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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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Name

学位論文題目：
Title of Dissertation

Microencapsulation of Flavors in *Saccharomyces cerevisiae* by Spray Drying and Characterization of Their Release Behavior
(噴霧乾燥法による酵母へのフレーバー包括粉末化と酵母粉末からのフレーバー徐放特性)

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Dissertation Summary

The final acceptability and consumers' satisfaction to choose any food are affected by the flavor compounds of that product. It becomes a major challenge for food processor to add flavor compounds with minimal losses or degradation. However, high volatility and poor stability against oxygen or heat contact of those compounds make difficulties to reduce losses or degradation during application in food products. Microencapsulation is the powerful solution to reduce degradation of aroma compounds in a healthier way (Pham-hoang et al., 2016). The main purposes of flavor encapsulation are to stabilize flavor compound, convert liquid flavor in solid state, improve technological properties, handling properties, safety and control the release of core compounds (Uhlemann et al., 2002). Besides carbohydrate, protein or liposome, active or dead yeast cell itself (whole) or its other parts (cell wall, β-glucan) are also gained tremendous interests to the researchers as encapsulant (Young and Nitin, 2019; Liu et al., 2018; Mokhtari et al., 2017) due to economic cost, health benefits and simple technique to complete encapsulation. It widens the scope of aroma encapsulation by providing the benefits of longer stability against temperature, oxygen and other environmental degradations (Normand et al., 2005; Shi et al., 2008). Spray drying is one of the oldest, mostly used and effective technique for flavor encapsulation, which produces high quality powder in very short time. In this research, two flavor compounds with different log P such as *d*-limonene (log P=4.8) and ethyl hexanoate (log P=2.8) were encapsulated by spray drying in *Saccharomyces cerevisiae*, from which β-glucans had been partially extracted. The release kinetics of encapsulated flavors were also investigated in this study.

The formation of encapsulated slurry was prepared by incubating the mixture of flavor compounds, yeast cells and water. The optimize incubation time, temperature, mass ratio of flavors to yeast cells and spray drying conditions were investigated to obtain maximum encapsulation efficiency. Another two flavor compounds namely citral and ethyl propionate were also encapsulated as guest compounds. Finally, the encapsulated slurry was dried using a mini spray dryer B-290 (Büchi, Nihon Büchi K.K.,

Japan) to make flavor encapsulated powder. The flavor contents in the incubation time could be correlated with the first-order encapsulation equation as follows (Fogler, 1999):

$$C_t = C_0 + \{(C_m - C_0)(1 - \exp(-kt))\} \quad (1)$$

Where, C_t: flavor content at the incubation time, t (h); C_o: initial flavor content without incubation; C_m: equilibrium flavor content; and k: encapsulation rate constant (h^{-1}).

Figure 1 shows the effect of incubation time on flavor content after spray drying. The content of *d*-limonene increased with incubation time and reached the equilibrium value at about 4 h. Conversely, content of ethyl hexanoate reached the equilibrium value at about 2 h.

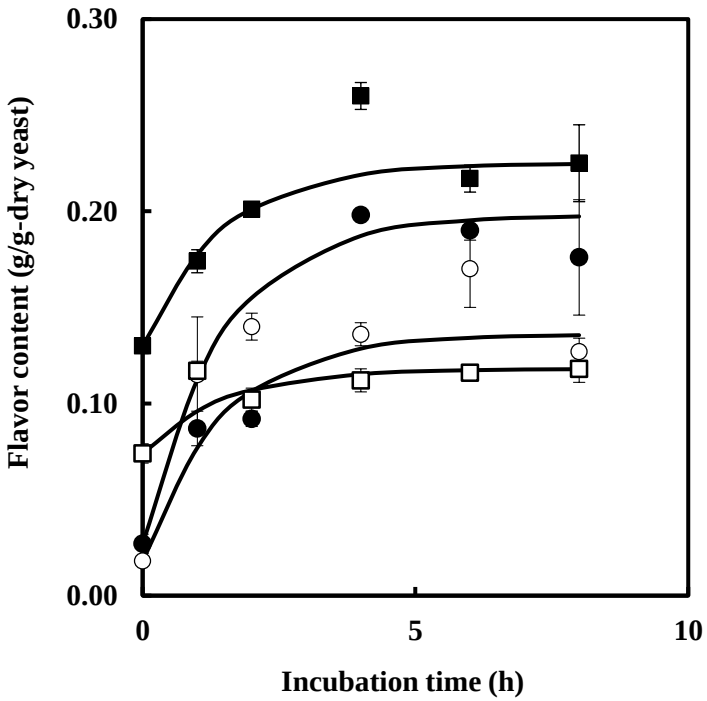

Figure 1. Effect of incubation time on flavor content in yeast at 40°C incubation temperature and 200°C inlet air temperature (● yeast: *d*-limonene = 2:1, ○ yeast: *d*-limonene = 4:1, ■ yeast:ethyl hexanoate = 2:1, □ yeast:ethyl hexanoate = 4:1)

The rate of encapsulation for both flavors was found 0.69 h^{-1}. From the research of Moyo et al. (2012), the rate constant (around 2.3 h^{-1}) for adsorption capacity of phenol can be calculated using the same equation. The rate constant might vary based on the properties of compounds. Highest encapsulation efficiency for both flavors were found at 40°C incubation temperature for 4 h and an inlet temperature 200°C of spray drying. The maximum flavor contents for *d*-limonene and ethyl hexanoate

were found around 37 wt% and 49 wt%. The total extracted flavor content obtained was higher for yeast to flavor ratio 2:1 compared to 4:1. Inlet air temperature did not influence the encapsulation efficiency of ethyl hexanoate, while *d*-limonene content increased gradually with an increase in the inlet air temperature. The encapsulation efficiency varied for citral and ethyl propionate which might be influenced due to different log P, molecular structure or other physico-chemical properties.

Controlled release and stability are important characteristics of encapsulated powder during storage. Controlled release has the beneficial effect of active compounds being released at controlled rates over prolonged times and can also reduce ingredient losses during cooking and processing (Dziezak, 1988). To prepare encapsulated powder, a pilot scale spray dryer (Ohkawara-L8; Ohkawara Kakohki Co., Ltd., Yokohama, Japan) equipped with a centrifugal atomizer was used instead of a mini spray dryer (B-290; Büchi, Japan) in this study. *d*-Limonene encapsulated emulsified maltodextrin (MD) (DE=19) powders with small and big oil-droplet were prepared to compare the stability of *d*-limonene in those powders with yeast powder. Flavor release rate constants were correlated using Gaussian distribution of the activation energy (ΔG) of the release rate constant. The equation is as follows (Yoshii et al., 2003; Ishido et al., 2003):

$$\phi = \frac{RT}{\sqrt{2\pi}\sigma}\int_{-\infty}^{\infty} \exp\left[-\frac{R^2T^2(lnk_1 - lnk_{10})^2}{2\sigma^2}\right]\exp(-k_1 t)\, d(lnk_1) \qquad (2)$$

Where, ϕ: flavor retention; k_1: release rate constant; T: absolute temperature, and R: gas constant, t: time; k_{10}: average release rate constant; and σ: standard deviation of activation energy distribution.

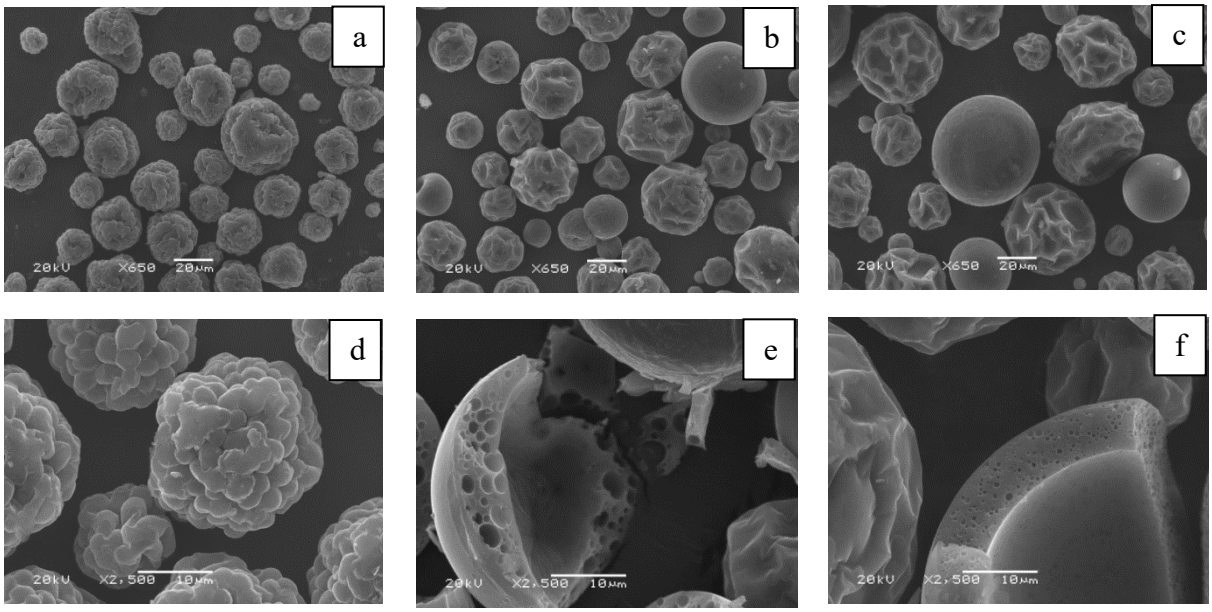

Figure 2. SEM images of spray-dried powders (a, d: yeast powder; b, e: MD1 powder: having larger oil-droplet; c, f: MD2 powder: having smaller oil-droplet)

The morphological images of flavor encapsulated spray-dried MD powders and yeast powder are shown in Figure 2. The surface of the yeast powder had an aggregated shape, whereas the MD powders were spherical. Some of the MD particles had a smooth surface and some had a dented surface. However, the broken part shows the bigger oil droplets for MD1 (using a mechanical homogenizer) and smaller droplets for MD2 (using a high-pressure homogenizer). The morphology of MD powder may depend on several factors such as DE value, spray drying condition, homogenization pressure etc.

The effects of dry heating (105, 120, and 140°C) and wet heating (40, 60, 80, and 105°C) to release encapsulated flavors were also investigated. Different amounts of water (0, 50, 100, and 200% of powder) were added to investigate release behavior.

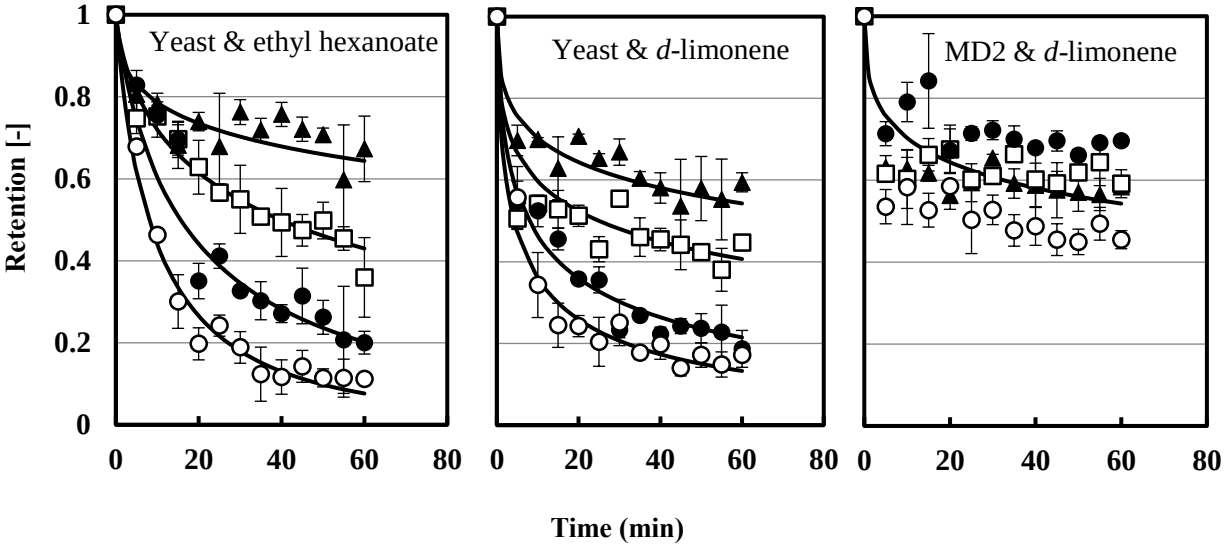

Figure 3. Effect of storage temperature on flavor release from yeast powder and MD powder (▲ 40ºC; □ 60ºC; ● 80ºC; ○ 105ºC with 200% water added to powder)

Figure 3 shows the effect of storage temperature on the release behavior of encapsulated flavor from the yeast powder and MD powder. In this experiment, small oil droplets containing MD powder (MD2) were used with an average oil-droplet diameter of 0.65 μm. The release kinetics of encapsulated *d*-limonene and ethyl hexanoate were investigated at 40, 60, 80, and 105ºC (using 200% water added to the powder). For the yeast powder, the release rate was faster at a higher temperature compared with a lower temperature. The release profile for yeast depended completely on the temperature. Both types of flavor were released in two steps. The first step was a very rapid release, whereas the second step was a very slow release of flavor. The release behavior of *d*-limonene from the MD2 powder was different from the yeast powder at different temperatures. This finding might be the reason for particle aggregation by adding water directly to the powder. The release rate constant of the MD2 powder for all temperatures was 0.006 min^{-1} and the standard deviation of activation energy distribution was 4.0.

Flavor release from yeast cell was almost stable in a dry heating condition (without addition of water), even at very high temperature (140ºC). The addition of water affected the release of flavor. However, the release rate was not noticeably different with the addition of different amount of water. Oliveira et al. (2006) pointed out that the oxidation of liquid limonene can proceed by two pathways: epoxidation (limonene oxide is the main product) and allylic oxidation or autoxidation. Oxidation of *d*-limonene results in the formation of limonene oxide, carveol, and carvone. Limonene oxide and carvone were chosen as the indicator of *d*-limonene oxidation in this study. A slower formation rate of limonene oxide and carvone was found for yeast powder after the incubation of 2 months at 30ºC. However, a faster formation rate of those two compounds were found for both types of maltodextrin powder. The better stability of yeast powder might be due to the antioxidation properties of mannoproteins and β-glucans present in yeast cell wall (Wu et al., 2015; Križková et al., 2001; Jaehrig et al., 2007).

The flavor release and stability of encapsulated powder are influenced by the glass transition temperature (T_g) of the powder. At the T_g, the molecular movement in the powder increases and the amorphous material changes from a glassy to a rubbery state, with consequences of stickiness and collapse of the powder (Levine and Slade, 1986). Furthermore, the stickiness of the powder observed at the T_g results in poor quality and low yields during drying, handling and storage condition (Shrestha et al., 2007). In this study, a method for the measurement of the T_g of flavor-encapsulated spray-dried emulsified powders and yeast powders is proposed using a simple instrument named aroma sensor. The rapid increment in flavor release from the encapsulating powders was monitored using a ramping method (linear programmed temperature gradient).

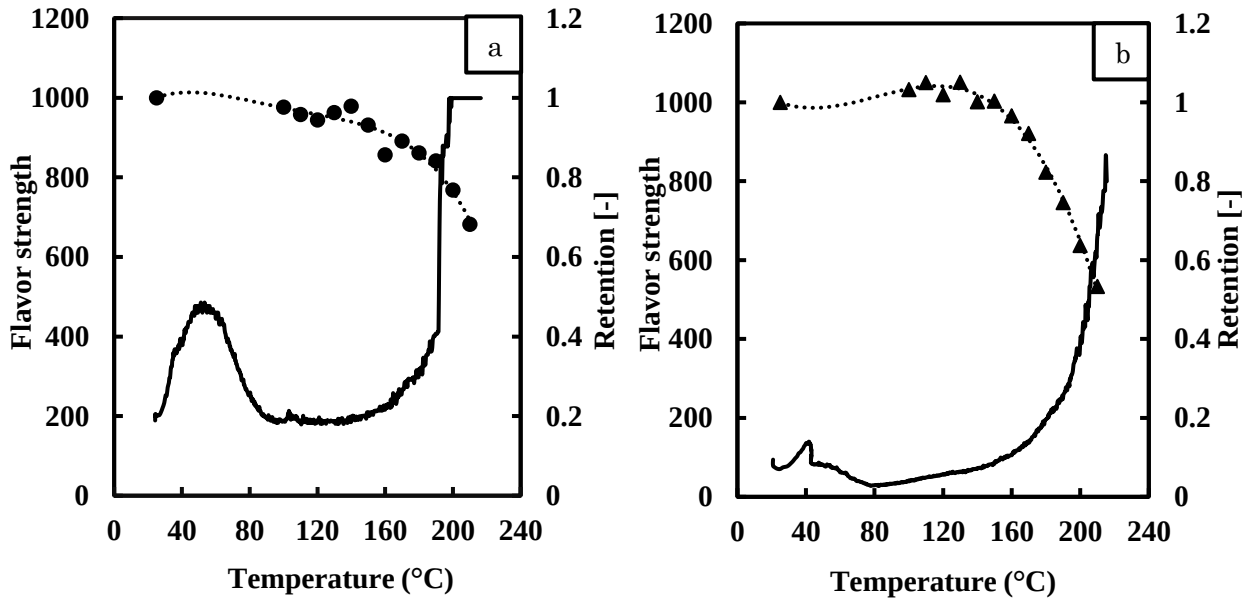

Figure 4. Release behaviors of (a) ethyl hexanoate and (b) *d*-limonene from yeast powders (solid lines: flavor strength; dotted lines: retention [-] of flavor)

Figure 4 illustrates the release behavior of ethyl hexanoate and *d*-limonene from yeast cells with the increasing temperature at 1°C/min. The initial high flavor strength possibly corresponds to the surface flavor of the yeast powder. After that, the flavor strength decreases and finally increases again at very high temperatures (180–190°C). The complex structure of yeast, i.e., the presence of different sugars, proteins, and other species, hampers the accurate monitoring of the flavor release. To confirm the apparent T_g of the flavor-encapsulated yeast powders, *d*-limonene and ethyl hexanoate were extracted from yeast cells under ramping temperature conditions (at 1°C/min) and analyzed by GC-FID. The retention of the encapsulated flavor was calculated following the method by Sultana et al. (2018). The obtained intercept point was very similar to the apparent T_g value found using the aroma sensor. The presence of trehalose, sucrose, and other sugars in yeast cells may be the reason behind the high apparent T_g of yeast cells.

Encapsulated flavors release profiles under a home-built dynamic vapor sorption instrument (DVS) in a stepwise changed humidity condition were analyzed in this study. The relative humidity (RH) was stepped to 50, 60, 70, and 80% from 20% at individual incubation temperature of 30, 40, 50, and 60°C. The RH was stepped after leaving the sample for 2 h at 20%. The flavor release flux of the encapsulated powder was calculated using Eq. 3 and 4 (Yamamoto et al., 2012) as follows:

$$F = N/(A \times m) \tag{3}$$

$$N = C_g \times V \tag{4}$$

Where, F: flavor release flux (mg/s·m^2·g-powder); N: flavor release rate (mg/s); C_g: flavor concentration (mg/mL N_2); V: N_2 flow rate (mL/s); A: area of the sample plate (1.3×10^{-4} m^2); and m: mass of the sample (g).

The maximum release flux was found at 60°C with 80% RH condition. The Weibull distribution function was well fitted to the experimental data to analyze release kinetics. An integrated release was found using above-mentioned equation (Eq. 5).

$$\ln\left[-\ln\left(\frac{M_\infty - M_t}{M_\infty}\right)\right] = n\ln k + n\ln t \tag{5}$$

Where, t: time from the stepped RH change; k: release rate constant; n: release mechanism parameter. M_t: integrated released flavor (mg/m^2-g powder) and M_∞: total amount of released flavor after stepwise change of RH (mg/m^2-g powder).

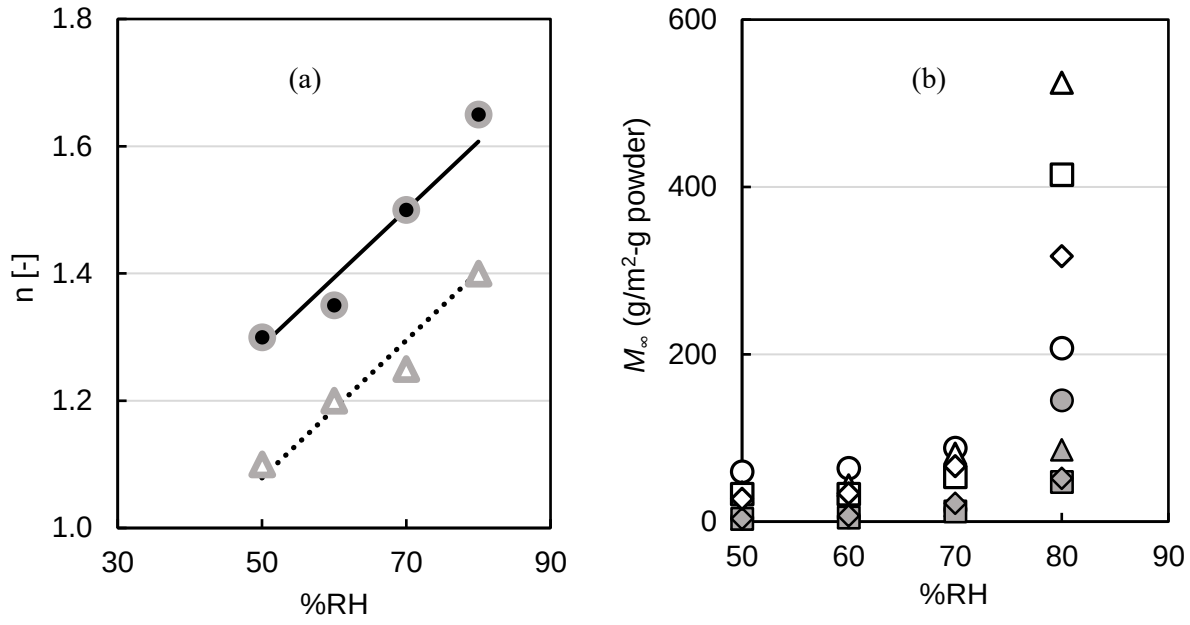

Figure 5. Graphical illustration of (a) release mechanism number (● *d*-limonene; △ ethyl hexanoate) and (b) maximum integrated release (●○ 30°C, ▲△ 40°C, ■□ 50°C, and ◆◇ 60°C; solid keys: *d*-limonene and open keys: ethyl hexanoate) as a function of RH

Figure 5 shows the mechanism number *n* and the total amount of released flavor after stepwise RH change M_∞ as a function of RH. As expected, M_∞ increased sharply with RH. The increasing amount of flavor release with RH is due to the moisture sorption properties of the yeast cell. Water molecules migrate to the deeper side via the protein layer and glucan layer of the yeast cell wall (Dardelle et al., 2007). The formation of gel or solubilization of β-glucan by absorbing water perhaps initiates the flavor release behavior of yeast cells. According to Bouquerand et al. (2004), flavor release from particles to water occurs when polymer is completely solvated or forms a hydrogel. The values of *n* (release mechanism parameter) for both the flavors were greater than 1.0. A release with induction period (initial constant stage or initial slow stage of release flux) occurs if the value of *n* is higher than 1.0 (Furuta et al., 2011).

It can be concluded that yeast (*Saccharomyces cerevisiae*) cell is a very good encapsulating vehicle for flavor compounds. The maximum load of flavor compounds is dependent on several factors such as physico-chemical properties of flavor compounds, incubation time, temperature or spray drying conditions. However, the prolong release of flavor and better stability of those compounds play the beneficial role of using yeast cells.

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