Autoxidation of Linoleic Acid Encapsulated with Polysaccharides of Differing Weight Ratio

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Linoleic acid was encapsulated with pullulan, maltodextrin and gum arabic at various weight ratios of the fatty acid to wall material by the hot-air-drying method. The antioxidative process of the encapsulated linoleic acids was observed at 37°C and at a relative humidity of 75%. The weight ratio strongly affected the antioxidative process, autoxidation being more suppressed with smaller ratios. The antioxidation process of encapsulated linoleic acid leveled off at fraction Y∞ of the oxidized substrate within 15 days. The Y∞ value strongly depended on both the ratio and the wall material, and steeply decreased near the ratio of 0.75 for every wall material. The dependence of the Y∞ value on the weight ratio was analyzed by the two- and three-dimensional models for percolation theory. The two-dimensional model expressed the experimentally observed dependence.

Key words: autoxidation; linoleic acid; encapsulation; percolation theory

The encapsulation of liquid lipid into a powdery matrix of saccharide or protein provides the lipid with some new functions. One of them is suppression of the autoxidation of polyunsaturated fatty acids or their esters (PUFAs), most of which are liquid at room temperature. Many factors affect the antioxidative process of encapsulated PUFAs; e.g., the combination of PUFA to be encapsulated and the wall material, single- or double-encapsulation, storage conditions such as temperature and humidity, and the drying method used for preparing encapsulated PUFA.7,8

The weight (or volumetric) ratio of PUFA to the wall material would also affect the antioxidative process of encapsulated PUFA. We encapsulated linoleic acid, ethyl eicosapentaenoate and ethyl docosahexaenoate with cyclodextrins at various molar ratios, and examined the antioxidative process of each.9 The level at which further autoxidation did not proceed strongly depended on the molar ratio. Cyclodextrins have the feature of incorporating hydrophobic substances within their molecular cavities. Therefore, this feature could be concerned with the stability of PUFAs against autoxidation.9 However, the effect of the molar or weight ratio on the antioxidation of encapsulated PUFA has hardly been studied for other wall materials.

In this context, we examined the antioxidative processes of linoleic acid encapsulated with pullulan, maltodextrin and gum arabic at various weight ratios by hot-air drying. The percolation theory was then applied to analyze the effect of the weight ratio on the fraction of unoxidized linoleic acid at which the antioxidative process leveled off.

Materials and Methods

Materials. Linoleic acid and methyl palmitate were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), their purity being more than 90% and 95%, respectively. They were stored in the dark at a temperature lower than −20°C until needed, and were used without further purification. Methyl palmitate was used as an internal standard in the gas chromatographic determination of unoxidized linoleic acid.

Pullulan, maltodextrin and gum arabic were each used as the wall material for encapsulating linoleic acid. Pullulan (PI-20) was obtained from Hayashibara (Okayama, Japan), its molecular mass being about 2 × 10⁶. Maltodextrin, the dextrose equivalent of which was 2 to 5, and gum arabic were purchased from Matsutani Chemical Industries (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), and were of analytical grade.

Encapsulation of linoleic acid by saccharides. Pullulan, maltodextrin or gum arabic was dissolved in distilled water while gently heating to give a concentration of 15% (w/v). The solution was put into a test tube (1.2 cm i.d.), and then linoleic acid was added to give a linoleic acid/saccharide ratio in weight of 0.2, 0.5, 0.75, 1.0 or 2.0 and to adjust the total volume of the mixture to about 3.0 ml. This mixture was homogenized for 2 min by an NS-50 homogenizer (Nichi-on, Tokyo, Japan) consisting of a stator and a rotor with a blade. During the homogenization procedure, the tube was cooled by immersing it into water.

An aliquot of the emulsion (5 μl) was suspended on a glass filament by using a micropipette. The filaments suspending the emulsions were placed in a drying equipment.7 The suspended emulsion droplets were dried by air introduced upward into the equipment at 50°C and at a velocity of about 0.4 m/s for 30 min to prepare microcapsules. The diameter of each microcapsule pre-

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pared was about 2–3 mm.

_Autoxidation of the encapsulated linoleic acid._ The microcapsules were stored at 37°C in a desiccator, the relative humidity in which was regulated to 75% by placing on the base a Petri dish filled with a saturated sodium chloride solution. At appropriate intervals, a microcapsule was taken out of the desiccator, and dissolved with 1 ml of 0.1 N NaOH in a test tube. A mixture of methanol (2.5 ml) and chloroform (2.5 ml), in which methyl palmitate had been dissolved at a concentration of 0.01% (v/v) as an internal standard for gas chromatographic analysis, was then added to the test tube. Lipophilic substances were extracted from the aqueous solution to the mixture under vigorous agitation. After that, 1 ml of 0.1 N HCl and 0.25 ml of distilled water were added, and the tube was again agitated. After centrifugation at 3000 rpm for 5 min to separate the organic and aqueous phases, the lower organic phase was carefully pipetted out. A mixture (2 ml) of chloroform, methanol and water (3:48:47 in volume) was added to the organic phase. After agitation and subsequent centrifugation, the lower organic phase was recovered and evaporated. To the resulting residue, 0.5 ml of methanol, 2 ml of benzene and 0.1 ml of 2 mol/l (trimethylsilyl)diazomethane in hexane were added, and the mixture was held at room temperature for 1 h to convert linoleic acid in the residue to its methyl ester.

After evaporation, the residue was dissolved in 0.3–1.0 ml of hexane. The extracted lipids were analyzed by a GC-7A gas chromatograph (Shimadzu, Kyoto, Japan) with a hydrogen ionization detector. The hexane solution (5 μl) was injected into the glass column, the dimensions of which were 3.2 mm in diameter and 3.1 m in length, with 5% Advance-DS on Shinnchrom A. The injection and column temperatures were 230°C and 180°C, respectively, while the N2 gas flow rate was 50 ml/min.

The ratio of the amount of methyl linolate, which was derived from unoxidized linoleic acid, to that of methyl palmitate was obtained from the areas under the peaks. The fraction of unoxidized substrate was calculated from this ratio.

**Results and Discussion**

Figures 1(a), (b), and (c) show the antioxidative processes of linoleic acid encapsulated with pullulan, maltodextrin and gum arabic, respectively. The ratio in weight of linoleic acid to the wall material ranged from 0.2 to 2 for each wall material. Linoleic acid autoxidized quickly in the early stage of storage, before reaching a level at which no further autoxidation seemed to proceed. This level is denoted as \( Y_\infty \). The \( Y_\infty \) value strongly depended on the ratio of linoleic acid to the wall material, and the autoxidation of linoleic acid encapsulated at lower ratios leveled off at higher \( Y_\infty \) values. A similar result had been observed for linoleic acid and ethyl docosahexenoate encapsulated with cyclodextrins.

Among the wall materials tested, gum arabic was the most effective to suppress the autoxidation of linoleic acid. Pullulan followed, and maltodextrin was the least effective. This order is consistent with our previous observation, for which the ratio was fixed at 1 for all the wall materials.

Figure 2 shows the relationships between the \( Y_\infty \) value and the weight ratio of linoleic acid to the wall material for microcapsules prepared with each wall material. All the microcapsules gave a steep decrease in the \( Y_\infty \) value near the ratio of 0.75. This indicates that the condition of the fatty acid in the microcapsule changed near this ratio, and that there would be two states of the fatty acid: easily oxidizable and hardly oxidizable ones. However, the reason for the existence of these two states and their nature are unknown.

Oxygen is consumed during the autoxidation of an unsaturated fatty acid, and is supplied from air surrounding the microcapsule. The diffusion coefficient of oxygen
in lipid is in the order of $10^{-10}$ m$^2$/s, being much larger than that through the dehydrated layer of wall material which is in the order of $10^{-12}$ m$^2$/s. Autoxidation of PUFAs is a radical chain-reaction, and the propagation step in autoxidation rapidly progresses. We presumed from this that the linoleic acid leading to the surface of the microcapsule would be easily oxidized, but that fatty acid isolated within the microcapsule would be hardly oxidized, although the suppressed autoxidation of encapsulated PUFA could not, in many cases, be explained only by the diffusional resistance of the dehydrated layer of wall material. Based on these suppositions, we simulated the $Y_\infty$ values for various volumetric fractions of linoleic acid in the microcapsule according to the percolation theory.  

Two- and three-dimensional models of percolation for the autoxidation were considered based on simple square and cubic models (Fig. 3). The square and cube were divided into $N \times N$ and $N \times N \times N$ equal lattices, where $N$ is the number by which a side of the square or cube is divided. The lattices occupied by linoleic acid are shown shaded. The lattice occupied by the fatty acid was allotted a random number in a range of 0 to 1, the random number being generated by the public domain program named Mersenne Twister. When the random number for a lattice was smaller than the volumetric fraction of linoleic acid in a microcapsule, the lattice was allocated as being occupied by the fatty acid.

In the two-dimensional model, autoxidation of the fatty acid was considered to take place by penetration from all the four sides of a square. When the lattices occupied by the fatty acid border each other on one side, autoxidation was considered to take place by penetration. On the other hand, autoxidation was assumed not to take place by penetration when the lattices contacted at a vertex. The number of lattices in which autoxidation was possible from all four sides was counted, and the quotient of the number to $N \times N$ is regarded to correspond to the $Y_\infty$ value. Generation of the random numbers, count of the oxidizable (penetratable) lattices, and calculation of the $Y_\infty$ value were repeated 100 to 1000 times, depending on $N$, for each volumetric fraction, and the $Y_\infty$ values for the fraction were averaged. The $Y_\infty$ value was calculated over the range for the fraction of 0.01 to 1. If it is assumed that autoxidation is limited to the surface lattices and cannot penetrate into the inner lattices, the $Y_\infty$ value is given by $1 - 4(N - 1)/N^2$, depending on the $N$ value.

In the three-dimensional model, autoxidation was considered to take place by penetration from all the six faces of the cube inwards. Only when two lattices occupied by the fatty acid border each other on one face, autoxidation was assumed to be possible by penetration. The evaluation of the $Y_\infty$ value was made in a manner similar to that for the two-dimensional model. In the three-dimensional model, the $Y_\infty$ value at the surface of the cube is given by $1 - (6N^2 - 12N + 8)/N^3$.

The $N$ value should be selected to reflect the size of the lipid droplet within the microcapsule. However, this value was treated as a parameter in this study because we had insufficient information for estimating the value. The solid and broken curves in Fig. 4 show the relationships between the $Y_\infty$ value and the volumetric fraction of linoleic acid, these being calculated based on the two- and three-dimensional models, respectively. The $Y_\infty$ value decreased steeply near the volumetric ratio of 0.5 with the two-dimensional model, while this occurred near the ratio of 0.3 with the three-dimensional model.

To compare the calculated and experimentally observed relationships between the $Y_\infty$ value and the volumetric fraction of linoleic acid, the abscissa of Fig. 2 must be converted to the volumetric fraction. The density of linoleic acid was 0.88 g/cm$^3$; however, it was difficult to estimate the density of the dehydrated layer of the wall material, so the density was assumed to be 1.5 g/cm$^3$ for all the wall materials used. This value is a
rough approximation of the density of mono- to trisaccharides. Using these density values and assuming the additivity in volume of linoleic acid and the wall material, the ratio in weight of linoleic acid to the wall material was converted to the volumetric fraction of linoleic acid in a microcapsule. Then, the $Y_{\infty}$ values were plotted against the volumetric fractions (Fig. 4). In this figure, the $Y_{\infty}$ values for linoleic acid encapsulated with $\beta$-cyclodextrin are also shown, although they were obtained for microcapsules prepared by vacuum drying.

The experimentally observed $Y_{\infty}$ values did not fall to zero even at high volumetric fractions of linoleic acid. This would be ascribable to the existence of hardly oxidizable linoleic acid, as already presumed. We then compared the calculated and experimentally observed $Y_{\infty}$ values, paying attention only to the easily oxidizable linoleic acid in a microcapsule.

The $Y_{\infty}$ values greatly decreased near the volumetric fraction of 0.5 when pullulan, maltodextrin and gum arabic were used as wall materials. This range of fractions coincided well with the fraction for which the $Y_{\infty}$ value decreased steeply, not in the three-dimensional model, but in the two-dimensional model of the percolation theory. This fact prompts us to believe that linoleic acid existed plane-geometrically in the radial direction within a microcapsule and that the planes were isolated from each other by the dehydrated layer of wall material. The drying front migrated from the surface of the emulsion droplet into the inside, and removing water left its paths in the dehydrated layer of the wall material. The critical surface tension of the layer was almost the same as the surface tension of linoleic acid.

The emulsion droplet shrank during its drying step, and this shrinkage would result in stress on the emulsion particles. Therefore, there is a possibility that the fatty acid would be spread along the paths. This supposition and the foregoing comparison indicate that the emulsion droplet would shrink two-dimensionally, and would not maintain its three-dimensional shape.

The $Y_{\infty}$ values observed experimentally at the smaller fractions lie under the calculated curves. A fully random and homogeneous distribution of linoleic acid was assumed in this calculation. However, the small $Y_{\infty}$ values suggest that the distribution was not homogeneous, but that some of the fatty acid was localized near the surface of a microcapsule.

On the other hand, the calculated $Y_{\infty}$ values are almost zero for the fractions greater than 0.7, while the experimentally observed $Y_{\infty}$ values are much higher than the calculated values for these fractions. No interaction between the fatty acid and wall material has been taken into consideration. The fact that linoleic acid encapsulated by the wall material at large volumetric fractions gave high $Y_{\infty}$ values indicates that the fatty acid would interact with the wall material and be stable against antioxidation.

When linoleic acid was encapsulated with $\beta$-cyclodextrin, the $Y_{\infty}$ value decreased at the volumetric fraction of about 0.3, which is almost the same as the fraction for which the $Y_{\infty}$ value decreased steeply in the three-dimensional model of percolation theory. However, the interpretation of this fact is difficult because there is the possibility that $\beta$-cyclodextrin formed an inclusion complex with linoleic acid so that the additivity in volumes might not hold.

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**References**


