Solvent-Extraction Processes of Linoleic Acid Encapsulated with Gum Arabic, Pullulan or Maltodextrin at Different Weight Ratios

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Linoleic acid was encapsulated with gum arabic, pullulan or maltodextrin at various weight ratios of the fatty acid to the wall material, and the fraction of the linoleic acid extracted from the microcapsule with chloroform was observed as a function of time. A certain amount of the linoleic acid could not be extracted even if the microcapsule was immersed in chloroform for a long period. The fraction of extractable linoleic acid was lower for the microcapsule having the lower weight ratio, and significantly changed between the weight ratios of 0.5 and 1.0 for gum arabic and pullulan. It was also shown that the fraction of extractable linoleic acid correlated fairly well with the fraction of easily oxidizable linoleic acid. An increase in the fraction was observed with 1-day storage of linoleic acid encapsulated with gum arabic at the ratio of 0.5, and the initial extraction rate linearly increased with the period of storage.

Keywords: encapsulation, linoleic acid, extraction, wall material

Encapsulation of a liquid lipid into the powdery matrix of a saccharide or protein can provide the lipid with some new functions (Matsuno & Adachi, 1993). The location or distribution of the lipid within the microcapsule can play an important role in the availability of these functions, however, the direct determination of its location or distribution is difficult. Therefore, the fraction of the lipid exposed at the surface of the microcapsule, the so-called surface oil, is a measure of the product evaluation for an encapsulated lipid.

It has been reported (Imagi *et al.*, 1992; Onwulate *et al.*, 1994) that the fraction depended on the kind of wall material used for the encapsulation. When orange peel oil or carrot carotene was encapsulated with maltodextrins of different dextrose equivalents (DEs), DE affected the fraction (Anandaraman & Reineccius, 1987; Wanger & Warthesen, 1995). The drying method used in the preparation of the microcapsule also affected the fraction (Desorbry *et al.*, 1997). The double-encapsulation of butteroil could reduce the fraction (Onwulate *et al.*, 1998). Furthermore, Shimada *et al.* (1991) showed that the fraction of methyl linoleate encapsulated with amorphous lactose-gelatin matrix increased during storage.

Although the fraction would depend on the extraction time, the fraction was determined by washing the microcapsule with or immersing it in an organic solvent for a given period in the above studies. Kim and Morr (1996) demonstrated that the fraction depended on the extraction time.

In this study, we encapsulated linoleic acid with gum arabic, pullulan or maltodextrin at various weight ratios of the acid to the wall material, and followed the solvent-extraction processes with chloroform as a function of time. It will

be demonstrated that part of the linoleic acid cannot be extracted even if the microcapsule is immersed in the chloroform for a long period. The relationship between solvent-extraction and the autoxidation processes of linoleic acid will also be discussed.

Materials and Methods

Materials Linoleic acid (purity: >90%) and methyl palmitate (>95%) were purchased from Tokyo Kasei Kogyo, Tokyo and were used as received. Methyl palmitate was used as an internal standard during the gas chromatographic determination of linoleic acid. Pullulan with a molecular mass of about 2×10^5 (PI-20) was obtained from Hayashibara, Okayama. Maltodextrin, the DE of which was 2 to 5, was purchased from Matsutani Chemical Industries, Osaka. Gum arabic was purchased from San-ei Chemical Industries, Osaka. Other chemicals were purchased from Wako Pure Chemical Industries, Osaka or Nacalai Tesque, Kyoto, and were of analytical grade.

Encapsulation of linoleic acid with saccharide Encapsulation of linoleic acid with a saccharide was carried out using the single-droplet method with hot-air-drying (Charlesworth & Marshall, 1960; Minemoto *et al.*, 1997). A wall material was dissolved in distilled water to give a concentration of 15% (w/v) in a test tube (1.2 cm i.d.), and linoleic acid was added to produce a linoleic acid/wall material weight ratio of 0.2, 0.5, 1.0 or 2.0. The volume of the mixture was adjusted to 3.0 ml with the addition of distilled water. The mixture was homogenized for 2 min using an NS-50 homogenizer (Nichi-on, Tokyo). The tube was cooled by immersing it in water during the homogenization.

The emulsions (5 μ l) were suspended on a glass filament using a micropipet. The suspended emulsion droplet was dried by air introduced upward into a drying instrument at

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50°C and at a velocity of about 0.4 m/s for 30 min to prepare a microcapsule, the diameter of which was about 2 to 3 mm.

Solvent-extraction of linoleic acid from microcapsule

A microcapsule was immersed in 2.5 ml of chloroform, which included 0.01% (v/v) methyl palmitate as the internal standard for the gas chromatographic analysis, for a specified period (1 s to 24 h) at room temperature. After the chloroform had evaporated, 0.5 ml of methanol, 2.0 ml of benzene and 100 μ l of (trimethylsilyl) diazomethane were added to convert the linoleic acid in the residue to its methyl ester. The methylation was completed by allowing the mixture to stand at room temperature for 1 h, and the solvent was then removed by evaporation. The residue was dissolved with 0.5 ml of hexane, and the methyl linoleate was analyzed by gas chromatography (Hashimoto *et al.*, 1981).

The amount of linoleic acid which was not extracted and remained within the microcapsule was determined as follows: the microcapsule removed from the chloroform was dissolved using 1.0 ml of 0.1 N NaOH. Methanol (2.5 ml) and chloroform (2.5 ml) were added to the solution and mixed well. One milliliter of 0.1 N HCl and 0.25 ml of distilled water were then added to the solution. After again mixing well, the mixture was centrifuged at 3000 rpm for 5 min to separate the aqueous and organic phases. The lower organic phase was carefully recovered, and mixed with 2.0 ml of a mixture of chloroform, methanol and water (3:48:47 in volume). The mixture was agitated well, and centrifuged again at 3000 rpm for 5 min. The chloroform layer was carefully recovered. The extracted linoleic acid was then determined by the method described above.

Change in extractability during storage Linoleic acid was encapsulated with gum arabic at the weight ratio of 0.5. Many microcapsules with such compositions were stored at 37°C in a dessicator filled with N₂ gas. The relative humidity in the dessicator was regulated at 75% by placing a petri dish filled with a saturated sodium chloride solution. Some microcapsules were taken out of the dessicator every day, and

the solvent-extraction process of linoleic acid was measured in the above described manner.

Results and Discussion

Figure 1 shows the solvent-extraction processes of linoleic acid from microcapsules prepared with gum arabic, pullulan and maltodextrin at various weight ratios of the acid to the wall materials. The ordinate represents the fraction of extracted linoleic acid to the total acid. A microcapsule was used for measurement of each fraction. The amount of total acid was experimentally determined from the sum of the amount of the acid extracted with chloroform and that of the acid not extracted, which was measured after dissolution of the microcapsule with 0.1 N NaOH. The solvent-extraction process largely depended on both the kind of wall material used and the weight ratio of linoleic acid to the wall material. When maltodextrin was used as the wall material, the fraction of extracted linoleic acid significantly increased during the early stage of extraction and reached certain levels where further extraction seemed not to proceed. For the microcapsules prepared with gum arabic and pullulan, the fraction gradually increased even at extraction times longer than 5 h. In any case, it seemed that all the linoleic acid encapsulated within the microcapsules was not always extracted even if the extraction time was prolonged, and that the fraction of extracted linoleic acid, E , leveled off. The level was denoted as E_{∞} . This would indicate that linoleic acid within a microcapsule can be classified into two states: easily extractable and hardly extractable. The E_{∞} corresponds to the fraction of easily extractable linoleic acid. To estimate the E_{∞} value, the E values at extraction times longer than 10 min were empirically fitted to the following equation:

$$E = \frac{E_{\infty}t}{a+t} \quad (1)$$

where t is the extraction time and a is a constant. The solid curves in Fig. 1 were drawn using Eq. (1) and the E_{∞} and a

Fig. 1. Solvent-extraction of linoleic acid encapsulated with (a) gum arabic, (b) pullulan and (c) maltodextrin at various weight ratios of the acid to wall material. The weight ratios of linoleic acid to wall material were ($\diamond$) 0.2, ($\triangle$) 0.5, ($\circ$) 1.0 and ($\square$) 2.0. Linoleic acid was extracted in chloroform at room temperature. The solid curves were calculated using Eq. (1) and the estimated parameters, E_{∞} and a .

values estimated.

Figure 2 shows the changes in the fraction of extracted linoleic acid with time during the first 1 min. The initial extraction rates were estimated by fitting the fractions at 1 and 3 s and the origin to the Lagrangian binomial interpolation formula (Yabushita, 1977), and then by differentiating the formula at the extraction time $t=0$:

$$\left. \frac{dE}{dt} \right|_{t=0} = -\frac{t_1+t_2}{(t_0-t_1)(t_0-t_2)}E_0 - \frac{t_0+t_2}{(t_1-t_0)(t_1-t_2)} \times E_1 - \frac{t_0+t_1}{(t_2-t_0)(t_2-t_1)}E_2 \quad (2)$$

where E_0 ($=0$), E_1 and E_2 are the fractions of extracted linoleic acid at the extraction times $t_0=0$, $t_1=1$ and $t_2=3$ s, respectively. The slopes of the solid lines in Fig. 2 represent

Fig. 3. Dependencies of the maximum fraction of extractable linoleic acid, E_∞ , and the initial extraction rate on the weight ratio of linoleic acid to wall material. The wall materials used were (Δ , $\blacktriangle$) gum arabic, ($\square$, $\blacksquare$) pullulan and ($\circ$, $\bullet$) maltodextrin. The open and closed symbols indicate the E_∞ and the initial extraction rate, respectively.

the initial extraction rates estimated in such a manner, which would include some extent of errors because only three points were used in the estimation. The dotted curves in the figure were empirically drawn.

The E_∞ values and initial extraction rates estimated above are plotted versus the weight fraction of linoleic acid to wall material in Fig. 3. The E_∞ values for the microcapsules prepared with gum arabic and pullulan significantly increased between the ratios of 0.5 and 1.0. The E_∞ values for the microcapsules prepared with maltodextrin changed greatly at the smaller ratio (between 0.2 and 0.5) compared to those for gum arabic and pullulan. As shown above, the E_∞ values were larger for the larger ratios of linoleic acid to wall material. If extractable linoleic acid homogeneously distributed within the microcapsules and did not interact with the wall materials, the microcapsule with higher E_∞ should give the higher initial extraction rate. However, the experimental results showed an opposite tendency. This would indicate the heterogeneous distribution of linoleic acid within the microcapsules. Based on these facts, we postulated that particles of linoleic acid were isolated from each other and were separately covered with a dehydrated layer of wall material when the weight ratio was low, and that the particles were susceptible to coalescence that made paths leading to the surface at the higher weight ratios. Linoleic acid, which led to the surface of a microcapsule, would be easily extractable and also be easily oxidizable. We reported the autoxidation processes of linoleic acid encapsulated with the wall materials at the same weight ratios as in this study (Minemoto *et al.*, 1999). Autoxidation processes also depend on the weight ratio of linoleic acid to wall material. Linoleic acid encapsulated with a wall material autoxidized with time in air. However, not all of the linoleic acid autoxidized, and the autoxidation leveled off at a fraction of unoxidized linoleic acid, Y_∞ . The relationship between the fraction of the easily extractable linoleic acid, E_∞ , and the fraction of the easily

Fig. 2. Change in the fraction of extracted linoleic acid with time in an early stage of extraction with chloroform. The symbols are the same as in Fig. 1. The slopes of the solid lines indicate the initial extraction rates, and the dotted curves were empirically drawn.

Fig. 4. Relationship between the E_{∞} values and the fractions of easily oxidizable linoleic acid, $1 - Y_{\infty}$. Y_{∞} is the fraction of unoxidized linoleic acid where further autoxidation seemed not to proceed. The Y_{∞} values were cited from our previous work (Minemoto *et al.*, 1999). The symbols Δ , $\square$ and $\circ$ indicate that gum arabic, pullulan and maltodextrin, respectively, were used as wall materials.

Fig. 5. Solvent-extraction of linoleic acid with chloroform from the microcapsules that were stored at 37°C and at a relative humidity of 75% under a nitrogen atmosphere for a given period. The symbols Δ , $\square$ and $\diamond$ represent solvent-extraction of linoleic acid from the microcapsules stored for 1, 2 and 3 days, respectively. The symbol $\circ$ indicates freshly prepared microcapsules, that is, the storage period was 0 day. The solid curves were drawn using Eq. (1).

oxidizable one, $1 - Y_{\infty}$, is shown in Fig. 4. There was a fairly good correlation between them except for the microcapsules prepared with gum arabic at high weight ratios. This indicated that we could, to some extent, predict the level of hardly oxidizable linoleic acid during a long storage period by observing the solvent-extraction process, which could be done in a much shorter time than observing the autoxidation process.

Figure 3 also shows the relationship between the initial extraction rate and the weight ratio of linoleic acid to wall material. There was a tendency for the initial extraction rate to be faster for the microcapsule with the smaller ratio. This would indicate that the distribution of linoleic acid within a microcapsule was not homogeneous and that a considerable

Fig. 6. Changes of ($\circ$) the E_{∞} and (Δ) the initial extraction rate with the storage period.

amount of it was located near the surface of the microcapsule when the microcapsule was prepared at the small weight ratio.

Figure 5 illustrates the solvent-extraction processes of linoleic acid from the microcapsules stored for a certain number of days. The microcapsules were prepared with gum arabic at the weight ratio of 0.5. The solid curves were drawn to fit the experimental points at the extraction times longer than 10 min to Eq. (1). The fraction of easily extractable linoleic acid, E_{∞} , for freshly prepared microcapsules was much lower than those for the stored microcapsules. The initial extraction rates were also estimated in the above described manner.

The changes in E_{∞} and the initial extraction rate during storage are shown in Fig. 6. The E_{∞} value greatly increased within one day of storage, but scarcely changed during further storage. It is possible that exudation of linoleic acid from its particles covered with a dehydrated layer of wall material through the layer occurred during the short storage period. The initial extraction rate linearly increased with storage time, and the surface of the microcapsule appeared to the eye to be oily at storage periods longer than 1 day. This would indicate that linoleic acid gradually migrated from the inside of the microcapsule toward its surface although the driving force for the migration could not be elucidated.

As mentioned above, observation of the solvent-extraction processes of an encapsulated lipid is one method of estimating the state of the lipid within a microcapsule. The maximum fraction of extractable lipid could be correlated fairly well with the level where further autoxidation did not proceed during a long storage period. Observation of the solvent-extraction process can be completed in a much shorter time than that of the autoxidation process.

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