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Oxidation of Arachidonoyl Glycerols Encapsulated with Saccharides

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Two types of lipids rich in arachidonic acid content were encapsulated using various saccharides as the wall material, and the oxidation processes of the encapsulated lipids were measured at 50℃ and 12% relative humidity. One of the lipids contained arachidonoyl residue at a content of *ca.* 40%, and another was a structured lipid, the major component of which was 1,3-octanoyl-2-arachidonoyl glycerol. Micro-encapsulation effectively retarded their oxidation. In particular, among the tested saccharides, soluble soybean polysaccharide and gum arabic were the best two wall materials for the suppression of both lipids.

Keywords: Arachidonic acid, Encapsulation, Oxidation, Structured lipid, Spray-drying

Introduction

Arachidonic acid is an n-6 polyunsaturated fatty acid and is a promising component of functional foods because of its various physiological functions (Calder, 2001). However, it is prone to oxidation due to its high degree of unsaturation (Adachi *et al.,* 1995; Ishido *et al.,* 2002). Encapsulation of an unsaturated fatty acid or its ester is an efficacious technique to suppress or retard its oxidation (Lin *et al.,* 1995; Moreau and Rosenberg, 1996; Minemoto *et al.,* 2002). The oxidative stability of arachidonic acid was significantly improved by encapsulating it with gum arabic (Watanabe *et al.,* 2004).

Recently, much attention has been paid to a structured lipid, which is a triacyl glycerol that has a predetermined composition and distribution of fatty acyl groups on the glycerol backbone of the molecule (Lee, 2005). We have developed an enzymatic method to produce structured lipids of arachidonic acid (Nagao *et al.,* 2003). However, even if arachidonic acid is position- and number-specifically acylated with glycerol, its oxidation is inevitable.

In this context, we encapsulated two types of structured arachidonic acids, which differ in terms of the content and position of the arachidonoyl residue, with saccharide in order to retard the oxidation of the arachidonoyl residue. Because the wall material largely affects the oxidation of an encapsulated lipid (Imagi *et al.,* 1990), many types of wall materials were examined in order to identify materials suitable for suppressing oxidation.

Materials and Methods

Materials Two types of arachidonoyl glycerols were used. One was SUNTGA40S, which contained the arachidonoyl residue at *ca.* 40% and mixed-tocopherols. The other was SUN8A8-E, which contained structured 1,3-octanoyl-2-arachidonoyl glycerol prepared through enzymatic transesterification of triacyl glycerol containing *ca.* 40% arachidonic acid and medium chain triacyl glycerols with *Rhizopus delemer* lipase (Nagao *et al.,* 2003). The fatty acid compositions of the structured lipids are shown in Table 1. The content of mixed-tocopherols in SUNTGA40S was 48.5 mg/100 g. Wall materials used for encapsulating either of the lipids were soybean soluble polysaccharide (abbreviated SSPS; Fuji Oil, Osaka, Japan), gum arabic (Nacalai Tesque, Kyoto, Japan), cluster cyclodextrin (Esaki Glico, Osaka), maltodextrin (Matsutani Kagaku, Osaka), trehalose (Hayashibara, Okayama, Japan), maltose (Nacalai), lactose (Nacalai), and α-cyclodextrin (Ensuiko Seito, Yokohama, Japan). For use as the wall material, trehalose, maltose, lactose, or α-cyclodextrin was mixed with maltodextrin at a weight ratio of 1: 1. For simplicity, the di- or hexasaccharide is designated merely by its name, although it was a mixture.

Encapsulation of structured arachidonoyl glycerol with saccharide The wall material was dissolved with water at 15% (w/v) and a lipid (30 g) was mixed with the solution (1000 g). The weight ratio of the lipid to the wall material was 0.2 throughout this study. The mixture was homogenized using a rotor/stator homogenizer (PT 20SK, Kinematica, Lucern, Switzerland) at 2.0×10^4 rpm for 3 min to produce an oil-in-water (O/W) emulsion. The emulsion was fed into an LB-8 spray-dryer with an atomizer (Ohkawara, Yokohama) at a flow rate of 3.0 kg/h to produce microcapsules of the lipid. The rotational speed of the atomizer was 3.0×10^4 rpm, and the temperatures of air at inlet and outlet were 180℃ and *ca.* 90℃,

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Table 1. Composition of fatty acids in SUNTAG40S and SUN8A8-E.

Fatty acids	SUNTAG40S	SUN8A8-E
8:0	–	59.2
16:0	10.6	6.1
18:0	7.6	3.2
18:1 n-9	6.6	2.7
18:2 n-6	10.0	4.0
18:3 n-6	2.4	1.0
20:4 n-6	41.5	16.7

respectively.

Sizes of oil droplets in O/W emulsion and of microcapsules The size distribution of oil droplets in the O/W emulsion was measured using a laser diffractive particle size analyzer (SALD-2100, Shimadzu, Kyoto) with distilled water as a dispersion medium. The median diameter was calculated from the volume-based distribution. The analyzer was also used to measure the size distribution of microcapsules with methanol as the dispersion medium.

Stability of O/W emulsion The O/W emulsion was stored at 25℃, and a portion of the emulsion was sampled every day during 4- to 10-day storage. The stability of the emulsion was estimated by appropriately diluting the sample with 0.1% (w/v) sodium dodecyl sulfate and measuring the absorbance at 500 nm (Pearce and Kinsella, 1978).

Microscopic observation of microcapsules Microcapsules were sputtered with gold and observed at 1000-fold magnification using a 3D real surface view microscope (VE-9800, Keyence, Osaka).

Oxidation of encapsulated arachidonoyl glycerol Microcapsules (10 mg) of the structured lipid, SUNTGA40S or SUN8A8-E, were weighed in a flat-bottom glass cup (15 mm I.D.×30 mm); 15 to 20 cups were prepared for each sample. The cups were placed in a plastic container where the relative humidity was regulated at 12% with a saturated LiCl solution, and the container was stored in the dark at 50℃. At appropriate intervals, a cup was removed from the container and the microcapsules were dissolved with 1.0 mL of 0.1 mol/L NaOH. Methanol and chloroform (2.5 mL each) were added to the solution, and 1 mL of 0.1 mol/L HCl and 0.25 mL of distilled water were further added there and well mixed. After centrifugation at 3.0×10^3 rpm (LX-120, Tomy, Tokyo, Japan) for 5 min, the lower layer was recovered using a Pasteur pipette, and the chloroform in the sample was evaporated under reduced pressure. The remainder was dissolved with 1 mL of 0.5% (w/v) sodium methoxide in methanol and heated at 70℃ for 30 min. Acetic acid (20 μL) was added to the solution to stop the reaction, and the methanol was evaporated under reduced condition. The remainder was dissolved with 500 μL of hexane, and 5 μL of the solution was applied to a GC-16B gas chromatograph (Shimadzu) with a hydrogen ionization detector to determine the unoxidized arachidonoyl residue for both SUNTGA40S and SUN8A8-E. The amount of unoxidized linoleoyl residue was also determined by the same methods for arachidonoyl residue. The fraction of unoxidized arachidonoyl or linoleoyl residue was calculated from the ratio of the peak area of methyl arachidonate or linoleate to that of methyl palmitate, which was originally derived from palmitoyl residue in the sample lipid. The separation column was a glass column (3.2 mmϕ×3.1 m) packed with Thermon-3000 on Shincarbon A60–80 (Shimadzu). The injection and column temperatures were 230℃ and 200℃, respectively. The carrier gas was nitrogen at 50 mL/min. The pressures of hydrogen gas and air were 0.6 and 0.5 kg-f/cm^2, respectively.

Table 2. Median diameters of oil droplets in an oil-in-water emulsion containing a saccharide in its aqueous phase and of microcapsules prepared by spray-drying the emulsion.

Saccharide	Oil droplet [μm]	Microcapsule [μm]
Soluble soybean polysaccharide	2.57	17.0
Gum arabic	3.97	22.8
Maltodextrin	5.00	18.0
Cluster dextrin	3.48	17.2
Trehalose*	9.47	33.9
Maltose*	5.33	29.4
Lactose*	4.22	33.6
α-Cyclodextrin*	3.91	23.0

SUNTGA40S was used as the oil phase or core material.

* mixed with maltodextrin in a weight ratio of 1:1.

Results and Discussion

Emulsifying and emulsion-stabilizing abilities of wall materials The size of the oil droplets in the O/W emulsion affected both the emulsion stability and the oxidative stability of the encapsulated lipid. For both SUNTGA40S and SUN8A8-E, the size of the oil droplets in an emulsion was measured in order to evaluate the median diameter (Table 2). The oil droplets in the emulsions prepared with SSPS and gum arabic, which are both reported to have an emulsifying ability (Maeda, 2000; Randall *et al.,* 1988), were small, at approximately 3 μm. However, disaccharides and α-cyclodextrin mixed with maltodextrin seemed to have no emulsifying ability because the oil droplets were relatively large.

The median diameters of the microcapsules are also shown in Table 2. They ranged from 20 to 30 μm with some exceptions. We reported that the rotational speed of the atomizer was a crucial parameter in determining the particle size (Fang *et al.,* 2005). According to our previous study, the diameters seemed to be reasonable at the rotational speed.

Figure 1 shows the storage stability of the emulsions with aqueous solutions of the wall materials. The oil phase of the emulsions was SUNTGA40S. The emulsion prepared with SSPS was the most stable among the tested emulsions, indicating that SSPS has both emulsion-stabilizing emulsifying abilities. The emulsion prepared with cluster cyclodextrin was also stable for the first two days. The other wall materials had no emulsion-stabilizing

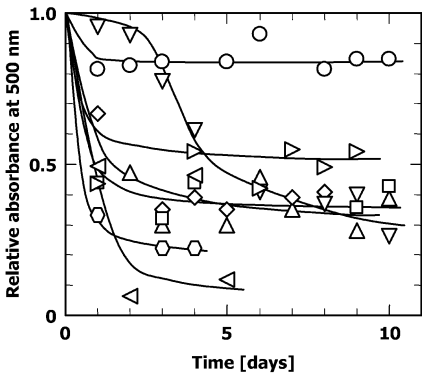

Fig. 1. Stability at 25℃ of O/W emulsions prepared with SUNTGA40S and aqueous solutions of (○) soluble soybean polysaccharide, (△) gum arabic, (◇) maltodexterin, (▽) cluster cyclodextrin, (□) trehalose, (▷) maltose, (⬡) lactose and (◁) α-cyclodextrin. Disaccharides and α-cyclodextrin were mixed with maltodextrin at the weight ratio of 1: 1.

ability.

Microscopic images of the microcapsules Figure 2 shows the photos of microcapsules prepared with SSPS, gum arabic, and cluster cyclodextrin using SUN8A8-E as the core material. No remarkable difference in appearance was observed among the microcapsules. The surface was wrinkled due to formation of a dehydrated skin on the surface and subsequent shrinkage of the particle or microcapsule during spray-drying. The microcapsules prepared with the polysaccharides using SUNTGA40S as the core material had almost the same appearance as those prepared using SUN8A8-E. Therefore, the type of core material had a limited effect on the appearance of the microcapsules.

Oxidation of encapsulated SUNTGA40S SUNTGA40S was encapsulated with various wall materials, and the microcapsules were stored at 50℃ and 12% relative humidity. The changes in the fractions of unoxidized arachidonoyl and linoleoyl residues over time are shown in Figs. 3 and 4, respectively. In a bulk system, both the arachidonoyl and linoleoyl residues of SUNTGA40S were fully oxidized within 5 days. However, the oxidation of SUNTGA40S was significantly retarded by encapsulating it with any wall material. Among the tested materials, SSPS and gum arabic exhibited remarkable retardation of the oxidation of the lipid. The oxidative stability was higher for the microcapsules prepared with smaller oil droplets (Minemoto *et al.,* 2002) although the specific surface area was larger for the smaller oil droplets. As shown in Table 2, the oil droplets in O/W emulsions prepared with SSPS and gum arabic were small. This is considered to be a reason for the retarded oxidation of the lipid. For both arachidonoyl and linoleoyl residues in SUNTGA40S encapsulated with any wall material, the fractions of unoxidized arachidonoyl and linoleoyl residues leveled off at a longer storage period. This is one of the features of the oxidation of encapsulated lipid (Minemoto *et al.,* 1999) and can be explained by the diversity in the strength of interaction between the lipid and the wall

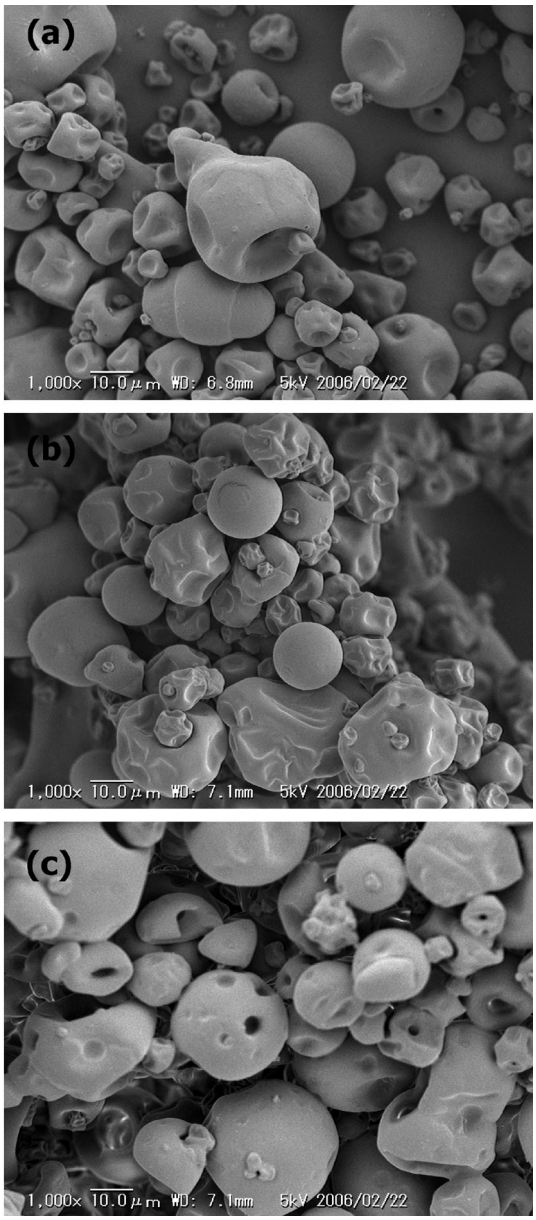

Fig. 2. Scanning electron microscopic images at 1000-fold magnification of the microcapsules prepared using (a) soybean soluble polysaccharide (SSPS), (b) gum arabic and (c) cluster cyclodextrin as the wall materials. The core material was SUN8A8-E.

material (Ishido *et al.,* 2003). Although this feature was remarkable for the SUNTGA40S encapsulated with the disaccharides and α-cyclodextrin, the reason for this remains unclear.

Figure 5 shows the relationship between the fraction of unoxidized arachidonoyl residue and that of unoxidized linoleoyl residue during the oxidation of SUNTGA40S encapsulated with various wall materials. The relationship for SUNTGA40S in the bulk system is also shown in the figure. Most of the plots lie over the diagonal line, indicating that the linoleoyl residue is oxidized more slowly than the arachidonoyl residue.

Oxidation of encapsulated SUN8A8-E SUN8A8-E, which was free of tocopherol, was also encapsulated with vari-

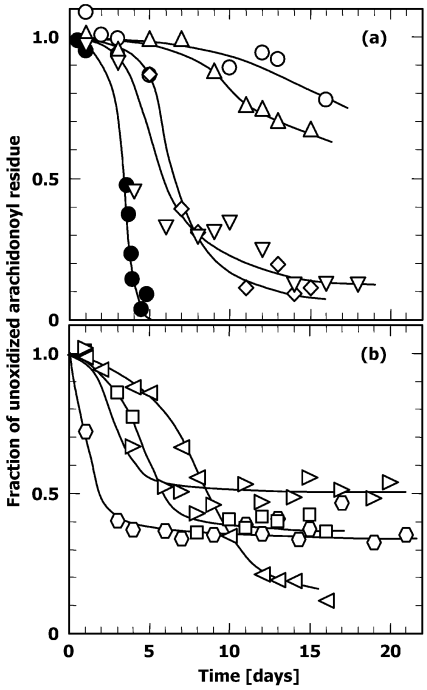

Fig. 3. Oxidation processes at 50℃ and 12% relative humidity of the arachidonoyl residue of SUNTGA40S encapsulated with various wall materials. The closed circle indicates the oxidation of the arachidonoyl residue of SUNTGA40S in a bulk system. Other symbols are the same as in Fig. 1.

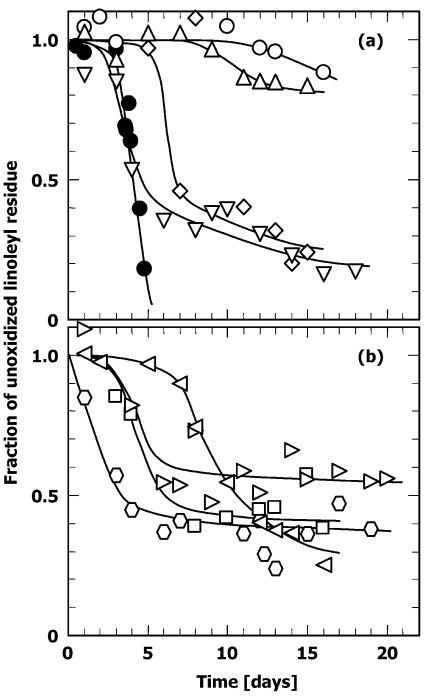

Fig. 4. Oxidation processes at 50℃ and 12% relative humidity of the linoleoyl residue of SUNTGA40S encapsulated with various wall materials. The closed circle indicates the oxidation of the linoleoyl residue of SUNTGA 40S in a bulk system. Other symbols are the same as in Fig. 1.

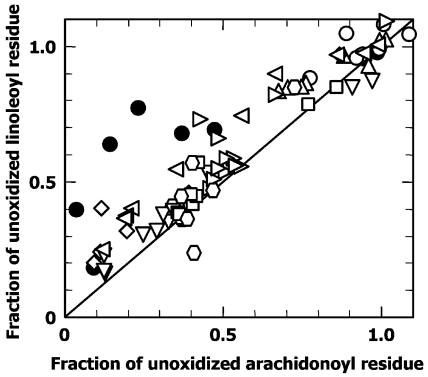

Fig. 5. Relationship between the fraction of unoxidized arachidonoyl and linoleoyl residues during the oxidation of SUNTGA40S encapsulated with various wall materials. The symbols are the same as in Fig. 1. The closed circle indicated the oxidation of SUNTGA40S in a bulk system.

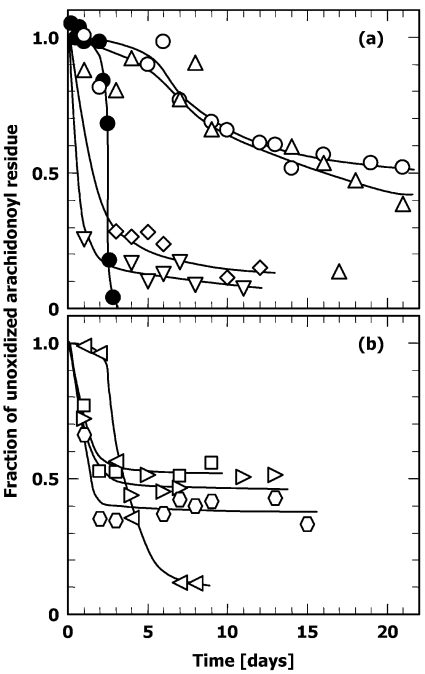

Fig. 6. Oxidation processes at 50℃ and 12% relative humidity of the arachidonoyl residue of SUN8A8-E encapsulated with various wall materials. The closed circle indicates the oxidation of the arachidonoyl residue of SUNT8A8-E in a bulk system. Other symbols are the same as in Fig. 1.

ous wall materials, and its oxidation processes were observed (Fig. 6). The oxidation of SUN8A8-E proceeded slightly more rapidly than that of SUNTGA40S. The oxidation of unsaturated fatty acid proceeded faster at its higher content in a mixed lipid (Ishido *et al.*, 2001). The content of unsaturated fatty residues in SUNTGA40S was higher than that in SUN8A8-E. Although, in that sense, SUNTGA40S seemed to be oxidized faster than SUN8A8-E in their bulk systems, the difference in the susceptibility to oxidation was small. The mixed-tocopherols in SUNTGA 40S would counteract the progress of its oxidation, and

there was apparently a small difference in the oxidation between SUNTAG40S and SUN8E8-E. There was a large difference in the composition of saturated fatty acids between the lipids. Although the composition did not seem to affect the oxidation of arachidonoyl residue, the supposition was not conclusive because of the limited results.

The oxidation pattern for encapsulated SUN8A8-E was almost similar to that of encapsulated SUNTGA40S irrespective of the wall material, and SSPS and gum arabic are good wall materials for suppressing the oxidation of SUN8A8-E. This fact would be also ascribed to the similar susceptibility to oxidation of SUNTGA40S and SUN8A 8-E in their bulk systems.

Conclusion

Soluble soybean polysaccharide had an emulsion-stabilizing ability for the emulsion containing SUNTGA40S as the oil phase. Among the tested saccharides, soluble soybean polysaccharide, and gum arabic could effectively suppress the oxidations of both SUNTGA40S and SUN8A 8-E by encapsulating them. The type of lipids had no significant effect on the oxidation pattern of the microencapsulated lipids.

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