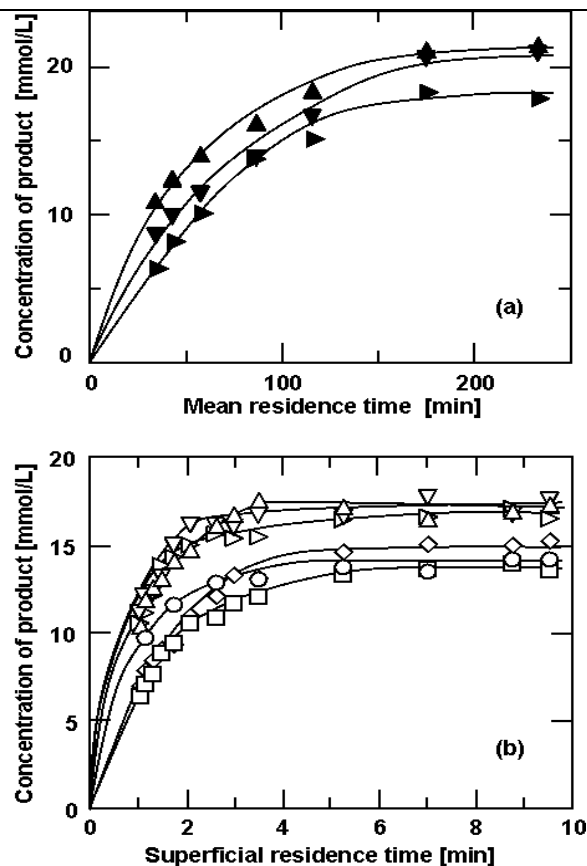


Figure 6. (a) Outcome of mean residence time, τ , and the concentration of (▲) decanoyl, (▼) lauroyl or (►) myristoyl ascorbate in the CSTR at 50°C. (b) Relationship between the superficial residence time, τ , and the concentration of (○) arachidonoyl, (□) oleoyl, (◇) linoleoyl, (△) decanoyl, (▽) lauroyl or (▷) myristoyl ascorbate using the PFR at 50°C. In both reactor systems, the fatty acid concentration in the feed was 200 mmol/L.

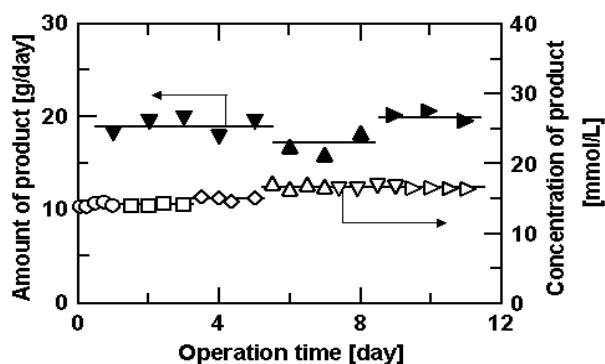


Figure 7. Continuous production of (▲, △) decanoyl, (▼, ▽) lauroyl or (►, ▷) myristoyl, (○) arachidonoyl, (□) oleoyl, and (◇) linoleoyl ascorbate using the CSTR (closed symbols) or PFR (open symbols) with immobilized lipase, Chirazyme® L-2 C2, at 50°C.

4 SOLUBILITY OF ACYL ASCORBATE IN WATER AND OIL

The solubilities of the saturated acyl ascorbates in water or soybean oil show specific behavior. The acylation of ascorbic acid improves its solubility in soybean oil but decreased the solubility in water. The temperature dependence of the solubilities, S , of the decanoyl, lauroyl, myristoyl, palmitoyl and stearoyl ascorbates in water or soybean oil (Figure 8) could be expressed by the following van't Hoff equation:

$$\frac{dS}{d(1/T)} = -\frac{\Delta H}{R}$$

where ΔH is the dissolution enthalpy, R is the gas constant, and T is the absolute temperature. The dependence of the solubility in water on the acyl chain length of the acyl ascorbate was much stronger than that of the solubility in soybean oil. The plots in Figure 8 shows a straight line for each acyl ascorbate. The dissolution enthalpy, ΔH , was evaluated from the slope. The relationship between the acyl chain length and ΔH for the solubilization of the saturated acyl ascorbates in soybean oil or water is unique.

5. APPLICATIONS : ACYL ASCORBATE FOR LIPID MICROENCAPSULATION

Microencapsulation of a lipid with a wall material is a promising technology in the food and other industries. Microencapsulation of polyunsaturated fatty acid or its acylglycerol suppresses or retards lipid oxidation.

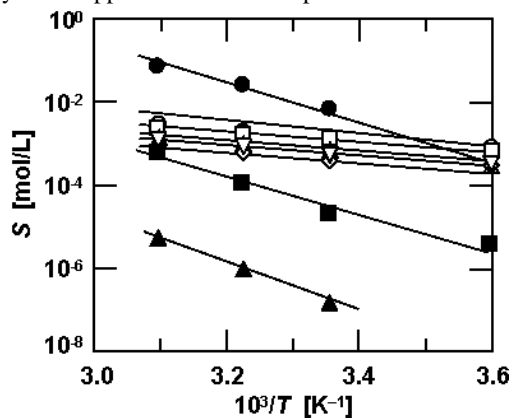


Figure 8. van't Hoff plots for the solubilities, S , of (○, ●) decanoyl, (□, ■) lauroyl, (△, ▲) myristoyl, (▽) palmitoyl and (◇) stearoyl ascorbates in soybean oil (open symbols) and water (closed symbols).

Microencapsulation is emulsification of lipid core with a polysaccharide wall followed by drying of the emulsions (Spray-dried microcapsules). As illustration, linoleic acid can be mixed with acyl ascorbates and microencapsulated with maltodextrin or gum arabic by spray-

drying with monitoring antioxidative ability of the ascorbates toward the encapsulated linoleic acid (see Figure 9). Microencapsulation needs optimal conditions spray-dryer flow rate of 3.0 kg/h, centrifugal atomizer at ca. 3×10^4 rpm, temperatures of air 200°C and 100 - 110°C, flow rate of air was ca. 7.5 m³/min, relative humidity 12%, dark at 37°C.

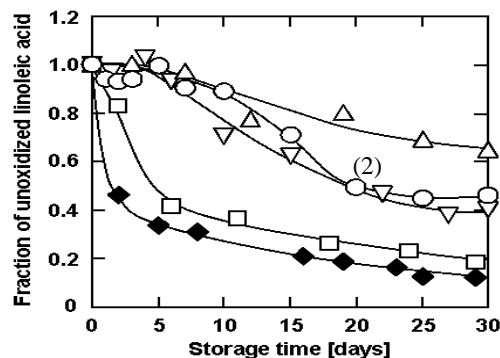


Figure 9. Effect of the addition of various saturated acyl ascorbates to linoleic acid on the oxidation of linoleic acid encapsulated with maltodextrin; (◇) no addition, (□) octanoyl, (△) decanoyl, (▽) lauroyl and (○) palmitoyl ascorbate. The molar ratio of the saturated acyl ascorbate to linoleic acid was 0.1. Temperature 37°C and a relative humidity 12%.

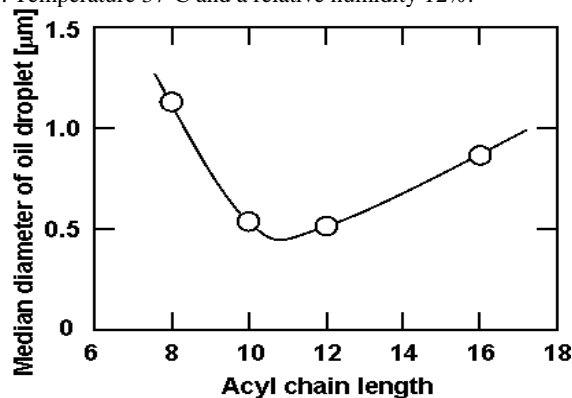


Figure 10. Example of oil droplets in emulsions prepared with various saturated acyl ascorbates depend on radii.

The oxidative stability of the microencapsulated arachidonoyl ascorbate and arachidonic acid is crucial at 37°C and 12% relative humidity.

6 CONCLUSION

Acyl ascorbate is synthesized through lipase-catalyzed-condensation of ascorbic acid with fatty acid. Enzymatic synthesis of acyl ascorbate is more advantageous than a chemical method. The application of acyl ascorbate for microencapsulation of a lipid as antioxidative emulsifier is useful technology for suppressing or retarding the oxidation of polyunsaturated fatty acid.

7 REFERENCE

<http://www.scribd.com/doc/25799862/Biotechnology-and-Nanotechnology/>