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Encapsulation of Citral Isomers in Extracted Lemongrass Oil with Cyclodextrins: Molecular Modeling and Physicochemical Characterizations

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The complexation between two isomers of citral in lemongrass oil and varying types of cyclodextrins (CDs), α-CD, β-CD, and HP-β-CD, were studied by molecular modeling and physicochemical characterization. The results obtained revealed that the most favorable complex formation governing between citrals in lemongrass oil and CDs were found at a 1:2 mole ratio for all CDs. Complex formation between *E*-citral and CD was more favorable than between *Z*-citral and CD. The thermal stability of the inclusion complex was observed compared to the citral in the lemongrass oil. The release time course of citral from the inclusion complex was the diffusion control, and it correlated well with Avrami's equation. The release rate constants of the *E*- and *Z*-citral inclusion complexes at 50 °C, 50% RH were observed at $1.32 \times 10^{-2}\,h^{-1}$ and $1.43 \times 10^{-2}\,h^{-1}$ respectively.

Key words: lemongrass oil; *E*-citral; *Z*-citral; cyclodextrin; inclusion complexes

Lemongrass (*Cympopogon citratus* L.) oil has been widely reported due to its biological activities including anti-bacterial,[1)] anti-fungal,[2,3)] and insecticidal.[4)] These properties are useful in both food and pharmaceutical applications. Lemongrass oil is a rich source of citrals, a mixture of *E*-citral (geranial) and *Z*-citral (neral) isomers.[5)] The aldehyde group of *E*-citral is in *trans* configuration, allowing better accessibility of it to water. The aldehyde group of *Z*-citral is in *cis* configuration, and hence it is less accessible. These differences in molecular structures lead to different physicochemical properties. For example, *E*-citral has an intense lemon odor while *Z*-citral has a milder, sweeter lemon odor. Although lemongrass oil has shown high potential as a broad-spectrum anti-microbial agent,[2)] its practical use remains limited due to its poor aqueous solubility and low stability against of high temperature, oxygen, and light. To overcome these problems, a stabilization process is therefore necessary.

Among various encapsulation techniques, inclusion complex by cyclodextrins (CDs) has been proposed to be one of the most effective method to increase the stability of many volatile compounds.[6–9)] CDs are natural starch derivatives resulting from enzymatic degradation by cyclodextrin glycosyltransferase. They constitute a family of cyclic oligosaccharides having six (α-cyclodextrin), seven (β-cyclodextrin), or eight (γ-cyclodextrin) glucose units linked by α-(1,4) glucopyranose subunits with a hydrophilic outer surface and a hydrophobic center cavity.[10)] The hollow molecular shape can form inclusion complexes with a wide variety of organic compounds.[11)] The inclusion complexes with hydrophobic compounds have been attributed to hydrophobic interactions between guest molecules and the walls of the cavities of CDs and other forces, such as Van der Waals and dipole-dipole interactions.[12,13)] CDs can also act as flavor carriers protecting against oxidation and light and heat degradation and improving stability during long-term storage.[8,11,13,14)] Moreover, inclusion complexes with CDs turn the essential oils into water-dispersible, easy-to-handle powders, which also allows better control of their volatility.[13)]

The use of CDs to encapsulate flavors has been widely studied. In recent years, there have been reports on the application of CDs in the encapsulation of flavors, including citral. The formation binding constant for 1:1 complexes of different types of CDs of pure aroma, including *E* and *Z* citral, has been reported.[15,16)] Weisheimer *et al.*[17)] also reported the preparation of β-CD and derivative microparticles containing lemongrass oil by spray drying and precipitation methods, but some aspects of citral complexation by CDs and their characteristics must be better understood as well as the influence of isomers of citral on the obtained inclusion complexes. Furthermore, there have been a few reports on the inclusion of mixed flavors from natural extracted essential oils.

The aim of this study was to employ molecular modeling to obtain insight complex formation of both citral isomers in inclusion complex formation into three CDs as α-CD, β-CD, and HP-β-CD. The effect of the ratio of citral to CDs in solution on the inclusion ratio of citral/CD has been investigated with comparison to the

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Abbreviations: α-CD, alpha-cyclodextrin; β-CD, beta-cyclodextrin; CDs, cyclodextrins; HP-β-CD, 2-hydroxypropyl-beta-cyclodextrin

modeling results. The inclusion ratio of each citral isomer (*E*- and *Z*-citral) in the lemongrass oil with CDs was also investigated. The formation of a citral/CD inclusion complex was confirmed by XRD and DSC. The thermal stability of the citral/CD complex was investigated. Furthermore, the release characteristics of each citral isomer inclusion complex were studied at 50 °C, 50% RH, and the release rate constants of the various citral isomers were determined.

Materials and Methods

Materials. α-CD, β-CD, and HP-β-CD were purchased from Wacker Chemicals (South Asia). Lemongrass oil was from Thai-China Flavours and Fragrances Industry (TCFF). The major components of the extracted lemongrass oil were citrals at 74 wt %. The contents of *E*-citral and *Z*-citral in the lemongrass oil were determined to be 42 wt % and 32 wt % respectively, similar to the values reported in the literature.[2)] Standard citral (*E* and *Z* isomers) was obtained from Sigma-Aldrich. Hexane was from Carlo Erba. The water used for all experiments was purified water obtained from a MilliQ Plus (Millipore, Germany). All other reagents used were commercially available and of analytical grade.

Computer modeling: Thermodynamic properties of the inclusion complex in the water system. All the molecular dynamic simulations were performed using Material Studio ver.4.3 (Accelrys, USA). A COMPASS force field was used throughout the study. The structures of α-CD, β-CD, HP-β-CD, and citral (*E*- and *Z*-form) were constructed and subjected to geometry optimization. The optimized structures of the citrals were then subjected to an absorption locator module with various CDs as absorption substrates. The models were constructed with varying mole ratios of *E*- and *Z*-citral to CDs (1:2, 1:1 and 2:1). The free energy of complex formation ($E_{complex}$) or binding energy for the models, a summation of intra-molecular distortion ($E_{distortion}$) and inter-molecular interaction ($E_{non\text{-}bond}$) energy terms, were first calculated under vacuum conditions.

The amorphous structures of the guest:host complexes were generated under periodic boundary conditions. The lengths of the sides of the cell were over 20.0 Å × 20.0 Å for the citral/CD complexes with the addition of 500 molecules of water to adjust the water box in the system. The densities of CD and citral in the systems were maintained in synthia units at 1.27 and 0.87 g/cm^3 at 25 °C, respectively. Of 10 configurations, only one system was chosen according to its lowest energy as given by molecular mechanics calculations as the initial configuration. To remove unfavorable interactions in the initial configuration, 5000 steps of energy minimization were employed using the Smart algorithm. Molecular dynamic simulation for 100 ps was carried out using normal pressure and temperature conditions with a time step of 1 fs in a range of 273–310 K. A cut-off distance of 9.0 Å and a buffer of 0.5 Å were adopted to minimize the calculation of the non-bond interactions. The electrostatic charges of the model were calculated by the charge equilibration method. The duration of the equilibration dynamics was equal to 100 ps. The models of inclusion complexes were then subjected to molecular dynamics for at least 1,000 ps. Each of the equilibrated citral/CD/water systems was then subjected to free energy calculation. The total free energy (ΔG) of each complex model in the water-explicit system was calculated by the 9 procedure,[18)] as briefly described below. According to this method, ΔG is calculated follows

$$\Delta G = \Delta G_{\text{bind}} + \Delta G^{Co}{}_{\text{sol}} - \Delta G^{Ci}{}_{\text{sol}} - \Delta G^{CD}{}_{\text{sol}} \quad (1)$$

where ΔG_{bind} is the complex formation energy or the binding energy ($E_{complex}$) between citral and CD, and $\Delta G^{Co}{}_{\text{sol}}$, $\Delta G^{Ci}{}_{\text{sol}}$, and $\Delta G^{CD}{}_{\text{sol}}$ are the solvation free energy for the complex, citral, and CD respectively.

All energetic analyses were performed only for a single molecular dynamic trajectory of the citral/CD complexes considered, with unbound CD and citral snapshots taken from the snapshots of that trajectory.

Preparation of citral/CD inclusion complexes. One g of CD was dissolved in the 30 mL of deionized water at 50 °C until a clear solution was obtained. After cooling of the solution to 40 °C, lemongrass oil was added at varying citral/CD mole ratios in solution. The mixtures were shaken at 250 rpm at 25 °C for 4 h. Clear and turbid solutions were collected, except for the oil excess. The inclusion mixture was frozen at −20 °C and then dried overnight under vacuum (CRYODOS-80, Telstar, Spain). The powders obtained were stored in gas-tight bottles at −20 °C until further analysis.

Measurement of citral encapsulated in the inclusion complexes. The powders (0.1 g) of the inclusion complexes were weighed in glass tubes and mixed with 2 mL of distilled water and 4 mL of hexane to extract the encapsulated citral. Then the mixture was kept in a water-bath at 85 °C for 30 min with intermittent shaking. The glass tube was cooled to room temperature. The organic phase was separated and stored at about 4 °C in a vial until GC-MS analysis.

For GC-MS analysis, a sample was injected twice into GC (7890A, Agilent Technologies, USA) interfaced to a mass spectrometer (5975C inert XL EI/CI MSD, Agilent Technologies, USA) equipped with a 30 m × 0.25 mm × 0.25 µm film DB-5MS fuse silica capillary column (J&W Scientific, USA). Helium was used as the carrier gas at a flow rate 1.11 mL/min. The injection volume was 1 µL. The column temperature was programmed initially at 60 °C for 5 min, followed by heating to 120 °C at a rate of 5 °C/min, then to 250 °C at a rate of 10 °C/min. Mass spectra were recorded over a 30–650 amu range at 1 scan/s with an ionization energy of 70 eV and an ion source temperature of 230 °C. The relative amounts of individual components were based on peak areas obtained. *E*- and *Z*-citrals were selected as the major markers of lemongrass oil. The external standard method was used to calculate the contents of citrals. Measurements were done in triplicate, and average values are reported.

Determination of crystallinity and inclusion complex structure. The crystallinity properties of β-CD and the inclusion complexes were determined by X-Ray diffractometer (JDX-3530, JEOL, Japan). The difference in crystallinity of β-CD and its inclusion complexes was determined by X-Ray diffraction (XRD) patterns which the formation of inclusion complexes confirmed. X-Ray diffraction (XRD) patterns were obtained using the Cu Kα radiation wavelength (λ = 1.54 Å) at 40 kV, 30 mA, and a scanning rate of 3°/min.

Determination the thermal properties of the inclusion complex. Differential scanning calorimeter (DSC) studies were performed to evaluate the formation of the complex and its stability after thermal oxidation. Thermal behavior of the citral inclusion complexes with β-CD were investigated using DSC (DSC823e/400, Mettler Toledo, Switzerland). A 2.0–5.0 mg powder sample was sealed into an aluminum pan and then heated at a rate of 5 °C/min from 0 to 400 °C. The analysis was done under both inert and oxidative conditions. Under the inert condition, nitrogen gas (N_2) flowed into the furnace at a rate of 20 mL/min. On the other hand, to study the stability of the inclusion complex against thermal oxidation, the samples were weighed in aluminum pans with a hole in the lid and heated under oxygen (O_2) at a flow rate of 20 mL/min. An empty standard aluminum pan was used as reference. The measurements were made in duplicate.

A release study of citrals from the inclusion complexes. The inclusion complex at 0.75:1 loading mole ratio of total citrals in lemongrass oil to β-CD (0.1 g) was spread in a thin layer in a 20 mL (16^{ϕ} × 100 mm) glass bottle and stored under 50% relative humidity (RH) at 50 °C controlled by constant climate chamber (KBF720, Binder, Germany). At each time point, the samples were removed and the *E*- and *Z*-citrals amounts in the powder were analyzed by the GC-MS technique, as described above. The relative amounts of individual components were based on the peak areas obtained. As a control experiment, the lemongrass oil was also placed in a glass tube and its release time course was also determined.

To evaluate the release rate constant of the *E*- and *Z*-citrals from the citral/β-CD complex, Avrami's equation was applied to the release time courses of the encapsulated citrals:[7,19)]

$$R = \exp[-(kt)^n] \quad (2)$$

where R is the retention of the *E*- and *Z*-citrals in essential oil, which was determined by the ratio of the remaining inclusion citrals in the CDs at a time t, to the initial inclusion of citrals in the CDs. t is the storage time (h), k is the release rate constant (h^{-1}), and n is a parameter representing the release mechanism.

Results and Discussion

Thermodynamic properties of the inclusion complexes in the water system

Table 1 shows the binding energy between two forms of citral and various types of CDs at varying mole ratios under vacuum conditions. The negative binding energy changes upon complexation clearly indicated that all the CDs formed stable inclusion complexes with both *E*- and *Z*-citrals for inclusion ratios of 1:2 and 1:1. Complexation at an inclusion ratio of 2:1 resulted in a positive free energy change, suggesting unfavorable interaction between the two citral isomers and the CDs (data not shown).

Additional models of citral/CD complexes in water explicit systems were subjected to molecular dynamic simulation, and the results obtained are presented. The total free energy (ΔG) of each complex was calculated. All energetic analysis was based on a snapshot of the molecular dynamic trajectories of the citral/CD complexes. As in Table 1, negative energetic terms resulted not only from non-bond interactions between citral and CD cavities, but also from the solubility of CD and its final complexes. The remarkable energetic terms described the energy required for desolvation of CD and the solvation energy of the inclusion complexes. The total free energy changes in the inclusion complexes between the citrals and α-CD, β-CD, and HP-β-CD were negative at the inclusion ratios (1:2 and 1:1). Furthermore, the models of the equilibrated citral/CD complexes of α-CD, β-CD, and HP-β-CD were observed to be stable complexes throughout the simulated period. The results suggest that all the CDs spontaneously formed inclusion complexes with the citrals at inclusion ratios of 1:2 and 1:1, but more favorable complex formation was observed at a ratio of 1:2. According to the total free energy on complex formation, the inclusion complexes of the *E*-citral/CDs were significantly more favorable than those of the *Z*-citral/CDs. These calculation results accord with Decock *et al.*[15] who reported that the formation constant of *E*-citral/CDs was higher than that of the *Z*-citral/CDs complexes. The formation constant of citrals/β-CD showed the highest value.

Alignments of the citral molecules (*E* or *Z* isomer) in the CD cavities of α-CD, β-CD, and HP-β-CD were done. The binding geometry of all the CDs with the two forms of citral was illustrated at an inclusion ratio of 1:2 and 1:1. A molar ratio of 1:1, the guest molecule is located in the hydrophobic cavity. While at a molar ratio of 1:2, the guest molecule was situated at the pocket between CD molecules, not the hydrophobic cavity, but the calculated total energy was more thermodynamically favorable than at a ratio of 1:1 as shown in Table 1. Intermolecular hydrogen bonds within inclusion complexes were observed (data not shown). In the inclusion complexes of α-CD and β-CD at a 1:2 inclusion ratio, CD rings were aligned into the same direction, while the CD rings of HP-β-CD formed inclusion complexes in the opposite orientation. At a 1:1 inclusion ratio, the hydroxyl groups of both *E*-citral and *Z*-citral are left out of the hydrophobic cavity of α-CD because of the cavity size limitation. On the other hand, the hydroxyl groups of both citrals were included in the cavities of β-CD and HP-β-CD, and were less exposed to solvent molecules. In the case of HP-β-CD, *E*- and *Z*-citrals conformers bind in similar manners, but in the case of β-CD, *E*-citral was perfectly incorporated in a hydrophobic cavity, while *Z*-citral molecule was partially inserted.

Table 1. Free Energy Terms of Citral/CD Inclusion Complexes at 1:2 and 1:1 Mol Ratio

Inclusion complex	Total free energy (kJ/mol)	Binding energy (kJ/mol)	Solvation energy (kJ/mol)
Citral/α-CD (1:2)			
E-form	−2706.02	−177.33	−2528.69
Z-form	−2555.13	−146.01	−2409.12
Citral/β-CD (1:2)			
E-form	−3071.84	−269.62	−2802.22
Z-form	−2724.90	−235.92	−2488.98
Citral/HP-β-CD (1:2)			
E-form	−3176.36	−286.79	−2889.58
Z-form	−2904.44	−226.66	−2677.78
Citral/α-CD (1:1)			
E-form	−508.15	−93.94	−414.22
Z-form	−421.74	−102.40	−319.34
Citral:β-CD (1:1)			
E-form	−526.39	−112.30	−414.10
Z-form	−468.51	−105.33	−363.18
Citral/HP-β-CD (1:1)			
E-form	−516.09	−104.27	−411.81
Z-form	−450.31	−90.37	−359.93

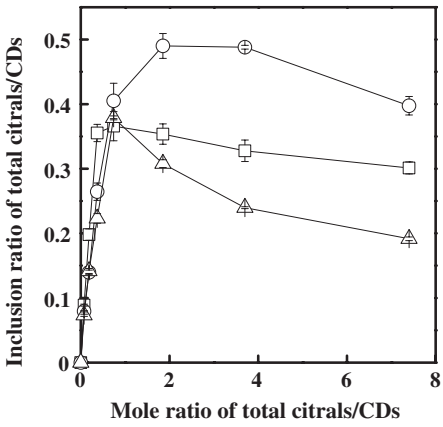

Fig. 1. The Inclusion Ratio of Citrals to CDs of the Complexes at Various Citrals to CDs in Solution (□: α-CD, ○: β-CD, △: HP-β-CD).

The inclusion ratio of total citrals in lemongrass oil to CDs

The inclusion complexes between total citrals and various CDs were obtained as a powder after the freeze-drying process. The effect of the mol ratio of total citrals to CDs in the solution on the obtained inclusion ratio was investigated at molar ratios of 0.1:1 to 8:1 (Fig. 1). At low mol ratios, the inclusion ratios of total citrals increased in response to increases in the mol ratios of total citral/CD in solution. The inclusion ratio increased

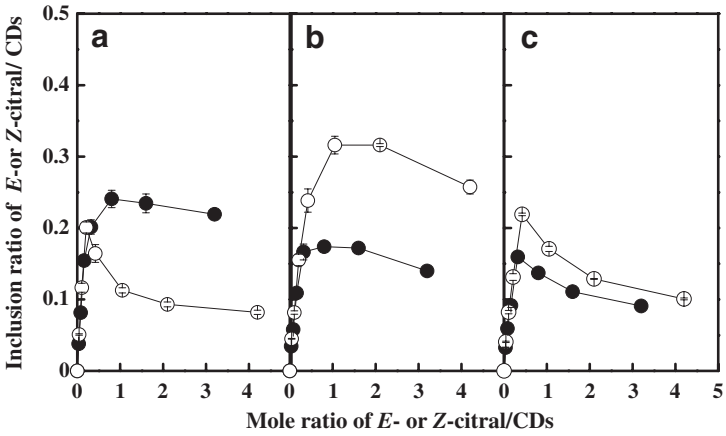

Fig. 2. The Mol Ratio of Citrals Isomer:CDs Solution on Content of Inclusion Complxes of *E*-Citral (open symbols) and *Z*-Citral (closed symbols) with α-CD (a), β-CD (b), and HP-β-CD (c).

up to certain values, after which leveling off occurred despite a continual rise in the loading mol ratio. For α-CD, β-CD, and HP-β-CD, maximum inclusion ratios of about 0.4–0.5 were achieved at mol ratios in solution of about 0.5–1.0. This indicates that one molecule of citral is likely to be encapsulated within two cavities of α-CD, β-CD, and HP-β-CD. However, the total citral/CDs inclusion ratio was slightly lower than 0.5, because lemongrass oil has other components beside the citrals, at about 27 wt%. These components, such as limonene, citronellal, borneol, neryl acetate, and *Z*-caryophyllene,[2] might also be form inclusion complexes with CDs, resulting a lower inclusion ratio of citral. The experimental results obtained here accord with the simulation study in that the formation of inclusion complexes between citrals and CDs can occur favorably at a molar ratio of 1:2 (guests to host).

Inclusion ratios of citral isomers in CDs

Figure 2 shows the effects of the initial ratio of each citral isomer in the lemongrass oil to CDs as to inclusion ratio. Considering the inclusion ratio for each citral isomer, the maximum inclusion ratios were found at 0.20:1, 0.31:1, and 0.22:1 mol of *E*-citral/mol of the CD for α-CD, β-CD, and HP-β-CD respectively, while the inclusion ratios of *Z*-citral/CD were found at 0.24:1, 0.18:1, and 0.18:1 for α-CD, β-CD, and HP-β-CD respectively. Furthermore, the inclusion ratio decreased with increasing citral/CDs ratios in the initial solution. This might have been due to the capacity of the other components of the lemongrass oil to form inclusion complexes.

Figure 3 show the ratios of *E*-citral/*Z*-citral in the inclusion complex at various mol ratios of total citral/CDs. The initial mol ratio of *E*- to *Z*-citrals in the lemongrass oil was 1.30. When the inclusion complexes were formed at a mol ratio of total citral/CDs lower than 1:1, the ratios of *E*- to *Z*-citrals increased slightly from the initial value. These experimental results accorded with the molecular simulation in that the inclusion complexes of *E*-citral/CD were significantly more favorable than the *Z*-citral/CD inclusion complexes at ratios 1:2 and 1:1. However, when the amount of citrals were excessive in the system (ratio citrals/CDs >1), the ratios of *E*- to *Z*-citrals in the inclusion complex were different for the various type

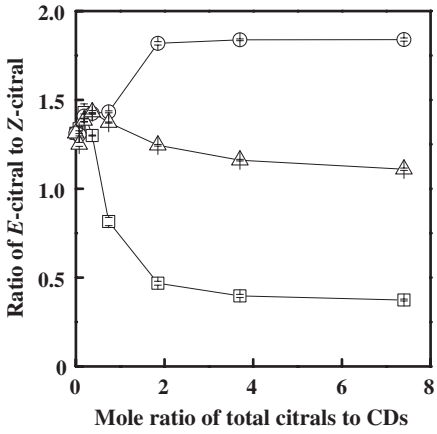

Fig. 3. The Ratio of *E*-Citral/*Z*-Citral in the Inclusion Complex at Various Citrals to CDs in Solution (□: α-CD, ○: β-CD, △: HP-β-CD).

of CDs (Fig. 3). For the inclusion complex with β-CD, the ratio of *E*- to *Z*-citrals increased with increasing ratio of citral/β-CD up to about 1.8. β-CD can selectively form inclusion complexes with *E*-citral better than *Z*-citral. This suggest that β-CD might be useful for separating *E*-citral from *Z*-citral. Furthermore, the ratios of *E*- to *Z*-citrals decreased with increasing initial citral contents to 1.1 and 0.4 for inclusion with HP-β-CD and α-CD respectively. This also suggests that α-CD might be useful to concentrate *Z*-citral from the *E*- and *Z*-citral mixture, as in the extracted lemongrass oil.

The crystalline of the inclusion complex

To confirm inclusion complex formation, the diffraction patterns of β-CD and inclusion complexes prepared from 0.38:1, 0.75:1 and 1.85:1 mol ratios of total citrals in lemongrass oil to β-CD were as shown in Fig. 4. Several sharp diffraction peaks appearing at 9.5°, 12.8°, 13.3°, and 18.1° were seen, suggesting the crystalline character of β-CD.[20] The XRD diffraction spectra of the citral/β-CD inclusion complexes were different than that of β-CD. The peaks of the inclusion complexes were shifted to a lower 2θ angle, at which broad peaks were seen. This indicates that the crystalline patterns of β-CD changed when a guest molecule was included in the cavity of the host molecule.[21]

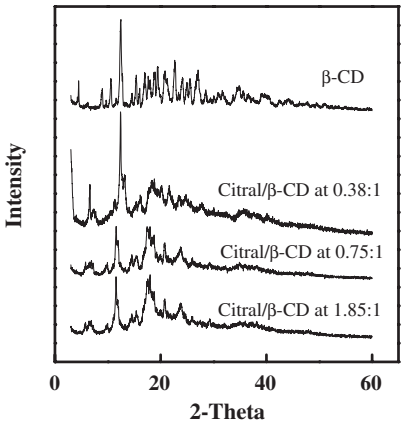

Fig. 4. Powder X-Ray Diffraction Patterns of Uncomplexed β-CD and the Citral/β-CD Inclusion Complexes at 0.38:1, 0.75:1, and 1.85:1 Mol Ratio.

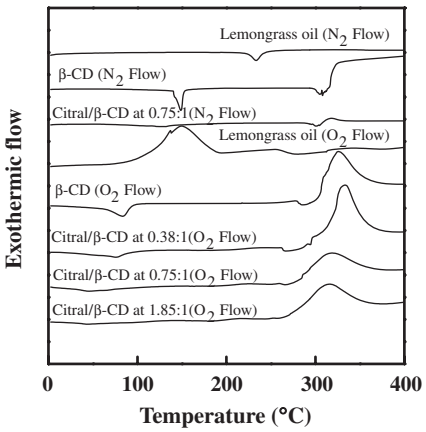

Fig. 5. DSC Thermograms of Lemongrass Oil, Ucomplexed β-CD and the Citral/β-CD Inclusion Complexes at 0.38:1, 0.75:1, and 1.85:1 Mole Ratio.

Thermal properties analysis

The formation of the citral/β-CD inclusion complex was studied by obtaining thermograms. The respective thermograms obtained under inert and oxidative conditions are presented in Fig. 5. In the thermogram of the extracted lemongrass oil under nitrogen, one endothermic peak appeared at 230 °C, probably due to evaporation of the citral (the normal boiling point of citral is about 229 °C). β-CD also exhibited endothermic peaks at about 130 and 330 °C, possibly due to the elimination of water molecules and the thermal decomposition of β-CD respectively. All these peaks were absent in the thermogram of citral/β-CD complex, indicating that inclusion took place.[22)]

Evaluation of the stability against thermal oxidation of the inclusion complexes was done by differential scanning calorimetry (DSC) under an oxygen atmosphere. Figure 5 also shows DSC thermograms under the oxidation condition for lemongrass oil, β-CD, and the inclusion complexes. The thermograms of the inclusion complex ratios were almost the same. The thermograms indicate that thermal oxidation of the lemongrass oil occurred in a range of 100–180 °C as the exothermic peak, while in the thermograms of the citral/β-CD complexes no exothermic event was observed in the same range temperatures. Thus it is evident that the β-CD cavity provided protection against thermal oxidation in the encapsulated citral in the lemongrass oil. The endothermic peak of about 100 °C and the exothermic peak about 300 °C of β-CD and its inclusion complexes were observed, possibly due to the elimination of water and thermal oxidative degradation of the CDs.

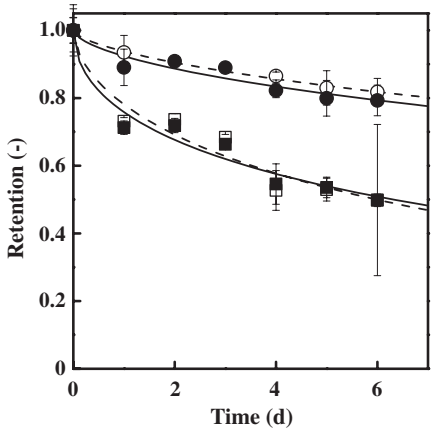

Fig. 6. The Release Time Course of Free Citral Isomers (■, □) Comparing to the Inclusion Complex β-CD (●, ○) at 0.75:1 Mol Ratio in Solution under 50 °C 50% RH: *E*-Citral (open symbols) and *Z*-Citral (closed symbols).

Release characteristics of the citrals from the inclusion complex

The retention of citral from the β-CD inclusion complexes at a 0.75:1 loading mol ratio was evaluated under 50% RH at 50 °C controlled by a constant climate chamber, as shown in Fig. 6. The contents of *E*-citral and *Z*-citral in the inclusion complexes in the same sample were 0.24 and 0.17 mol citral/mol β-CD respectively. A comparison of the relative content of free citral and the citral/β-CD inclusion complex was made. The citrals were released slowly from the inclusion complexes as compared to those of the free citrals. The inclusion complex within β-CD can protect citral under the temperature and relative humidity imposed. Furthermore, considering the type of citral isomers in the inclusion complex, the rate of release of *E*-citral from β-CD was insignificantly different from *Z*-citral.

To evaluate the retention rate constants of both citrals in free form and in the inclusion complex, the data were fitted to Avrami's equation and kinetic parameters (k and n values), as shown in Table 2. The k value, the release rate constant, of free *E*- ($8.84 \times 10^{-2}\,h^{-1}$) and *Z*-citral ($7.58 \times 10^{-2}\,h^{-1}$) was higher than those in the inclusion complexes ($1.32 \times 10^{-2}\,h^{-1}$ and $1.43 \times 10^{-2}\,h^{-1}$ respectively), suggesting better retention of essential oil from the β-CD inclusion complex. A better retention of inclusion citral in *E*-citral than in *Z*-citral was confirmed, lower release rate constant. These releasing results also agreed well with the molecular simulation according to which the inclusion complexes of *E*-citral/CDs were significantly more favorable than the *Z*-citral/CD inclusion complexes. Furthermore, compared to the work reported in the literature,[19)] the obtained release rate constant at 50 °C 50% RH was higher than *d*-limonene ($0.27 \times 10^{-2}\,h^{-1}$) and *l*-menthol ($1.4 \times 10^{-2}\,h^{-1}$), and lower than AITC ($1{,}200 \times 10^{-2}\,h^{-1}$) and ethyl-*n*-butyrate ($360 \times 10^{-2}\,h^{-1}$). In

Table 2. Release Rate Constants and Parameters Representing the Release Mechanism of Citral Isomers from the Inclusion Complex

Sample	Citral	k ($\times 10^{-2}$) (h^{-1})	n
Inclusion complex	*E*-form	1.32 ± 0.01	0.63 ± 0.06
	Z-form	1.43 ± 0.01	0.60 ± 0.16
Lemongrass oil	*E*-form	8.84 ± 0.02	0.58 ± 0.12
	Z-form	7.58 ± 0.02	0.50 ± 0.10

addition, the n values from Avrami's parameter were related to the release mechanism.[7,23,24] The n values obtained were in a range of 0.50–0.63 for both *E*- and *Z*-citrals, suggesting that the release of citral was controlled by a diffusion mechanism. The diffusion mechanism might be due to the diffusion of guest molecules through the crystal structure of the CDs. Even the guest molecule was excluded from the CD molecules.

Conclusion

Citral is a naturally occurring aliphatic aldehyde mixture. It usually consists of two terpenoid isomers, *E*-citral and *Z*-citral. In this study, different affinities on complex formation between cyclodextrins (α-CD, β-CD, and HP-β-CD) and two types of citrals were observed. It is suggested that citrals can form spontaneously inclusion complexes with cyclodextrin at a citral/CD molar ratio of 1:2 or 1:1. Although citrals could be translocated into hydrophobic cavities of cyclodextrin at citral/CD molar ratio of 1:1, they did not fit well and there were unsatisfied Van der Waals contacts. Hydrophobic portions of citrals were partially exposed, contributing to a less favorable solvation energy. The experimental data for complex formation between the citrals and the CDs correlated with the free energy calculated from computational modeling. At a citral/CD molar ratio of 1:2, more stable citral-CD complexes were obtained by a molecule of citral encapsulated between two molecules of CD. The inclusion complex between *E*-citral and the CDs was significantly more favorable than *Z*-citral. It is also proposed that β-CD is the best candidate for selective a binding molecule for *E*-citral due to its suitable cavity size. Furthermore, a higher thermal stability of the citral/CD inclusion complex was observed compared to the citral components in lemongrass oil. The release rate constant of the *E*-citral inclusion complexes was lower than those of *Z*-citral complex, which was in agreement with the simulation results.

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