Assessment of the Antimicrobial Inclusion Complex of Four Essential Oils in β-Cyclodextrin

Deming Li, Xinyi Deng, Chuan Cao*

Anhui Vocational and Technical College, Hefei 230011, China

Abstract. By creating inclusion complexes with β-CD, the effort aims to increase the stability and release of four plant essential oils: camellia, lemon, laurel, and litsea cubeba. It is obvious from the antibacterial experiment that β-CD is the ideal wall material for CAEO to regulate the essential oils' delayed release and increase the antibacterial activity's duration. Studies on release kinetics show that the essential oil microcapsules' release behaviour at 37℃ and 65℃ follows the Peppas equation, indicating a non-Fickian diffusion mechanism. In conclusion, β-CD can be used as a wall volume to control the constant flow of essential oils and extend the persistence of the antibacterial effect.

Keywords: β-CD; essential oill inclusion complexes; release

1. Introduction

Global food safety faces significant problems, including food poisoning and foodborne illnesses brought on by foodborne bacteria. These problems are closely linked to both environmental and public health. The carcinogenicity, teratogenicity, and susceptibility to food poisoning of chemically synthesized antibacterial agents pose concerns for food safety. As a consequence, it's crucial to produce effective and safe natural antibacterial agents [1]. Terpenoids, phenols, and organic molecules containing nitrogen are examples of secondary metabolites that plants create as a means of fending off harmful environmental pressures[2]. These substances function as plant protectors. Among them, terpenoids-dominated plant essential oils are mostly used to protect against the invasion of pathogenic microbes and pests[3]. It has been demonstrated that natural plant essential oils exhibit significant antibacterial action against microorganisms as well as excellent membrane permeability, which can compromise the integrity of microorganism cell structure and membrane permeability and cause organic materials to seep out of the cells. Essential oils have been shown in numerous studies to have antibacterial qualities against foodborne pathogens such as Salmonella, Escherichia coli O157:H7, Staphylococcus aureus, and Botrytis cinerea [4]. However, there are still few reports on the comparative studies of the antibacterial properties of microcapsules of four essential oils. By investigating β-cyclodextrin's ability to regulate the continuous release of essential oils and extend its antibacterial properties, we hope to comprehend its use in food preservation and storage.

2. Experiment

2.1 Materials

Shanghai Yuanye Biotechnology Co., Ltd. provides the essential oils of Atractylodes macrocephala and Camellia sinensis, while China National Pharmaceutical Group Chemical Reagent Co., Ltd. is the source of β- CD. Shanghai McLean Biochemical Technology Co., Ltd. is the supplier of essential oils for lemon and laurel. Staphylococcus aureus (ATCC 6538) and Escherichia coli (ATCC 8739) were obtained from the Shanghai Conservation Biotechnology Centre, while Sinopharm Chemical Reagent Co., Ltd. supplied further analytical grade chemicals and reagents for the investigation.

2.2 Preparation of inclusion complexes

The inclusion complexes were prepared according to a previous report [5].

2.3 Extended release of essential oils and their microcapsules

2.3.1 Study on the release kinetics of microcapsules

The release kinetics of essential oils and their microcapsules were adjusted using Mehdi and Stefani's methods[6][7]. 1.5g of microcapsules should be weighed and added to a conical flask that has 120mLPBS ethanol (3:2V/V) solution in it that has a pH of 6.5. Utilizing a

* Corresponding author: 877542357@qq.com

magnetic stirrer provided by the collector, slowly stir at 37°C and 65°C constant temperatures. Measure the absorbance values of four distinct essential oils at various wavelengths after sucking off 4 mL of sample at predetermined intervals for analysis,collecting the supernatant after centrifuging for 10 minutes at 5000 rpm. Use PBS ethanol (3:2V/V) solution as a blank control. Four modelling approaches were taken into consideration in order to characterise the release kinetics of essential oils and their microcapsules: zero order, first order, Higuchi, and Peppas empirical models. The release kinetics equation was fitted using Origin2018.

$$Q = \sum_{t=0}^{t} \frac{Mt}{M0} \times 100 \quad (1)$$

where M0 represents the essential oil's initial mass in the sample and Mt represents the total amount of essential oil released throughout each sampling period.

2.3.2 Antibacterial activity

Essential oils and microcapsules have been shown to have altered antibacterial activity in accordance with the previously stated technique [8].Experiments were conducted three times using test strains of Escherichia coli and Staphylococcus aureus on a filter paper diffusion culture plate (90 mm in diameter). Following a day of cultivation, essential oil and microcapsules were dropwise placed onto filter paper after Escherichia coli and Staphylococcus aureus were inoculated onto LB medium. Subsequently, a sealing membrane was placed over the inverted culture dish. After inverting the culture for 3, 5, and 7 days at 37℃, measure the diameter of the microbial community using the cross technique. Neither essential oil nor microcapsule therapy were administered to the blank control group.

2.4 Statistical analyses

The statistical program SPSS version 21.0 (Chicago, Illinois, USA, SPSS Inc.) and the Duncan's multiple range test were used to examine the significant differences between the means ($P<0.05$).

3. Results and discussion

3.1 The essential oil microcapsules' release performance

The extent of the inhibition zone for the strains that were tested and suppressed by microcapsules and essential oils at various sustained release times is displayed in Figure 1. The bacterial diameter of the experimental group was significantly greater than that of the blank control group during different sustained-release time periods, suggesting that essential oils and microcapsules can effectively restrict bacterial growth. Furthermore, following three days of incubation, the essential oil treatment group's antibacterial ring was noticeably bigger than the microcapsule treatment group's, and essential oil had a stronger inhibitory impact. The four essential oils have the following order of inhibitory activity on Escherichia coli: LEEO>LAEO>LCEO>CAEO.The order of the four essential oils' inhibitory effects on Staphylococcus aureus is LCEO>LEEO>LAEO>CAEO. The enhanced inhibitory potential of microcapsules is revealed by longer cultivation times, particularly when it comes to CAEO microcapsules' ability to inhibit Staphylococcus aureus and Escherichia coli. This distinction suggests that pure essential oils are less potent and have shorter release durations when it comes to antibacterial activity than microcapsules. The main rationale might be that the CD wall material can both efficiently reduce the pace at which the core material volatilises and continuously release a certain amount of essential oil, which will impede the development of the experimental strain to varying degrees.

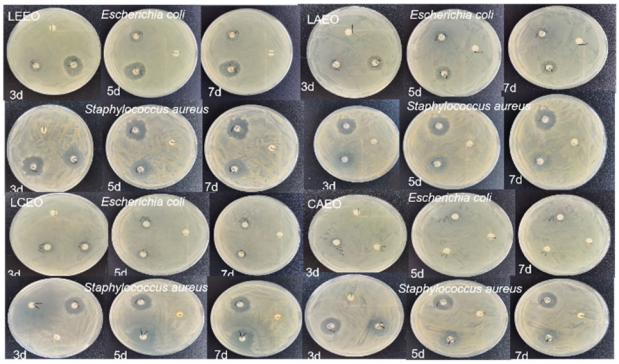

Figure 1. The effect of different sustained release times of four essential oils and inclusion complexes on the growth of tested microorganisms .(1 blank control group, 2 essential oil group, 3 microcapsule group)

3.2 Identifying the volatile curve of essential oil microcapsules

The temperature of the surrounding environment has a major impact on how well essential oil microcapsules operate for extended release. Figure 2 displays the essential oil's and its microcapsules' volatilisation curves at 37℃ and 65℃.The quick release stage, which is kept for seven days, correlates with the first stage. The four essential oils and the microcapsules release more than 80% and 60%, respectively, under 37 °C and more than 85% and 65% at 65 °C. The second stage (7–11 days) is characterized by a comparatively sluggish release rate, or maybe more accurately, a stable condition of essential oil release. Figure 6 illustrates the fact that the volatilisation rate in microcapsules that is less than that of essential oils, which may be due to the fact that microcapsule cavity prevents essential oils from escaping into the solvent. The main way that essential oils are released is through diffusion into the polymer matrix, which also forces materials with tight walls to expand and break down. Microcapsules reduce the amount of surface area that essential oils have in contact with the external environment by encapsulating them inside the wall material cavity[9] .

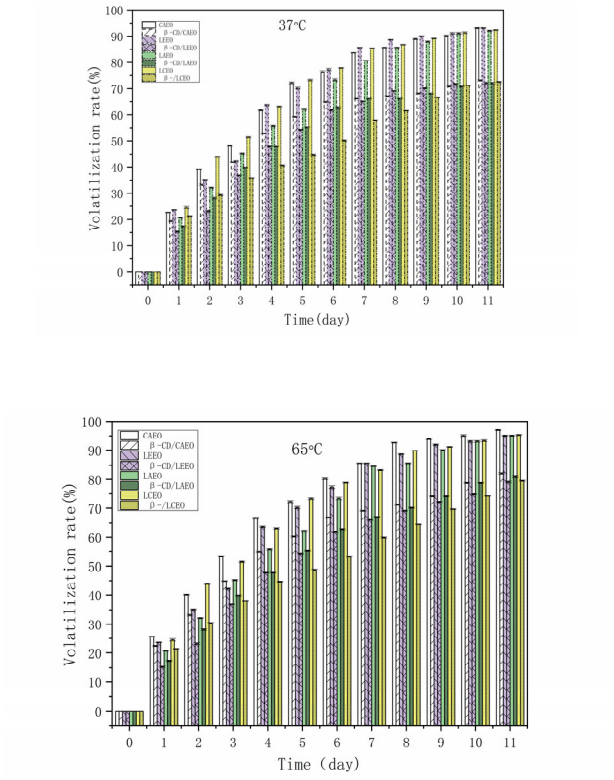

Figure 2 Volatilization curve of microcapsules at 37 and 65℃

3.3 Kinetics of essential oil microcapsules' release

The essential oil microcapsules' release kinetics were investigated using two different temperatures: 37 ℃ for the human body and 65 ℃ for food processing (Table 1). The essential oil release rate of each of the four microcapsules at 65 ℃ varies significantly ($p < 0.05$). At different temperatures, the essential oil release rate curve most nearly approaches the Peppas equation ($R^2 \geqslant 0.95$). The pace at which essential oils release is impacted by temperature changes without changing the release mechanism. N = 0.85 indicating zero-order release kinetics (Case of II transport) [8][9], n > 0.89 "supraII" type immigration [10], 0.43<n<0.85 unusual or non Fickian transport, and n ⩽ 0.43 is Fickian diffusion (the case I transport) are the categories into which the Peppas equation can classify the release mechanism according to its diffusion the index "n" of microcapsules. With n<0.43 at 65 ° C, this suggests that Fickian diffusion is taking place in the four essential oil microcapsule systems. A crucial element that isn't directly connected with wall material concentration is diffusion temperature. The main cause of the release rate is diffusion, which may be brought on by insufficient swelling and droplets of oil on the microcapsules' surface or outer layer. At 37 ° C, non-Fickian transport pathways are demonstrated by the four essential oil microcapsule systems. The unique non-Fickian expansions and diffusion transport processes that control the release rate are regulated by diffusion swelling. The release of essential oils is controlled by the expanding of biopolymers and the movement of oil through the matrix of biopolymer [11]. The temperature affects both the rate and the mode of release of essential oils in microcapsules. The rate and amount of essential oil release in microcapsules are directly correlated with temperature. The volatilisation curve's fitting results are consistent with the Higuch equation, the Peppas equation, and the first-order kinetic equation. At the same time, the Peppas equation has been more closely followed at cooler temperatures and the initial-order kinetic equation is more carefully followed through the release phase at higher temperatures.

Table.1 Kinetic release parameters of essential oil microcapsules at 37 and 65℃

Kinetic equation	65℃				37℃			
	β-CD/CAEO	β-CD /LCEO	β-CD /LEEO	β-CD /LAEO	β-CD/CAEO	β-CD /LCEO	β-CD /LEEO	β-CD /LAEO
Zero order release kinetics:Q=kt	Q=0.51t+21.68 R^2=0.79	Q=0.50t+25.16 R^2=0.75	Q=0.50t+22.65 R^2=0.76	Q=0.51t+23.14 R^2=0.78	Q=0.47t+10.53 R^2=0.91	Q=0.46t+10.34 R^2=0.91	Q=0.45t+11.44 R^2=0.90	Q=0.49t+12.43 R^2=0.91
First-order kinetic equation:$Q=1-etp^{(-kt)}$	Q=59.34(1-$etp^{-0.72t}$) R^2=0.88	Q=58.01(1-$etp^{-2.48t}$) R^2=0.87	Q=57.74(1-$etp^{-0.68t}$) R^2=0.86	Q=57.91(1-$etp^{-2.28t}$) R^2=0.87	Q=45.12(1-$etp^{-0.87t}$) R^2=0.78	Q=42.76(1-$etp^{-0.83t}$) R^2=0.80	Q=47.32(1-$etp^{-0.87t}$) R^2=0.78	Q=43.26(1-$etp^{-0.83t}$) R^2=0.80
Higuchi equation :$Q=kt^{0.5}$	$Q=6.87t^{0.5}$+3.62 R^2=0.94	$Q=6.84t^{0.5}$+6.64 R^2=0.94	$Q=6.91t^{0.5}$+3.17 R^2=0.93	$Q=6.69t^{0.5}$+6.28 R^2=0.94	$Q=5.87t^{0.5}$-2.12 R^2=0.97	$Q=6.15t^{0.5}$-3.21 R^2=0.99	$Q=5.71t^{0.5}$-2.32 R^2=0.98	$Q=6.25t^{0.5}$-2.51 R^2=0.99
Peppas equation :$Q=kt^n$	$Q=11.88t^{0.39}$-1.96 R^2=0.96	$Q=14.81t^{0.35}$-1.70 R^2=0.96	$Q=10.98t^{0.29}$-1.38 R^2=0.96	$Q=13.871t^{0.29}$-1.67R^2=0.96	$Q=5.24t^{0.52}$-1.43 R^2=0.98	$Q=5.27t^{0.53}$-1.43 R^2=0.99	$Q=5.10t^{0.52}$-1.51 R^2=0.98	$Q=5.33t^{0.53}$-1.27 R^2=0.99

4. Conclusions

Antibacterial experiments have shown that essential oils and their microcapsules have strong and broad-spectrum inhibitory effects on the tested strains. Microcapsule release studies have shown that β-CD is a suitable wall material for CAEO release and prolonging the inhibition period of strains. The release kinetics of microcapsules at different temperatures should be evaluated taking into account the effect of temperature on the rate and

mechanism of essential oil release. Considering the previously described results, it can be concluded that the use of β-CD improved the essential oils' regulated release, thermal stability and antimicrobial while also successfully creating microcapsules.

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Conflict of interest statement

There are not any conflicts of interest disclosed by the authors.

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