Encapsulation of Lemon Oil by Paste Method Using β-Cyclodextrin: Encapsulation Efficiency and Profile of Oil Volatiles

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Microencapsulation of lemon oil was undertaken by kneading with β-cyclodextrin, at a β-cyclodextrin to lemon oil ratio of 88:12 (w/w). The resulting paste samples of the complex were vacuum- or spray-dried. Ten selected lemon oil flavor volatiles (α-pinene, sabinene, β-pinene, β-myrcene, limonene, γ-terpinene, terpinolene, linalool, neral, and geranial) in the complex were analyzed periodically after 1, 2, 5, 10, 15, 20, and 30 min of kneading time. The results indicated that the levels of these volatiles were not significantly different ($P > 0.05$) irrespective of mixing time or type of the drying (vacuum- or spray-drying) used. An optimum mixing time was found to be 15 min, at which time the maximum encapsulation of lemon oil (97.7 mg/g of β-cyclodextrin) was obtained in the complex powder.

Keywords: *Microencapsulation; lemon oil; β-cyclodextrin; flavor volatiles; paste method*

INTRODUCTION

Among various flavor encapsulation techniques, molecular inclusion in a β-cyclodextrin molecule is the most effective one (Hedges et al., 1995; Szente and Szejtli, 1988). The cyclodextrin−flavor complex is highly stable against oxidation, heat, and evaporation (Pagington, 1985). The complexing of β-cyclodextrin with flavor molecules can be achieved by using three different methods (Pagington, 1986; Furuta et al., 1994). In the first method, the cyclodextrin and flavors are stirred in aqueous solution, sometimes in the presence of an amount of solvent (e.g., ethanol). In the second method, complexation is achieved by bubbling the flavors in vapor form through a solution of cyclodextrin. In the third method, the flavors are mixed with the cyclodextrin paste containing 10−40% water. This method is preferred as the use of solvent and excess amount of water is avoided; hence, less energy is needed for dehydration.

A proper balance of the encapsulated flavor volatiles is important in the final complex powder. Preferential encapsulation of some volatiles in the β-cyclodextrin molecule is a possibility as each compound can have various degrees of physicochemical interactions with the β-cyclodextrin cavity. There are general comments that the encapsulated volatiles in a β-cyclodextrin−flavor complex can have a different chemical profile from that of the original flavor material, although a number of studies mentioned that the flavor profile in the complex is similar to that in the original oil (Pagington, 1985; Westing et al., 1988; Shahidi and Han, 1993). Bhandari et al. (1998) studied the profile of eight major volatiles in a lemon oil−β-cyclodextrin complex prepared according to the solution method. It was found that the flavor profiles of these volatiles were similar in both the complex powder and original oil. In the paste method, the flavor oil is kneaded with the cyclodextrin−water paste. An assumption is that if some volatiles are preferentially encapsulated in β-cyclodextrin, they can be detected at the initial mixing time. The profile of the volatiles will change as the mixing progresses until equilibrium is reached. Few studies have been published on the efficiency of the paste method. Furuta et al. (1994) studied the optimum kneading time required to encapsulate δ-limonene in a β-cyclodextrin and maltodextrin mixture. However, this study did not consider a flavor oil composed of a number of volatiles.

The aim of this study was to evaluate the encapsulation profile of selected volatiles in the complex at various kneading times. In addition, the optimum time of kneading that produces maximum oil retention in the complex powder will be determined. The final drying of the wet complex will be undertaken using vacuum oven and spray-drying methods, and the powders obtained by using these two drying methods will be compared with respect to the volatiles profile and the encapsulation efficiency.

MATERIALS AND METHODS

Raw Materials. Cold-pressed lemon oil stored at 4 °C, β-cyclodextrin (Japan Food and Chemical Pty. Ltd., Tokyo), and distilled water were used as raw materials in the microencapsulation process. The concentration of volatiles in the lemon oil was 97.63% as determined by the gas chromatography/mass spectrometry (GC/MS) analysis using the internal standard (ISD) tetradecane. The moisture content of the β-cyclodextrin was 9.94% as determined by the vacuum-drying method (AOAC, 1990).

Complexation Procedure. One kilogram of β-cyclodextrin was moistened with 700 mL of distilled water to form a paste in a dough mixture, and then 120.8 g of lemon oil was added. The ratio of β-cyclodextrin to lemon oil was 88:12 (dry basis). In a previous study, Bhandari et al. (1998) had found that this ratio was optimum for the β-cyclodextrin−lemon oil in a solution method of complexation. To be able to compare the paste technique with the solution technique, the same ratio was chosen in this study. After being mixed gently, this mixture was immediately transferred into a Z-arm mixer

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(Morton Machine, Wishaw, Scotland). The kneading was continued for 30 min at a minimum speed of the mixer. The temperature of the mixture was measured during sampling but was not controlled. Samples (50 g) were drawn at 1, 2, 5, 10, 15, 20, and 30 min intervals. These samples were dried in a vacuum oven at 70 °C for 16 h [as described by Furuta et al. (1994)]. The remaining complex paste at the end of the mixing (at 30 min) was added with the water (2 L) to obtain a thin slurry consistency (~18% solids) and then spray-dried (APV Lab 1 spray drier, Denmark) using inlet and outlet air temperatures of 160 and 60 °C, respectively. Complexation was undertaken in three batches.

Moisture Determination. The moisture content of the β-cyclodextrin−lemon oil complex powder was analyzed by drying a powder sample (3−4 g) in a vacuum oven at 70 °C for 24 h, under pressure <6.7 kPa (AOAC, 1990).

Surface Oil Extraction. The volatile compounds present on the surface of the powder were determined by washing a sample of powder (3−5 g) with hexane (20 mL) using the method of Bhandari et al. (1992, 1998). This solvent−powder mixture was gently shaken manually for 20 min. The mixture was then filtered, and the residue was further washed with hexane (10 mL) successively three times. It was found that the third washing did not contain any flavor volatiles. For each treatment, hexane (1 mL) containing the ISD, tetradecane (0.991 mg), was added to the filtrate, which was then concentrated using a nitrogen stream to ~1 mL. This extract of the surface oil was stored at 0 °C until required for GC analysis.

Total Oil Extraction. The total amount of oil in the powder was determined according to the Clevenger hydrodistillation method as described by Anandaraman and Reineccius (1987). This method was validated also by Padukka et al. (1999) to determine the total oil and flavor profile in the β-cyclodextrin−lemon oil complex powder. Complex powder (10 g) was hydrodistilled with distilled water (250 mL) for 3 h. The volume of oil separated was read directly from the graduated arm and multiplied by the density for gravimetric determination (density of lemon oil was 0.856 g/cm^3 at 20 °C). The experiments were carried in duplicate for three separate batches.

GC Analysis. Analyses of the hydrodistilled lemon oil and concentrated surface oil extracts were undertaken using a Perkin-Elmer GC system with a flame ionization detector with helium as carrier gas at a linear flow velocity of 25 cm/s. Also, the GC/MS system was interfaced to a fused silica capillary column (50 × 0.22 mm i.d. coated with 5% phenylpolysilphenylene−siloxane; BPX-5, SGE Ltd., Melbourne, Australia). The column oven was temperature programmed to rise from 50 °C (1 min initial hold) to 200 °C at 3 °C/min; the injector and detector temperatures were maintained at 250 and 300 °C, respectively. For all injections, 1 μL of the extract was used in a splitless injection mode. Retention indices were determined by interpolation of the GC retention times to those of *n*-alkanes (C_5−C_{22} mixture) under identical conditions.

Quantification of Lemon Oil Flavor Volatiles. Quantification of the flavor compounds present in the flavor extracts of the complex and surface oil was accomplished using GC results together with an ISD and response factors. The response factor of limonene was determined and used for the quantification of all flavor compounds.

Statistical Analysis. Data were analyzed by using the SAS system (1992) for windows 3.95, release 6.08. Duncan's multiple-range test was used to detect significant differences between the batches and kneading times for the mean concentration of total oil, surface oil, and individual volatiles in three batches. Significant difference was reported with $P < 0.05$.

RESULTS AND DISCUSSION

Complex Preparation and Drying. The complexation was conducted in three batches. As mentioned earlier, the complex preparation according to the paste method was simpler than the solution method as described in our earlier publication (Bhandari et al., 1998). There are several kneading systems proposed in the literature. On the basis of this research, it was found that a Z-arm mixer is a suitable option. After the addition of the oil and subsequent kneading, there was a heat generation in the mixture. The temperature of the paste was measured each time when the sample was drawn. The temperature of the paste was gradually increased and reached to slightly above 30 °C from an initial temperature of 20 °C. The heat generation was obviously due to mechanical shear and positive heat of complexation. In addition, significant water condensation was observed on the cover of the Z-arm mixer. Initially, the water−β-cyclodextrin mix had a dough type of consistency. This state was turned into a paste-like softer consistency during kneading with lemon oil, which was due to reduced water interaction of the complex molecule as compared to β-cyclodextrin alone. After sufficient samplings until 30 min of kneading, the remaining complex paste was diluted with water to achieve a feasible viscosity for subsequent spray-drying. The samples dried using the vacuum oven method were in the form of agglomerates (cakes); as a consequence, light grinding was required to achieve a sufficiently flowable powder. On the other hand, the spray-dried complex powder was very fine, with an excellent flowability (subjective observation). However, due to its very fine particle size (size not measured), a bag filter was required in the cyclone to avoid losses during the spray-drying process. Use of a spray-drying system was much simpler and more quickly achieved a reasonable powder than drying in a vacuum oven. As the oil−β-cyclodextrin complex has a minimum interaction with water, most of the water in the paste will be in free form. This makes spray-drying more efficient even at lower drying temperature conditions.

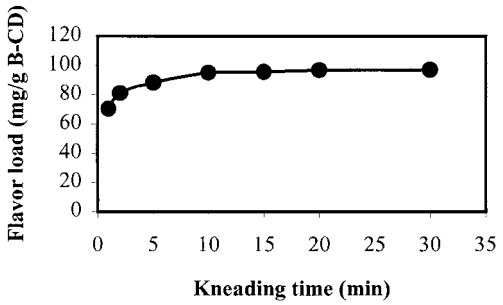

Figure 1. Flavor load of β-cyclodextrin as a function of kneading time (B-CD to lemon oil ratio = 88:12). The mean values at 15, 20, and 30 min were not significantly different at $P > 0.05$ as analyzed by Duncan's multiple-range test.

Inclusion Efficiency of β-Cyclodextrin (B-CD). The inclusion efficiency is expressed as the amount of oil included in the β-cyclodextrin molecule (hydrodistilled oil minus the surface oil). As shown in Figure 1, the maximum load of oil volatiles occurred at 30 min of kneading time (97.1 mg/g of B-CD). However, Duncan's multiple-range test indicated that the mean values were not significantly different ($P > 0.05$) at 15, 20, and 30 min of kneading time. This means that 15 min of kneading time was an optimum kneading period for this particular experimental condition. In an earlier paper, we (Bhandari et al., 1998) undertook experiments to investigate the maximum lemon oil load in β-cyclodextrin by a solution technique. A similar oil load (96.8 mg of oil/g of B-CD) in B-CD was found. The similarity demonstrates that 1 g of β-cyclodextrin has a molecular inclusion capacity of not more than 97 mg of lemon oil.

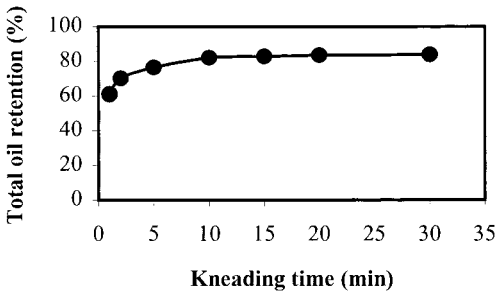

Figure 2. Retention of total flavor volatiles (as determined by hydrodistilltaion) as a function of kneading time (B-CD to lemon oil ratio = 88:12). The mean values at 15, 20, and 30 min were not significantly different at $P > 0.05$ as analyzed by Duncan's multiple-range test.

Table 1. Surface Oil in the Complex Powder (Vacuum-Dried) as a Function of Kneading Time

kneading time (min)	surface oil content (mg/g of complex)	
	mean[a]	SD
1	9.83 a	6.91
2	7.97 ab	4.71
5	7.03 abc	3.35
10	5.63 bcd	2.83
15	5.93 abcd	3.16
20	4.8 bcd	2.17
30	3.27 cd	1.42

[a] Means with the same letter are not significantly different at $P > 0.05$. SD, standard deviation.

Addition of any excess amount of lemon oil may result in an increase in the surface oil.

Total Oil Retention. The total oil retention is the amount of oil recovered by hydrodistillation in the complex powder compared to the initial amount added. The maximum amount of oil retention also occurred at 30 min of kneading time (Figure 2). Duncan's multiple-range tests indicated that the mean values at 15, 20, and 30 min were not significantly different ($P > 0.05$). This further proved that the optimum time for kneading was 15 min.

Surface Oil. The amount of surface oil present in the complex powder is presented in Table 1. However, the amount of surface oil in the powder varied largely in all three batches and at all kneading times. This is demonstrated by the high standard deviation values in Table 1. Additionally, the amount of unencapsulated oil remained as surface oil in the complex. This oil is normally evaporated during the drying step of the encapsulation process. The amount of evaporation will be influenced by the exposure of oil to the air. It was mentioned earlier that the resultant product from vacuum-drying was in the form of agglomerates. It is possible that some of the oil is encapsulated (entrapped) within the solid matrix of the agglomerates. This makes it difficult to control the amount of surface oil present in the complex powder. It is expected that the less the amount of encapsulated oil present in β-cyclodextrin, the more will be the amount of surface oil. When compared with the previous result (total oil retention), there should be a higher amount of surface oil at 1 min of kneading time and the lowest amount of surface oil at 30 min of kneading. The mean values follow this trend (Table 1). The surface oil in the complex powder prepared according to the paste method (this research) was similar to that of the complex powder recently prepared by using the solution method (Bhandari et al., 1998).

Table 2. Flavor Load and Total and Surface Oil Retention in Vacuum- and Spray-Dried Complex Powders (Dry Basis)

	vacuum-dried		spray dried	
	mean[a]	SD	mean[a]	SD
flavor load (mg/g of B-CD)	97.05 a	0.11	96.33 a	0.03
total oil retention (%)	83.81a	0.91	83.19 a	0.23
surface oil (mg/g of complex)	3.27 a	1.43	2.20 a	0.80

[a] Means with the same letter are not significantly different at $P > 0.05$. SD, standard deviation.

Spray-Dried versus Vacuum-Dried Complex Powder. At the end of 30 min of kneading and sampling, the remaining paste was diluted and dried by spray-drying. The comparative results for the samples dried using vacuum oven and spray-drying (same kneading times for both) are presented in Table 2. The results indicated that the complex powders obtained by using both methods were similar in compositional properties. The similar amounts of surface oil in both complex powders indicated that the extents of evaporation of unencapsulated oil during long-time vacuum-drying and ultra-short-time spray-drying processes were comparable. However, there was a difference in physical properties between the two products. Powder from spray-drying was very fine, whereas that from vacuum-drying was coarse even after light grinding of agglomerates.

Profile of Lemon Oil Volatiles for the β-Cyclodextrin Complex. Ten major volatiles in the total oil were analyzed and compared for effects of the various kneading times. The results are presented in Table 3. Duncan's multiple-range tests indicated no significant difference ($P > 0.05$) at any kneading time for sabinene, γ-terpinene, terpinolene, linalool, and neral. For the remaining volatiles, there were some significant differences ($P < 0.05$), although the differences in absolute values were not large. At 5 min of kneading time, α-pinene and limonene levels in the total oil were significantly different ($P < 0.05$) from the mean values at any other kneading times. This was not explainable. It was probably due to unexpected experimental errors. In the spray-dried complex powder, limonene was significantly lower ($P < 0.05$) than in the vacuum-dried samples; otherwise, other volatiles were similar to those of vacuum-dried samples. The results indicated that the encapsulation of all lemon oil volatiles takes place simultaneously right from the beginning and there was no preferential encapsulation of any of the volatiles considered in this study. All of these findings suggest that an unbalanced flavor profile of the microencapsulated product using β-cyclodextrin by the paste method may not occur. Similar results were found in a previous study using a solution method (Bhandari et al., 1998). However, it should be kept in mind that some minor volatiles were not analyzed in this research study. Sensory evaluation once again would have complimented this research. However, use of sensory evaluation was out of the scope of this study due to legal constraints in Australia involving the use of β-cyclodextrin as a food additive.

Conclusions. It was found that a lemon oil powder can be successfully produced according to the paste method using β-cyclodextrin. The flavor encapsulation profile in the complex was similar irrespective of the time of mixing (kneading). An excess amount of lemon oil to β-cyclodextrin was used in this research, which

Table 3. ANOVA of the 10 Main Volatiles in the Total Oil Extracts (Hydrodistilled) of the Lemon Oil to B-CD Complex Powders as a Function of Kneading Time

	mean concn of extracts[a] (%) at kneading time of							
							30 min	
volatile	1 min	2 min	5 min	10 min	15 min	20 min	vacuum-dried	spray-dried
α-pinene	5.07 a	5.04 a	4.84 b	5.19 a	5.03 a	5.13 a	5.02 a	5.11 a
sabinene	0.26 a	0.26 a	0.24 a	0.25 a	0.25 a	0.25 a	0.25 a	0.25 a
β-pinene	1.99 b	1.99 ab	1.95 b	2.01 a	1.98 ab	2.01 a	1.98 ab	2.01 a
β-myrcene	1.19 ab	1.19 ab	1.19 ab	1.20 a	1.19 ab	1.20 a	1.21 a	1.18 b
limonene	86.90 b	86.87 b	87.53 a	86.93 b	86.71 b	86.93 b	86.42 b	84.98 c
γ-terpinene	1.27 a	1.08 a	1.28 a	1.27 a	1.27 a	1.27 a	1.29 a	1.26 a
terpinolene	0.49 a	0.42 a	0.46 a	0.47 a	0.47 a	0.47 a	0.44 a	0.49 a
linalool	0.13 a	0.29 a	0.10 a	0.10 a	0.11 a	0.14 a	0.12 a	0.31 a
neral	0.80 a	0.98 a	0.71 a	0.77 a	0.77 a	0.75 a	0.75 a	0.81 a
geranial	1.67 a	1.53 a	1.44 b	1.63 ab	1.64 ab	1.56 ab	1.64 ab	1.78 ab

[a] Means with the same letter are not significantly different at $P > 0.05$. Note: paste complex samples for 1, 2, 5, 15, and 20 min kneading time were all vacuum-dried.

resulted in low recovery of the volatiles in the final product. A further investigation is needed to optimize the lemon oil to *β*-cyclodextrin ratio without affecting the encapsulation capacity of *β*-cyclodextrin.

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