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Suppressive Effect of Saturated Acyl L-Ascorbate on the Oxidation of Linoleic Acid Encapsulated with Maltodextrin or Gum Arabic by Spray-Drying

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6-*O*-Palmitoyl L-ascorbate was added to linoleic acid at various molar ratios of the ascorbate to the acid, the mixtures were emulsified with a maltodextrin or gum arabic solution, and the emulsions were spray-dried to produce microcapsules. At higher molar ratios, the oil droplets in the emulsions were smaller, and the oxidative stabilities of the encapsulated linoleic acid were higher for both the maltodextrin- and gum arabic-based microcapsules. 6-*O*-Capryloyl, caproyl, and lauroyl L-ascorbates, which were synthesized through lipase-catalyzed condensation in acetone, were also used for the microencapsulation of linoleic acid. Except for capryloyl L-ascorbate, the addition of a saturated acyl ascorbate, especially caproyl ascorbate, to linoleic acid was effective for preparing oil droplets of small particle diameter and for suppressing the oxidation of the encapsulated linoleic acid.

KEYWORDS: Saturated acyl L-ascorbate; encapsulation; oil-in-water emulsion; spray-drying; antioxidative ability

INTRODUCTION

Microencapsulation of a lipid with a wall material can provide the lipid with some new functions (*1*) and is a promising technology in food and other industries (*2*). Microencapsulation of a polyunsaturated fatty acid (PUFA) or its acylglycerol can suppress or retard its oxidation (*3*, *4*). Microencapsulation consists of two steps: one is emulsification of a core material such as a lipid with a dense solution of a wall material such as a polysaccharide, and another is drying of the emulsions. Spray-drying is the general technique for preparing microcapsules. We have reported that the properties of emulsions such as stability and droplet size affected the oxidative stability of the core material in the spray-dried microcapsules (*4*).

L-Ascorbic acid is a water-soluble vitamin (vitamin C) with high reductivity and is widely used as an additive in foods and cosmetics. Its lipophilic derivatives, 6-*O*-palmitoyl and 6-*O*-stearoyl L-ascorbates, which are chemically synthesized and are commercially available, are also used as an additive in foods rich in lipids. The enzymatic synthesis using lipase is preferred to the chemical synthesis because of the direct use of unmodified substrates, moderate reaction conditions, and high regiospecificity of the enzyme. Some studies have been published about the lipase-catalyzed synthesis of acyl L-ascorbates in an organic solvent (*5*−*9*), and we have also synthesized various 6-*O*-acyl L-ascorbates using an immobilized lipase (*10*−*13*). In addition, we evaluated the antioxidative ability of 6-*O*-acyl L-ascorbate for the autoxidation of PUFA (*12*).

There has been no report about the application of a saturated acyl L-ascorbate to the microencapsulation of lipids. 6-*O*-Acyl L-ascorbate has the enediol−lactone resonant structure and both a hydrophilic L-ascorbic acid moiety and a lipophilic acyl chain group. Therefore, it is expected that 6-*O*-acyl L-ascorbate acts both as an emulsifier in an emulsification process and as an antioxidant for the encapsulated lipid.

In this context, linoleic acid mixed with various 6-*O*-acyl L-ascorbates was microencapsulated with maltodextrin or gum arabic by spray-drying, and its oxidation process was evaluated to examine the antioxidative ability of the ascorbates toward the encapsulated linoleic acid. The oxidation process of the encapsulated linoleic acid mixed with methyl palmitate or unmodified L-ascorbic acid and then encapsulated with maltodextrin was also measured for comparison.

MATERIALS AND METHODS

Materials. Caprylic and capric acids, methyl myristate, and acetone were purchased from Wako Pure Chemical Industries, Osaka, Japan. Methyl myristate was used as an internal standard for gas chromatographic analysis of unoxidized linoleic acid. Lauric acid was purchased from Sigma Chemical, St. Louis, MO. L-(+)-Ascorbic acid and 6-*O*-palmitoyl L-ascorbate were obtained from Nacalai Tesque, Kyoto, Japan. Immobilized lipase, Chirazyme L-2 C2 from *Candida antarctica*, was purchased from Roche Molecular Biochemicals, Mannheim, Germany.

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Linoleic acid and methyl palmitate were purchased from Tokyo Kasei Kogyo, Tokyo, Japan, their purities being more than 90 and 95%, respectively. Maltodextrin with a dextrose equivalent of 2−5 and gum arabic were purchased from Matsutani Chemical Industries, Osaka, Japan, and San-ei Chemical Industries, Osaka, Japan, respectively. All of the other chemicals of analytical grade were obtained from either Wako Pure Chemical Industries or Nacalai Tesque.

Synthesis and Purification of Saturated Acyl L-Ascorbate. L-Ascorbic acid (0.04 mol) and caprylic, capric, or lauric acid (0.2 mol) were weighed into a glass bottle with a screw cap, and 20 g of Chirazyme L-2 C2 and 400 mL of acetone were added to the bottle. The headspace of the bottle was filled with nitrogen gas, and the bottle was tightly sealed, blowing the gas. The bottle was then immersed in a water bath at 55 °C with vigorous shaking to commence the condensation reaction. After ca. 24 h, each 6-*O*-acyl L-ascorbate was isolated from the reaction mixture according to the reported methods (*8*) with a slight modification.

Microencapsulation of Linoleic Acid. Forty-five grams of maltodextrin or gum arabic was dissolved in 300 mL of distilled water with slight heating. Nine grams of linoleic acid was mixed with the wall material solution. A specific amount of methyl palmitate, L-ascorbic acid, or 6-*O*-palmitoyl L-ascorbate was then added to the solution. For 6-*O*-capryloyl, caproyl, and lauroyl L-ascorbates, the mixture was prepared on a half-scale. The mixture was emulsified with a rotor/stator homogenizer (Polytron PT20SK, Kinematica, Switzerland) for 1 min at power control 8. The particle size distribution of the oil droplets in the emulsion was measured using a centrifugal particle size analyzer (SA-CP3L, Shimadzu, Kyoto) or a laser diffraction particle size analyzer (SALD-2100, Shimadzu, Kyoto). The emulsion was fed into an LB-8 spray-dryer (Ohkawara, Yokohama, Japan) at a flow rate of 3.0 kg/h and was atomized by a centrifugal atomizer operated at ca. 3×10^4 rpm. The emulsion in the reservoir was gently magnetically stirred to prevent flotation of the emulsion droplets. The temperatures of air at the inlet and the outlet were 200 and 100−110 °C, respectively. The flow rate of air was ca. 7.5 m^3/min. The microcapsules prepared were collected in a cyclone. Because spray-drying was completed within 3 or 6 min, no significant change in the size of oil droplets would occur during the drying.

GC Analysis of Unoxidized Linoleic Acid in a Microcapsule. Twenty milligrams of spray-dried microcapsules was weighed into a flat-bottom glass cup (1.5 cm i.d. and 3.0 cm height). About 15 cups were prepared for each sample. The cups were placed in a plastic container in which a Petri dish filled with a saturated lithium chloride solution was placed to regulate the relative humidity at 12%. The container was tightly closed and stored in the dark at 37 °C. Under the conditions the water contents of maltodextrin- and gum arabic-based microcapsules were estimated to be 0.044 and 0.052 g of water/g of wall material, respectively, from the sorption isotherms of water onto maltodextrin (unpublished data) and gum arabic (*14*).

At appropriate intervals, a cup was taken out of the container. The unoxidized linoleic acid in the microcapsules was extracted and determined by a GC-14B gas chromatograph (Shimadzu, Kyoto) with a hydrogen ionization detector according to our previous procedures (*4*). The fraction of unoxidized linoleic acid was calculated from the ratio of the peak area of methyl linoleate to that of methyl myristate.

RESULTS AND DISCUSSION

Effect of 6-*O*-Palmitoyl L-Ascorbate on Oxidative Stability of Linoleic Acid Encapsulated with Maltodextrin or Gum Arabic. Figure 1 shows the oxidation processes of linoleic acid to which 6-*O*-palmitoyl L-ascorbate was added at various molar ratios of the ascorbate to the acid and which was encapsulated with maltodextrin or gum arabic. Because linoleic acid encapsulated without the addition of the ascorbate was oxidized relatively quickly, there was a possibility that oxidation of the acid partially proceeded during emulsification and spray-drying, although the peroxide values of the acid before and after microencapsulation were not measured. Therefore, the present results are an overall estimate of the antioxidative ability of the ascorbate for all the steps of emulsification, spray-drying, and storage. The oxidative stabilities of the linoleic acid in both the maltodextrin- and gum arabic-based microcapsules were higher at the higher molar ratios. The induction period for oxidation of the encapsulated linoleic acid was elongated by the addition of palmitoyl L-ascorbate at high molar ratios due to its radical scavenging ability. Oxidation of the encapsulated linoleic acid quickly proceeded after the induction period and then reached a level where further oxidation seemed to proceed very slowly or to cease. The level was higher for the microcapsules with the higher ratios.

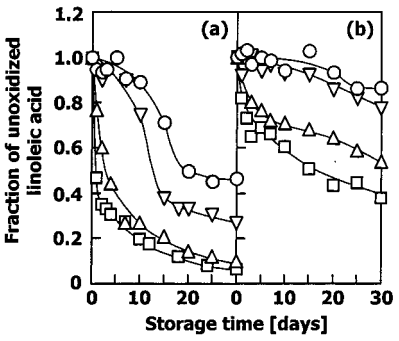

Figure 1. Oxidation processes at 37 °C and relative humidity of 12% of linoleic acid to which 6-*O*-palmitoyl L-ascorbate was added and which was encapsulated with (a) maltodextrin and (b) gum arabic. Molar ratios of ascorbate to linoleic acid were (□) 0, (△) 0.01, (▽) 0.05, and (○) 0.1.

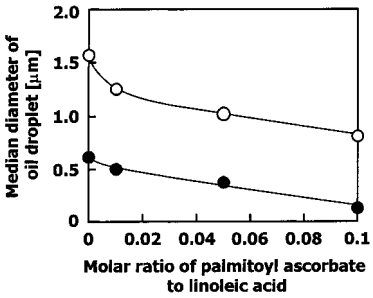

Figure 2. Effect of the molar ratio of 6-*O*-palmitoyl L-ascorbate to linoleic acid on oil droplet size in emulsions prepared with (○) maltodextrin and (●) gum arabic solutions.

The molar ratio would affect the particle size of the oil droplets in the oil/water emulsion because of the emulsification ability of palmitoyl L-ascorbate. Therefore, the distribution of the particle size was measured using the centrifugal particle size analyzer. The dependence of the median diameter of the oil droplet on the molar ratio is shown in **Figure 2**. As expected, the median diameter decreased with an increase in the ratio. We reported that the oxidative stability of linoleic acid in the microcapsule prepared from an emulsion with a smaller median diameter of oil droplets was higher because of high probability in interaction of the acid with the wall material within the microcapsule (*4*, *15*). The high oxidative stability of linoleic acid with the high molar ratio would be attributed to the synergistic effect of the small oil droplet in the emulsion and the antioxidative ability of palmitoyl L-ascorbate.

It has been reported, in a nonencapsulated system, that the addition of a large amount of a saturated fatty acid to a PUFA decreased the PUFA fraction in the mixture and delayed the oxidation of the PUFA (*16*). Thus, it is possible that the addition of 6-*O*-palmitoyl L-ascorbate to linoleic acid resulted in improved oxidative stability of the encapsulated linoleic acid by decreasing the fraction of linoleic acid in the mixture. To examine this possibility, methyl palmitate was added to linoleic

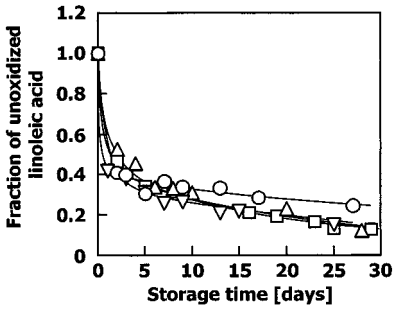

Figure 3. Oxidation processes at 37 °C and relative humidity of 12% of linoleic acid to which methyl palmitate was added and which was encapsulated with maltodextrin. Molar ratios of palmitate to linoleic acid were (□) 0, (△) 0.01, (▽) 0.05, and (○) 0.1.

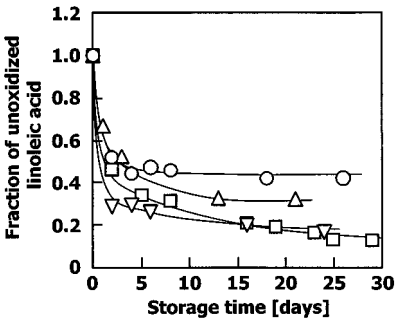

Figure 4. Oxidation processes at 37 °C and relative humidity of 12% of linoleic acid encapsulated with maltodextrin to which L-ascorbic acid was added to produce a specified molar ratio of ascorbic acid to linoleic acid. Molar ratios were (□) 0, (△) 0.05, (▽) 0.2, and (○) 1.

acid at various molar ratios, and the mixture was then microencapsulated with maltodextrin by spray-drying. The encapsulation efficiency of linoleic acid, which was defined as a ratio of the actual amount of the acid in the microcapsules to the one calculated from the composition of emulsion used for preparing the microcapsules, was 0.4−0.6. The efficiency was almost the same as that of the microcapsules prepared with no additive. **Figure 3** shows the oxidation processes of the encapsulated linoleic acid. At a ratio of methyl palmitate to linoleic acid of 0.1, the oxidation of linoleic acid was suppressed, but the extent was very small. Therefore, the suppressive effect of palmitoyl L-ascorbate on the oxidation of the encapsulated linoleic acid is not due to the decrease in the fraction of linoleic acid by the addition of the ascorbate.

To compare the effects of unmodified L-ascorbic acid and 6-*O*-acyl L-ascorbate on the oxidation of encapsulated linoleic acid, the oxidation processes of linoleic acid encapsulated with maltodextrin to which unmodified L-ascorbic acid was added to produce specified molar ratios of the L-ascorbic acid to linoleic acid were examined. The addition of unmodified L-ascorbic acid at any ratio had no effect on the encapsulation efficiency of linoleic acid. As shown in **Figure 4**, unmodified L-ascorbic acid slightly suppressed the oxidation of linoleic acid at a molar ratio of 1. Because this ratio was 10 times that for palmitoyl L-ascorbate, the antioxidative effect of unmodified L-ascorbic acid for encapsulated linoleic acid was very weak. Elongation of the induction period, which was observed for palmitoyl L-ascorbate, did not occur.

Suppression of Oxidation of Encapsulated Linoleic Acid by Addition of Various Saturated Acyl L-Ascorbates. It is expected that saturated acyl L-ascorbates with different acyl chain lengths also possess antioxidative ability for the encapsulated linoleic acid. Various saturated acyl L-ascorbates were

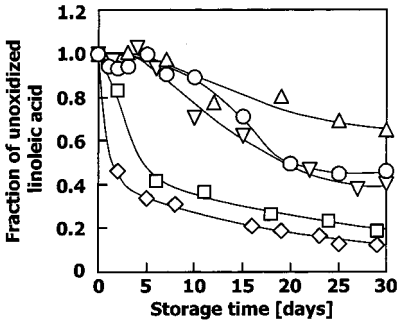

Figure 5. Effect of the addition of various saturated acyl L-ascorbates to linoleic acid on the oxidation of linoleic acid encapsulated with maltodextrin: (◇) no addition; (□) capryloyl (C8); (△) caproyl (C10); (▽) lauroyl (C12); (○) palmitoyl (C16) L-ascorbate. Molar ratio of saturated acyl L-ascorbate to linoleic acid was 0.1. Oxidation was observed at 37 °C and relative humidity of 12%.

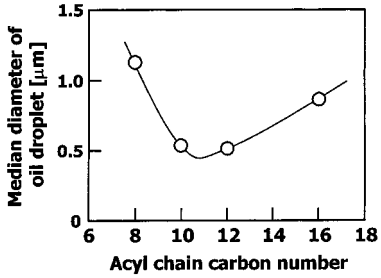

Figure 6. Median diameters of oil droplets in emulsions prepared with various saturated acyl L-ascorbates.

synthesized through the condensation of L-ascorbic acid and a saturated fatty acid (caprylic, capric, or lauric acid) by the immobilized lipase and purified. **Figure 5** shows the oxidation processes of linoleic acid mixed with various saturated acyl L-ascorbates at a molar ratio of 0.1 and encapsulated with maltodextrin. Mixing of any ascorbate significantly increased the encapsulation efficiency of linoleic acid, and the efficiency was >0.9. The median diameters of the oil droplets in the oil/water emulsions before microencapsulation are also shown in **Figure 6**. The median diameter was measured using the laser diffraction particle size analyzer. L-Ascorbates with acyl chain carbon numbers of ≥10 gave emulsions with small median diameters and significantly suppressed the oxidation of the encapsulated linoleic acid. Especially, caproyl L-ascorbate was superior both in emulsification ability (small diameter of emulsion) and in suppression of the oxidation. The oil droplet size of the emulsion prepared with capryloyl L-ascorbate was large, and the ascorbate was less effective than other ascorbates for suppression of the oxidation of the encapsulated linoleic acid.

The solubility of caproyl L-ascorbate in water was almost the same as that in soybean oil (data not shown). The saturated acyl L-ascorbates with acyl chain lengths of >10 are apt to dissolve in the oil, whereas the ascorbates with the lengths of <10 preferentially dissolve in water rather than in the oil. Therefore, when capryloyl L-ascorbate was used for microencapsulation of linoleic acid, most of the molecules would exist in the dehydrated wall material layer and could not scavenge the PUFA radicals generated in the oil phase. Because L-ascorbates that produced emulsions with small oil droplets were surface-active, they were apt to locate in the interface of linoleic acid and the wall material layer. Some linoleic acid molecules would be replaced with the saturated groups of the ascorbates,

and the replacement would increase the stability of linoleic acid toward the oxidation.

As mentioned above, the addition of a saturated acyl L-ascorbate to linoleic acid produced emulsions with small oil droplets and improved the oxidative stability of the encapsulated linoleic acid. The stability was higher at a higher molar ratio of the ascorbate to the acid. The ability of the acyl ascorbate to decrease the median diameter and to suppress the oxidation of the encapsulated linoleic acid depended on the acyl chain length. Caproyl ascorbate had the best ability among the ascorbates tested.

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