Encapsulation of Cinnamaldehyde from Cinnamon Essential Oils in Cyclodextrin

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Abstract. Cinnamaldehyde is the major compound in cinnamon essential oil which has effective inhibiting the microbial growth and revealed low physicochemical stability, low solubility in water. The aim of this study was the encapsulation of cinnamaldehyde in β-cyclodextrin in order to obtain a complex which can be used for preserving fresh-cut produce. The encapsulated complex cinnamaldehyde/β-cyclodextrin was prepared using the inclusion complex method. The various ratios of cinnamaldehyde/β-cyclodextrin on cinnamaldehyde releasing efficiency was investigated. The various ratios of cinnamaldehyde/β-cyclodextrin as 50:50, 40:60, 30:70 and 25:75 were monitored. Encapsulation efficiency (%EE) and encapsulation capacity (%EC) of encapsulated cinnamaldehyde powder were analyzed. The results showed that the ratio at 25:70 cinnamaldehyde/β-cyclodextrin showed the highest %EC and %EE when compared with other treatments. The ratio at 25:75 had faster control release in first 3 h. Antimicrobial activity was tested against two strains of gram-positive (Staphylococcus aureus and Bacillus cereus) and two strains of gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria. β-cyclodextrin with encapsulated cinnamaldehyde exhibited good inhibitory effect against all tested bacterial strains.

Keywords: Cinnamaldehyde, Cyclodextrin, Encapsulation

1. Introduction
Cinnamomum verum is an aromatic tree belonging to the family Lauraceae, commonly known as cinnamon, is one of the most important and famous spices widely used. Cinnamon is mainly used in the aroma and flavor industries due to its fragrance, which can be incorporated into different varieties of cuisine, perfumes, and medicinal products [1]. Previous reports have shown that cinnamaldehyde and eugenol are the major active component of cinnamon essential oil. Cinnamaldehyde are frequently used as flavors, but they are also becoming increasingly important as naturally occurring antimicrobial, antioxidant and antiseptic agents [2]. However, the natural and artificial flavors are a
normally liquids at room temperature and also are very sensitive to the effect of light, oxygen, humidity and high temperature. Moreover, these concentrates are not water-soluble, so it is necessary to transform them for their utilization [3]. Then, encapsulation technology has been remarkably developed upgrade their chemical and thermal stability and facilitate handling [4;5] which could increase the potential enhance delivery of antimicrobials. Cyclodextrin have been wildly used to prepare inclusion complexes to improve the stability and solubility. The type of cyclodextrin (α-β-γ-) indicates its size and therefore the size of the hydrophobic molecule that the cyclodextrin can entrap in its inner cavity [6]. The interaction between BCD (host) and active compounds (guests) may involve total inclusion or association with the hydrophobic or hydrophilic part of the molecule. Once oils with in the inclusion complexes, their sensory impact on food products can be reduced and their water solubility increased, providing sufficient contact with pathogens inhibit their growth, making food safe for human consumption [7]. The aim of this study was to prepare the inclusion complexes of cinnamaldehyde with β-cyclodextrin in various ratios and to determine the encapsulation efficiency and encapsulation capacity, morphological characteristics, control release and antimicrobial activities.

2. Methods

2.1. Materials

2.1.1. Raw materials for encapsulation process
Cinnamaldehyde (>98% purity, 3-Phenyl-2-propanal; Cinnamic aldehyde) and β-Cyclodextrin (98%, Acros Organics™; product of China) were obtained from Fisher Scientific (Loughborough, United Kingdom). The encapsulation process was used cinnamaldehyde and β-Cyclodextrin as raw materials. All reagents and solvents used in the experiment were of analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.1.2. Microorganisms
Four indicators bacteria used for testing antimicrobial activity were Escherichia coli TISTR 527, Pseudomonas aeruginosa TISTR 1287 (representative for gram-negative bacteria), Bacillus cereus TISTR 1527 and Staphylococcus aureus TISTR 2329 (representative for gram-positive bacteria). These bacteria strains were obtained from Thailand Institute of Scientific and Technological Research. All bacteria were cultivated in nutrient agar (NA) and incubated at 37 °C for 24 h. The indicator bacteria were kept in 30% glycerol (v/v) then stored at -80 °C until testing.

2.2. Methods

2.2.1. Preparation of Cinnamaldehyde Encapsulated in β-Cyclodextrin
An inclusion complex of cinnamaldehyde and β-cyclodextrin method was modified from Bhandari [2]. 4.5 grams of β-cyclodextrin were dissolved in 50 ml of an ethanol and double deionized water mixture (1:2 v/v). The solution was magnetically stirred at 55 °C for 30 min until the complete dissolution of β-cyclodextrin. A predetermined quantity of cinnamaldehyde were prepared in ethanol (10% w/v) was then slowly added to warm β-cyclodextrin solution. The following starting ratios of cinnamaldehyde to β-cyclodextrin were used: 25:75, 30:70, 40:60 and 50:50 (w/w). After addition of the cinnamaldehyde solution, the mixture was stirred for 4 h at room temperature (25 °C) without heating, while its temperature decreases spontaneously to 25 °C. The final solution was stored overnight at cooling temperature (4 °C). The cold precipitated cinnamaldehyde/ β-cyclodextrin complex was recovered by vacuum filtration. This precipitate was dried in hot air oven at 50 °C for 24 h. Finally, the cinnamaldehyde powders were stored in airtight glass containers under refrigeration (4 °C), prior to further analysis.

2.2.2. Total oil extraction
The total oil content in the complex powder was determined using hexane as a solvent extraction method was modified from Bhandari [8]. The cinnamaldehyde powder (5 g) was extracted by the mixed solution of deionized water (8 mL) and hexane (8 mL) in a glass beaker and sealed. The
solution was then heated in an ultrasonic bath at 70±2 °C for 20 min. The clear organic phase containing the volatile compound was decanted, and the aqueous phase was exhaustively extracted with hexane (2 mL) 3 times. The solvent was evaporated in a rotary evaporator. The amount of encapsulated cinnamaldehyde was measured gravimetrically.

2.2.3. Surface oil extraction

The volatile compounds absorbed on the surface of powder were determined by washing method [8]. Cinnamaldehyde encapsulated powders (0.5 g) were washing with hexane (20 mL) which was gently stirred for 20 min. The solvent mixture was filtered by Whatman filter paper (No.1) and the powder was collected on the filter was rinsed three times with 2 mL of hexane. The final extract was evaporated using a nitrogen steam. The amount of encapsulated cinnamaldehyde was measured gravimetrically. The difference between the total oil and the surface absorbed oil is the amount complexed in the β-cyclodextrin cavity.

2.2.4. Calculation of microencapsulation efficiency

The encapsulation efficiency (EE) was calculated from the quantitative determination detailed above as follows:

\[
EE = \frac{\text{weight of total oil} - \text{weight of surface oil}}{\text{weight of total oil}} \times 100
\]

2.2.5. Calculation of microencapsulation capacity

The encapsulation capacity was obtained from the quantitative analysis above as follows:

\[
EC = \frac{\text{weight of total oil} - \text{weight of surface oil}}{\text{weight of dry complex powder} - \text{weight of total oil}} \times 100
\]

2.2.6. Scanning electron microscopy (SEM)

The morphological analysis of β-cyclodextrin and cinnamaldehyde/β-cyclodextrin were analyzed by field emission scanning electron microscope JSM-7800F (JEOL Ltd., Tokyo, Japan). As the sample had no electrical conductivity, they were coated with a thin layer of gold by using a sputter-coater (Q150R, East Sussex, United Kingdom). The operation conditions setting at 2 kV LED accelerating voltage, 1000× and 5000× magnifications.

2.2.7. In vitro control release

The scanning for the absorbance spectra (λmax) of the cinnamaldehyde was determined. An amount of 0.1 mL of commercial cinnamaldehyde was dissolved in 9.9 mL deionized water, and then, the mixture was strongly shaken at 25 °C. UV-visible spectrophotometer (model UV 1800; Shimadzu; Kyoto, Japan) was used to monitored the absorbance spectra (λmax) of the cinnamaldehyde in water and the wavelength of absorbance ranged from 190-500 nm. Finally, the λmax absorbance of cinnamaldehyde was observed.

In vitro release experiments were performed for the cinnamaldehyde release profile from the formed cinnamaldehyde: β-cyclodextrin to be evaluated. This study, the release profile of the cinnamaldehyde was studied under specific media of silk cocoon protein solution. Cinnamaldehyde release profiles were assayed by a dialysis method. 1 mg of microcapsules with 4 mL of 0.02% sericin solution was added into a dialysis bag (molecules weight cut off 12 kDa, Cell-Sep T3, Membrane filtration products, USA), the bag was continuously placed into 10 mL of 0.02% sericin solution with magnetic stirring at 25 °C. The release solution was sucked out for analysis, and was replaced with an equivalent volume of fresh sericin solution. The amount of release cinnamaldehyde in the sericin solution was monitored by UV-visible spectrophotometer (model UV 1800; Shimadzu; Kyoto, Japan) at the λmax of cinnamaldehyde. The absorbance was compared to the standard of cinnamaldehyde.

2.2.8. Antibacterial activity
Antibacterial activity was determined by the disc diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*. Briefly, 100 µL of bacteria suspension (adjusted to 10⁸ CFU/mL) was spread on the dry agar medium plates. After drying, the sterilized filter paper Whatman No.1 (0.6 mm diameter) was placed on the agar medium. After that 100 µL of inclusion complex at the concentration 100 mg/mL was dropped on the sterilize filter paper, then incubated at 37 °C for 24 h. The antimicrobial activity was assessed by measuring the diameter of the inhibition growth zone (clear zone) of tested indicator microorganism around the filter paper.

2.2.9. **Statistical analysis**

The data was summarized as the mean ± standard deviation (SD). Statistical analysis was carried out using the ANOVA procedure in the general linear model procedure of the SPSS software (version 15.0; IBM Corp.; White Plains, NY, USA) for completely randomized design experiments. Significance was tested at p ≤ 0.05 using Duncan’s multiple range test.

3. **Results and Discussion**

This study was concerned with preparation of an inclusion complex of cinnamaldehyde with β-cyclodextrin. All of the treatments produced the powders of light-yellow to medium-yellow color, different from the white color of pure β-cyclodextrin powder. The difference in color is due to the interaction of cinnamaldehyde with β-cyclodextrin either as an inclusion of cinnamaldehyde pigments into the β-cyclodextrin cavity or absorption on the powder surface. The result shows the light-yellow to medium-yellow of the powders were found in 25:75 ratio followed by 30:70, 40:60 and 50:50 ratios respectively. This intensively color depending on the amount of cinnamaldehyde.

**Table 1.** Determination of the mean and standard deviation (Mean ± SD) of encapsulation efficiency (EE %) and encapsulation capacity (EC %).

| Sample                        | Encapsulation efficiency (EE %) | Encapsulation capacity (EC %) |
|-------------------------------|---------------------------------|-------------------------------|
| Cinnamaldehyde/ β-cyclodextrin (25:75) | 94.93a ± 0.16                   | 5.82b ± 0.48                  |
| Cinnamaldehyde/ β-cyclodextrin (30:70) | 50.32b ± 10.66                  | 3.55b ± 1.18                  |
| Cinnamaldehyde/ β-cyclodextrin (40:60) | 31.32c ± 11.40                  | 4.18b ± 1.86                  |
| Cinnamaldehyde/ β-cyclodextrin (50:50) | 48.63b ± 6.40                   | 10.01a ± 2.65                 |

Note: a-c Mean ± SD in each column within the same letter are not significant different

Table 1 shows the encapsulation efficiency (EE) and encapsulation capacity (EC) at various cinnamaldehyde with β-cyclodextrin ratios. The encapsulation efficiency (EE) reflects the presence of free oil on the surface of the particles within the powder and the degree to which the wall matrix can prevent extraction of internal oil through a leading process [9]. In our study, the EE of inclusion complex of cinnamaldehyde: β-cyclodextrin ranged from 48.63% to 94.93%. The highest EE obtained for the 25:75 ratio followed by 30:70, 50:50 and 40:60 respectively (p ≤ 0.01). However, the EE for the 30:70 and 50:50 ratios was not different. The encapsulation capacity (EC), % oil encapsulated by β-cyclodextrin on a dry weight basis, was chosen as the criterion for comparing the different oil determination methods. The 50:50 ratio had the highest EC (p ≤ 0.05) while, there was no statistical difference for the 25:75, 40:60 and 30:70 ratios were observed. There are several factors which may contribute to the loss of *Cinnamomum verum* essential oil: retention of the oil in the solution after forming the complex; equilibrium of flavors between the liquid and the complexed state; evaporation of surface oil during the long completion process and evaporation during the drying step [10]. EE is a more important parameter even that EC, because of the need to retain a high value of essential oil in the encapsulation process. From our results, the 25:75 and 50:50 showed a higher EE and EC than the other ratios, so there were used to do the next parameters.
Confirmation concerning the morphological particles of the encapsulated cinnamaldehyde was observed under a SEM to characterize the evidence of inclusion formation compared with free β-cyclodextrin. Figure 1 presents the SEM images of the cinnamaldehyde encapsulated in β-cyclodextrin at 25:75 (a), 50:50 (b) and β-cyclodextrin (c) respectively with different magnitudes (1: magnitude of 1000 and 2: magnitude of 5000). There was drastic change in particle size and shape among β-cyclodextrin and inclusion complexes. As can be seen, the particle of pure β-cyclodextrin were present in rectangular shape, in agreement with [11] and shows as crystalline particles with different size without a characteristic shape, as shown in Figure 1. c1 and c2. While, existing of the cinnamaldehyde: β-cyclodextrin presents a non-spherical morphology that resembles that of prisms, having parallel and rather smooth sides, resulting in the great uniformity in its crystal and regularity in its shapes. (Figure 1. a1, a2, b1, and b2). These results indicated that the addition of cinnamaldehyde would disturb the accumulative crystallization of β-cyclodextrin. In the process of forming inclusion complex, the interference might become deeper.
Figure 2. The release profile of the encapsulated cinnamaldehyde at specific media during 105 h (a) as well as at the first 3 h of the experiment (b).

The scanning of release solutions (standard curve of cinnamaldehyde, \( R = 0.9998, Y = 48071x - 0.0124 \), where \( Y \) = absorbance and \( X \) = cinnamaldehyde concentration in mg/mL), in the range of 190 to 500 nm, indicated maximum absorbance of cinnamaldehyde at 288 nm. The in vitro release study of cinnamaldehyde from cinnamaldehyde: \( \beta \)-cyclodextrin complex was carried out in the specific media silk cocoon protein solution at room temperature (25 °C) to: (i) confirm the success of cinnamaldehyde encapsulation, and (ii) understand the release mechanism and release kinetic of cinnamaldehyde from the microcapsules, which is important for further applications of cinnamaldehyde: \( \beta \)-cyclodextrin microcapsules. Control release of cinnamaldehyde encapsulated in \( \beta \)-cyclodextrin at 25:75 and 50:50 ratios is shown in Figure 2. As it can be observed in Figure 2a, the cinnamaldehyde release from \( \beta \)-cyclodextrin microparticles is characterized by two different phases; an initial relatively rapid release phase (0-2.5 h) is observed (“burst effect”) followed by a slower, more constant release phase (after 3 h) indicating a sustained release (“lag time”). The microcapsules with the weight ratio 25:75 that contained 1.5 g of cinnamaldehyde: 4.5 g of \( \beta \)-cyclodextrin had faster release rate in the initial phase (0-2.5 h), as shown in Figure 2b, after that the release rate becomes constant and lower than the weight ratio 50:50 that contained 4.5 g of cinnamaldehyde: 4.5 g of \( \beta \)-cyclodextrin. For the weight ratio 25:75, the release time was approximately obtained for 48 h, while
the weight ratio 50:50 had a long release time approximately 105 h. Our results indicate that the quantity (weight) of cinnamaldehyde had a pronounced effect on the release rate and release time of cinnamaldehyde from the complex.

Antimicrobial activity of tested cinnamaldehyde encapsulated in β-cyclodextrin against four indicator microorganisms is presented in Table 2. Essential oil and derivatives have been shown to be powerful natural antimicrobials against a variety of food borne pathogens [12]. Cinnamon extract and trans-cinnamaldehyde have been found to be some of the most effective antimicrobials against food born pathogens and it has been reported that spice extracts are more powerful inhibitors than purified compounds [13; 14]. This knowledge is particularly important to predict their effect on the different microorganisms, how they interact with food matrix components, and how they work in combination with other antimicrobial components. The results showed that microcapsules performed with cinnamaldehyde encapsulated in β-cyclodextrin at weight ratio 50:50 had a significant inhibitory (p ≤ 0.05) effect on gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). Whereas, the inhibitory effect on gram-positive bacteria (Bacillus cereus and Staphylococcus aureus) was not significantly different on both ratios.

Table 2. Antimicrobial activity of cinnamaldehyde encapsulated in β-cyclodextrin

| Sample                  | Diameter of inhibition clear zone (mm) |
|-------------------------|----------------------------------------|
|                         | Escherichia coli | Pseudomonas aeruginosa | Bacillus cereus | Staphylococcus aureus |
| Cinnamaldehyde/ β-cyclodextrin (25:75) | 1.42 ± 0.63b | 0.33 ± 0.26b | 4.00 ± 1.80 | 1.58 ± 0.29 |
| Cinnamaldehyde/ β-cyclodextrin (50:50) | 2.83 ± 0.58a | 1.25 ± 0.25a | 2.58 ± 1.01 | 1.71 ± 0.31 |

Note: Data are given as mean of inhibition zone (mm) of three replicates. The different letters are significant different at (p ≤ 0.05)

4. Conclusion

Our findings indicate that a cinnamaldehyde powder can be produced by a microencapsulation technique using inclusion complex method with β-cyclodextrin. Elucidation of the experimental results suggests that a maximum encapsulation efficiency (EE) and encapsulation capacity (EC) of β-cyclodextrin with cinnamaldehyde occurs for a starting ratio of cinnamaldehyde to β-cyclodextrin of 25:75 and 50:50. The results of SEM implied that cinnamaldehyde: β-cyclodextrin complex had different morphological characteristics compared to pure β-cyclodextrin. The weight ratio at 25:75 showed the faster cinnamaldehyde release from microcapsules within first 3 h. The cinnamaldehyde encapsulated powder could inhibit four indicators bacteria represent on gram-negative and gram-positive bacteria. The application of the cinnamaldehyde encapsulated in β-cyclodextrin at the ratio of 25:75 (cinnamaldehyde: β-cyclodextrin) could be further applied utilization in the functionalization of drug, food and agricultural product.

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