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Encapsulation of Flavors by Molecular Inclusion Using β-Cyclodextrin: Comparison with Spray-drying Process Using Carbohydrate-based Wall Materials

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Abstract Microencapsulation of flavor was carried out by molecular inclusion process using β-cyclodextrin (βCD). βCD-flavor complex was prepared at various flavor-to-βCD ratios (1:6–1:12) to determine the effect of βCD concentration on the inclusion efficiency. Maximum total oil retention and minimal surface oil content were obtained at flavors to βCD ratio of 1:10. The physical properties and controlled release pattern of flavors from βCD-flavor complex were measured and compared with spray-dried microcapsules prepared using carbohydrate wall system. βCD-flavor complex showed higher total oil retention and surface oil contents, smaller mean particle size, lower moisture uptake, and higher oxidation stability than spray-dried microcapsule. Oxidative stability of flavor was correlated with hygroscopicity of wall materials. The controlled release mechanism was highly affected by temperature and characteristics of wall materials.

Keywords: microencapsulation, molecular inclusion, β-cyclodextrin, flavor, controlled release

Introduction

Many types of food contain small concentrations of many organic compounds which give the food their characteristic flavor and aroma. However, many flavors are volatile liquids which are thermally labile and very susceptible to loss during food processing and storage (1). Therefore it is beneficial to encapsulate volatile flavors prior to use in foods. Numerous encapsulation processes and polymers, including carbohydrates and proteins, have been developed for the microencapsulation of flavors.

The ability of carbohydrates to bind flavors, low cost, and widespread use in foods make them the preferred choice for encapsulation. Although a variety of methods are used to manufacture encapsulated flavors, spray-drying, and extrusion are the most common techniques (1). In those techniques, the particles comprise droplets of core material dispersed in a continuous matrix of carrier material, or the core is continuous and surrounded by a shell of carrier (2).

Molecular inclusion is a process that occurs at the molecular level, that is, individual molecules of food or flavors are trapped or included within cavities present in individual molecules of carrier. The most well-known carriers of this type are the cyclodextrins (2). Cyclodextrins are a series of cyclic oligosaccharides that are produced enzymatically from starch by the action of the cyclodextrin transglycosylases (3,4). Three cyclodextrins are obtained: α, β, and γ having 6, 7, and 8 glucopyranose units, respectively. β-Cyclodextrin (βCD) has been known as the most common and suitable cyclodextrin for microencapsulation (5). Cyclodextrins have hydrophobic central cavity which can form inclusion complexes with a wide range of organic

and inorganic guest molecules (6). The inclusion complexation of these host-guest systems occurs through various interactions, such as hydrogen bonding, van der Waals, electrostatic, or hydrophobic interactions (7).

In the pharmaceutical, cosmetics, and food industries, cyclodextrins have been used as complexing agents for various compounds, such as drugs, vitamins, and food colorants, because this complex is quite stable to evaporation, oxidation, and light (6,8). βCD has been also known as a most common and suitable wall material for flavor application (3). The unique feature of the molecular inclusion using cyclodextrin compared with traditional encapsulation is that, this technology provides an effective protection for every single flavor constituents present in a multicomponent food system (9).

Several studies have been conducted for encapsulation of natural or synthetic flavors using βCD (2,4,10-16). However, most of the studies are limited to total flavor retention and stability during storage. Relatively few studies have been conducted on the controlled release pattern of flavors from microencapsulated powder and the comparison of physical properties with other types of microcapsules.

Encapsulation profiles of selected flavors in the βCD at various ratios of flavor mixtures to βCD were investigated. And physical properties and controlled release behaviors of βCD-flavor complex with spray-dried microcapsule prepared using carbohydrate-based wall materials were compared.

Materials and Methods

Materials Seven flavor compounds were selected based on the boiling point (90-200°C) and molecular weight (100 to 140 Da): ethyl propionate, butyl acetate, 2-heptanone, limonene, octanol-1, and citral (*cis*- and *trans*-form). All flavor compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). These flavor components were mixed with rapeseed oil (1 part flavor: 4 part oil).

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βCD was provided from Sewon Co. (Seoul, Korea). Maltodextrin (DE=15, Sewon Co.), gum arabic (TIC Gums, Belcamp, MD, USA), N-Lok (National Starch and Chemical Co., Bridgewater, NJ, USA) and gellan gum (Kelco, San Diego, CA, USA) were used as wall materials for the carbohydrate-based wall system of spray-dried microcapsule.

Preparation of βCD-flavor complex A hundred g of βCD were dissolved in 1,000 mL of a mixture of ethanol and water (1:2, v/v) at 55°C with vigorous stirring. A predetermined quantity of flavor complex was added dropwise to βCD solution with stirring and the temperature was maintained at 55°C. The resultant mixture was slowly cooled down to 22°C with stirring. The final solution was refrigerated at 4°C for 16 hr. The crystalline product was filtered with Whatman No. 4 paper, and freeze-dried at 10°C for 3 hr. The starting ratios of flavor complex to βCD were 1:6, 1:8, 1:10, and 1:12.

Preparation of spray-dried microcapsule The carbohydrate-based wall system consisted of a mixture of maltodextrin, gum arabic, N-Lok, and gellan gum at a ratio of 30: 26.4:39.6:4 (17). Each wall material was weighed and reconstituted in distilled water at a 30%(w/w) concentration using homomixer (Matsushita Electronic Industrial Co., Osaka, Japan). Coarse emulsion was prepared by blending core/wall (1:4) mixture for 10 min at 10,000 rpm with homomixer, and then homogenized at 20 MPa with a pressure-homogenizer (Rannie, APV, Abertslund, Denmark). This emulsion was spray-dried in disk type spray dryer (L-8; Ohkawara Kakohki, Yokohama, Japan) at an air inlet temperature of 180°C (outlet temperature=100°C) and nozzle speed of 15,000±100 rpm.

Total oil determination The total oil contents of encapsulated powders were determined using a Clevenger trap (18). Encapsulated powder (30 g) was dispersed in 200 mL of distilled water in 500-mL flat-bottomed flask. The Clevenger trap and a water-cooled condenser were fitted into the top of the boiling flask and distilled for 2 hr. The volume of oil collected in the trap was directly read from oil collecting arm of the Clevenger apparatus and multiplied by a density factor of 0.9 g/mL to calculate the weight of oil recovered from the sample.

Surface oil determination The surface oil content of microencapsulated powders was determined using a Soxhlet extraction apparatus according to the method of Reineccius *et al.* (18). Ten g of microencapsulated powders was weighed into an extraction thimble and extracted with 200 mL of pentane for 2 hr in a Soxhlet extractor. The extract was evaporated to a volume of approximately 25 mL. The concentration of oil (flavor mixture) in the pentane solution was determined by injecting 1 mL samples into gas chromatograph (GC 6890; Hewlett Packard, Wilmington, DE, USA) equipped with a flame ionization detector. Separation was achieved on a 25 m ×0.32 mm i.d., 0.17-μm thickness Ultra II column (Hewlett-Packard, Palo Alto, CA, USA). The oven

temperature was held at 35°C for 5 min, and then increased at 10°C/min to 180°C. The injection port and detector were maintained at 200 and 250°C, respectively. Hydrogen was used as a carrier gas at a column flow rate of 1 mL/min. Each flavor compound (0.2 mL) was mixed with 200 mL of pentane in a 500-mL flask and evaporated as above, for use as an external standard.

Moisture content The AOAC method (19) for flour was used for moisture determination. All samples were analyzed in triplicate.

Moisture uptake Each microcapsulated powder was placed in a petri dish and weighed. The moisture uptake of the powder was determined at 25°C and 80% relative humidity in an incubator (KCL-1000; Eyela, Tokyo, Japan). Measurements were carried out in triplicate.

Particle size Microencapsulated powers were dispersed in ethanol. The particle distribution and mean particle size were analyzed using a particle size analyzer (Analysette 22; Fritsch, Idar-Oberstein, Germany).

Oxidative stability Each microencapsulated powder was stored in an incubator at 37°C. Every 7 day, 0.3 g of powder was withdrawn and dissolved in 10 mL of high performance liquid chromatography (HPLC) grade water in a 50-mL centrifuge tube. The centrifuge tubes were sealed and shaken vigorously for 1 min using a vortex mixer. The tubes were then stored at 60°C for 1 hr. HPLC grade pentane (5 g) containing 1 mg of nonane as an internal standard was added to each tube, then the tubes were shaken as before. The tubes were transferred to a shaking incubator and maintained at 40°C for 1 hr, then centrifuged for 10 min at 3,000×g (Union 55R; Hanil Co., Seoul, Korea). The clear supernatant layer (1 μL) was analyzed by gas chromatography (GC) for determination of the surface oil. The formation of limonene 1,2-epoxide from L-limone during 37°C storage was measured as an indicator of oxidative stability in the single- and double-encapsulated powders.

Determination of flavor release from encapsulated powder For the determination of cumulative flavor release, each encapsulated powder was stored at 25°C and 65% relative humidity in an incubator. A manual solid phase microextraction (SPME) fiber holder and a 100-μm polydimethylsiloxane coated fiber were used (Supelco, Bellefonte, PA, USA). Every day, sample (1.5 g) was weighed into a 50-mL headspace vial and 28.5 mL of distilled water was added. The vial was sealed with a teflon-coated septa and an aluminum cap. The vial was immersed in a 30°C water bath. An SPME needle was inserted through the septum and the SPME fiber was allowed to equilibrate for 15 min with sonication. The SPME fiber was exposed in the GC inlet (250°C) for 15 sec during the desorption step. For the determination of controlled release by temperature, the samples were incubated at 25, 50, 75, and 100°C for 1 hr with sonication before analysis.

Table 1. Total oil retention and surface oil contents of βCD-flavor complexes as a function of the flavors to βCD ratios

Flavors:βCD	Mean concentration	
	Total oil retention (%)[1]	Surface oil content (mg/100 g)
1:6	58.2[b]	301.4
1:8	77.7[a]	240.3
1:10	79.1[a]	170.6
1:12	78.9[a]	209.8

[1]Values with different superscript letters within the same column are significantly different at $p<0.05$.

Table 2. Total oil retention and surface oil contents of each flavor compound encapsulated in βCD-flavor complexes (at flavors:βCD=1:10)

Flavor compound	Mean concentration	
	Total oil retention (%)	Surface oil content (mg/100 g)
Ethyl propionate	25.6	9.3
Butyl acetate	68.1	27.7
Heptanone	93.1	24.2
Limonene	97.7	25.3
Octanol	82.2	28.4
Citral-*cis* type	96.0	27.4
Citral-*trans* type	91.4	28.3

Results and Discussion

Optimum molar ratio of flavors to βCD for the complexation The molar ratio of the guest to cyclodextrin is the key factor for the preparation of inclusion complex (16). Microencapsulation of flavors was undertaken with βCD using complex inclusion method at the 4 flavors to βCD ratios (1:6, 1:8, 1:10, and 1:12) to determine the effect of flavor/βCD ratio on the inclusion efficiency. As shown in Table 1, the total oil retention reached a maximum at 1:10 treatment. This result was not significantly different ($p>0.05$) from the total oil retention of other treatments except 1:6 treatment. The amount of surface oil ranged from 171 to 301 mg/100 g of dried powder. The 1:10 treatment showed lowest surface oil content, while the highest value was found for the 1:6 treatment (301 mg/100 g). With these results, it could be concluded that the 1:10 treatment had a maximum inclusion capacity with flavor mixtures. These results were slightly different from results on lemon oil (14) and garlic oil (5). They reported that a maximum inclusion capacity of βCD with lemon oil or garlic oil occurs for a starting ratio of oil to βCD of 12:88 (approximately 1:8).

Various flavor compounds have different degrees of polarity, molecular size, geometry, and chemical compositions. Therefore, flavor compounds may possess a particular configuration of complex formation with βCD (9,14). Table 2 lists the flavor retention and surface oil content of each flavor complexed within βCD with 1:10 ratio. As looking at the pattern of total oil retention, the terpenes such as limonene and citral achieved the best retention with βCD. On the other hand, esters having lower boiling point

Table 3. Comparison in composition and properties of βCD-flavor complex and spray-dried microcapsule

	Spray-dried microcapsule	βCD-flavor complex
Total oil retention (%)	61.3	79.1
Surface oil content (mg/100 g)	4.3	170.6
Moisture content (%)	1.2	4.5
Particle size mean (μm)	30.7	22.3
Range (μm)	15.2-43.5	3.5-36.2

showed low retention within βCD; ethyl propionate and butyl acetate showed low retention. Some researches have also reported that short-chain esters are less retained than larger molecules (2,14). With these results, it could be recognized that the hydrophobic and long chain compounds can be fitted better within βCD than hydrophilic and short chain compounds (2,20).

Physicochemical characteristics The measured composition and physical properties of encapsulated powders are shown in Table 3. The total oil retention of βCD-flavor complex (79.2%) was higher than carbohydrate-based microcapsule (61.3%). During spray drying, highly volatile flavor components (those with low boiling points) are less effectively encapsulated and more quickly lost than less volatile components (1). However, the surface oil content showed a reverse result. Westing *et al.* (13) also reported that the surface oil content for orange oil-cyclodextrin complexes was much greater than the level of spray-dried product. The high level of surface oil content in βCD-flavor complex could be explained by the research of Padukka *et al.* (8) in which they carried out studies to compare the method for surface oil determination in βCD-lemon oil complex powder. They indicated that the Soxhlet method extracted more flavor than the washing method. For the Soxhlet method, the powder is washed repeatedly with fresh solvent for a long period of time, resulting in extraction of loosely encapsulated flavor molecules in addition to surface oil.

As shown in Fig. 1, the moisture uptake of spray-dried microcapsule sharply increased up to 60 min of incubation time, then remained constant. βCD is not hygroscopic itself, so moisture uptake of βCD-flavor complex was less than 0.3%. A remarkable advantage of cyclodextrin complexed flavor as comparing with other types of carbohydrate-based wall material is their negligible hygroscopicity under high humidity conditions (12).

Oxidative stability Limonene was selected from among several flavor compounds to use as an indicator of oxidative stability. When limonene is stored at elevated temperature, the 2 oxidation products of limonene, limonene-1,2-epoxide and carvone appear during storage. Since limonene-1,2-epoxide and carvone are the earliest compounds observed during oxidation, they were used as indicators of oil oxidation (10). As shown in Fig. 2, limonene 1,2-epoxide appeared to have an induction period where little or no epoxide formation occurs. Spray-dried microcapsule had induction period of 7 weeks. Beyond this period, the rate of limonene oxidation sharply increased.
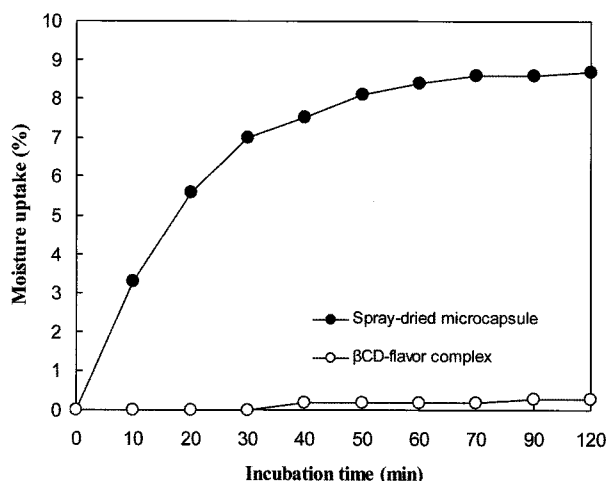
Fig. 1. Changes in moisture uptake of spray-dried microcapsule and βCD-flavor complex during incubation at 25°C and 80% relative humidity.
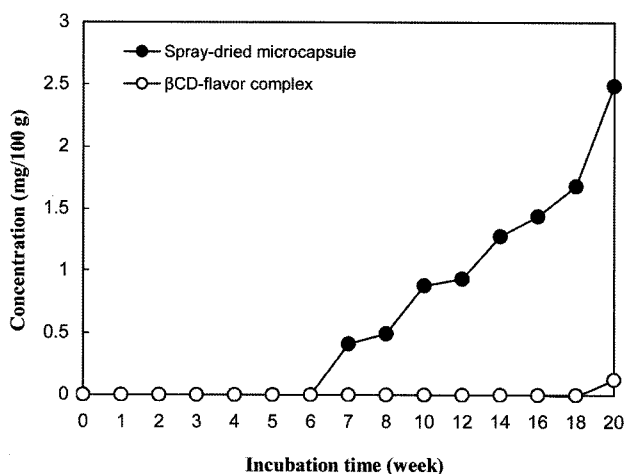

Fig. 2. Changes in concentration of limonene 1,2-epoxide formed from spray-dried microcapsule and βCD-flavor complex during storage at 37°C.

Table 4. Correlation analysis: Pearson correlation coefficients

	Particle size	Storage stability	Moisture uptake
Particle size	1.0000	0.5515	0.5743
Storage stability	0.5515	1.0000	0.9996
Moisture uptake	0.5743	0.9996	1.0000

On the other hands, βCD-flavor complex did not exhibit oxidation product of limonene up to 18 weeks, then reached a level of 0.13 mg/100 g limonene 1,2-epoxide after 20 week. The formation of carvone during storage was observed solely in spray-dried microcapsule, although with a lesser amount and a slower formation rate (data not shown). One of the remarkable properties of molecular encapsulation is protection against atmospheric oxidation (12).

Pearson correlation analysis was performed with the 3 variables of mean particle size, oxidative stability at 37°C incubation temperature and moisture uptake (Table 4). A strong correlation between the oxidative stability and moisture
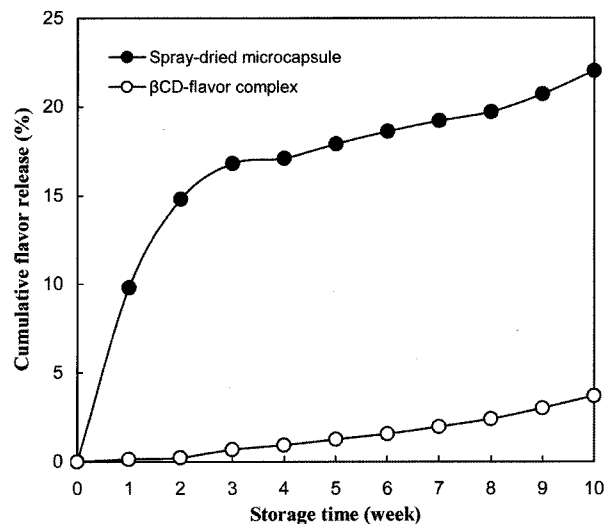

Fig. 3. Cumulative flavor release from spray-dried microcapsule and βCD-flavor complex during storage at 37°C and 60% relative humidity.
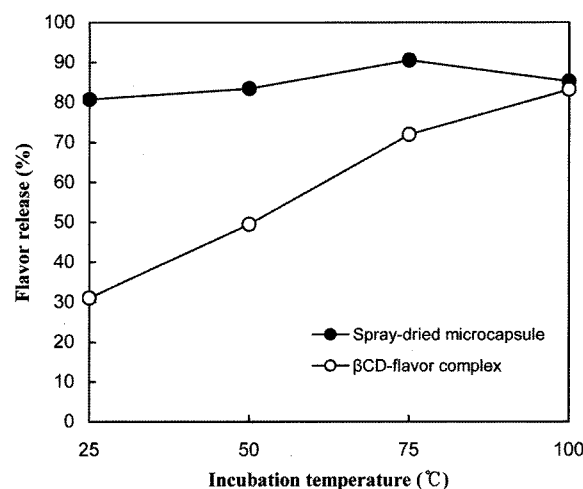

Fig. 4. Effect of temperature on release of flavors from spray-dried microcapsule and βCD-flavor complex.

uptake was detected, which indicated that microcapules having higher moisture uptake exhibited lower oxidative stability. No linear correlation was observed between moisture uptake or oxidative stability and the mean microcapsule particle size.

Controlled release The release characteristics of encapsulated flavors from the powder are quite important for estimating the storage period, as well as the controlled release applications in food (21). In general, release of the active compounds depends upon the type and geometry of the wall material used to form microcapsule (22). The cumulative release of flavor compounds as time progress is shown in Fig. 3. The flavor release was highly influenced by the hygroscopicity of wall materials. The initial release of spray-dried microcapsule followed first-order kinetics. As time progresses, the release rate shifted more towards half order release profile, indicating the steady release of a uniform concentration of core material. This initial 'burst

effect' could be interpreted as a fast release of flavors from the surface of encapsulated powders combined with a fast release of flavors having lower boiling point. When the moisture begins to penetrate spray-dried microcapsule, its surface walls are stressed, then cracks may appear. It allows complete release of flavors at or near the surface of the collapsing mass (22). Yoshii *et al.* (21) also showed similar release pattern of ethyl butyrate at 60% relative humidity.

The flavor release rate of βCD-flavor complex followed first-order release kinetics through the storage time. However, βCD-flavor complex had much slower release rate than spray-dried microcapsule, that is, it showed sustained-release pattern. This result was due to the low hygroscopicity of βCD under normal (50-60%) and extreme (96%) humidities at room temperature (23).

In order to study the effect of temperature on the release mechanism, flavor release from encapsulated powders was investigated at 25, 50, 75, and 100°C (Fig. 4). The release of flavors from spray-dried microcapsule was not influenced by temperature. As the wall materials used for spray-dried microcapsule are water soluble, microcapsule dissolves when in the presence of water and quickly liberates its contents. This kind of release mechanism is called as solvent-activated release which is by far the most common controlled release mechanism used in the food industry such as dry beverage and dry cake mix (24). On the other hand, the flavor release rate from βCD-flavor complex greatly accelerated by an increase in temperature, that is, βCD-flavor complex showed temperature-sensitive release pattern. This kind of release pattern is going to be limited to the unique ability of some materials to either collapse or expand at some critical temperature (24). The controlled release mechanism of βCD-flavor complex is applicable in the food industry such as microwavable foods and hot drinks which are heated prior to consumption.

In conclusion, βCD-flavor complex had a higher resistance to moisture and oxygen than spray-dried microcapsule. Each flavor-encapsulated power showed different mechanisms of controlled release. The release mechanism of spray-dried microcapsule was a diffusion-controlled or solvent-activated release, while the release mechanism of βCD-flavor complex was temperature-sensitive release. Therefore, the characteristics of carbohydrate wall materials were an important parameter for the controlled release mechanism of flavors. Different technologies and wall materials of capsules are available to meet the needs of a given application in the food industry.

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