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Emulsifier properties of saturated acyl L-ascorbates for preparation of O/W emulsions

Ko Kuwabara[a], Yoshiyuki Watanabe[a], Shuji Adachi[a,*], Kazuhiro Nakanishi[b], Ryuichi Matsuno[a]

[a]*Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*
[b]*Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Tsushima-naka 7-1-1, Okayama 700-8530, Japan*

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Abstract

Oil-in-water emulsions, the oil phases of which were soybean oil, were prepared using acyl L-ascorbates with even acyl carbon numbers of 8–16. The median diameters of oil droplets in the emulsions and the stability of the emulsions were examined. Caproyl and lauroyl L-ascorbates were adequate for the preparation of fine and stable emulsions. The emulsions were more stable at higher concentrations of the emulsifiers. The emulsions were prepared using citrate buffers with various pH values as an aqueous phase. The stability of the emulsions was higher at higher pH values of the aqueous phase. The high stability was ascribed to the largely negative surface-charge of oil droplets in the emulsions.

Keywords: Acyl L-ascorbate; Emulsifier; Emulsion stability; O/W emulsion

1. Introduction

L-Ascorbic acid is a water-soluble vitamin (vitamin C) and is widely used as an additive in foods and cosmetics. Its derivatives with hydrophobic groups, such as acyl residues, seem to be promising emulsifiers with both surface-activity and reductivity. The stability of ascorbic acid or the ascorbyl moiety of palmitoyl L-ascorbates has been examined in an oil-in-water (O/W) emulsion system (Gallarate, Carlotti, Trotta, & Bovo, 1999; Špiclin, Gašperlin, & Kmetec, 2001). However, 6-*O*-palmitoyl and stearoyl L-ascorbates, which are commercially available, are practically so insoluble in water that they would be difficult to use as an emulsifier for preparation of O/W emulsions.

Some reports have recently been published on the lipase-catalyzed synthesis of 6-*O*-acyl L-ascorbates in organic solvents with low water content (Hemeau, Girardin, Coulon, & Miclo, 1995; Stamatis, Sereti, & Kolisis, 1999; Tang, Zhang, Shehate, & Sun, 2000; Yan, Bornscheuer, & Schmid, 1999). We also reported the synthesis of saturated or unsaturated acyl ascorbates (Watanabe, Adachi, & Matsuno, 1999; Watanabe, Adachi, Nakanishi, & Matsuno, 2001). Acyl L-ascorbates with shorter acyl chains would be more amphiphilic than palmitoyl and stearoyl ascorbates. The ascorbates with short acyl chains would be more water-soluble and have more potential as emulsifiers for preparing O/W emulsions than those with long acyl chains.

In this context, we have synthesized L-ascorbates with even carbon numbers of acyl chains, from 8 to 14, through immobilized-catalyzed condensation of ascorbic acid and the respective fatty acids and have then examined their emulsifier properties for preparation of O/W emulsions, using soybean oil as an oily phase. The effects of the acyl chain length of L-ascorbates on the median diameter of oil droplets in O/W emulsion and the stability of the emulsion were estimated. The effects of pH and ion strength of the aqueous phase on the stability of emulsions were also examined.

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* Corresponding author. Tel.: +81-75-753-6287; fax: +81-75-753-6285.

E-mail address: adachi@kais.kyoto-u.ac.jp (S. Adachi).

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2. Materials and methods

2.1. Materials

Soybean oil, palmitoyl L-ascorbate and other chemicals of analytical grade were purchased from Wako Pure Chemical Industry (Osaka, Japan). Capryloyl, caproyl, lauroyl and myristoyl L-ascorbates were synthesized through an immobilized-lipase-catalyzed condensation of L-ascorbic acid and the respective fatty acids in acetone according to our previous procedure (Watanabe et al., 1999) with some modifications.

2.2. Preparation of O/W emulsions

An appropriate amount of an acyl L-ascorbate, which was specified to give a final concentration of 5×10^{-7} to 5×10^{-3} mol/kg solution (water plus soybean oil), was weighed into an amber glass vial, and 19 g of distilled water and 1 g of soybean oil were put into the vial. When the effects of pH of the aqueous phase on the stability and ζ-potential of the emulsion were examined, distilled water was replaced by a buffer solution. The buffer solutions used were 0.1 or 0.01 mol/l sodium citrate-HCl for pH = 2, 3 and 4, 0.1 or 0.01 mol/l sodium citrate for pH = 5, and 0.1 or 0.01 mol/l sodium citrate–NaOH for pH = 5.5 and 6. The mixture was emulsified to produce an O/W emulsion with a rotor/stator homogenizer (Physcotron NS-50, Nichion Irika, Tokyo, Japan) for 1 min at a power control setting of 70. The emulsion was further homogenized to produce a fine O/W emulsion with small oil droplets using a high-pressure homogenizer (PEL-20, Nanomizer, Tokyo, Japan) at a pressure of ca. 98 MPa.

The particle size distribution of the oil droplets was measured using a laser diffraction particle size analyzer (SALD-2100, Shimadzu, Kyoto, Japan) after appropriate dilution with distilled water.

2.3. Stability of emulsion

The stability of the emulsion was estimated by a turbidity method (Pearce & Kinsella, 1978). The emulsion was stored at 30 °C with gentle stirring. At appropriate intervals, aliquots of the emulsion were removed and diluted 50 and 400 times for the emulsions with 0.1% (w/v) sodium dodecyl sulfate. The absorbance at 500 nm of the diluted emulsion was then measured using a Shimadzu UV-1600 spectrophotometer (Kyoto, Japan).

2.4. ζ-Potential of oil droplets

Aliquots of the emulsion were diluted 4000 times with the buffer solution which had been used in the emulsification. The ζ-potentials of at least 50 oil droplets were measured at room temperature with a zeta-potential analyzer (Zeecom, Microtech Nichion, Tokyo, Japan) at an applied voltage of 50 V. The potentials were calculated by the Smoluchowski equation using software provided by the manufacturer and were averaged.

3. Results and discussion

3.1. Median diameter of oil droplets in O/W emulsion

Fig. 1 shows the median diameters of oil droplets in the O/W emulsions prepared at various concentrations of acyl L-ascorbates with different acyl chain lengths. Except for palmitoyl L-ascorbate, the median diameter decreased with increasing emulsifier concentration. The inner figure of Fig. 1 shows the relationship between the median diameter, d_p, observed at an emulsifier concentration of 0.5 mmol/kg and the carbon number of the acyl chain of the emulsifier, n. The emulsifiers with acyl chains shorter than or equal to 14 gave fine emulsions with median diameters of ca. 1 μm.

3.2. Stability of O/W emulsions prepared using L-acyl ascorbates with different acyl chain lengths

O/W emulsions consisting of distilled water and soybean oil were prepared at various concentrations of acyl L-ascorbates with different acyl chain lengths, and their stabilities were measured at 30 °C by the turbidity method (Fig. 2). The emulsions prepared using capryloyl, myristoyl and palmitoyl L-ascorbates as the

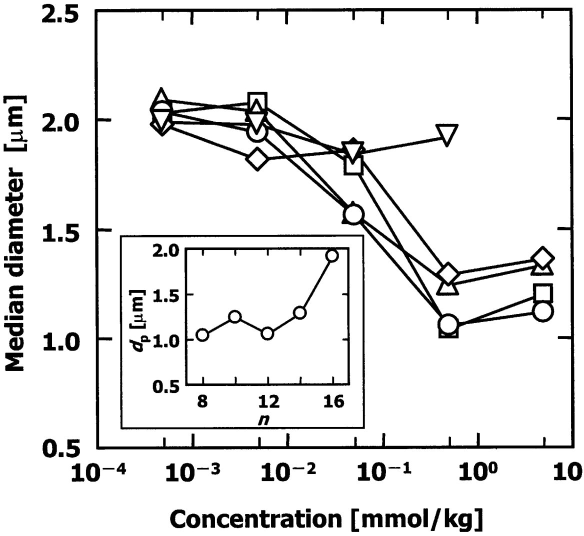

Fig. 1. Median diameter of oil droplets in O/W emulsions prepared using acyl L-ascorbates with different acyl chains at various concentrations as emulsifiers. The emulsifiers used were (□) capryloyl, (△) caproyl, (○) lauroyl, (◇) myristoyl and (▽) palmitoyl L-ascorbates. The inner figure shows the dependence of the median diameter, d_p, of oil droplets in the emulsions prepared at an emulsifier concentration of 0.5 mmol/kg on the carbon number of the acyl chain, n.

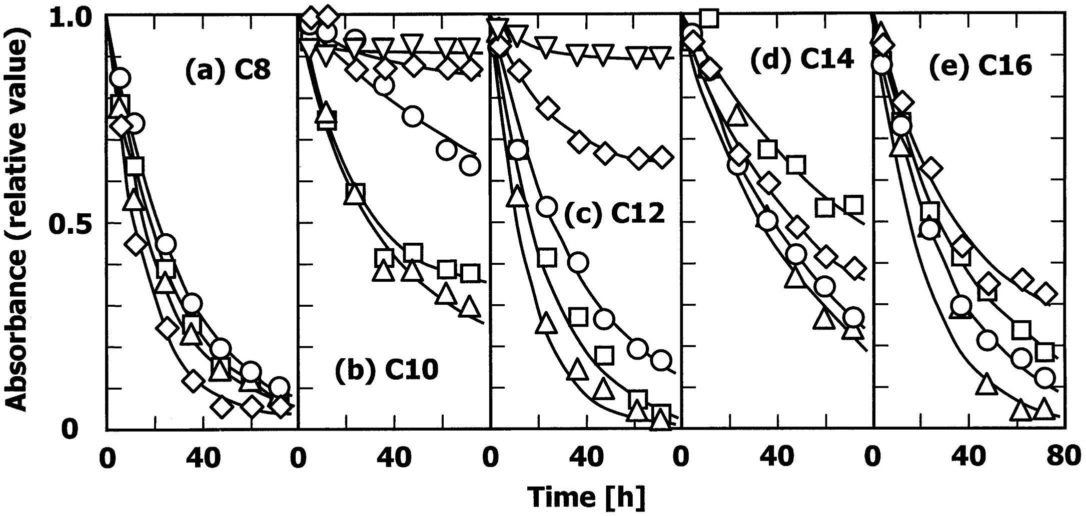

Fig. 2. Stability at 30 °C of O/W emulsions prepared using (a) capryloyl, (b) caproyl, (c) lauroyl, (d) myristoyl and (e) palmitoyl L-ascorbates at various concentrations as emulsifiers. The concentrations of each acyl L-ascorbate were (□) 0.0005, (△) 0.005, (○) 0.05, (◇) 0.5, and (▽) 5.0 mmol/kg. The ordinate indicates the absorbance at 500 nm relative to the initial one.

emulsifiers were unstable at the concentrations tested. To examine the effect of the emulsifier concentration on the stability, the relative absorbance at 48 h is plotted versus the concentration for each acyl ascorbate in Fig. 3. The stabilities of the emulsions prepared with caproyl and lauroyl L-ascorbates depended on the emulsifier concentrations and increased with higher concentrations.

The hydrophilic–lipophilic balance (HLB) numbers of capryloyl, caproyl, lauroyl, myristoyl and palmitoyl L-ascorbates, which were evaluated according to the Davies' equation (Davies, 1957), are 11.8, 10.9, 9.90, 8.95 and 8.00, respectively. McClements (1999) reported that the maximum stability of O/W emulsions is obtained using surfactants with an HLB number around 10–12. This criterion was almost applicable for acyl ascorbates, and caproyl and lauroyl L-ascorbates produced fine and stable O/W emulsions.

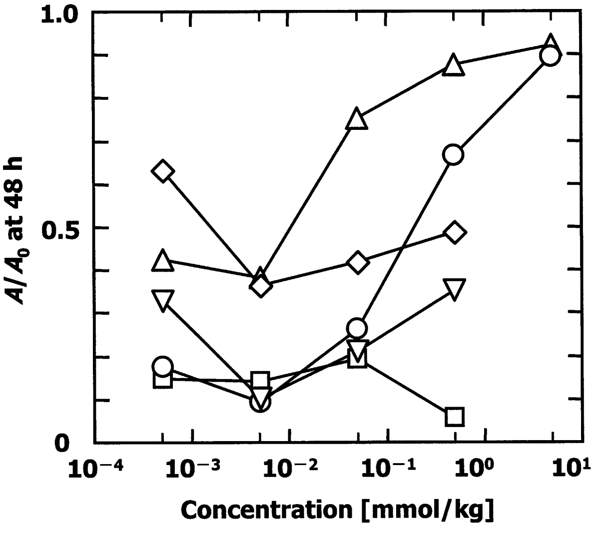

Fig. 3. Dependence of the relative absorbance at 48 h on the emulsifier concentration. The keys are the same as in Fig. 1.

3.3. Effect of pH of aqueous phase on the stability of O/W emulsions

The pH and ionic strength of the aqueous phase of O/W emulsion are factors affecting the stability. Their effects on the stability were examined for the emulsions prepared using lauroyl L-ascorbate at a concentration of 0.5 mmol/kg. As shown in Fig. 4a, the buffer concentrations of 0.1 mol/l were high, and the emulsions were very unstable at any pH value. The instability may be ascribed to the short Debye length surrounding the oil droplets. When the buffer concentrations were 0.01 mol/l (Fig. 4b), the stability of the O/W emulsions greatly depended on the pH value of the aqueous phase, and the emulsions were more stable at higher pH.

The pK_a value of L-ascorbic acid is ca. 3.77 (Kröger-Ohlsen & Skibsted, 1997). Acyl ascorbates, which adsorb on the surface of oil droplets in O/W emulsions, also seem to have similar pK_a values. Therefore, the surface charge of the oil droplets would be affected by the pH of the aqueous phase. The ζ-potentials of oil droplets in the O/W emulsions, prepared using caproyl and lauroyl L-ascorbates at a concentration of 0.5 mmol/kg, were measured at various pH values of the aqueous phase (Fig. 5). The ζ-potentials of the oil droplets in the emulsions prepared with no emulsifier were also measured. For the emulsions with caproyl and lauroyl ascorbates, the potential largely decreased at pH vaules higher than 4. The largely negative surface charges at pH = 5 and 6 would be the reason for the high stability of the emulsions at these pH values.

As mentioned above, caproyl and lauroyl L-ascorbates, which were enzymatically synthesized through

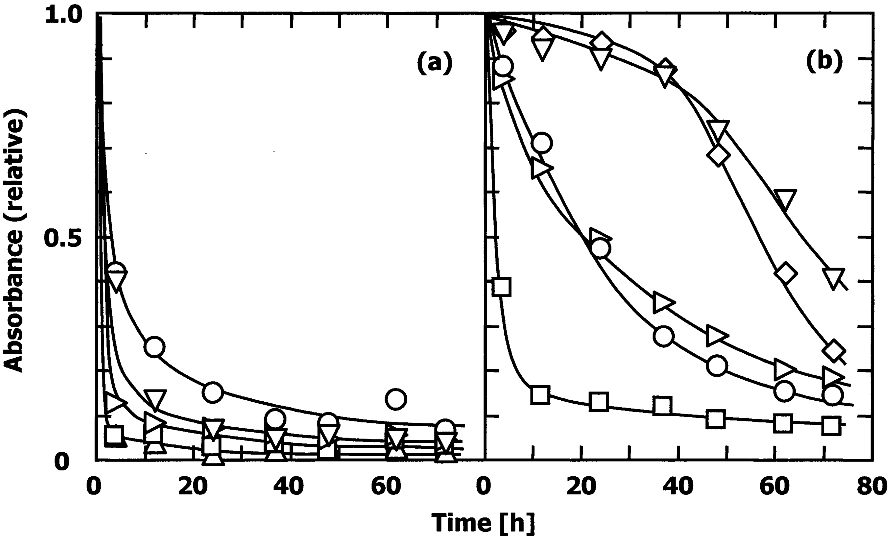

Fig. 4. Effect of the pH of aqueous phase on the stability of O/W emulsions. The concentrations of citrate buffers of pH = (△) 2, (□) 3, (▷) 4, (○) 5, (◇) 5.5, and (▽) 6 were (a) 0.1 and (b) 0.01 mol/l.

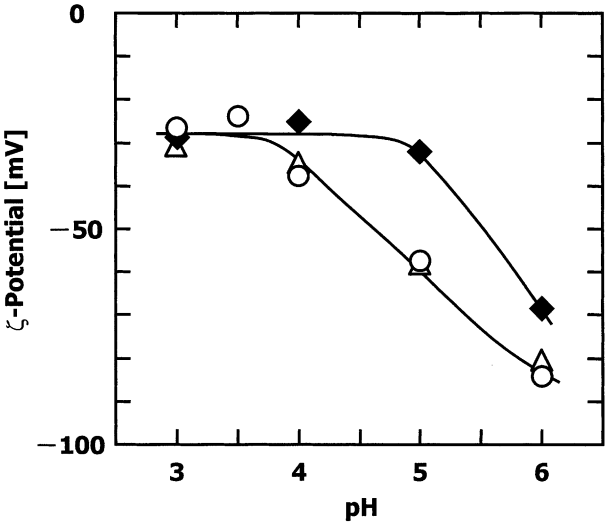

Fig. 5. pH-dependence of the ζ-potential of oil droplets in O/W emulsions prepared with (△) caproyl and (○) lauroyl L-ascorbates and (◆) without any emulsifier. The concentrations of caproyl and lauroyl L-ascorbates were 0.5 mmol/kg.

immobilized-lipase-catalyzed condensation in acetone, are useful emulsifiers for O/W emulsions, although only palmitoyl and stearoyl L-ascorbates are commercially available.

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