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Original Research Article

Optimization of Preparation Process and Sustained-Release Properties of A-Terpineol Microcapsules

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Abstract: To overcome the limitations associated with the high volatility, pungent odor, and restricted application of α-terpineol in food packaging, α-terpineol-loaded microcapsules were prepared using the ionic gelation method, with α-terpineol as the core material and chitosan (CS) as the wall material. Based on single-factor experiments, the preparation process was further optimized using response surface methodology. The results demonstrated that the mass ratio of CS to α-terpineol, the mass ratio of CS to sodium tripolyphosphate (TPP), and the pH of the chitosan solution significantly affected the encapsulation efficiency, with the degree of influence ranked as follows: CS: α-terpineol > CS: TPP > pH. The optimal preparation conditions were determined as follows: CS: α-terpineol ratio of 1:0.75, CS:TPP ratio of 2.2:1, and pH 4.9, under which the encapsulation efficiency reached 73.85%. Under these optimized conditions, the microcapsules exhibited superior sustained-release behavior in phosphate buffer solution (pH 7.4), with a cumulative release rate of only 35.8% after 312 h. The developed microencapsulation system effectively retarded the diffusion rate of α-terpineol, providing an important basis for its broader application in food packaging systems.
Keyword: α-terpineol; microcapsules; response surface methodology; sustained-release performance.

INTRODUCTION

α-Terpineol is a naturally occurring monocyclic monoterpene tertiary alcohol widely distributed in various plant species, including sweet orange, mango, and mint (Liu *et al.,* 2026). Previous studies have demonstrated that α-terpineol possesses broad-spectrum antimicrobial activity against a variety of bacteria and fungi (Kong *et al.,* 2019), highlighting its considerable potential for industrial applications. Consequently, it has been extensively used as a fragrance component in perfumes, cosmetics, and food products (Jing, Tao, Jia, & Zhou, 2015) However, the practical application of α-terpineol in food packaging is severely limited by its high volatility, pungent odor, and poor water solubility (Garcia *et al.,* 2024). Therefore, the development of effective encapsulation strategies to improve the stability and applicability of α-terpineol has become a critical issue in the field of active food packaging.

Microencapsulation technology, as an important functional encapsulation approach, enables the incorporation of active substances (core materials) into natural or synthetic polymeric matrices (wall materials) to form microcapsules with core–shell structures(Chaudhari *et al.,* 2020). The formation of microcapsules essentially involves the construction of a functional barrier between the core and wall materials, thereby protecting the encapsulated compounds from degradation caused by harsh environmental factors such as light, oxygen, and chemical agents, while reducing the loss of bioactivity(Yu *et al.,* 2024). Under specific conditions, the wall material can further regulate the sustained release of the core material through controlled-release mechanisms, thereby prolonging the activity of bioactive molecules and achieving long-term preservation or targeted delivery(Calvo, Castaño, Hernández, & González-Gómez, 2011; Z. Zhang *et al.,* 2025). Therefore, microencapsulation is considered an effective strategy for protecting thermosensitive bioactive compounds such as α-terpineol. At present, the commonly used methods for microcapsule preparation mainly include spray drying, complex coacervation, and ionic gelation. Among these approaches, ionic gelation has attracted considerable attention due to its significant advantages, including operational simplicity, mild reaction conditions, the absence of high-temperature treatment, and low toxicity. Consequently, it has been

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widely applied in the fields of drug delivery, food preservation, and encapsulation of functional ingredients(Fitri, Sri, Aprilia, & Supriyadi, 2022).

Based on the above considerations, α-terpineol-loaded microcapsules were prepared in this study using α-terpineol as the core material and chitosan as the wall material via the ionic gelation method. The preparation process was optimized through single-factor experiments combined with response surface methodology, and the sustained-release properties of the obtained microcapsules were further investigated. This study provides theoretical support and a technical basis for the application of α-terpineol microcapsules in the food field.

1. MATERIALS AND METHODS

1.1 Materials and Reagents

α-Terpineol, chitosan, and 2.5% glutaraldehyde fixative solution were purchased from Adamas Reagent Co., Ltd.; sodium tripolyphosphate (TPP), Tween 80, and glacial acetic acid were obtained from Macklin Biochemical Co., Ltd**.**

1.2 Instrumentation

An adjustable high-speed homogenizer was supplied by Hangzhou Jingfei Instrument Technology Co., Ltd.; a high-speed refrigerated benchtop centrifuge was purchased from Shanghai Luxiangyi Centrifuge Instrument Co., Ltd.; a heating magnetic stirrer was obtained from Shenzhen Dingxinyi Experimental Equipment Co., Ltd.; a UV–visible spectrophotometer was provided by Shimadzu Corporation; and a vacuum freeze dryer was purchased from Ningbo Scientz Biotechnology Co., Ltd.

1.3 Preparation of α-Terpineol-Loaded Chitosan Microcapsules

The preparation was conducted according to the method of (Chaudhari *et al.,* 2020) with appropriate modifications. An appropriate amount of chitosan (CS) was dissolved in 1% (v/v) acetic acid solution under magnetic stirring at room temperature overnight to obtain a 1% (w/v) CS solution, and the pH was adjusted using 2 M NaOH. Subsequently, 1% (v/v) Tween 80 was added to the CS solution, followed by magnetic stirring at 45 °C for 2 h. α-Terpineol was dissolved in 2 mL of anhydrous ethanol at a predetermined ratio and then added to 20 mL of the CS mixture, followed by homogenization in an ice bath for 10 min. Subsequently, an appropriate volume of 0.5% (w/v) sodium tripolyphosphate (TPP) solution was added dropwise according to the CS/TPP mass ratio, and the reaction was allowed to proceed for 45 min. The suspension was centrifuged at 8000 rpm for 30 min at 4 °C. The supernatant was discarded, and the precipitate was washed three times with anhydrous ethanol. The precipitate was resuspended in deionized water, frozen at −80 °C overnight, and then lyophilized to obtain powdered microcapsules. The obtained microcapsules were sealed and stored at 4 °C for subsequent controlled-release analysis.

1.4 Determination of Encapsulation Efficiency of α-Terpineol-Loaded Chitosan Microcapsules

A volume of 10 μL of α-terpineol was transferred into a 10 mL volumetric flask and diluted to volume with anhydrous ethanol, followed by thorough mixing. Aliquots of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the prepared solution were further diluted to 10 mL with anhydrous ethanol to obtain a series of standard solutions. The absorbance was measured at 204 nm using a UV–Vis spectrophotometer, and a calibration curve was constructed. The content of α-terpineol encapsulated in the microcapsules was determined according to the method of (Yousefi, Khorshidian, Mortazavian, & Khosravi-Darani, 2019)with slight modifications. Briefly, an appropriate amount of microcapsules was ultrasonically extracted with anhydrous ethanol, followed by centrifugation at 8000 rpm for 15 min at 4 °C. The absorbance of the supernatant was measured at 204 nm, and the encapsulated α-terpineol content was calculated based on the calibration curve. The encapsulation efficiency (EE, %) was calculated using Equation (1)：

$$\text{EE}\ (\%) = \frac{\text{encapsulated α-terpineol content}}{\text{total α-terpineol added}} \times 100\% \quad (1)$$

1.5 Preliminary Single-Factor Experiments for Optimization of Microcapsule Preparation

Encapsulation efficiency (EE) was used as the evaluation index, and single-factor experiments were conducted to investigate the effects of the CS: α-terpineol (w/w), CS: TPP (w/w), and CS pH on the encapsulation efficiency of the microcapsules. In each experiment, only one factor was varied while the other conditions were kept constant.

Table 1: Single-factor test level

Factors	Levels				
CS: α-terpineol (w/w)	1: 0.4	1: 0.6	1: 0.8	1: 1	1:1. 2
CS: TPP (w/w)	1.25: 1	2.5: 1	3.75: 1	5: 1	6.25: 1
CS pH	3.8	4.2	4.6	5	5.4

1.6 Box–Behnken Design (BBD) for Optimization of α-Terpineol-Loaded Chitosan Microcapsules

To determine the optimal encapsulation conditions for the microcapsules, a Box–Behnken design (BBD) was employed based on the results of single-factor experiments. The factor levels used in the response surface experiments are presented in Table 2.

Table 2 Factors and levels of response surface methodology

Factors	Levels		
	-1	0	1
A CS: α-terpineol (w/w)	1: 0.6	1: 0.8	1: 1
B CS: TPP (w/w)	1.25: 1	2.5: 1	3.75: 1
C CS pH	4.2	4.6	5

1.7 Validation Experiment

Based on the optimal encapsulation conditions obtained from the response surface methodology (RSM), validation experiments were conducted. The experimental values were compared with the predicted values to evaluate the validity and reliability of the model.

1.7 Determination of Controlled Release Behavior of Microcapsules

Referring to the method reported by (Keawchaoon & Yoksan, 2011) with slight modifications, 100 mg of freeze-dried microcapsule powder was dispersed separately in 20 mL of phosphate buffer solution (pH 7.4) and acetate buffer solution (pH 3.0). The buffer systems were incubated at 25 °C. At predetermined time intervals, the samples were centrifuged at 8000 rpm for 10 min at 4 °C, and a certain volume of the supernatant was collected for absorbance measurement at 204 nm. An equal volume of fresh buffer solution was subsequently added to maintain a constant release medium volume. The cumulative release rate of α-terpineol at each time interval was then calculated.

$$\text{Release rate}(\%)=\frac{M_1}{M_0}\times 100\% \quad (2)$$

where M_1 represents the amount of α-terpineol released at each time interval (mg), and M_0 represents the total encapsulated amount of α-terpineol in the microcapsules (mg).

1.7 Statistical analysis

All experiments were conducted in triplicate. Experimental data were organized and statistically processed using Microsoft Excel. One-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics 26 software. Data visualization and graphical analysis were conducted using Origin 2021, while response surface analysis was carried out using Design-Expert 13.0 software.

2. RESULTS AND DISCUSSION

2.1 Analysis of Single-Factor Experiments

2.1.1 Effect of Chitosan (CS) to α-Terpineol (w/w) Ratio on Encapsulation Efficiency

As shown in Fig. 1(a), the CS: α-terpineol (w/w) ratio had a significant effect on the encapsulation efficiency of α-terpineol. When the mass ratio increased from 1:0.4 to 1:0.8, the encapsulation efficiency increased from 64.38% to 73.63% ($P < 0.05$), indicating that an appropriate increase in core material content can enhance encapsulation performance. This improvement may be attributed to enhanced interactions between the wall and core materials, resulting in more effective incorporation of active compounds within the microcapsule matrix (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). However, when the mass ratio exceeded 1:0.8, encapsulation efficiency decreased significantly due to the limited encapsulation capacity of the wall material, which hindered the formation of a compact coating structure and led to leakage of free α-terpineol. Therefore, a CS: α-terpineol ratio of 1:0.8 was considered optimal for subsequent experiments(Zhang, Zhu, Yan, & Li, 2025).

2.1.2 Effect of Chitosan (CS) to Tripolyphosphate (TPP) Ratio on Encapsulation Efficiency of α-Terpineol Microcapsules

As shown in Fig. 1(b), the CS: TPP (w/w) ratio had a significant effect on the encapsulation efficiency of α-terpineol. When the CS: TPP ratio increased from 1.25: 1 to 2.5: 1, the encapsulation efficiency increased from 68.64% to 73.47% ($P < 0.05$), indicating that an appropriate increase in TPP content enhances encapsulation performance. This improvement may be attributed to ionic crosslinking between TPP and amino groups in chitosan, leading to the formation of a dense and stable three-dimensional network structure that enhances core material entrapment. However, excessive CS:TPP ratios resulted in insufficient crosslinking, preventing the formation of a stable network and thereby reducing encapsulation efficiency (Sruthi, M, M, & R, 2018; Yan, Yuhang, & Yakov, 2015). Therefore, a CS: TPP ratio of 2.5:1 was selected as the optimal preparation condition.

2.1.3 Effect of Chitosan Solution pH on the Encapsulation Efficiency of α-Terpineol Microcapsules

As shown in Fig. 1(c), the pH of the chitosan (CS) solution had a significant effect on the encapsulation efficiency of microcapsules. When the pH increased from 3.8 to 5.4, the encapsulation efficiency gradually increased from 67.33% to 76.27%, with significant differences among treatments ($P < 0.05$),

indicating that an appropriate increase in pH can enhance encapsulation performance. This improvement may be attributed to the protonation behavior of amino groups in chitosan under acidic conditions. At lower pH, strong electrostatic repulsion between protonated polymer chains hinders the formation of a compact crosslinked network structure(Sacco *et al.,* 2016). As the pH increases, the degree of protonation decreases, reducing electrostatic repulsion and promoting ionic crosslinking between chitosan and TPP. This results in a more stable and dense microcapsule structure, thereby improving encapsulation efficiency (Wongput, Liou, Yang, & Hung, 2026). However, when the pH exceeded 5.0, obvious phase separation of the prepared microcapsules was observed after short-term standing. Therefore, under the present experimental conditions, pH 5.0 was selected as the optimal value for maximum encapsulation efficiency.

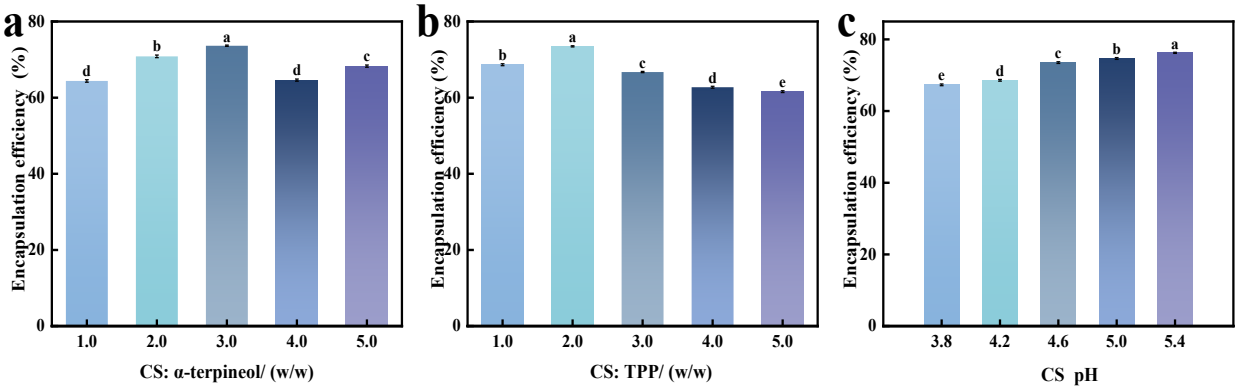

Fig. 1: The influence of different factors on the encapsulation efficiency
Note: a, CS: α-terpineol (w/w); b, CS: TPP (w/w); c, pH of CS solution.

2.2 Optimization of α-Terpineol Microcapsule Preparation via Response Surface Methodology Based on Ionic Gelation

2.2.1 Establishment and Statistical Analysis of the Quadratic Response Surface Regression Model

The results of the response surface design are shown in Table 3. A total of 17 experimental runs were analyzed using Design-Expert 13.0 software, and a quadratic polynomial regression model describing the relationship between the response variable and independent factors was established as follows: $Y = 73.32 - 1.34\,A - 0.4787\,B + 0.75\,C + 0.8325\,AB - 0.155\,AC - 0.475\,BC - 3.57\,A^2 - 2.88\,B^2 - 1.22\,C^2$. The significance test and analysis of variance (ANOVA) for the regression model are presented in Table 4.

Table 3: Design and results for response surface experimental

Run order	Factors			Encapsulation efficiency /%
	A（CS: α-terpineol）	B（CS: TPP）	C（CS pH）	
1	1:0.8	1.25:1	4.2	68.65
2	1:0.6	2.5:1	5	70.27
3	1:0.8	2.5:1	4.6	73.43
4	1:0.8	1.25:1	5	71.56
5	1:0.6	3.75:1	4.2	67.04
6	1:1	3.75:1	4.6	65.47
7	1:0.6	2.5:1	4.6	72.98
8	1:0.6	3.75:1	4.2	67.84
9	1:0.6	2.5:1	4.6	73.59
10	1:1	2.5:1	4.2	67.10
11	1:1	2.5:1	4.6	73.55
12	1:1	3.75:1	5	68.85
13	1:1	2.5:1	5	67.83
14	1:0.8	2.5:1	4.6	73.07
15	1:0.6	1.25:1	4.6	69.94
16	1:1	1.25:1	4.6	65.04
17	1:0.6	2.5:1	4.2	68.92

Table 4: Analysis of response surface regression equation

Source	DF	S	MS	F-value	F-value	Significance level
Model	131.17	9	14.57	68.59	<0.0001	**
A	14.39	1	14.39	67.73	<0.0001	**
B	4.49	1	4.49	21.11	0.0025	**
C	4.50	1	4.50	21.18	0.0025	**
AB	2.77	1	2.77	13.05	0.0086	**
AC	0.0961	1	0.0961	0.4523	0.5228	NS
BC	0.9025	1	0.9025	4.25	0.0783	NS
A^2	53.76	1	53.76	253.00	<0.0001	**
B^2	34.88	1	34.88	164.15	<0.0001	**
C^2	6.27	1	6.27	29.53	0.0010	**
Residual	1.49	7	0.2125			
Lack of fit	1.17	3	0.3905	4.94	0.0783	NS
Pure error	0.3159	4	0.0790			
Total	132.66	16				
R^2	0.9888					
Adj R^2	0.9744					
Lack of fit value	1.17					

Note: * indicates a significant difference ($P < 0.05$), and ** indicates an extremely significant difference ($P < 0.01$)

The results showed that the model F-value was 68.59 ($P < 0.0001$), indicating that the regression model was highly significant and could reliably describe and predict the response variable based on the experimental data (Andrade *et al.,* 2024). The coefficient of determination (R^2) was 0.9888, indicating a high goodness-of-fit of the model. The adjusted R^2 (Adj R^2) was 0.9744, which was close to R^2, suggesting that 97.44% of the variation in encapsulation efficiency could be explained by the model. In addition, A, B, C, AB, A^2, B^2, and C^2 had extremely significant effects on encapsulation efficiency ($P < 0.01$). Based on the comparison of F-values, the influence of factors on encapsulation efficiency followed the order: CS: α-terpineol > CS: TPP > CS solution pH. The interaction effects followed the order: AB > BC > AC.

2.2.2 Evaluation of Interaction Effects among Independent Variables in the RSM Model

The interaction effects of different factors on encapsulation efficiency are shown in Fig. 2. The response surface plots and contour plots visually illustrate the interactions among variables and their influence on encapsulation efficiency (A, Gizem, Hande, Esra, & Neşe, 2023). For the interaction between A and B, the response surface exhibited a pronounced convex shape, and the contour plot showed an elliptical distribution, indicating a significant interaction between the two factors ($P < 0.01$). For the interactions between A and C as well as B and C, the response surfaces showed noticeable curvature, whereas the contour plots were nearly circular or slightly elliptical. Combined with the ANOVA results, these patterns were mainly attributed to significant quadratic effects rather than true interaction effects, suggesting that the response was primarily governed by individual factors (Yang, Zheng, & Chen, 2025).

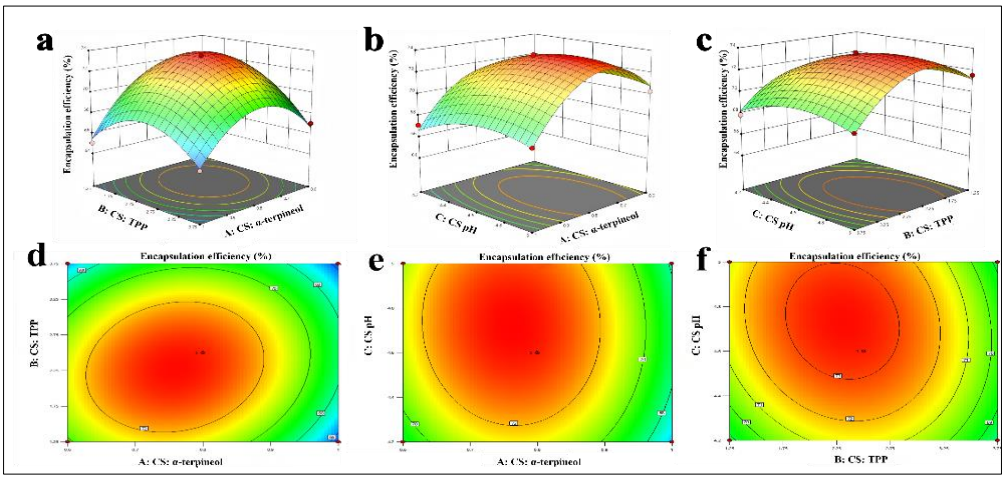

Fig. 2: Effect of different factor interactions on encapsulation efficiency

Note: a, 3D response surface plot of the interaction between factors A and B; b, 3D response surface plot of the interaction between factors A and C; C, 3D response surface plot of the interaction between factors B and C; D, 2D contour plot of the interaction between factors A and B; e, 2D contour plot of the interaction between factors A and C; f, 2D contour plot of the interaction between factors B and C.

2.2.3 Model Validation Experiment

Based on model analysis, the optimal encapsulation conditions were determined as CS: α-terpineol = 1:0.7533, CS: TPP = 2.2067: 1, and CS pH = 4.9428, with a predicted encapsulation efficiency of 73.38%. Considering practical operability, the conditions were adjusted to CS: α-terpineol = 1: 0.75, CS: TPP = 2.2: 1, and CS pH = 4.9. Under these optimized conditions, the experimentally measured encapsulation efficiency was 73.85%, which closely matched the predicted value. The low deviation between experimental and predicted results confirmed the reliability of the response surface model and its suitability for optimizing the preparation conditions of α-terpineol microcapsules.

2.3 Controlled Release Behavior of α-Terpineol Microcapsules

The release profiles of α-terpineol-loaded chitosan microcapsules (α-TCSM) in phosphate buffer solution (pH 7.4) and acetate buffer solution (pH 3.0) are shown in Figure 3. As illustrated in the figure, the release behavior of α-terpineol could be divided into two distinct stages. The first stage was characterized by a rapid burst release. Specifically, the release rate of α-TCSM in acetate buffer solution (pH 3.0) reached 49.98% within the first 24 h, whereas that in phosphate buffer solution (pH 7.4) was 35.8%. This phenomenon may be attributed to the instantaneous diffusion of unencapsulated α-terpineol adsorbed on the surface of the microcapsules (Keawchaoon & Yoksan, 2011). After 24 h, the microcapsules gradually entered a sustained and slow-release stage, eventually reaching a relatively stable state. The release of the core material from the microcapsules mainly depended on the concentration gradient between the internal and external media. With increasing storage time, the amount of α-terpineol remaining inside the microcapsules gradually decreased, resulting in a reduced concentration gradient in the buffer system and consequently a slower release behavior (Luo, Zhang, Whent, Yu, & Wang, 2011).Meanwhile, the release rate of α-TCSM was closely associated with the pH of the release medium. According to the experimental results, the release rate of α-TCSM in acetate buffer solution (pH 3.0) was significantly higher than that in phosphate buffer solution (pH 7.4). This phenomenon may be related to the structural characteristics of chitosan. Under acidic conditions, the amino groups on the chitosan molecular chains become protonated, generating electrostatic repulsion among similarly charged chains, which leads to swelling and loosening of the microcapsule network structure and thereby accelerates the outward diffusion of encapsulated α-terpineol (Li *et al.,* 2025). In contrast, under the neutral condition of pH 7.4, the amino groups of chitosan are deprotonated, strengthening intermolecular interactions and resulting in a more compact and dense network structure, which suppresses the release rate of α-terpineol (Shikuku *et al.,* 2024).

These results indicated that the prepared microcapsules exhibited a faster release behavior under acidic conditions, whereas a superior sustained-release effect was observed under neutral conditions.

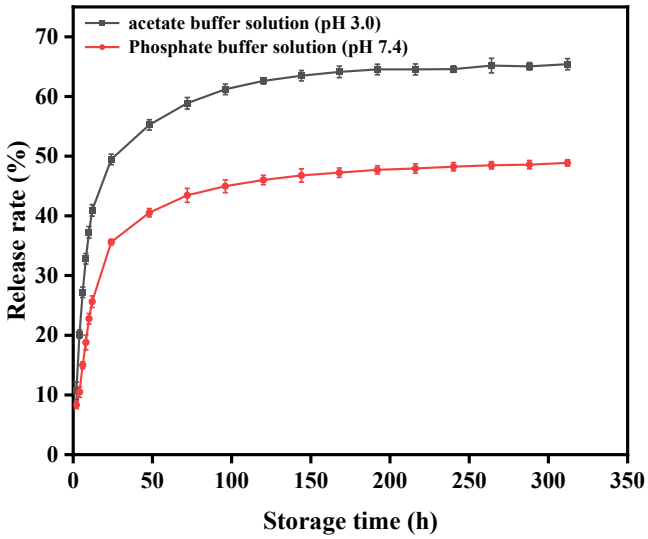

Fig. 3: Release rate of α-TCSM in different buffer solutions

3. CONCLUSIONS

In this study, an efficient sustained-release system for α-terpineol microcapsules was successfully developed through process optimization. The results demonstrated that the optimal preparation conditions obtained by response surface methodology were as follows: CS: α-terpineol (w/w) ratio of 1: 0.75, CS: TPP (w/w) ratio of 2.2: 1, and CS pH of 4.9. Under these conditions, the encapsulation efficiency of α-terpineol reached 73.85%. Sustained-release studies further revealed that the prepared microcapsules exhibited continuous release behavior over 312 h in both phosphate buffer solution (pH 7.4) and acetate buffer solution (pH 3.0). Moreover, the microcapsules showed superior sustained-release performance in phosphate buffer

solution (pH 7.4), indicating enhanced stability and controlled-release capability under neutral conditions. These findings provide a theoretical basis and technical support for the development of active food packaging systems with improved thermal stability and controllable sustained-release properties.

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