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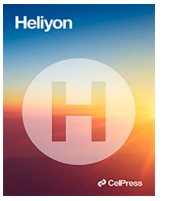

Research article

Preparation of *Perilla frutescens* L. essential oil hydrogel beads and preservation application research in strawberry

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ABSTRACT

Perilla frutescens L. essential oil (PLEO) has antibacterial and antioxidant properties, which can effectively maintain the quality of fruits and extend their shelf life. In this study, sodium alginate and chitosan were used as wall materials, and PLEO microcapsule powder was used as the core materials to prepare PLEO hydrogel beads. The best results were obtained by using 2%w/v sodium alginate and 1.5%w/v chitosan as wall materials, with a core-to-wall ratio of 2:1 and homogenized for 15 min producing PLEO hydrogel beads with encapsulation efficiency of 82.61 %. For strawberries preservation, PLEO hydrogel beads preservation group had a better effect after 5 d of storage, showing a lower decay rate (15.71 %), better maintaining the hardness of 1.75 kg/cm^2, and a weight loss of 3.29 %. Furthermore, organic acids and total phenols were retained more in this group, the number of microorganisms was significantly reduced, and sensory qualities were improved, especially taste and color. This study provides important insights into the application of natural preservatives in the food industry and promotes sustainable practices in food preservation.

1. Introduction

Plant essential oils (PEOs) are aromatic substances extracted from leaves, flowers, seeds and fruits of plants [1]. The chemical composition of PEOs is extremely complex and usually contains a variety of different compounds [2], such as terpenes, aldehydes, ketones, phenols and ethers. These components give the unique aroma and properties of essential oils determine their use in food, cosmetics [3], and medicine [4]. Especially in the field of food and fruit preservation, essential oils are widely used because of their antimicrobial activity, which helps to extend the shelf life of food and reduce microorganisms spoilage [5]. For example, rose is used to preserve minced beef, extending its shelf life to 6 d [6]. Ginger essential oil and green tea extract were used to preserve strawberry, and the shelf life was extended to 4 d and 8 d under normal temperature and refrigeration conditions [7]. *Monarda didyma* L. essential oil is used to preserve blueberries after harvest and shows excellent antibacterial activity [8].

Perilla frutescens L. is an annual plant widely utilized in food and medicine in China, celebrated for its antioxidant, antimicrobial, and preservative properties [9]. *Perilla frutescens* L. essential oil (PLEO), a pale-yellow liquid with a fragrant aroma, is extracted from the leaves of the Perilla frutescens plant. Gas chromatography-mass spectrometry (GC/MS) analysis has identified 150 compounds in

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PLEO, with the main components being 2-hexylfuran (27.14 %) and perillaldehyde (19.21 %) [10]. Additionally, the composition of PLEO is rich in monoterpenes and sesquiterpenes that contribute to its bioactivity, including caryophyllene, D-limonene, and α-phellandrene [11].

The effectiveness of PLEO in preserving fruits can be attributed to the antibacterial properties of its specific compounds, particularly polyphenols. These polyphenols disrupt the cell walls and membranes of microorganisms, inhibit enzyme activity and nucleic acid synthesis, and alter cell permeability [12]. In addition, compounds within PLEO contribute antioxidant activities that scavenge free radicals, thereby delaying the aging and decay of fruits [13]. Therefore, the application of PLEO in the field of fruit preservation shows great potential. For example, Tai [14] successfully employed PLEO microcapsules to extend the shelf life of peaches. Moreover, these microcapsules have been shown to effectively inhibit strawberry rot and preserve the freshness of fish fillets [15], demonstrating their broad applicability as a natural preservative.

The effectiveness of PEOs compounds can be negatively influenced by external factors such as oxidation, light, and temperature, leading to degradation and volatilization of the compounds [16]. Microencapsulation technology addresses this challenge by enclosing active substances within a protective matrix or shell material, aimed at shielding these components from adverse environmental effects and enabling controlled release or stable storage of the active ingredients [17]. Depending on the type and amount of shell material used, this technology can be further categorized into single-layer and multi-layer encapsulation. This encapsulation not only prevents interactions between additives and food ingredients, thereby preserving the integrity and aroma of the core substances, but also facilitates the controlled release of PEOs components. Such control enhances the stability of active ingredients in PLEO and manages their release rates, which are essential for providing continuous antiseptic and antibacterial effects, extending the shelf life of fruits [18], and effectively masking the distinctive odor of PLEO to enhance the sensory acceptance of treated fruits [19]. The versatility of encapsulation strategies, including single-layer and multi-layer techniques, allows for tailored approaches to maximize PLEO's effects based on specific preservation needs [15].Previous studies by our research group, which focused on the preparation and characterization of PLEO hydrogel beads [20]. primarily addressed the impact of the core-to-wall ratio on encapsulation efficiency through limited gradient experiments. These foundational studies pave the way for further exploration to optimize the process and fully assess the application potential of PLEO hydrogel beads.

Strawberries are non-climacteric fruits that must be picked fully ripened [21]. Due to their high-water content and soft fruit structure, they are particularly susceptible to microbial contamination, which makes them prone to softening and rotting during transportation and storage. Current strawberry preservation technologies primarily utilize refrigeration processing [22], coating [23], controlled atmosphere preservation [24], and natural preservation methods including essential oil preservation [25]. However, these methods often require high technical expertise and controlled environmental conditions, and chemical preservatives can leave residues on the surfaces of fruits and vegetables, posing additional concerns. In contrast, plant essential oils serve as natural preservatives and offer a more environmentally friendly and safer alternative for fruit preservation. Therefore, there is an urgent need to develop a safe and effective essential oil-based preservative for strawberries.

In this study, we aim to optimize the preparation process of PLEO hydrogel beads to enhance their effectiveness in strawberry preservation. Utilizing a single-factor experimental design, we examined the impacts of chitosan concentration, sodium alginate concentration, core-to-wall ratio, and homogenization time on the encapsulation efficiency of the hydrogel beads. We then assessed the efficacy of both multi-layer and single-layer encapsulated PLEO formulations in preserving strawberries against common spoilage metrics. The study aimed to substantiate the extended shelf life and quality preservation afforded by PLEO-based hydrogel beads.

2. Materials and methods

2.1. Chemicals and reagents

Perilla frutescens L. essential oil (PLEO, density = 0.925 g/mL) was purchased from Xi'an Hele Biotechnology Co., Ltd (Xi'an, China). Sodium starch octenyl succinate (degree of substitution ranging between 0.01 and 0.03) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Chitosan (deacetylation degree of 95 % and viscosity: 100–200 mPa s) was purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China).Sodium alginate (viscosity: 1.05–1.15 mPa s, and pH: 6.8–8.0) was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Gallic acid was acquired from Fuchen Chemical Reagents Company (Tianjin, China). Folin-Ciocalteu's phenol reagent was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Chromatographic grade oxalic acid, tartaric acid, malic acid, citric acid, and succinic acid were all obtained from Aladdin Reagent Company (Shanghai, China). All other chemicals and solvents used in this study were of analytical grade.

2.2. Preparation of PLEO microencapsulated beads

2.2.1. Single factor experiments

One of the essential chemical modification methods is octenyl succinic anhydride (OSA) esteri cation to enhance surface active properties of starch, which is mainly through incorporating the hydrophobic alkenyl groups from OSA into the hydrophilic starch molecule. Using OSA as the wall material and PLEO as the core material, the mixture is placed in a water bath at 80 °C for 10 min to achieve gelatinization. After cooling to room temperature, PLEO is added and thoroughly mixed. The mixture is then dispersed at 9000 rpm for a specified period of time, and the resulting emulsion is spray-dried to obtain the solid microcapsule powder. The spray drying conditions refer to the method of Bhattacharya [26] with appropriate modifications: the inlet temperature is 170 °C, the outlet temperature is 100 °C, and the material flow rate is 500 mL/h. The optimal parameters of the preparation process are: the purity of

PLEO is 81 %, the wall material mass fraction is 20 %, the wall-to-core ratio is 3:1, and the encapsulation efficiency under these conditions is 80.19 %.

First, sodium alginate with different mass fractions (0.5 %, 1 %, 1.5 %, 2 %, 2.5 % w/v) was used as the wall material, and PLEO microcapsule powder was used as the core material. Mixed with different core-to-wall ratios (1:1, 2:1, 3:1, 4:1, 5:1), then homogenize at 9000 r/min for a certain time (3, 6, 9, 12, 15 min). Finally chitosan (0.5 %, 1 %, 1.5 %, 2 %, 2.5 % w/v) and calcium chloride solutions were dropped into the above solution by syringe to form PLEO hydrogel beads. When conducting each factor level test, the remaining factors were fixed as sodium alginate mass fraction 1.5 % w/v, core-to-wall ratio 3:1, homogenization time 3 min, chitosan mass fraction 1.5 % w/v, chlorine Calcium solution 1.5 % w/v, to examine the effects of various factors on the encapsulation rate of PLEO microencapsulated powder.

2.2.2. Response surface methodology (RSM) design

Building upon the single factor experiments, a central composite design was employed to examine the effects of three independent variables: sodium alginate concentration (X_1), chitosan concentration (X_2), and core-to-wall ratio (X_3) on the encapsulation efficiency (Y). This design included a three-factor, three-level experiment (see Table 1). The RSM approach was utilized to optimize the conditions for encapsulation and to statistically analyze the interaction between these variables. This method is critical for understanding the combined effects of various factors on the encapsulation efficiency and for guiding the optimization of the microencapsulation process.

2.3. Determination of encapsulation efficiency

In this study, PLEO was mixed with anhydrous ethanol to extract the oil. The mixture was scanned at a wavelength range of 200–800 nm to determine the maximum absorption wavelength of PLEO, which was found to be 291 nm. To accurately quantify PLEO, different concentrations of the oil (5, 10, 15, 20, and 25 mg/L) were prepared. The absorbance of these solutions was measured at 291 nm using a UV mini 1240 spectrophotometer (Shimadzu, Kyoto, Japan). A standard curve was then constructed, with PLEO concentration (mg/L) as the x-axis and absorbance as the y-axis, resulting in the equation $y = 34.48x - 0.047$ ($R^2 = 0.9975$). This curve was used to calculate the content of PLEO in the solution after separation of the hydrogel beads by centrifugation, facilitating the determination of encapsulation efficiency (EE), expressed as follows [27]:

$$EE\ (\%) = \frac{W_{TO}\text{-}W_{SO}}{W_{TO}} \times 100 \quad (1)$$

Where, W_{TO}: total oil content; W_{SO}: surface oil content.

2.4. Raw strawberry pre-treatment

A total of 1.5 kg of fresh strawberries from the same batch was randomly divided into five groups, with each group containing approximately 20 strawberries, and packaged in specialized fruit preservation boxes. Each group was matched for quantity, quality, and ripeness. The groups were designated as follows: control group (CK), 4 g PLEO hydrogel bead group (F0), 2 g PLEO microcapsule powder group (F1), 4 g PLEO microcapsule powder group (F2), and an ethanol preservation card control group (FJ). The preservative materials for groups F0, F1, and F2 were placed in custom non-woven fabric bags and then together with the strawberries into the preservation boxes. The strawberries were stored at a consistent 23 °C with 50 % relative humidity for 5 days, with daily sampling. Each sampling involved selecting at least three strawberries to evaluate the quality under various preservation conditions, including morphological changes, weight loss, decay rate, firmness, organic acids, total phenols, total microbial count, and sensory evaluation. The experiment was replicated five times.

2.5. Decay Rate

The decay rate was determined using the decay index method [28] (see Table 2). The extent of surface decay on strawberries was categorized into different grades as shown in Table 2. The decay rate over a period of 5 d of storage was calculated by observing the decayed area and the number of samples at each level using the following formula.

$$\text{Decay Rate}(\%) = \frac{\sum (i \times n_i)}{m} \times 100 \quad (2)$$

Table 1
Factors and levels table of response surface design.

	Factors	Levels		
		−1	0	1
X_1	sodium alginate mass fractions (%)	1.5	2	2.5
X_2	chitosan concentrations (%)	1	1.5	2
X_3	core-to-wall ratios	1	2	3

Table 2
Decay grade and corresponding area of strawberry.

Rotting Level	Rotting Level	Area of Rot
0	No Rot	No Rot
1	Mild	<1/4
2	Moderate	1/4–1/2
3	Severe	>1/2

Where, i: Decay grade, corresponding directly to the Rotting Level.; n: Number of samples at that decay grade; m: Total number of samples.

2.6. Weight loss rate

The weight loss rate was determined by differential weight method [29]. The weight loss rate over a period of 5 d of storage was calculated using the following formula.

$$\text{Weight Loss Rate } (\%) = \frac{M_0\text{-}M_1}{M_0} \times 100 \tag{3}$$

Where, M_0: Initial weight of the strawberries before storage; M_1: Weight of the strawberries after storage.

2.7. Hardness

The hardness of the samples was measured using a digital fruit hardness tester [30] (Model: GY-4, Manufacturer: Edelburg Instruments Co., Ltd., Location: Leqing City, China). For each measurement, the strawberry was positioned directly beneath the indenter of the hardness tester to ensure alignment. The indenter was then pressed into the strawberry at a consistent speed of 5 mm/s until a deformation of 5 mm was achieved. The hardness, measured in Newtons (N), was recorded directly from the digital display when the indenter reached the predefined scale line. A total of 30 strawberries were tested, with five measurements taken per strawberry to ensure consistency and reproducibility of the data.

2.8. Organic acids

Strawberry pulp was thoroughly ground and then transferred into a centrifuge tube. The mixture was first incubated at 37 °C for 1 h in a water bath to facilitate the extraction of organic acids. After incubation, the mixture was centrifuged at 6743 g for 10 min, and the supernatant was carefully collected. This extraction process was repeated to ensure comprehensive collection of organic acids. The combined supernatants were then diluted tenfold with distilled water and centrifuged again at 6743 g for 10 min to further clarify the solution. The resultant supernatant was then filtered through a 0.22 μm aqueous phase filter membrane. HPLC analysis was performed using a Thermo Scientific™ Ultimate™ 3000 system (USA). Chromatographic conditions were as follows: Hypersil Gold column (250 × 4.6 mm, 5 μm), mobile phase of 0.01 mol/L KH_2PO_4–H_3PO_4 buffer solution (pH = 2.9), flow rate of 0.6 mL/min; injection volume of 20 μL, detection wavelength of 210 nm; and a column temperature of 35 °C [31].

To prepare the organic acid standard mother solution, accurately weigh the following amounts of each acid: 20.36 mg of oxalic acid, 80.12 mg of tartaric acid, 160.64 mg of malic acid, 80.80 mg of citric acid, 160.60 mg of succinic acid, and 6.40 mg of pyruvic acid. Dissolve these quantities in 20 mL of deionized water. Subsequently, prepare serial dilutions of this mother solution at 0, 2, 4, 6, 8, and 16 times to facilitate a range of concentration measurements. During the HPLC analysis, record the retention time and the peak area for each dilution. Construct standard curves for each organic acid using the peak area (measured in milli-absorbance units, mAU) as the ordinate and the corresponding concentration (mg/mL) as the abscissa. The equations for the standard curves, along with their coefficient of determination (R^2), are as follows: oxalic acid $y = 159.6x + 2.929$ ($R^2 = 0.9996$), tartaric acid $y = 29.318x + 1.2239$ ($R^2 = 0.9998$), malic acid $y = 13.74x + 0.5794$ ($R^2 = 0.9995$), citric acid $y = 16.493x + 1.5377$ ($R^2 = 0.9991$), and succinic acid $y = 9.1104x + 0.3271$ ($R^2 = 0.9991$).

2.9. Total phenols

The total phenol content in strawberries was determined using the Folin-Ciocalteu (FC) method, as modified by Gómez-Martínez [32]. To begin, 1 mL of strawberry extract or a Gallic acid standard solution (used for expressing the results as Gallic acid equivalents, GAE) was mixed with 0.5 mL of Folin-Ciocalteu reagent. To this mixture, 1.5 mL of 7 % Na_2CO_3 and 7 mL of distilled water were added. The mixture was thoroughly mixed and allowed to react in the dark for 2 h. The absorbance was measured at 765 nm. The results were quantified in mg of GAE per gram of fresh strawberry weight, representing the concentration of phenols as equivalents to Gallic acid. A standard curve was constructed with the mass concentration of GAE as the abscissa and the absorbance as the ordinate, yielding the equation $y = 2.9531x+0.0076$ ($R^2 = 0.9994$).

2.10. Total microbial count

The total number of microorganisms was determined according to the plate count method [33]. Prior to applying the PLEO treatments, the strawberries were rinsed under running water to remove superficial contaminants. This ensured that any microbial load measured originated predominantly from the intrinsic properties of the strawberries rather than external contaminants. Serial dilutions of strawberry juice were then plated, and colony counts were performed after incubation at 36 ± 1 °C for 48 ± 2 h. The total microbial count was calculated using the following specific formula, providing insight into the microbial load in different sample groups:

$$N = \frac{\sum C}{(n_1 + 0.1n_2) \times 10} \tag{4}$$

Where, N: The number of bacteria in the sample; $\sum C$: The sum of bacterial counts on plates (plates containing an appropriate range of bacterial counts); n_1: The number of plates diluted 10 times (lower dilution factor); n_2: The number of plates diluted 100 times (higher dilution factor).

2.11. Sensory quality assessment

A sensory evaluation was conducted by 100 randomly selected participants, who assessed the strawberries for acceptability, odor, taste, firmness, and color. Each attribute was scored on a scale of 0–10, with the method outlined in Table 3. This evaluation provided a comprehensive assessment of the strawberries' overall sensory quality [34]. The study did not involve any invasive procedures or health risks to the participants; hence, it was classified under non-invasive research. According to the regulations governed by China's Ministry of Health, such non-clinical, non-invasive sensory evaluations do not require formal ethical approval. Our procedures were strictly observational, involving commonly consumed food products, and were conducted in a controlled, safe environment ensuring that all participant interactions adhered to general food safety guidelines.

2.12. Statistical analysis

Three parallel samples were taken for each group, and the average and standard deviation were calculated. SPSS software was used for data statistics and significance analysis, $P < 0.01$ highly significant; $0.01 < P < 0.05$ significant, and the data graphs in the paper were drawn using Origin 2022 software.

3. Results and discussion

3.1. Study on the preparation process of PLEO hydrogel beads

3.1.1. Single factor analysis

Fig. 1A depicts the effect of varying sodium alginate concentrations on the encapsulation efficiency. Initially, as the concentration of sodium alginate increased, the encapsulation efficiency showed an upward trend, followed by a decrease. The encapsulation efficiency started to decline when the sodium alginate concentration exceeded 2.0%w/v. Therefore, the optimal concentration of sodium alginate was identified as 2.0%w/v. As shown in Fig. 1B, similar to sodium alginate, the encapsulation efficiency increased with an increase in chitosan concentration up to a certain point, after which it began to decrease. The study found that chitosan concentrations above 1.5%w/v were not conducive to the preparation of PLEO hydrogel beads, establishing 1.5%w/v as the optimum concentration of chitosan. The encapsulation efficiency gradually increased from a homogenization time of 3 min–15 min, reaching its peak at 12 min. However, when the homogenization time exceeds this point, no significant change in encapsulation rate is observed, indicating that the most effective homogenization time is 15 min. The extension of the mean time has no effect on the encapsulation rate, so the extension of the mean time has no effect on the encapsulation rate, so this item is not selected as RSM. The study also investigated the effect of different core-to-wall ratios on the encapsulation efficiency, ranging from 1:1 to 5:1. The encapsulation efficiency decreased for ratios greater than 2:1, likely due to an excess of PLEO microcapsule powder in relation to the wall material, leading to inadequate encapsulation. Thus, the optimal core-to-wall ratio was determined to be 2:1.

Table 3
Sensory score scale.

Score	Acceptance	Odor	Taste	Hardness	Coloration
0–2	Hard to accept	Strong odor	Bitter and hard to swallow	Serious softening with water stains	Mold spots, severe browning
3–5	Unacceptable	Slight odor	Slightly bitter	Beginning to soften	Darkened color, moderate browning
6–8	Acceptable	Normal, no odor	Normal, no odor	Good strawberry elasticity, firm flesh	Normal color, slight browning
9–10	Highly recognized	Fragrant odor	Deliciously sweet with strong aroma	Good elasticity, firm flesh	Bright color, no browning

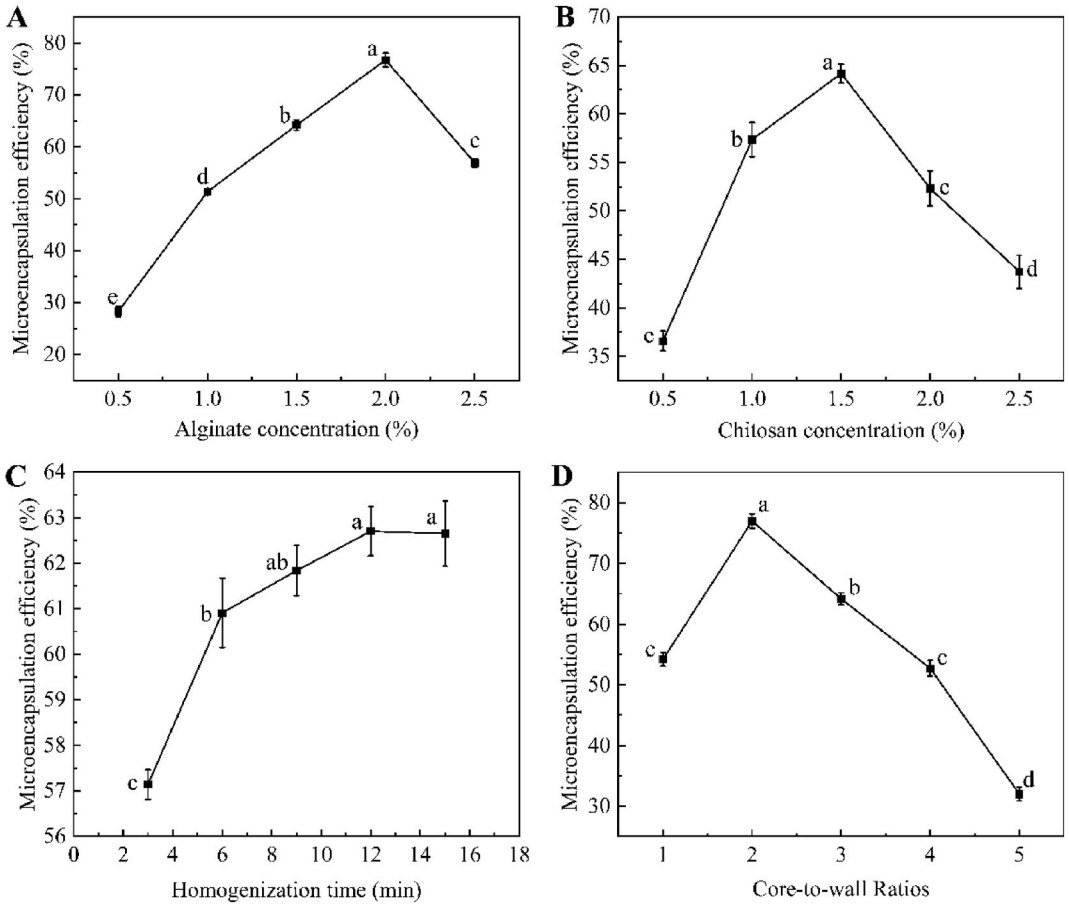

Fig. 1. The influences of various factors on the encapsulation efficiency
(A: Alginate concentration; B: Chitosan concentration; C: Homogenization time; D: Core-to-wall ratio).

Table 4
Box-Behnken Design and observation response of PLEO entrapment efficiency.

Run	X_1	X_2	X_3	Microencapsulation efficiency (%)
1	1	1	0	64.67
2	0	0	0	87.57
3	0	0	0	90.37
4	−1	−1	0	60.27
5	0	−1	1	64.5
6	1	0	1	61.42
7	−1	0	1	63.96
8	1	−1	0	63.2
9	−1	0	−1	51.79
10	0	0	0	89.48
11	0	0	0	90.56
12	0	−1	−1	55.32
13	0	1	1	60.43
14	0	0	0	87.39
15	−1	1	0	59.88
16	1	0	−1	58.59
17	0	1	−1	61.86

The Analysis of Variance (ANOVA) for this quadratic polynomial model, as shown in Table 5, indicated high statistical significance ($P < 0.01$), affirming a notable correlation between the independent variables (sodium alginate concentration, chitosan concentration, core-to-wall ratio) and the encapsulation efficiency of PLEO hydrogel beads. The lack-of-fit was non-significant with a P-value of 8.31 (>0.05), suggesting an appropriate fit of the model. The model's coefficient of determination, R^2, was 0.9943, and the adjusted R^2 was 0.9869, elucidating that the model could explain 98.69 % of the variance in encapsulation efficiency. The quadratic terms X_1^2, X_2^2, and X_3^2 showed a significant effect on the encapsulation efficiency ($P <$ 0.01).

3.1.2. Second-order polynomial model

Using Design-Expert software, a regression analysis of the data from Table 4 was conducted for the encapsulation efficiency of PLEO hydrogel beads. This analysis led to the establishment of the following second-order polynomial model for optimizing the hydrogel beads process:

$$Y = +90.49 + 0.87X_1 + 0.57X_2 + 2.34X_3 + 0.46 \times 1 \times 2 + 0.41 \times 1 \times {}_3 + 0.098X_2X_3 - 17.03X_1^2 - 12.94X_2^2 - 16.26 \times \frac{2}{3}$$

Here, X_1 represents the concentration of sodium alginate, X_2 the concentration of chitosan, and X_3 the core-to-wall ratio.

The results of the ANOVA, as outlined in Table 5, demonstrate that the quadratic polynomial model is an effective predictive tool for optimizing the preparation conditions of PLEO hydrogel beads, playing a pivotal role in enhancing their encapsulation efficiency.

3.1.3. RSM analysis

Fig. 2 illustrates the response surface plots showing the effects of pairwise interactions of various factors on the entrapment efficiency of PLEO. These plots are critical for visualizing and understanding the complex interplay between the encapsulation process variables and guiding the optimization of microencapsulation conditions for PLEO. The RSM was employed to elucidate the interaction effects of sodium alginate concentration (X_1) and chitosan concentration (X_2) on the encapsulation efficiency of PLEO hydrogel beads, maintaining a constant core-to-wall ratio (2:1). As depicted in Fig. 2A, the interaction between sodium alginate and chitosan concentrations was not statistically significant ($P > 0.05$), with the encapsulation efficiency initially increasing and then decreasing as the concentration of sodium alginate increased. In a fixed homogenization time (12 min) setting, the influence of sodium alginate concentration and core-to-wall ratio on the encapsulation efficiency is shown in Fig. 2B. Fig. 2C reveals the effect of chitosan concentration and core-to-wall ratio on the encapsulation efficiency at a constant sodium alginate concentration (2%w/v). The interaction effects in these groups were statistically significant ($P < 0.05$).

3.1.4. Optimization of conditions

Based on the established second-order polynomial model, the optimal preparation conditions for hydrogel beads were determined. The ideal conditions were found to be a sodium alginate concentration of 2.31%w/v, a chitosan concentration of 1.52%w/v, and a core-to-wall ratio of 2.08:1. Under these conditions, the predicted encapsulation efficiency was 84.77 %. For practical application, these optimal conditions were slightly modified to more convenient values: sodium alginate concentration at 2%w/v, chitosan concentration at 1.5%w/v, and a core-to-wall ratio of 2:1. The experimental encapsulation efficiency under these adjusted conditions was 82.61 %, which closely aligned with the predicted value. This slight adjustment in the optimal conditions demonstrates the robustness of the model and its applicability in practical scenarios. This optimization process highlights the importance of precise control over formulation parameters in the microencapsulation process, ensuring maximal encapsulation efficiency and practical feasibility in the production of PLEO hydrogel beads [35].

3.2. Appearance characteristics of strawberries

Appearance is a crucial quality attribute for fresh produce selection. As illustrated in Fig. 3A, after 5 d of storage at room temperature, the CK and the F1 and FJ groups showed significant mold growth and widespread rotting on the strawberries' surfaces. In contrast, the F0 and F2 groups exhibited no signs of mold. The application of PLEO hydrogel beads and microcapsule powder evidently mitigated rotting, positively influencing the preservation of strawberries' appearance during storage at room temperature [36]. Additionally, we quantitatively assessed the preservation effect using three parameters: decay rate, weight loss rate, and firmness.

Table 5
ANOVA for the quadratic polynomial mode.

source	Sum of squares	df	Mean square	*F* value	*P*-value[b] Prob *F*	Significance
Model	3037.34	9	337.48	135.31	<0.0001	[a]
X_1	17.94	1	17.94	7.19	0.0314	[b]
X_2	1.58	1	1.58	0.63	0.4529	
X_3	64.7	1	64.7	25.94	0.0014	[a]
X_1X_2	0.86	1	0.86	0.35	0.5744	
X_1X_3	21.81	1	21.81	8.74	0.0212	[b]
X_2X_3	28.14	1	28.14	11.28	0.0121	[b]
X_1^2	864.42	1	864.42	346.58	<0.0001	[a]
X_2^2	683.48	1	683.48	274.04	<0.0001	[a]
X_3^2	1051.88	1	1051.88	421.75	<0.0001	[a]
Residual	17.46	7	2.49			
Lack of fit	8.31	3	2.77	1.21	0.4136	
Pure error	9.15	4	2.29			
Cor total	3054.8	16				
R^2	0.9943					
R^2_{Adj}	0.9869					

[a] $P < 0.01$ highly significant.
[b] $0.01 < P < 0.05$ significant.

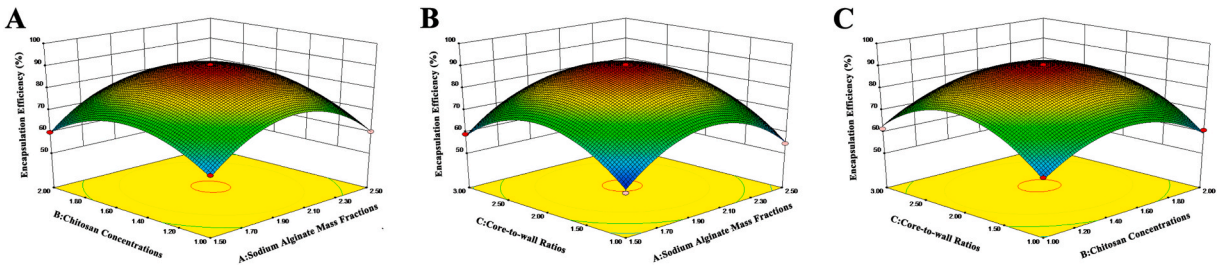

Fig. 2. RSM of the effects of pairwise interaction of various factors on the encapsulation efficiency of PLEO hydrogel beads (A: Interaction between sodium alginate concentration and chitosan; B: Interaction between sodium alginate concentration and core-to-wall ratios; C: Interaction between chitosan concentration and core-to-wall ratios).

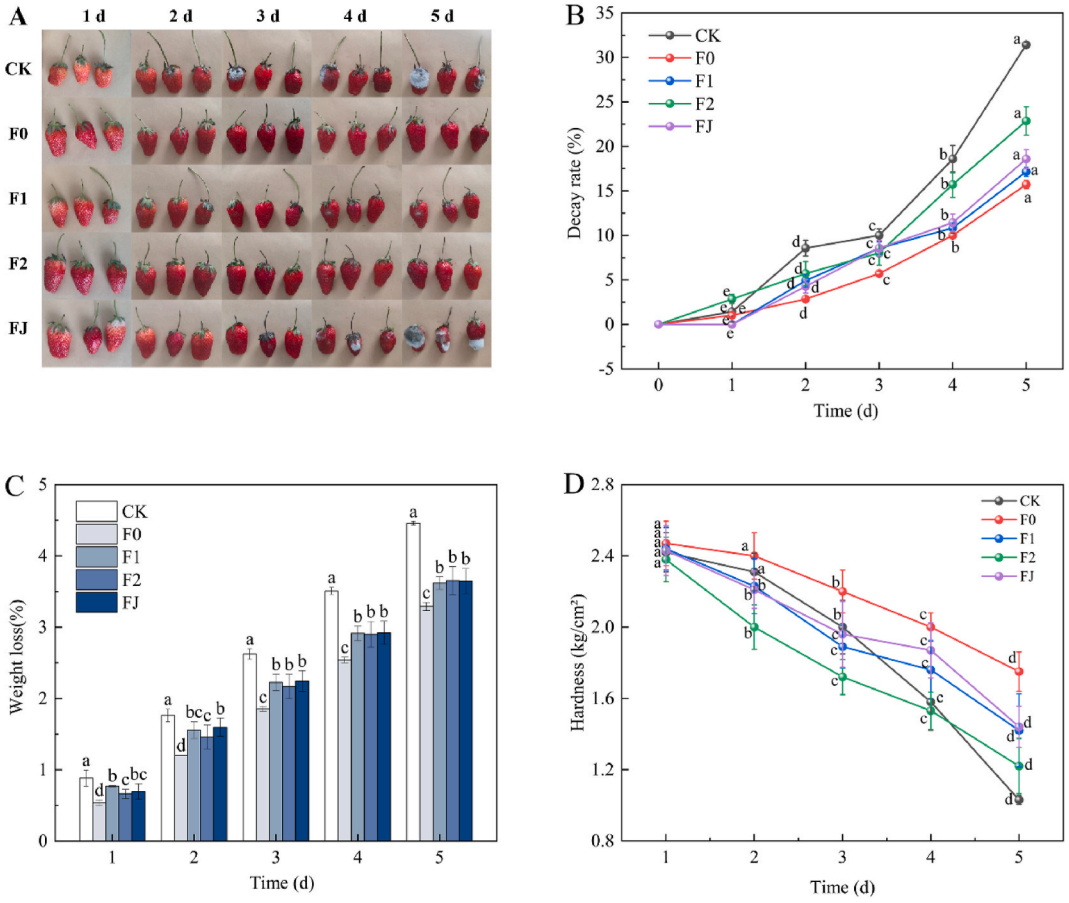

Fig. 3. Morphological characteristics and Indexes changes of Strawberry
(A: Morphological characteristic diagram; B: Decay rate; C: Weight loss rate; D: Hardness.

Strawberries are highly perishable, and their decay rate increases over time. On the first day of storage, there was no significant increase in decay rate for CK, F0, F1, and FJ groups. However, after 5 d, all groups showed a significant increase in decay rate, with the most pronounced decay observed in the CK group (31.43 %). The F0 group exhibited a decay rate of 15.71 %.

Blank control group (CK), 4 g of PLEO hydrogel beads group (F0), 2 g of PLEO microcapsule powder (F1), 4 g of PLEO microcapsule powder (F2) and alcohol fresh card control group (FJ))

3.3. Weight loss rate analysis

Weight loss is a key indicator of fruit aging acceleration [37]. Over time, the strawberries experienced an increase in weight loss rate, similar to trends observed in other fruit preservation studies [38]. After 5 d, the strawberries in the CK group shrank, with a

weight reduction of 4.46 %. This is attributed to moisture loss and accelerated cellular aging due to respiration and evaporation during storage [39]. Conversely, the experimental groups showed less weight loss under the same storage conditions, with the F0 group displaying the least weight loss at 3.29 % (Fig. 4B). This confirms the efficacy of PLEO microcapsule beads in preventing moisture evaporation and maintaining the fruit's texture [40].

3.4. Firmness analysis

The firmness of strawberries decreased during storage, with the CK group experiencing the fastest softening, from 2.42 kg/cm^2 to 1.03 kg/cm^2. Groups treated with preservatives showed a delay in softening, particularly the F0 group, where the firmness on the fifth day was 1.75 kg/cm^2. PLEO may enhance fruit firmness by reducing enzyme activity that leads to softening, suppressing the expression

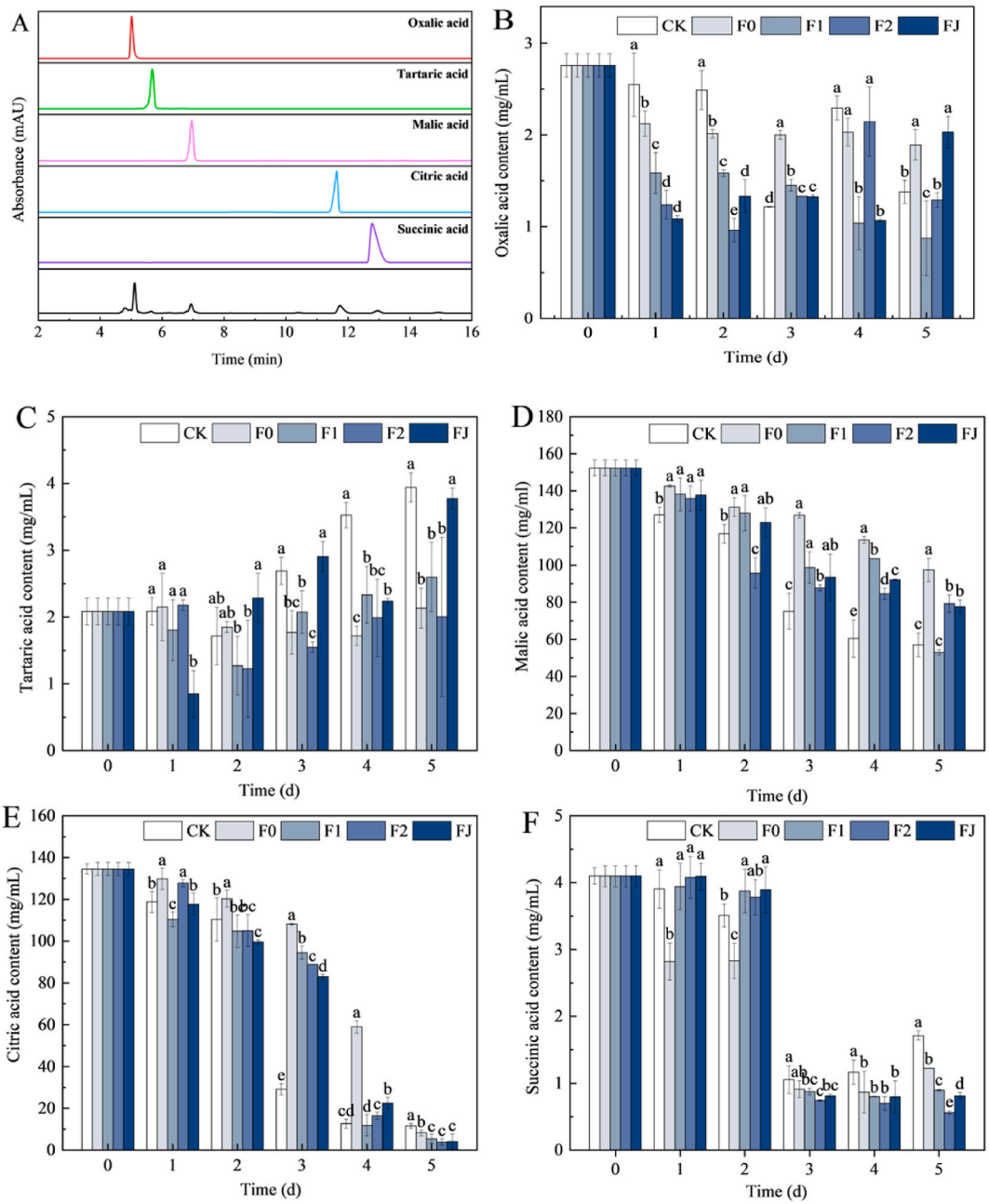

Fig. 4. Changes of organic acid content in strawberry during storage
(A: Organic acids of strawberries; B: Oxalic acid; C: Tartaric acid; D: Malic acid; E: Citric acid; F: Succinic acid).

of cellulase genes, delaying cell wall degradation, and modulating changes in strawberry tissue structure [41].

3.5. Organic acids contents analysis

Organic acids are crucial indicators of fruit ripeness, varying in content and types across different fruits [42]. During the ripening process, due to respiration and metabolic activities, the content of organic acids gradually decreases. After preservation treatment, the content of organic acids decreased slowly, and there was the same trend in the Zhang [43] study. As observed in Fig. 4A, strawberries contain many organic acids, with malic acid having the highest content, followed by succinic acid, oxalic acid, citric acid and tartaric acid. The contents of these acids are related to the taste of strawberries [44].

Oxalic acid, a notable organic acid in fruits and vegetables, is known for its antioxidant properties and its role in improving fruit and vegetable quality [45]. As shown in Fig. 4, apart from tartaric acid, the other four organic acids showed a gradual decrease over the storage period. The CK group had relatively lower organic acid content compared to the groups treated with preservatives, particularly the F0 group (Fig. 4D and E). This suggests that PLEO hydrogel beads effectively inhibit microbial growth, slow down the respiration rate of strawberries, and reduce enzyme activity. The content of tartaric acid, however, gradually increased (Fig. 4C), possibly due to environmental factors and biochemical reactions leading to its synthesis and accumulation in strawberry cells [46].

Statistical analysis of the data involved an ANOVA test, followed by Tukey's Honestly Significant Difference (HSD) test to identify significant differences among treatment groups at a 0.05 significance level. These analyses helped to clarify which treatment effects were statistically significant. The occasional increases in organic acid concentrations at certain storage days could be attributed to natural variability in fruit composition and the dynamic nature of biochemical reactions occurring during storage.

3.6. Total phenols contents analysis

Phenolic compounds, as secondary metabolites, play an essential role in the nutritional value and antioxidant capacity of fruits [47]. During post-harvest storage, changes in environmental conditions lead to a gradual decomposition and decrease of these compounds [48]. As illustrated in Fig. 5, the total phenolic content in all experimental groups was higher than that in the CK. The most rapid decline in total phenolic content was observed in the CK group, which dropped to 3.40 mg/mL by the fifth day. In contrast, the F0 group maintained the highest phenolic content at the same time point (4.10 mg/mL), indicating that PLEO microcapsule beads effectively slowed down the reduction of total phenols. The F1 and F2 groups also demonstrated similar effects. Previous studies [15] have shown that PLEO exhibits strong antioxidant properties, possibly contributing to the observed reduction in free radical formation and subsequent preservation of total phenols in the F0, F1, and F2 groups. The slightly lower performance of F1 and F2 compared to F0 might be due to the direct exposure of PLEO microcapsule powder to air, whereas the hydrogel beads provided a controlled release effect [49].

3.7. Total microbial contents analysis

Fig. 6 illustrates the total microbial count in strawberries during storage. The CK was more susceptible to microbial invasion and rot [50]. The microbial count in CK group increased significantly over 5 d of storage, reaching 2.2×10^4 CFU/mL, which was 18.3 times higher than on the first day (1200 CFU/mL). On the fifth day, the total microbial count, in ascending order, was F0 < F2 < F1 < FJ < CK. This suggests that the PLEO hydrogel beads were highly effective in inhibiting microbial growth, which is consistent with the observed appearance of strawberries in Fig. 3A. The above results show that PLEO hydrogel beads can have antibacterial effects within

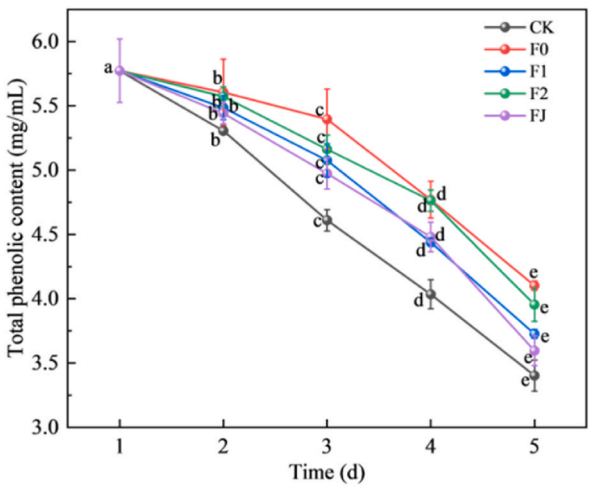

Fig. 5. Changes of Total Phenol content of Strawberry during Storage.

5 d of storage, indicating that they have a sustained-release effect after being embedded.

3.8. Sensory quality

The sensory analysis, as shown in Fig. 7, indicated that the experimental groups exhibited superior sensory quality compared to the CK, especially in terms of consumer acceptability, taste, and color. Among all the strawberry groups, the CK group had the lowest sensory scores. Compared to the F1 and F2 groups, the F0 group outperformed in color retention, fruit firmness, taste, and overall acceptability, demonstrating the significant effect of PLEO hydrogel beads in maintaining fruit quality. However, it should be noted that despite the excellent performance of the PLEO hydrogel beads-treated groups in multiple sensory attributes, there was a slight deficiency in odor, likely due to the distinct smell of PLEO. Nevertheless, the overall sensory scores of the experimental groups were still higher than the CK group, but slightly lower than the group treated with PLEO hydrogel beads. These results highlight the unique advantages of PLEO hydrogel beads not only in extending the shelf life of fruits but also in enhancing their overall sensory quality.

4. Conclusion

In this study, the encapsulation process of PLEO hydrogel beads was optimized. The most effective method involved encapsulating PLEO microcapsule powder using sodium alginate (mass fraction 2 w/v%) and chitosan (mass fraction 1.5 w/v%) as wall materials, with a core-to-wall ratio of 2:1, homogenize for 15 min. The encapsulation efficiency under this condition is 82.61 %. PLEO hydrogel beads significantly improved the preservation effect of strawberries. After 5 d of storage, the appearance was good, and all indicators were better than those of the CK group. The decay rate (15.71 %), weight loss rate (3.29 %) and bacterial colony count were all smaller, the hardness (1.75 kg/cm^2) was the largest, and organic acids and The total phenolic content decreased slowly and the overall sensory evaluation was advantageous. Overall, this study highlights the potential of PLEO hydrogel beads as a natural preservative, providing a new method to extend the shelf life and improve the quality of fresh produce, thereby providing Significant contributions have been made to the field of sustainable food preservation.

Ethics declarations

Approval by an ethics committee was not needed for this study because the study did not involve any invasive procedures or health risks to the participants; hence, it was classified under non-invasive research. As per the guidelines outlined by local legislation, our sensory study on the preservation of strawberries using Perilla frutescens L. essential oil did not require formal ethical review. Specifically, the legislation "《涉及人的生命科学和医学研究伦理审查办法》" (Regulations on Ethical Review of Human Life Sciences and Medical Research) and "《科技伦理审查办法（试行）》" (Measures for the Review of Science and Technology Ethics - Trial Implementation) exempts non-invasive research involving generally recognized safe substances, such as food products, from ethical review. Here are the links:

(Regulations on Ethical Review of Human Life Sciences and Medical Research)http://www.nhc.gov.cn/qjjys/s7946/202302/c3374c180dc5489d85f95df5b46afaf5.shtml.

[Measures for the Review of Science and Technology Ethics (Trial Implementation)]https://www.gov.cn/gongbao/2023/issue_10826/202311/content_6915814.html.

According to these regulations and those governed by China's Ministry of Health, such non-clinical, non-invasive sensory evaluations do not require formal ethical approval. Our procedures were strictly observational, involving commonly consumed food products, and were conducted in a controlled, safe environment ensuring that all participant interactions adhered to general food safety guidelines.

Respondents provided informed consent via the statement, "I am aware that my responses are confidential, and I agree to participate in this survey," where an affirmative reply was required to enter the survey. They were informed of their right to withdraw from the survey at any time without providing a reason. All products tested were safe for consumption and complied with existing food safety regulations.

Data availability

Data associated with our study has not been deposited into a publicly available repository and data will be made available on request.

CRediT authorship contribution statement

Yanbo Wang: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Yana Zhao:** Writing – review & editing, Validation, Investigation, Conceptualization. **Yurong Guo:** Software, Formal analysis. **Wanyu Han:** Software, Formal analysis. **Zhijun Zhang:** Supervision, Resources, Project administration, Methodology, Conceptualization. **Tianyu Hou:** Validation, Supervision, Resources, Project administration, Investigation. **Huizhen Li:** Supervision, Resources, Project administration. **He Li:** Supervision, Resources, Project administration. **Qinqin Wang:** Software, Formal analysis.

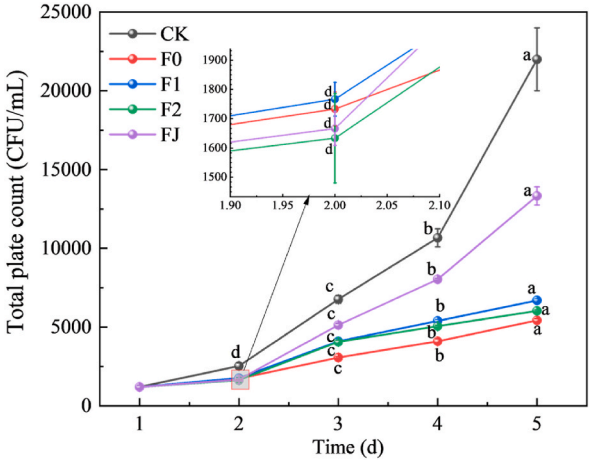

Fig. 6. Changes of total bacterial count of strawberries during storage.

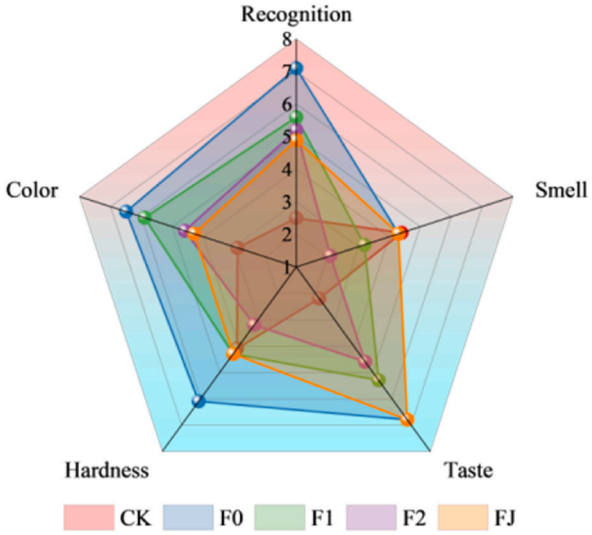

Fig. 7. Sensory evaluation radar map of strawberry after storage.

Declaration of competing interest

There are no conflicts of interest to declare.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33689.

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