

Effect of Water Activity on the Release Characteristics and Oxidative Stability of D-Limonene Encapsulated by Spray Drying

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The stability of encapsulated D-limonene, which was prepared by spray drying, was studied in view of the release characteristics and oxidation stability. Gum arabic, soybean water-soluble polysaccharide, or modified starch, blended with maltodextrin were used as the wall materials. The powders were stored under the conditions of 23–96% relative humidity at 50 °C. The release rate and the oxidation rate were closely related to the relative humidity. The relationship was not simple. Initially, the release rate and the oxidation rate increased with increasing water activity, but around the glass transition temperature, the rates decreased sharply to increase again at a further increase of water activity. The results could be explained by a change in the powder structure, where a glass capsule matrix was changed into rubbery state during storage.

KEYWORDS: Microencapsulation; spray drying; retention; release; oxidation stability

INTRODUCTION

Spray drying is the most common way to perform microencapsulation of a flavor (i.e., to perform transformation of a liquid state (solution, dispersion, emulsion) into a solid state). The advantages of this technology are numerous; it provides protection against degradative reactions and prevents the loss of flavors during storage. In addition to the primary means of stabilization and protection of flavors, a controlled or extended flavor release at an appropriate time may also be required. The oxidation stability and the release characteristics of encapsulated flavors in powder matrixes are also important for estimating the shelf life of the product, as well as the controlled release applications in foods. During processing and storage, the lowering or inhibition of the loss and flavor decomposition is dependent on the ability to maintain the structural integrity that retains flavors within the powder particles, as well as to control molecular diffusion through the wall matrixes.

Several mechanisms have been proposed for the controlled release of flavor. Whorton (1) provided an overview of the basic release mechanisms in various capsule matrixes. The release of flavor from the spray-dried powder may be considered to be

associated with the diffusion mechanisms of both flavor and water, because the solubilization of the wall matrixes with water would be followed by subsequent release of the encapsulated flavor. Relatively few studies have been published on the release characteristics of encapsulated flavors. Rosenberg et al. (2) observed that the release of an encapsulated flavor ester during storage increases with an increase in relative humidity. They suggested that water uptake at a high relative humidity destroys the capsule structure. The effect of relative humidity on the release of flavor indicates that high retention of flavor is maintained as long as the individual structure of the capsule is intact. Whorton and Reineccius (3) evaluated the mechanism associated with the controlled release of the flavor encapsulated in maltodextrins of different dextrose equivalents (DE). The flavor release increased with the increase in water activity up to the point where collapse occurred. However, a discrepancy in the water activity effect on the release rate of flavor has also been observed. Further, they correlated the retention of flavor with the glass transition temperature and the collapse temperature of the wall materials. Recently, Yoshii et al. (4) observed the release of the encapsulated ethyl-*n*-butyrate during storage. The release rates were dependent on not only the relative humidity but also the composition of the wall materials. They also evaluated the release rate constant of flavor using Avrami's equation.

In addition to the release of flavor through the capsule matrixes, the oxidation of encapsulated flavor is also used as an index of the encapsulated flavor stability. Anandaraman and

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Reineccius (5) studied the shelf life of encapsulated orange oil and its linkage with the capsule materials. They used limonene-1, 2-epoxide (limonene oxide) and carvone as the marker of oxidation compounds. Furthermore, many investigations have been performed to examine the dependence of the shelf life of the encapsulated flavors on the wall material (6), the emulsion size (7), the type of volatile compounds (8), the particle size (9), and the type of atomizer and the drying air temperature (10). The effect of water activity on the stability to oxidation has also been studied. Anker and Reineccius (11) and Beristain et al. (12) showed that the oxidation rates generally become lower with an increase of water activity in the range of 0.001–0.536 and 0.108–0.628, respectively. Hardas et al. (13) observed better protection against oxidation of an encapsulated milk fat when the powder was stored at water activities of 0.14 and 0.44, compared with a water activity of 0.52. Thus, these results, which are not in agreement with each other, have been reported. The effect of water activity on the oxidation process may be analogous to that on the release of flavors, if it is the diffusion of target molecules that is the rate-limiting step for both phenomena. In a low-moisture food, the diffusion of molecules has been related to a glass transition phenomenon (14). Labrousse et al. (15) successfully correlated the oxidation of encapsulated lipids with the glass transition temperature, when sugar crystallization and a structural change occurred. Whorton and Reineccius (3), Beristain et al. (12), and Gunning et al. (16) also mentioned that the release and oxidation of the encapsulated flavors have been related to collapse and crystallization. A better understanding of the effect of storage relative humidity on the properties of capsule matrixes would be useful in the quality control and applications of these powders.

The present study focused on the effect of the relative humidity on the release characteristics and the oxidative stability of encapsulated D-limonene by spray drying. The rates of release and oxidation were investigated with structural changes and glass transition temperature of the capsule matrixes.

MATERIALS AND METHODS

Materials. D-Limonene and gum arabic (GA) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Maltodextrin with ca. 20 DE (MD, Amycol No. 1) and modified starch (HI-CAP 100) were gifts from Nippon Starch Chemicals Co., Ltd. (Osaka, Japan), and Nippon NSC Co., Ltd. (Tokyo, Japan), respectively. Soybean soluble polysaccharide (SSPS), a new type of emulsifier which is extracted and refined from soybean (17), was a gift from Fuji Oil Chemical Co., Ltd. (Osaka, Japan). The organic chemicals used in the analyses were of analytical grade.

Preparation of Spray-Dried Powder Containing a Liquid Flavor Emulsion. D-Limonene was used as the model flavor. The carrier solution was prepared by blending and rehydrating the solid powders in warm distilled water, followed by cooling to room temperature. Four different solid systems were prepared: blending of GA and MD (mass ratio = 1:3), blending of SSPS and MD (1:3), blending of HI-CAP 100 and MD (1:3) and HI-CAP 100. The total concentration of dissolved solids in the carrier solutions was 40% w/w on the wet basis as 0.24 kg of water and 0.16 kg of solid powder. After that, D-limonene was added to the carrier solution to produce a D-limonene mass ratio to the total solids of 0.25 to 1. An emulsion was prepared by homogenizing the mixture of D-limonene and the carrier solution using a Polytron homogenizer (PT-10, Kinematica GA, Littau, Switzerland) at dial position 8 for 3 min, followed by use of a Microfluidizer (model 110T, Microfluidics Corp., Newton, MA.) at 12 000 psig (82.8 MPa). The emulsion was spray dried in an Ohkawara-L8 spray dryer (Ohkawara Kakouki Co., Ltd., Yokohama, Japan), equipped with a centrifugal atomizer. The operational conditions of the spray drying were air inlet

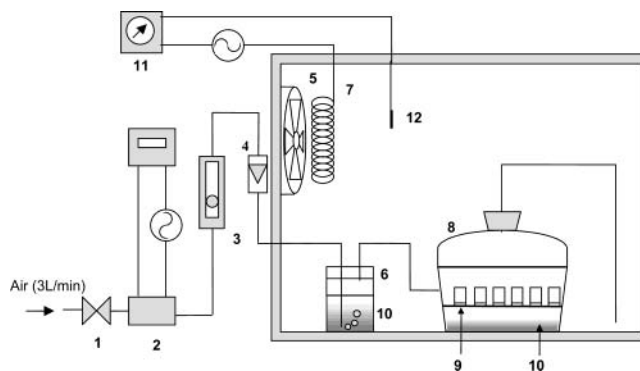


Figure 1. Experimental equipment for measuring the release and oxidation of encapsulated D-limonene and moisture sorption isotherms of the spray-dried powder. 1. Flow control valve, 2. air pump, 3. flow meter, 4. valve, 5. fan, 6. bubbling bottle, 7. heater, 8. desiccator, 9. sample powder, 10. saturated salt solution, 11. temperature regulator, 12. thermostat.

temperature of 200 °C, air outlet temperature of 110 ± 10 °C, rotational speed of the atomizer of 30 000 rev/min, feed rate of 45 mL/min, and air flow rate of 110 kg/h.

The Stability of Spray-Dried D-Limonene. The stability of encapsulated D-limonene during storage was investigated to determine the release and the oxidation of D-limonene at constant temperature and humidity. The experimental equipment is illustrated in **Figure 1**. About 0.1 g of the spray-dried powder was weighed and spread in a thin layer in a 15-mL (20 × 48-mm) glass bottle, and stored in a desiccator. The relative humidity (RH) inside the desiccator was constant of 23, 51, 75, and 96% at 50 °C. Constant RH was created using saturated salt solutions of potassium acetate, magnesium nitrate, strontium chloride, sodium chloride, potassium chloride, and potassium sulfate (18). The desiccator was placed in an air bath to keep the temperature at 50 °C for 24 h to reach equilibrium. During equilibration, the temperature and humidity inside the desiccator was continuously measured by a thermo recorder (TR-72S, T&D Corporation, Nagano, Japan), and the humidity was adjusted to the values mentioned above within $\pm 5\%$ RH.

Sample bottles were placed in a desiccator to study the release and oxidation kinetics for 25 or 30 days. At fixed time intervals, the bottles were removed from the desiccator to extract and measure the residual amounts of D-limonene and the oxide compounds. Furthermore, humid air of the same RH was blown into the desiccator to purge the gas for 10 min at 6 h intervals.

Extraction of D-Limonene and the Oxidation Products from the Powder. The storage powder was dispersed in 4 mL of water in a glass bottle, and then 2 mL of hexane was added, followed by violent mixing in a vortex mixer for 1 min. To extract the emulsified D-limonene into the organic solvent, the mixture was heated on a heating block with intermittent shaking at 90 °C for 30 min. Heating hexane in the closed bottle at 90 °C can be dangerous. The experiment should be carefully done and the safety guard should be used. After cooling to room temperature in chilled water, the extracted mixture was then centrifuged at 3000 rev/min for 10 min to separate the organic phase from water. The organic phase solution was injected to a gas chromatograph to determine the quantity of D-limonene and the oxide compounds as described in the following section. The retention of D-limonene was defined as the ratio of the remaining amount of D-limonene to the initial one. The amount of the oxide compound was expressed by the mass ratio of the oxide to the retained D-limonene. All of the samples were analyzed in duplicate and the data were presented as an average.

Gas Chromatographic Analysis. A 1- μ L sample of the organic phase after the extraction was injected twice for each sample into a gas chromatograph (GC-2010, Shimadzu Corporation, Kyoto, Japan) equipped with a 30-m × 0.32-mm × 0.25- μ m film thickness, fused silica capillary column (DB-17, J&W Scientific, Folsom, California.) using a flame ionization detector at 230 °C with N_2 of 60 kPa. A splitless mode was used with a 2 min sampling time, and then the

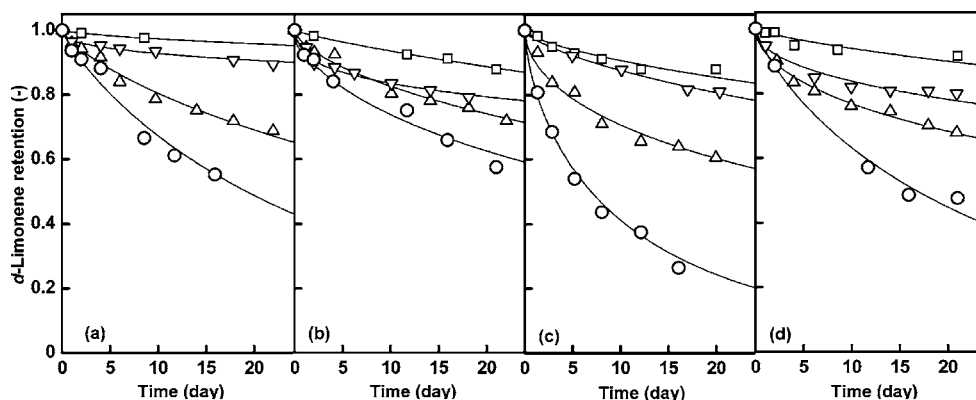


Figure 2. Release time-courses of encapsulated D-limonene in spray-dried powders stored at various relative humidities and 50 °C. (a) Blend GA-MD, (b) blend SSPS-MD, (c) blend HI-CAP 100-MD, (d) HI-CAP 100. □, 23%RH; △, 51%RH; ▽, 75%RH; ○, 96%RH.

split ratio was controlled at 1:30. The column temperature was programmed initially at 50 °C for 2 min, followed by heating to 130 °C at a rate of 10 °C min⁻¹, then to 150 °C at a rate of 5 °C min⁻¹, and finally to 190 °C at a rate of 30 °C min⁻¹ with 3 min of holding time. The external standard method was used to calculate the D-limonene, limonene oxide, and carvone quantities. Two bottles of the standard 1 μL/mL of flavors in hexane were prepared. A 1-μL sample of the standard was injected into the gas chromatograph, twice for each bottle of standard. The average peak area was used to calculate the amount of the compounds.

Moisture Sorption Isotherms. To calculate the moisture contents of the powders at the desired RH, the moisture sorption isotherm of the spray-dried powders was determined. For this purpose, spray-dried powders without D-limonene were prepared. About 0.1 g of the powder was weighed and spread as a thin layer in a 15 mL (20 × 48-mm) glass bottle and placed in a desiccator containing a saturated salt solution as described above. The desiccator was then stored in an air bath at constant temperatures of 20 and 50 °C. The sample powders were weighed periodically up to an equilibrium for about 1 week. The initial moisture content in the powder was measured using a MKS-510N Karl Fischer Titrator (Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan). The powder sample, 0.03–0.08 g, was weighed and added to Hayashi solvent FM containing 26% methanol and <0.2 mg H₂O/ml (Hayashi Pure Chemical Ind., Co., Ltd., Osaka, Japan), and then titrated against a 5 mg/mL Hydranal composite solvent (Riedel-deHaën, Sigma-Aldrich, Seelze, Germany). The water content was reported as % w/w of the sample. The samples were measured 5 times and the averages were used.

Glass Transition Determination. Glass transition temperatures were determined with a Mettler DSC 820 (Dietikon, Switzerland) differential scanning calorimeter equipped with a liquid nitrogen cooling system. A sample of 10 mg was weighed in an aluminum pan and sealed. Heating and cooling rates were 10 °C min⁻¹. Glass transition points were measured after equilibrating the samples over a salt solution at 20 °C and at relative humidities of 33, 51, and 75% with the salt solutions of magnesium chloride, magnesium nitrate, and sodium chloride, respectively (18). The temperature range of the scans was varied between -100 °C and 130 °C depending on the water content. All samples were first heated to destroy the thermal history of the sample, then cooled and reheated. Glass transition temperature was taken as the midpoint of the baseline shift in the second scan. Duplicate measurements were performed.

Scanning Electron Microscopy (SEM). A JSM 5800 model (JEOL Co., Ltd., Tokyo, Japan) scanning electron microscope was used to investigate the outer structure changes in the spray-dried powder during storage. The powders were placed on the SEM stubs using a two-sided adhesive tape (Nisshin EM Co. Ltd., Tokyo, Japan). The specimens were subsequently coated with Pt-Pd using a Model MSP-1S magnetron sputter coater (Vacuum Device Inc., Tokyo, Japan). The coated samples were then analyzed using an SEM that operated at 15 kV. Twenty frames of pictures of each sample were taken to represent the structure of the powders.

RESULTS AND DISCUSSION

Influence of Relative Humidity on the Release of D-Limonene from the Powder. The release time-courses of D-limonene from the spray-dried powder were measured at 50 °C and at 23, 51, 75, and 96% relative humidity (RH). The effects of RHs on the release of D-limonene during storage are shown in **Figure 2** for four types of capsule matrixes: blend GA-MD, blend SSPS-MD, blend HI-CAP 100-MD, and HI-CAP 100. In **Figure 2**, the relative humidity greatly affected the release rate of D-limonene. The dependence, however, was not simple. Considering only 23 and 96% RH, the release of D-limonene increased with increasing RH. However, when comparing the 51 and 75% RH, the release of D-limonene at 51% RH was higher than that observed at 75% RH. These results suggested that the release of D-limonene was closely related at least to the water activity of the powder, which is in agreement with many earlier observations. The loss of D-limonene during storage may be caused by two mechanisms: diffusion of D-limonene through the matrixes of the wall material and the oxidation of D-limonene. However, as will be mentioned in the following section, the loss by oxidation was at most 5–6% of the initial D-limonene content. Therefore, the release of D-limonene may result mainly from diffusion as suggested by Whorton (1). As reported in our previous work (4), the release of ethyl butyrate also increased with increasing RH. However, when comparing the two model flavors, the release rate was observed to be slower for D-limonene in this study.

Analysis of the Release Rate by Avrami's Equation. To evaluate the release rate constant of D-limonene in the powder, Avrami's equation (eq 1) was applied to the release time-courses of the encapsulated D-limonene as reported in the previous work (4).

$$R = \exp[-(kt)^n] \quad (1)$$

where R is the retention of D-limonene, t is the storage time, k is the release rate constant, and n is a parameter representing the release mechanism. The release time-courses could be correlated well with Avrami's equation, as shown by the solid line in **Figure 2**. Eq 1 is also called the Weibull distribution function, which is successfully applied to describe the shelf-life failure (19). The release rate constants, k , and n value were calculated from eq 1 by a linear plot of $\ln(-\ln R)$ against $\ln t$. The relation between the release rate constant and water activity (a_w), which was calculated from the relative humidity ($a_w = \text{RH}/100$), is shown in **Figure 3**. The release rate constants first increased with the increase in a_w , followed by a decrease at an

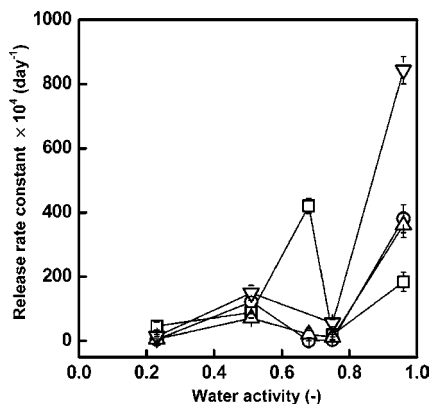


Figure 3. Effect of water activity on the release rate constant of encapsulated D-limonene at 50 °C. ○, Blend of GA-MD; □, blend of SSPS-MD; ▽, blend of HI-CAP 100-MD; △, HI-CAP 100. The error bars indicate 95% confidence levels.

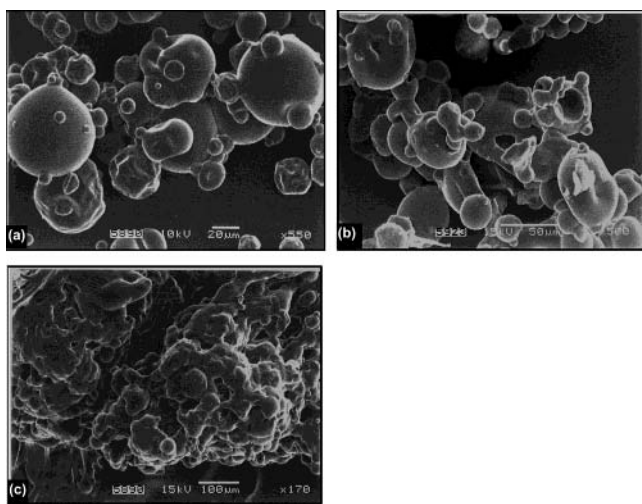


Figure 4. Outer structural changes in the spray-dried powder of the blend GA-MD as wall capsules stored at 50 °C. (a) Storage at 23% RH for 1 week, (b) storage at 51% RH for 1 week, (c) storage at 75% RH for 1 day.

a_w of around 0.70. At a higher a_w range, the release rate tended to increase again because the powder matrixes were destroyed. To explain the results from the structural changes in the powder at different a_w , SEM was used to observe the outer structure of the powder during storage as shown in **Figure 4**. At a low a_w (≈ 0.23) the spray-dried particles remained in the original shape, and the carrier solid seemed to be still in the glassy state, as shown in **Figure 4a**. This implies that the slower release in the low water activity region is most likely due to the lower mobility of D-limonene molecules in the glassy state of the capsule matrixes (1). As compared with the release rate at a_w of 0.23, the higher rate was observed at around a_w of 0.5 as shown in **Figure 3**, though the structural changes cannot be observed clearly (**Figure 4b**). This can again possibly be the result of the higher mobility of D-limonene (i.e., the capsule matrixes start to be plasticized as will be discussed later). When a_w increased up to a value of around 0.7, the powders began to be rehydrated (**Figure 4c**). At this stage, it may be assumed that the effective surface area decrease resulted in a decrease of D-limonene evaporation from the surface of the powder particles. Most particles were detected to be clumped and adhered together into a paste like mass, which explains the rubbery form of the capsule matrixes. At a very high a_w , the release rate increased (**Figure 3**). The most likely explanation is that the emulsion

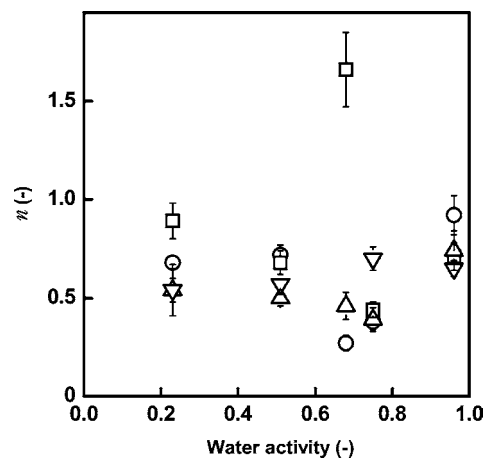


Figure 5. Effect of water activity on the release mechanism factor of encapsulated D-limonene at 50 °C. ○, Blend of GA-MD; □, blend of SSPS-MD; ▽, blend of HI-CAP 100-MD; △, HI-CAP 100. The error bars indicate 95% confidence levels.

Table 1. Glass Transition Temperature (T_g)^a of Capsule Matrixes after Equilibration at 20 °C as a Function of Water Activity (a_w)

wall materials	glass transition temperature, T_g (deg C)		
	$a_w = 0.33$	$a_w = 0.54$	$a_w = 0.75$
GA-MD	79.3 ± 0.5	51.2 ± 0.6	14.3 ± 0.3
SSPS-MD	73.4 ± 0.2	47.7 ± 0.3	9.2 ± 0.3
HI-CAP-MD	64.8 ± 0.2	38.0 ± 0.4	-2.9 ± 0.5
HI-CAP	42.6 ± 0.4	12.7 ± 0.4	-30.2 ± 0.4

^a Measurements were done in duplicate.

droplet of D-limonene in the powder crushed due to the destruction of the capsule matrixes. A similar speculation was also proposed by Whorton and Reineccius (3).

Avrami's parameter n relates to the release mechanism. As indicated in the previous work (4), $n = 1$ represents the first-order release mechanism, and $n < 1$ (theoretically $n = 0.54$) means that the molecular diffusion of D-limonene is rate limiting. For $n > 1$, the release shows a rapid release with an induction period. The influence of a_w on n is shown in **Figure 5**. From the results, the n values are almost in the range of 0.30–0.80 over a wide range of a_w , except for the blend SSPS-MD at an a_w of 0.68. This implies that the release of encapsulated D-limonene was generally controlled by the diffusion mechanism.

Glass Transition Temperature and Water Sorption Isotherm of Capsule Matrixes. The results of the effect of water activity (or relative humidity) on the release characteristics of D-limonene indicate that the release of encapsulated D-limonene is closely related to the structural changes of capsule matrixes. Glass transition temperature, T_g , was used as the phase transition index and defined to be the temperature at which the materials soften due to the onset of long-range coordinated molecular motion (14). Therefore, the effects of a_w on the T_g 's of the capsule matrixes were measured at 20 °C and various relative humidities as shown in **Table 1**. The T_g 's at the desired moisture contents were estimated using the following Gordon-Taylor equation.

$$T_g = \frac{x_1 T_{g1} + z x_2 T_{g2}}{x_1 + z x_2} \quad (2)$$

where z is a constant, x_1 and x_2 are the mass fraction of

Table 2. GAB Equation Parameters^a of Capsule Matrixes after Equilibration at 20 °C

wall materials	GAB model		
	m_m	C	K
GA-MD	8.01 ± 0.43	0.87 ± 0.01	15.98 ± 7.20
SSPS-MD	6.74 ± 0.23	0.95 ± 0.01	14.81 ± 5.09
HI-CAP-MD	7.49 ± 0.46	0.90 ± 0.01	5.91 ± 1.48
HI-CAP	10.01 ± 0.92	0.88 ± 0.02	3.47 ± 0.85

^a The range indicates 95% confidence levels of the fitting values.

Table 3. GAB Equation Parameters^a of Capsule Matrixes after Equilibration at 50 °C

wall materials	GAB model		
	m_m	C	K
GA-MD	5.26 ± 1.52	0.99 ± 0.05	2.63 ± 2.02
SSPS-MD	5.37 ± 0.76	0.91 ± 0.03	2.61 ± 0.93
HI-CAP-MD	6.52 ± 0.13	0.88 ± 0.05	1.77 ± 0.90
HI-CAP	8.65 ± 3.05	0.85 ± 0.06	1.41 ± 0.78

^a The range indicates 95% confidence levels of the fitting values.

Table 4. Gordon–Taylor Equation Parameters^a of Capsule Matrixes

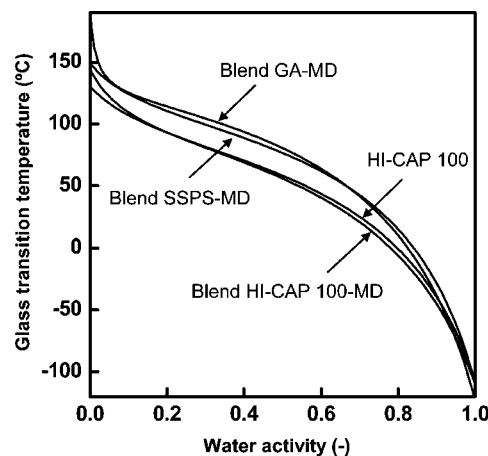
wall materials	Gordon–Taylor model	
	z	T_{g1}
GA-MD	5.21 ± 0.02	461.13 ± 0.43
SSPS-MD	4.26 ± 0.03	422.34 ± 0.60
HI-CAP-MD	5.13 ± 0.53	417.43 ± 10.51
HI-CAP	3.86 ± 0.47	403.26 ± 10.73

^a The range indicates 95% confidence levels of the fitting values.

components 1 and 2, and T_{g1} and T_{g2} are the glass transition temperatures of components 1 and 2. In this study, components 1 and 2 are the capsule matrixes and water, respectively. The glass transition temperature of pure water (T_{g2}) was taken from literature to be -135 °C (14). Two parameters in eq 2, z and T_{g1} , could be obtained by correlating the experimental data of T_g with eq 2 at different relative humidities (i.e., x_2). To determine the relation between the mass fraction of water x_2 and the relative humidity (water activity, a_w), the Guggenheim–Andersson–DeBoer (GAB) equation (eq 3) was used.

$$\frac{m}{m_m} = \frac{a_w K C}{(1 - a_w C)[1 + a_w C(K - 1)]} \quad (3)$$

where m is the water content (g/100 g solids), m_m is the monolayer value, K and C are constants, and a_w is the water activity. Three parameters in eq 3, m_m , K , and C , were calculated from the experimental water sorption isotherm data and are listed in **Tables 2 and 3** at 20 and 50 °C, respectively. Using the parameters in **Table 2**, the water mass fractions x_2 at different a_w values in **Table 1** could be estimated, and the correlation parameters in eq 2, z and T_{g1} , could then be determined as listed in **Table 4**. Because the values z and T_{g1} in **Table 4** can be assumed to be independent of temperature, T_g 's at 50 °C and at different RH values can be estimated by the same procedure. **Figure 6** shows the T_g 's against a_w at 50 °C for four capsule matrixes. The blend of GA-MD was found to show a higher T_g than the blends of SSPS-MD and HI-CAP 100-MD. The blending of MD with HI-CAP 100 showed an increase in the glass transition temperature and a decrease in the sorption of water compared to HI-CAP 100.

**Figure 6.** Prediction of glass transition temperature of the capsule matrixes equilibrated at 50 °C under variable water activity with the Gordon–Taylor equation and the GAB sorption equation.

Correlation of the Release Rate Constants with the Glass Transition Temperature. The release characteristics of the encapsulated D-limonene were observed to be closely related to the structural changes of capsule matrixes, therefore, the release rate constants k were presented as a function of the storage temperature (T) minus the glass transition temperature (T_g) of the capsule matrixes, $T - T_g$, as shown in **Figure 7**. Labrousse et al. (15) successfully applied an analogous correlation to the oxidation of encapsulated lipids in the function $T - T_g$ when sugar crystallization and a structural change occurred. In **Figure 7**, the release rate constants increased with increase in $T - T_g$ up to the point, at which $T - T_g$ was roughly equal to zero, followed by a decrease and then an increase again with increasing $T - T_g$. For the blend of GA-MD as capsule matrixes, although the release rate constant showed the maximum at $T - T_g$ of less than zero, the behavior of the release rate against $T - T_g$ is much the same as that of other wall materials. The observed high release rate near the glass transition temperature could be explained by saying that the mobility of D-limonene molecules increased. Furthermore, low release rate constants were observed in the range of $0 < T - T_g < 50$, when the powders were changed into a rubbery state. In this region, higher mobility of D-limonene should occur, but at the same time, the collapse of the powders occurred to clump and adhere the particles together, resulting in closing of the pore spaces between the particles and decreasing the surface area for D-limonene evaporation. Whorton and Reineccius (3) also showed similar results and defined the state as the re-encapsulation of flavor.

The Oxidation of Encapsulated D-Limonene during Storage. In addition to the release of flavors through the wall of the dried capsules, oxidation of the encapsulated flavors was also used as an index of its stability. Many oxidation products are known to be formed in the oxidation reactions of D-limonene (8). In this study, limonene oxide (limonene-1, 2-epoxide) and carvone were chosen as indicators of the oxidation. The formations of carvone and limonene oxide are the parallel reactions as reported by Anandaraman and Reineccius (5). The formation time-courses of limonene oxide and carvone in the powder were studied at 50 °C and 23–96% RH for the matrixes of GA-MD and HI-CAP 100 in **Figures 8 and 9**, respectively. The formation of the oxides markedly depended on RH and increased initially during storage. However, the formation of oxides at higher RH tended to decrease over a longer storage time. This might be explained by the accelerated degradation

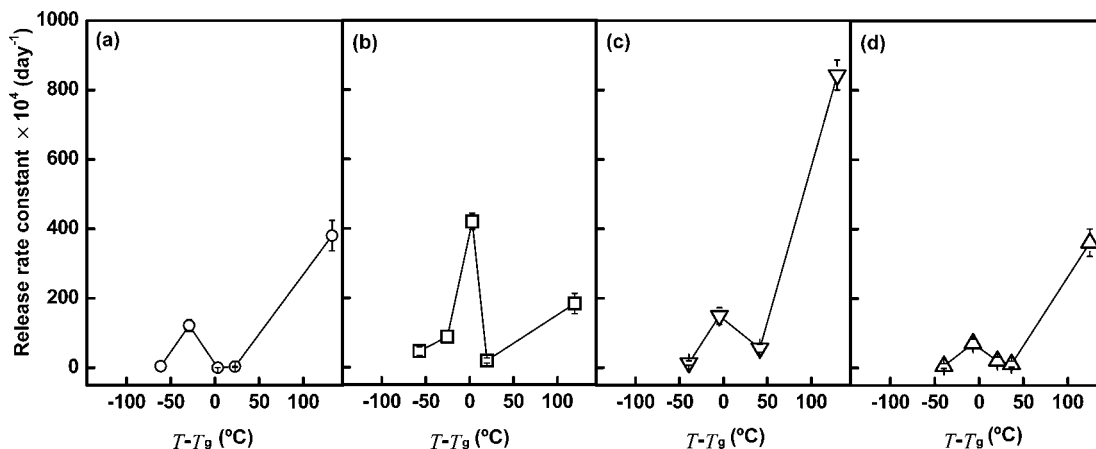


Figure 7. Influence of phase transition parameter $T - T_g$ of capsule matrixes on the release rate constant of encapsulated D-limonene at 50 °C. (a) Blend GA-MD, (b) blend SSPS-MD, (c) blend HI-CAP 100-MD, (d) HI-CAP 100. The error bars indicate 95% confidence levels.

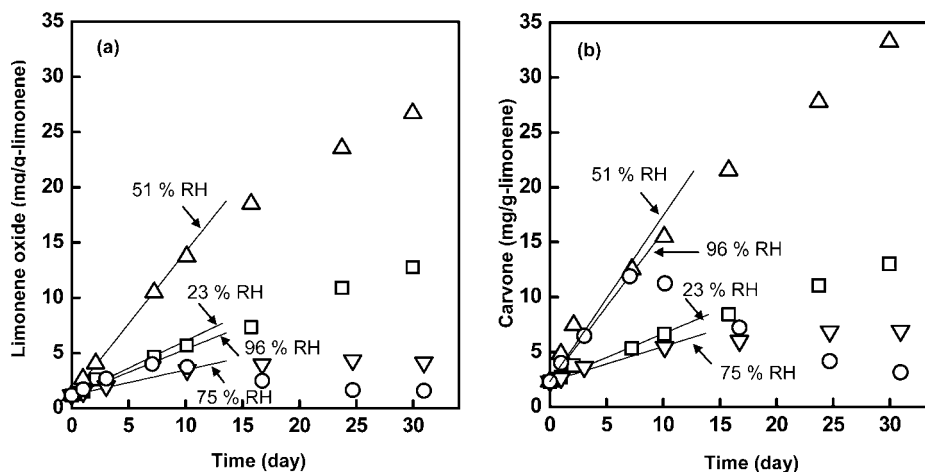


Figure 8. Formation time-courses of limonene oxide and carvone in spray-dried powder stored under various humid conditions and 50 °C when the blending of GA-MD was used as the wall material. (a) Limonene oxide, (b) carvone. \square , 23% RH; Δ , 51% RH; ∇ , 75% RH; \circ , 96% RH.

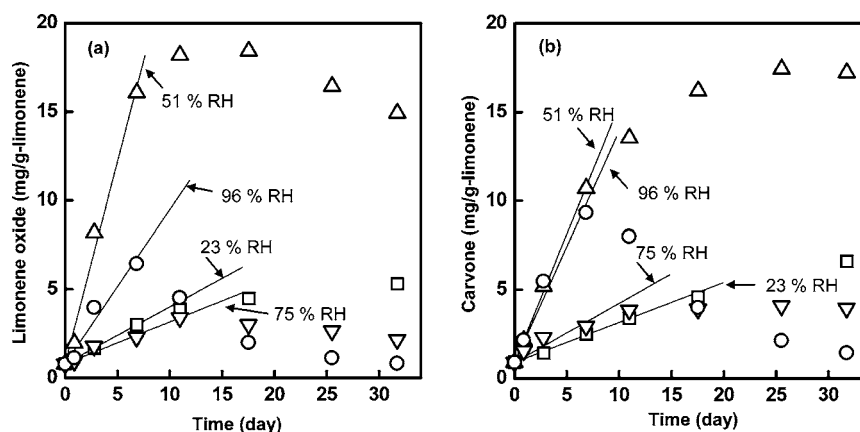


Figure 9. Formation time-courses of limonene oxide and carvone in spray-dried powder stored under various humid conditions and 50 °C when HI-CAP 100 was used as the wall material. (a) Limonene oxide, (b) carvone. \square , 23% RH, Δ , 51% RH; ∇ , 75% RH; \circ , 96% RH.

to form other oxide compounds and release of the oxides into the surroundings. Further, the decrease of oxygen concentration at the higher water content, which was caused by the low solubility of oxygen in the water, could also be considered to play a role to the phenomena. During the initial period, however, they increased linearly with time, so that the apparent oxidation rate constant was calculated on the basis of the zero order kinetic reaction scheme (5) as shown in the linear regression line in Figures 8 and 9.

Figure 10, parts a and b shows the effect of a_w on the rates of oxide formation for the matrixes of GA-MD and HI-CAP 100, respectively, during 50 °C storage. The changes in the oxidation rate constant with a_w are quite similar to those of the release rate constants shown in Figure 3. The oxidation rate constants increased with increasing a_w and reached a maximum at the a_w where the powder matrixes structure began to change from a glass to a rubbery state. However, when a_w increased around 0.75, the oxidation rate constant decreased and reached

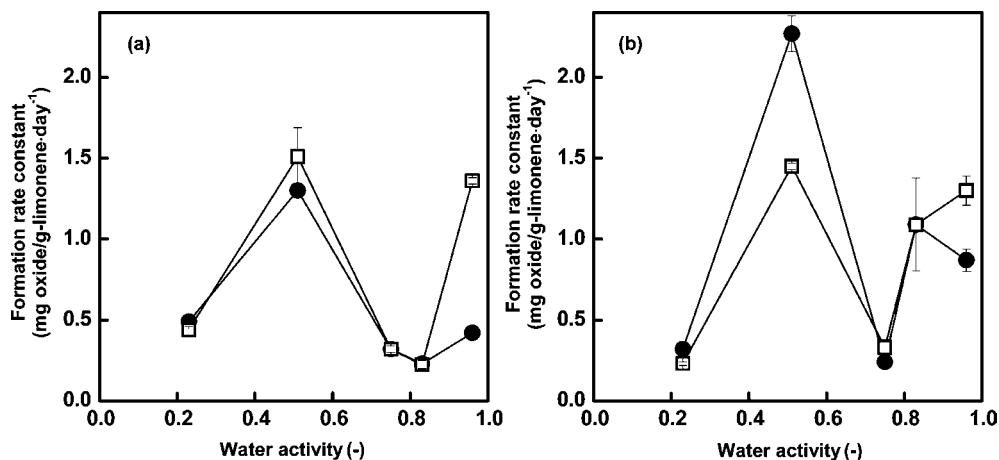


Figure 10. Effect of water activity on the formation rate constant of limonene oxide and carvone in the spray-dried powders stored at 50 °C. (a) Blend GA-MD was used as the wall material, (b) HI-CAP was used as the wall material. ●, Limonene oxide; □, carvone. The error bars indicate 95% confidence levels.

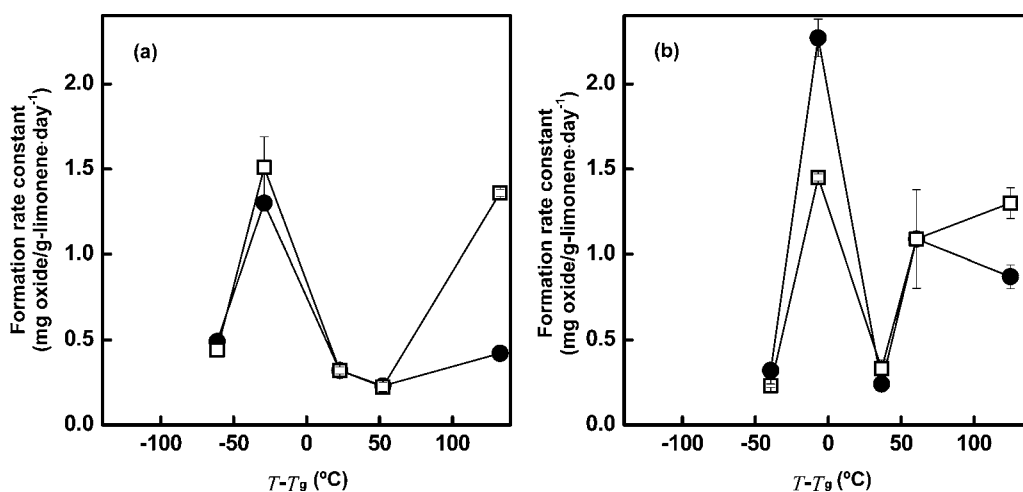


Figure 11. Influence of phase transition parameter $T - T_g$ of capsule matrixes on the formation rate constant of limonene oxide and carvone in spray-dried powders stored at 50 °C. (a) Blend GA-MD was used as the wall material, (b) HI-CAP was used as the wall material. ●, Limonene oxide; □, carvone. The error bars indicate 95% confidence levels.

a minimum value. This might be explained by the rehydration of the powder and the beginning of particle adhesion, which resulted in restriction of the oxygen supply from the environment. In the higher a_w range above 0.8, the powder matrix was totally destroyed, and the oxidation reaction would be promoted again.

Figure 11 shows the oxidation rate constants against $T - T_g$. The oxidation rates had maximum values at $T - T_g = -30$ and 0 °C for the matrixes of GA-MD and HI-CAP 100, respectively. These results were similar to the release rate constants (**Figure 7**) as explained above, which were directly related to the structure change in the dried capsule powder. As shown in **Figure 11**, around the glass transition temperature, the oxidation rate constant showed the maximum value. However, at a high value of $T - T_g$, the oxidation rate constant once decreased, and followed by an increase again. This trend is analogous to the dependence of the release rate constant on $T - T_g$.

In conclusion, the release rate and the oxidation rate of encapsulated D-limonene by spray drying increased with the increasing in water activity. However, at around the glass transition temperature, the rates decreased sharply to increase again at a further increase in water activity. The same results were reported in the effect of water activity on the release of en-

capsulated ethyl-*n*-butyrate (4). Therefore, the shelf life of the encapsulated flavors, which can be related to their release characteristics and oxidation, directly depends on the glass transition temperature of wall materials. Further, the release rate also depended on the composition of the wall materials and the kind of the flavor. This implied that the release rate was controlled by the interactions between the flavors and the surrounding matrixes.

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