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Optimised formulation and characterisation of oregano essential oil edible composite films by response surface methodology

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Electronic Supplementary Material

Supplementary Tables S1–S4
Supplementary Figures S1–S2

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Table S1. Consistency inspection results

Maximum eigenvalue	Consistency index value	Random index value	Consistency ratio value	Consistency test result
5.378	0.095	1.120	0.084	passed

Consistency inspection results. Our analytic hierarchy process (AHP) model's consistency was evaluated using the consistency index and ratio. The consistency index was 0.095, not equal to 0, while the consistency ratio was 0.084, below the acceptable threshold of 0.1 (Table S1). These findings confirm the matrix's adherence to the consistency test, indicating the reasonableness and effectiveness of the weight coefficients.

Film thickness. Five points on the film were randomly selected, and the average values were calculated to determine the thickness. The addition of oregano essential oil (OEO) increased film thickness compared to the control film, although the difference was not statistically significant ($P > 0.05$).

Wavelength selection. Accurately weigh 0.1000 g of carvacrol in a 100 mL volumetric flask, dilute it to the mark with 95% (*v*/*v*) ethanol, and shake thoroughly to obtain a 1 mg·mL^{-1} carvacrol solution. Transfer 4 mL of the 1 mg·mL^{-1} carvacrol solution into a 10 mL volumetric flask, add 95% (*v*/*v*) ethanol, and shake well to achieve a 0.4 mg·mL^{-1} carvacrol solution. Take 1 mL of the 0.4 mg·mL^{-1} mg·mL^{-1} carvacrol solution into a 10 mL volumetric flask, dilute it to the mark with 95% (*v*/*v*) ethanol, and shake well to prepare a 40 μg·mL^{-1} carvacrol solution. Using 95% (*v*/*v*) ethanol as the blank control, determine the absorbance of the solution in the wavelength range of 200 to 400 nm. The maximum absorption of carvacrol is observed at λ = 210 nm (Figure S1). However, to mitigate the influence of interference factors such as end absorption, λ = 276 nm is ultimately selected for subsequent experiments' measurement wavelength.

Drawing of standard curve of carvacrol and precision test. Accurately weigh 1.0078 g of carvacrol into a 100 mL volumetric flask, dilute it with 95% (*v*/*v*) ethanol, and shake thoroughly to achieve a 10 mg·mL^{-1} carvacrol solution. Various volumes of the carvacrol solution (50, 75, 100, 125, 150, 175, and 200 μL) were transferred into 25 mL volumetric flasks, filled with 95% (*v*/*v*) ethanol, and thoroughly shaken to prepare a series of concentrations (20, 30, 40, 50, 60, 70, 80 μg·mL^{-1}). The absorbance of 95% (*v*/*v*) ethanol was measured at λ = 276 nm, and the experimental data were recorded. Additionally, the absorbance of the 30 μg·mL^{-1} carvacrol solution was measured at the same wavelength six times to assess instrument precision. The results indicated an average absorbance of 0.309 with an RSD of 0.78%, reflecting excellent precision. A standard curve for the carvacrol solution was plotted using absorbance (*A*) as the vertical coordinate and concentration (*C*) as the horizontal coordinate. The linear regression equation and correlation coefficients were determined as $A = 0.0104C + 0.0049$ ($R^2 = 0.9998$). The results, depicted in Figure S2, demonstrate a robust linear relationship for carvacrol within the concentration range of 20.00 to 80.00 μg·mL^{-1} in the standard solution.

Repeatability test. From the same batch of temporarily prepared composite films, six portions were cut into 2 × 2 cm^2 pieces each. The absorbance of the prepared solution was measured, and then the linear regression equation was applied to calculate the average carvacrol content and relative standard deviation (RSD) values. The results in Table S2 indicate that the

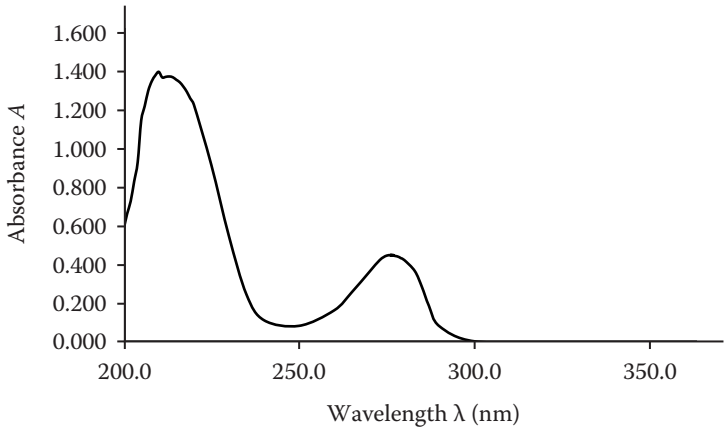

Figure S1. Ultraviolet absorption spectrum of carvacrol

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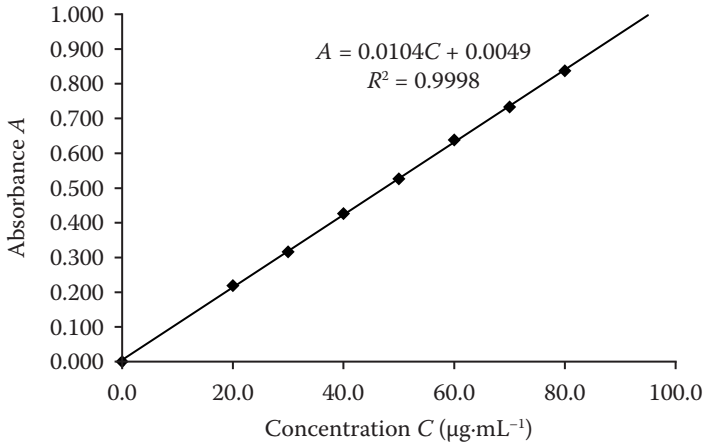

Figure S2. Standard curve of carvacrol

average carvacrol content is 2.88 mg, with RSD values of 1.64%, demonstrating good repeatability.

Recovery test. Six portions of the temporarily prepared composite films were combined to form a sample solution. After filtering, the initial filtrate was discarded, and the refill filtrate was retained for absorbance measurement at the wavelength λ = 276 nm. Subsequently, 5 mL of the refill filtrate was transferred into a 10 mL volumetric bottle and set aside. Accurately weighing 0.9989 g of carvacrol into a 100 mL volumetric flask, 95% (*v*/*v*) ethanol was added and thoroughly shaken to prepare a 10 mg·mL^{-1} carvacrol solution. Using a pipette gun, 75 μL of this solution was drawn into a 25 mL volumetric flask, and 95% (*v*/*v*) ethanol was added to the mark, followed by thorough shaking. The control solution was prepared at a concentration of 30 μg·mL^{-1}, and 5 mL of the control solution was mixed to the 10 mL above the volumetric bottle, and its absorbance was measured. The regression equation was then applied to calculate carvacrol's average content and RSD. The results in Table S3 demonstrate an average recovery of 100.32% and RSD of 1.69%, indicating good recovery.

Stability test. Triplicate copies of the prepared films were exposed to air and stored away from light for different durations (0, 3, 6, 9, 12, and 24 h). The sample solution was prepared, and the absorbance was measured at λ = 276 nm. The content of carvacrol and RSD were obtained by substituting them into the linear regression equation. As shown in Table S4, the results indicate that the CS/PVA composite OEO film exhibits good stability.

Supplementary data for the response surface methodology (RSM) experiments.

Table S2. Repeatability test of CS/PVA composite oregano essential oil film

Number	Absorbance (*A*)	Carvacrol content (mg per film)	Average content (mg per film)	RSD (%)
1	0.613	2.924	2.880	1.640
2	0.591	2.818		
3	0.616	2.938		
4	0.600	2.861		
5	0.596	2.842		
6	0.607	2.895		

CS – chitosan; PVA – polyvinyl alcohol; RSD – relative standard deviation

Table S3. Recovery test of CS/PVA composite oregano essential oil film

Sample size (μg·mL^{-1})	Reference quantity (μg·mL^{-1})	Measured quantity (μg·mL^{-1})	Recovery rate (%)	Average recovery rate (%)	RSD (%)
50.01	30.00	80.30	100.97	100.32	1.69
63.47	30.00	93.95	101.60		
40.97	30.00	70.49	98.40		

CS – chitosan; PVA – polyvinyl alcohol; RSD – relative standard deviation

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Table S4. Stability test of CS/PVA composite oregano essential oil film

Number	Content (mg·film^{-1})						RSD (%)
	after 0 h	after 3 h	after 6 h	after 9 h	after 12 h	after 24 h	
1	2.69	2.73	2.74	2.87	2.66	2.72	2.64
2	2.54	2.56	2.65	2.53	2.43	2.50	2.85
3	2.61	2.65	2.55	2.70	2.54	2.60	2.32

CS – chitosan; PVA – polyvinyl alcohol; RSD – relative standard deviation