# Comparison of Oxidation of Methyl Linoleate Encapsulated with Gum Arabic by Hot-Air-Drying and Freeze-Drying

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Methyl linoleate was encapsulated with gum arabic by two drying methods, hot-air-drying and freeze-drying. The oxidation of methyl linoleate encapsulated by both methods depended on the relative humidity during storage. Methyl linoleate encapsulated by freeze-drying was more slowly oxidized than that encapsulated by hot-air-drying at any relative humidity. The initial fraction of nonencapsulated lipid in the hot-air-dried microcapsule was about 1%, and the fraction increased quickly in the early stage of storage at a high relative humidity. On the other hand, the fraction of nonencapsulated lipid in the freeze-dried microcapsule was about 10%, but it did not change during storage at any relative humidity. Scanning electron micrographic observation of microcapsules prepared by hot-air-drying and freeze-drying showed that their morphologies were greatly different. These results suggested that the state of the lipid encapsulated by freeze-drying was different from that encapsulated by hot-air-drying.

**Keywords:** *Microencapsulation; gum arabic; hot-air-drying; freeze-drying; oxidation*

## INTRODUCTION

Microencapsulation of liquid lipid into powdery matrixes of saccharides or proteins includes two unit operations: emulsification of the lipid with an aqueous solution of wall material and drying of the emulsions. Both hot-air-drying and freeze-drying methods have been used for encapsulation of the lipid. A difference in morphology exists between the microcapsulates prepared by hot-air-drying and freeze-drying methods. In evaporating dehydration such as spray-drying, microcapsules shrank during drying. When gum arabic was used as a wall material, hot-air-drying of emulsions resulted in formation of dents on the microcapsules (Rosenberg et al., 1985; Imagi et al., 1990). On the other hand, freeze-drying, which is sublimating dehydration, maintained the form of microcapsules because of fixation by freezing (Nagata, 1996). Thus, the microcapsules prepared by the hot-air-drying and freeze-drying methods were different in their morphology, and the morphology of the microcapsules affected the stability of the lipid against oxidation, flavor retention, and other characteristics. However, there have been few reports on the effects of the drying method on lipids.

The fraction of nonencapsulated lipid in the microcapsules, which included the lipid exposed at the surface, was often used to estimate the quality of the microcapsules (Imagi et al., 1992a; Lin et al., 1995). Although there have been many reports on the fraction (Gejl-Hansen and Flink, 1977; Anandaraman and Reineccius, 1987; Shimada et al., 1991; Wanger et al., 1995), the methods for measurement of the fraction were different among the reports. Therefore, it is difficult to compare their values.

In this context, we prepared microcapsules of methyl linoleate with gum arabic by the hot-air-drying and freeze-drying methods, and measured the oxidative processes of methyl linoleate in the microcapsules and the change in the fraction of nonencapsulated lipid during storage. The morphologies of microcapsules prepared by the methods were also observed by scanning electron microscopy. In the preparation of the microcapsules, a single-droplet-drying method (Charlesworth and Marshall, 1960) was used in both drying methods. Nonencapsulated lipid was extracted by immersing a microcapsule into a solvent. This method has often been adopted by some researchers (Shimada et al., 1991; Imagi et al., 1992a). Optimum immersion time was specified from the time course of the fraction of lipid extracted. The location of the oxidized lipid can be discussed from the relationship between the fraction of oxidized lipid and that of the nonencapsulated material.

## MATERIALS AND METHODS

**Materials.** Methyl linoleate, methyl palmitate, and methyl myristate were purchased from Tokyo Kasei Kogyo, Tokyo, Japan. The lipids were used without further purification, their purities being 95, 95, and 98%, respectively. Methyl palmitate and methyl myristate were used as internal standards in gas chromatographic determination of unoxidized methyl linoleate and of nonencapsulated lipids, respectively. Gum arabic from San-ei Chemical Industries (Osaka, Japan) was used as a wall material in encapsulation.

**Emulsification.** Gum arabic was dissolved in distilled water with slight heating at a concentration of 25% (w/v). The solution (3.0 mL) was placed into a test tube, and 0.75 g of a mixture of methyl linoleate and methyl palmitate of equal weight was added. The mixture was homogenized for 2 min by a homogenizer (Physcotron NS-50, Nichi-on, Tokyo, Japan) consisting of a stator and a rotor with a blade. During the homogenization, the tube was cooled by immersion in water.

**Drying of Emulsions.** Five microliters of the emulsions was suspended onto a glass filament using a micropipet, and then placed in the drying section of the apparatus shown in Figure 1a (Imagi et al., 1992b). The emulsions were dried by air introduced upward into the

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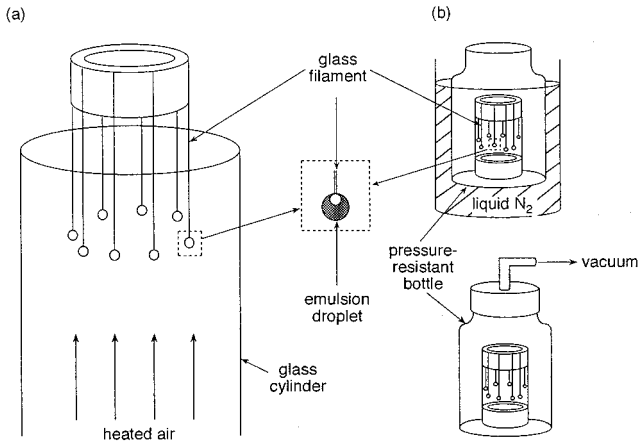


**Figure 1.** Schematic drawings of equipment for (a) hot-air-drying and (b) freeze-drying. In hot-air-drying, the air was heated at 50 °C. In freeze-drying, the emulsion droplet was frozen in liquid $N_2$ and then dried at room temperature.

section at 50 °C and at a velocity of about 0.4 m/s for 30 min to prepare a microcapsule.

In a freeze-drying method, the emulsions were suspended on a glass filament as mentioned above and then placed in the freezing section of a pressure-resistant bottle (Figure 1b). The bottle with the suspensed emulsions was immersed in a liquid $N_2$ to freeze the emulsions below −150 °C over 30 min. The frozen emulsions were freeze-dried below $6.6 \times 10^{-4}$ atm and at room temperature over 3 h.

All the dried samples held on the glass filaments were stored at 37 °C in a desiccator. The relative humidity in the desiccator was controlled with a saturated LiCl, $K_2CO_3$, NaCl, or $K_2SO_4$ solution at 12, 44, 75, or 96%. For measurement of nonencapsulated lipids, the samples were stored under $N_2$ atmosphere.

**Determination of Unoxidized Substrate.** A dried sample was taken out of the desiccator. The sample with the glass filament was dissolved in 1 mL of 0.1 N NaOH. The lipids were extracted from the aqueous phase into a mixture of methanol (2.5 mL) and chloroform (1.25 mL) under vigorous agitation. Then 1.25 mL of chloroform was added to the mixture. After agitation, 1 mL of 0.1 N HCl and 0.25 mL of distilled water were added, then agitated.

After centrifugation for separation of the organic and aqueous phases, the lower organic phase was carefully pipeted out. A mixture (2 mL) of chloroform, methanol, and water (3:48:47 in volume) was added to the organic phase. After the mixture was shaken and then centrifuged, the lower phase was recovered and evaporated (Bligh and Dyer, 1959). The residue was dissolved in 0.5 mL of hexane.

The extracted lipids were analyzed by a Shimadzu GC-7A gas chromatograph (Kyoto, Japan) with a hydrogen ionization detector. Five microliters of the hexane solution was injected into a glass column, the dimensions of which were 3.2 mm in diameter and 3.1 m in length, with 5% Advance-DS on Shinchrom A. The injection and column temperatures were 230 °C and 180 °C, respectively. The $N_2$ gas flow rate was 50 mL/min.

The ratio of the amount of unoxidized methyl linoleate and that of methyl palmitate was obtained from the areas under their peaks. The fraction of unoxidized substrate was calculated from the ratio.

**Observation of the Microcapsules by Scanning Electron Microscopy.** The surfaces of the microcapsules prepared by hot-air-drying and freeze-drying were coated with Pt−Pd using an ion coater (IB-3, Eiko, Tokyo) and observed by an Akashi ALPHA-9 (Tokyo, Japan) scanning electron microscope at 15 kV.

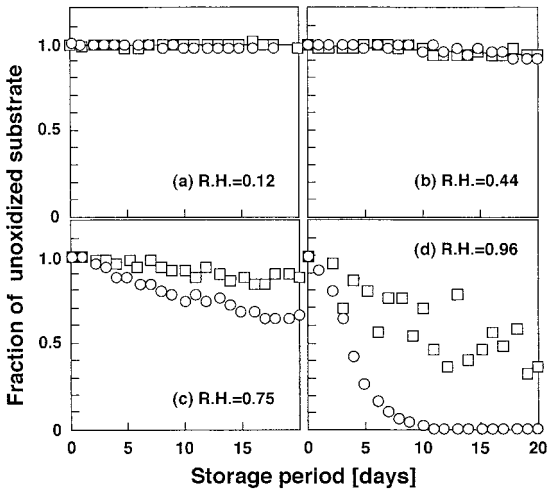


**Figure 2.** Oxidative processes of methyl linoleate encapsulated with gum arabic by (○) hot-air-drying and (□) freeze-drying during storage at 37 °C and at various relative humidities. R.H., relative humidity in fraction.

**Determination of Nonencapsulated Lipids.** A dried sample was immersed in 2.5 mL of chloroform, which included methyl myristate at a concentration of 0.01% (v/v) as an internal standard in gas chromatographic determination, for a specified period. After evaporation, the residue was dissolved in 0.5 mL of hexane, then analyzed by gas chromatography. The conditions for the analysis were the same as those mentioned above. The fraction of nonencapsulated lipids was calculated from the areas under the peaks for methyl linoleate, methyl palmitate, and methyl myristate.

**Measurement of Sorption Isotherm of Water onto Gum Arabic.** Gum arabic was dried at 150 °C to remove water completely. The dried gum arabic (5−10 mg) was placed in a 6 mm ∅ × 2.5 mm platinum cell. The cell was placed at 37 °C for at least 3 days in a desiccator, in which a beaker filled with a saturated salt solution was placed to regulate the relative humidity. The salts used were LiCl (12), $CH_3COOK$ (23), $MgCl_2$ (33), $K_2CO_3$ (44), $NH_4NO_3$ (55), NaCl (75), KCl (86) and $K_2SO_4$ (96). The figures in parentheses indicate the percent relative humidities of the saturated solutions. After the sorption had reached equilibrium, the cell was taken out of the desiccator and immediately placed in a Shimadzu TGA-50H thermogravimeter. The gum arabic was heated at a rate of 5 °C/min from room temperature to 150 °C and then kept at this temperature for at least 30 min under flowing $N_2$ at a rate of 20 mL/min. The amount of water sorbed at 37 °C was calculated from the difference in weight before and after heating.

## RESULTS

**Oxidative Processes of Methyl Linoleate Encapsulated by Hot-Air-Drying and Freeze-Drying.** Figure 2 shows the oxidative processes of methyl linoleate encapsulated with gum arabic by hot-air-drying and freeze-drying. The processes largely depended on both the relative humidity during storage and the drying method. At lower relative humidities, oxidation of methyl linoleate in microcapsules prepared by both methods was suppressed. At higher humidities, the

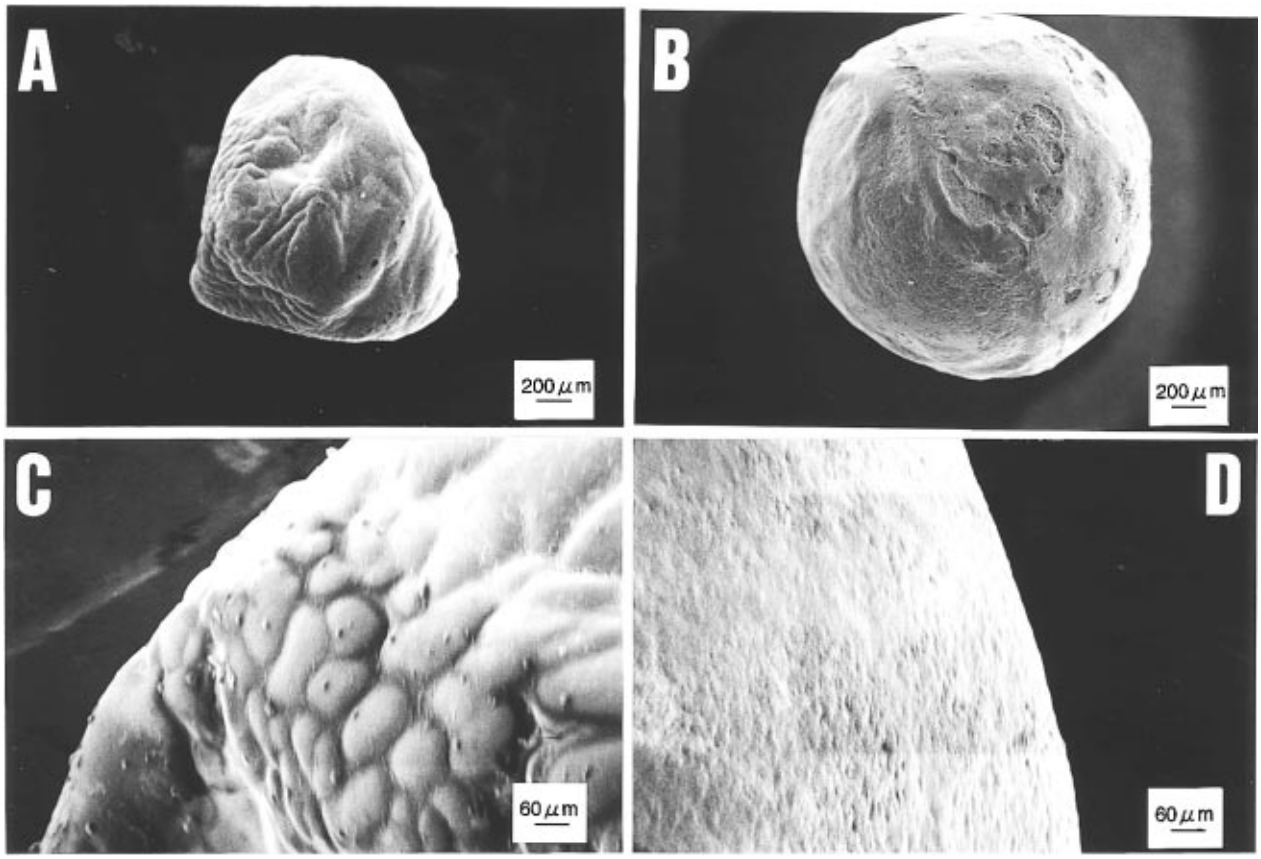


**Figure 3.** Scanning electron micrographs of the microcapsules prepared by hot-air-drying (A and C) and freeze-frying (B and D). Micrographs A and B show the whole images, and C and D show the surface images.

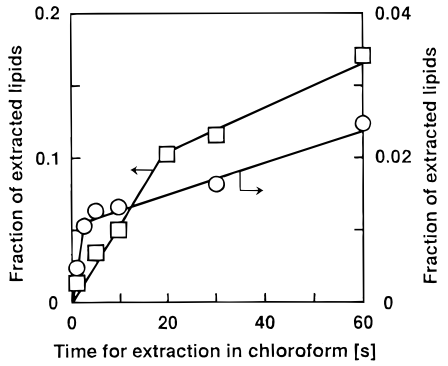


**Figure 4.** Changes in the fraction of lipids extracted by chloroform for the microcapsules prepared by (○) hot-air-drying and (□) freeze-drying. Extraction was carried out at room temperature.

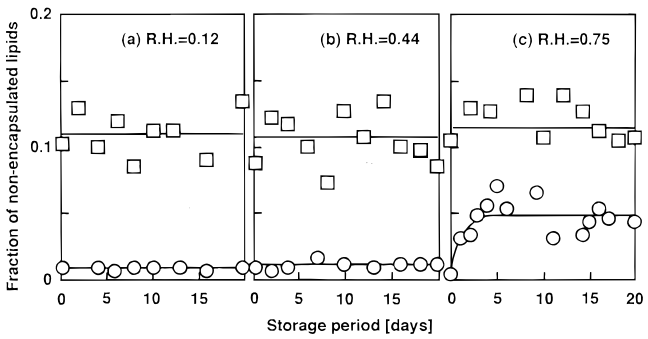


**Figure 5.** Changes in the fraction of nonencapsulated lipids during storage under $N_2$ atomosphere, at 37 °C and at various relative humidities for the microcapsules prepared by (○) hot-air-drying and (□) freeze-drying. R.H., relative humidity in fraction.

method for preparation of the microcapsules greatly affected the oxidative process of methyl linoleate. Methyl linoleate in the microcapsules prepared by freeze-drying was more resistant than that in the microcapsules prepared by hot-air-drying.

**Morphology of the Microcapsules.** Figure 3 shows the whole and surface images of microcapsules prepared by hot-air-drying and freeze-drying. The freeze-dried microcapsule was as spherical as a droplet and its surface was smooth. On the other hand, the hot-air-dried microcapsule shrank to form dents, and there were irregular protrusions on the surface.

**Extraction of Nonencapsulated Lipids.** Figure 4 shows the fraction of lipids extracted for various periods. The lipids were extracted by immersing a microcapsule in chloroform. There were inflection points in the time courses at 3 and 20 s for the microcapsules prepared by hot-air-drying and freeze-drying, respectively. The lipids extracted before the inflection point would be nonencapsulated. Hereafter, the fractions of the lipids extracted for 3 and 20 s will be regarded as the fractions of nonencapsulated lipids for the microcapsules prepared by hot-air-drying and freeze-drying, respectively. The fraction of nonencapsulated lipids in the microcapsules prepared by freeze-drying was about 10 times that of the nonencapsulated lipids in the microcapsules prepared by the hot-air-drying.

**Change in the Fraction of Nonencapsulated Lipids during Storage.** Changes in the fraction of nonencapsulated lipids during storage at various relative humidities are shown in Figure 5. The fraction of nonencapsulated lipids in the microcapsules prepared by hot-air-drying did not change at the relative humidities of 12 and 44%, but it increased at the early stage of storage at the relative humidity of 75%, then leveled

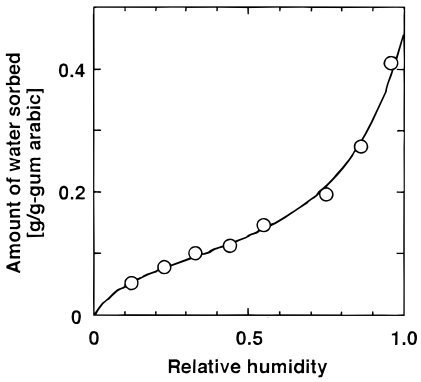


**Figure 6.** Sorption isotherm of water onto gum arabic at 37 °C. The solid curve was drawn by fitting the experimental points to the Guggenheim−Anderson−de Boer equation. Relative humidity was expressed in fraction.

off. On the other hand, the fraction of nonencapsulated lipids in the microcapsules prepared by freeze-drying did not change during storage at any relative humidity.

**Sorption Isotherm of Water onto Gum Arabic.** Figure 6 shows the sorption isotherm of water onto gum arabic at 37 °C. The experimental points were fitted to the following Guggenheim−Anderson−de Boer equation:

$$q = \frac{q_m ckp^*}{(1 - kp^*)\{1 + (c - 1)kp^*\}} \quad (1)$$

where $q$ is the amount of water sorbed onto gum arabic, $p^*$ is the relative humidity in fraction, $q_m$ is the $q$ value corresponding to monolayer coverage, $c$ is an experimental parameter, and $k$ is a correction factor. The $q_m$, $c$, and $k$ values were determined to be 0.0842 g water/g dry material, 12.7, and 0.821, respectively. The solid curve in Figure 6 was drawn using these parameters.

## DISCUSSION

The stability of methyl linoleate encapsulated with gum arabic against oxidation was dependent on both the relative humidity of atmosphere during storage and the method for preparation of the microcapsules. Rosenberg et al. (1990) reported that volatile retention in microcapsules prepared with gum arabic by spray-drying depended on the relative humidity and that easy release of volatiles at higher relative humidities was ascribed to uptake of water by gum arabic, which would make the matrix semifluid. This supposition could be applied to the oxidative process of methyl linoleate encapsulated with gum arabic by hot-air-drying and stored at higher relative humidities. Figure 6 shows that the amount of water sorbed onto gum arabic largely depended on the relative humidity and that the amounts at the relative humidities of 12 and 44% were less than or almost equal to the $q_m$ value. At those relative humidities, methyl linoleate encapsulated by either of the drying methods was scarcely oxidized during 20-day storage. When the microcapsules were stored at the relative humidities at which gum arabic sorbed water over the monolayer coverage, methyl linoleate was oxidized. The deterioration rate was faster at higher relative humidities. However, there was a large difference in the oxidative stability of methyl linoleate in the microcapsules prepared by hot-air-drying and freeze-drying (Figure 2), although both the microcapsules became almost fluid during storage at the relative humidity of 96% because of water uptake.

In hot-air-drying, a dense matrix layer was formed at the surface of the microcapsules at the initial stage of drying (Imagi et al., 1992a) and dents were formed after complete drying (Figure 3A; Rosenberg et al., 1985; Imagi et al., 1990). The formation of dents was due to shrinkage of the microcapsules during drying (Kim et al., 1996). Therefore, the microcapsules prepared by hot-air-drying were rigid. In freeze-drying, the microcapsule maintained its form because the emulsion droplets were frozen and their form was fixed prior to drying (Figure 3B). The microcapsule had a porous structure because cavities formed by ice crystals during freezing remained within it (Figure 3D), and it was fragile. Considering the structures of the microcapsules, we found that oxygen could diffuse more easily and methyl linoleate would be oxidized faster within the microcapsule prepared by freeze-drying than within the microcapsule prepared by hot-air-drying. However, the experimental results showed that methyl linoleate encapsulated by freeze-drying was more slowly oxidized than that encapsulated by hot-air-drying. This would suggest that the oxidative stability of methyl linoleate encapsulated with the wall material is greatly related to the state of the lipid in the microcapsules.

Emulsification forms the lipid into small particles. In hot-air-drying, the emulsion droplet shrinks during drying. This shrinkage would cause the aggregation of the lipid particles in the microcapsule. Once oxidation of the lipid is initiated in a part of this lipid assembly, it propagates within the assembly. On the other hand, each lipid particle in the microcapsule prepared by freeze-drying would be isolated and covered with gum arabic because the form of the emulsion droplet is fixed by freezing prior to drying. Therefore, oxidation of the lipid would be completed within a small particle and would not propagate to other particles. Although these are suppositions and we do not have direct evidence for them, scanning electron micrographic observation seems to be consistent with the suppositions.

As shown in Figure 4, the fraction of nonencapsulated lipids in the microcapsule prepared by freeze-drying was about 10 times that in the microcapsule prepared by hot-air-drying. This difference in the fraction is due to whether the microcapsules form dense matrix at their surface during drying. In hot-air-drying, the dense matrix is formed at the surface of the microcapsule (Imagi, 1992), so that only the lipid exposed at the surface would be extracted as the nonencapsulated lipid. On the other hand, the microcapsule prepared by freeze-drying had a porous structure (Figure 3D). Therefore, solvent can easily penetrate inside the microcapsule and extract the lipid located on the wall of the pores. The reason for the long time needed to extract the lipid from the microcapsule prepared by freeze-drying would be explained by the same supposition. As shown in Figure 5, the fraction of nonencapsulated lipids on the microcapsule prepared by hot-air-drying increased in the early stage of storage when the microcapsule was stored at the relative humidity of 75%. Sorption of water onto the matrix of dehydrated gum arabic would make the matrix softened or fluid-like and allow the lipid to migrate toward the surface. This migration might result in the increase in the fraction at the relative humidity. In the microcapsule prepared by freeze-drying, lipid particles would be covered with gum arabic and would be isolated from each other, so that the migration would be difficult.

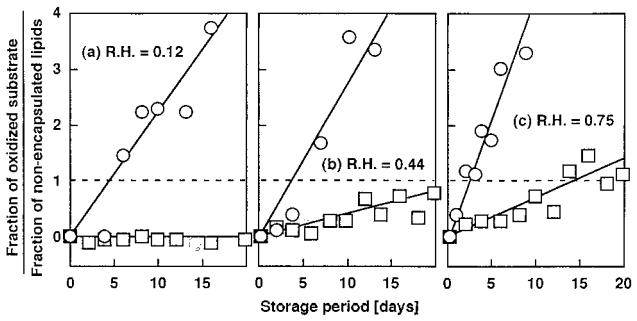


**Figure 7.** Changes in the ratio of the fraction of oxidized substrate to that of nonencapsulated lipids during storage at 37 °C and at various relative humidities for the microcapsules prepared by (○) hot-air-drying and (□) freeze-drying. R.H., relative humidity in fraction.

Thus, the fraction of nonencapsulated lipid would scarcely change during storage at any relative humidity.

The ratio of the fraction of oxidized substrate to that of the nonencapsulated lipids should provide information on the location of the oxidized substrate within the microcapsule. When it is assumed that only the nonencapsulated lipid is oxidized, the ratio should be less than or equal to unity. A ratio higher than unity indicates that the encapsulated lipid, that is, the lipid covered with the dehydrated layer of the wall material, is also oxidized. Figure 7 shows the changes in the ratio of the microcapsules prepared by hot-air-drying and freeze-drying during storage at various relative humidities. The ratios for the microcapsules prepared by hot-air-drying exceeded unity much earlier than those for the microcapsules prepared by freeze drying at any relative humidity. Interestingly, even nonencapsulated lipid in the microcapsule prepared by freeze-drying was resistant to oxidation. The figure also indicates that storage at higher relative humidities facilitated the oxidation of lipid located inside the microcapsules prepared by both drying methods.

Methyl linoleate encapsulated with gum arabic by freeze-drying was more slowly oxidized than that encapsulated by hot-air-drying, especially at higher relative humidities. The fraction of nonencapsulated lipid also depended on both the drying method and the relative humidity of the storage atmosphere. The fraction for the microcapsules prepared by freeze-drying did not change during storage. Thus, freeze-drying was better than hot-air-drying for encapsulation of methyl linoleate with gum arabic. However, it is unkown whether this is true for other combinations of lipids and wall materials. This is now under investigation. The reason why the fraction of nonencapsulated lipid in the microcapsule prepared by freeze-drying was high (about 10%) remains unclear. This should also be investigated further.

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