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Construction of *Lactobacillus casei*-loaded Water-in-oil High Internal Phase Emulsion and its Study on Anti-breast Cancer Properties

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Abstract: *Objective:* To prepare an oil-in-water high internal phase emulsion containing *Lactobacillus casei*, and to optimize and characterize the preparation process. To explore the therapeutic effect of the probiotic-carrying emulsion on in situ breast cancer in mice. *Methods:* Using corn oil, sodium alginate, polyglycerin ricinoleate and *L. casei* as raw materials, the high internal phase emulsion containing probiotic oil-in-water was prepared by high-speed homogenization method. The optimal preparation process was obtained through single factor investigation, microstructure characterization, stability investigation and in vitro simulation digestion experiment. Ten female BALB/c mice (SPF grade, 18–20 g) were selected. After the *in situ* breast cancer model was successfully established, the mice were randomly divided into experimental group and control group, with 5 mice in each group. The mice were administrated with probiotic-loaded emulsion and normal saline respectively, and were administrated with the stomach every other day for 10 days. The change of tumor volume during treatment and tumor mass at the end of treatment were recorded. Results: The optimum process of emulsion was: With the concentration of 5% Polyglycerin ricinolate, 75% aqueous volume, 3000r/min homogenizing speed, 30 s homogenizing time and 2% aqueous sodium alginate concentration, a high internal phase emulsion with a particle size of 9.33 ± 1.74 μm and an appearance of milky paste was prepared. The inclusion rate of probiotics reached 90.97 ± 27.09%. Breast cancer anti-tumor experiment showed that the tumor inhibition rate of the experimental group reached 33.78% compared with the control group. Conclusion: Water-in-oil high internal phase emulsion can effectively protect L. casei and reduce the loss of live probiotic when probiotics pass through the digestive fluid environment. Oral intragastric high internal phase emulsion containing L. casei oil-in-water has a certain therapeutic effect on breast cancer.

Keywords: *Lactobacillus casei*; High interior phase emulsion; Process optimization; Probiotic embedding; Anti-breast cancer

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1. Introduction

Lactobacillus casei belongs to the genus *Lactobacillus*, Gram-positive, facultative anaerobes. Together with *L. acidophilus* and *Bifidobacterium*, it is known as the three major probiotics. After entering the intestinal,

L. casei can colonize the intestinal tract and live in great quantities, regulate the intestinal flora balance, and improve human gastrointestinal function [1]. At the same time, the study showed that *L. casei* can lower blood pressure, lower cholesterol, improve lactose intolerance, regulate the body's immune, cancer inhibition [2]. Usually, probiotics for oral drug delivery way, while probiotics to temperature, environmental factors including oxygen, humidity, pH value of highly sensitive, oral delivery process will in turn through the stomach and small intestine, colon, of which the low pH of the gastric fluid can seriously affect the survival of probiotic. This process will lead to serious loss of probiotic activity at the same time, research has shown that only a sufficient number of viable probiotics ($\geq 10^7$ CFU/g) released into the human gut can exert its probiotic effect [3]. Therefore, due to the characteristics of high safety of biomaterials and good adaptability *in vivo*, it will be an effective method to solve this problem to equip intestinal probiotics with appropriate biomaterials to give them a protective effect and enable probiotics to target the colon [4].

High internal phase emulsion preparation process because of its moderate and more applicable probiotic protection effect [5]. The concept of high internal phase emulsion (HIPEs) was put forward by Lissant in the 1960s and refers to the dispersed phase volume fraction of 74.05% or more of the emulsion [6]. HIPEs discussed in recent years as a pharmaceutical, food, cosmetics and hot research topic in the field of oil industry. Most sub-embedding methods have greater damage to probiotics, such as the microgel process. Due to dehydration and high temperature, the activity of probiotics was greatly damaged by spray drying. In contrast, high internal phase emulsion is more suitable for probiotic protection due to its mild preparation process. Sodium alginate and can bind with bivalent cation (Ca^{2+}, Ba^{2+}) according to the characteristics of the formation of three dimensional network gel due to its high biocompatibility and is often used for the package of probiotic microgel preparation [7]. Besides, sodium alginate gel and thickening properties, biodegradable, cheap accessible features, which makes sodium alginate have extensive application value. Sodium alginate is also associated with lowering serum cholesterol, triglycerides, and blood glucose, thus preventing diseases such as hypertension, diabetes, and obesity.

Based on the above content, Michelle CL *et al.* (2019) chose a model by *L. casei*, polyglycerol castor alkyd resin (PGPR) and sodium alginate (SA) as the emulsifier and thickener respectively [8]. The preparation of a load of probiotics water-in-oil high internal phase emulsion (w/o HIPEs), at the same time on the storage stability, microbial load capacity, investigation and get the best preparation technology of *in vitro* digestion conditions. At the same time, the anti-tumor effect of the probiotic emulsion was further investigated in the breast cancer model mice implanted with 4T1 cells.

2. Materials and methods

2.1. Animal feeding

BALB/c mice (female, 18–20 g, SPF grade, License No.: SCXK (E) 2021-0027) were purchased from Hubei Beient Bio-Technology Co., Ltd. They were housed at the SPF Animal Experimental Center of Hubei Provincial Institute for Food and Drug Control (Optics Valley Campus). All animal experimental procedures followed the guidance principles of the Animal Care and Use Committee of the Hubei Provincial Institute for Food and Drug Control and were conducted under aseptic conditions in an SPF environment.

2.2. Materials and equipment

L. casei (Chinese Typical Culture Preservation Center, strain preservation number AB 2011138); Reagent grade Corn oil (Lot No. C805618), Shanghai Maclin Biochemical Technology Co., Ltd. Sodium alginate (Lot No. C13554334), Shanghai Maclin Biochemical Technology Co., Ltd.; Polyglycerin ricinoleate (PGPR, Lot No. C14639818), Shanghai Maclin Biochemical Technology Co., Ltd. MRS Broth (Lot No. HB0384-

1), Haibo Biotechnology Co., Ltd. Pepsin (pig source), Shanghai Maclin Biochemical Technology Co., Ltd. Trypsin (Biotechnology level), Shanghai Maclin Biochemical Technology Co., Ltd. Pharmaceutical grade TWEEN 80, Shanghai Aladdin Biochemical Technology Co., Ltd. Aseptic PBS (pH 7.4, 500 mL), Gibco, USA; Purified water, laboratory made. High-speed Homogenizer (FSH-2A), Xicheng Xinrui Instrument Factory, Jintan District; Inverted fluorescence microscope (DP72), OLYMPUS Corporation, Japan; pH meter (PHS-3E), Shanghai Leimi Instrument Co., Ltd. Petri dish (90 mm × 15 mm), Beijing Lanjieke Technology Co., Ltd. 50 mL centrifugal tube, 15 mL centrifugal tube, Corning Life Science (Wujiang) Co., Ltd. Disposable sterile syringe (1 mL), Shanghai Jinta Medical Equipment Co., Ltd.

2.3. The preparation of probiotic HIPEs

The preparation method of HIPEs in the experiment was improved by previous studies. The specific process was as follows:

(1) A one-step emulsification method was used to prepare probiotic emulsion and an appropriate amount of sodium alginate was added into deionized water to prepare 2% sodium alginate solution for use;
(2) Take 4 mL of probiotic solution (incubated in a 37℃ constant temperature incubator and MRS Broth for 2–3 days) and centrifuge it three times;
(3) After centrifugation, rinse with an equal volume of PBS buffer solution with the probiotic solution;
(4) Centrifuge again to discard the supernatant, mix 15 mL of 2% sodium alginate with probiotics to prepare a water phase, and then set aside for use;
(4) Take 5 mL corn oil and an appropriate amount of 1 mL PGRP and mix well to form the oil phase, set aside;
(5) The water phase is slowly poured into the oil phase at one time and then the high-speed homogenizer is used to stir and homogenize at 3,000 r/min for 30 s to obtain the probiotic-carrying w/o high internal phase emulsion.

2.4. HIPEs preparation technology of the single-factor investigation

According to the results of the preliminary experiment, the blank emulsion was taken as the experimental object, and the appearance of the emulsion after its preparation and standing at room temperature for 24 h was taken as the investigation index. A single-factor experiment was designed to investigate the effects of water phase ratio, PGRP concentration, homogenizing machine speed, homogenizing time and sodium alginate concentration on the preparation of the emulsion, and screen out the factors that have a greater impact on the preparation of the emulsion.

2.5. Morphological characterization of HIPEs

2.5.1. The microstructure of emulsion

Using an optical microscope microscopic morphology of the latex was characterized, and the blank emulsion a few points on the slide, pressing with the cover glass to remove bubbles, let stand slides to the emulsion to fill the space between the glass and cover glass, through appropriate magnification to observe the microscopic structure of the emulsion.

2.5.2. Particle size measurement

In order to measure the average particle size of droplets in HIPEs samples, the study used ImageJ software (ImageJ 1.53 K, Nation Institutes of Health, USA) for image analysis. The software calculated the droplet diameter by analyzing the particle size of the emulsion droplet under the optical microscope through image

pixels. At least 100 particles per sample were analyzed.

2.6. Stability test

2.6.1. Placement stability test

Studying the stability of the emulsion in a certain storage period is necessary because the emulsion is unstable in thermodynamics, there is a tendency of coalescence. Puts emulsion samples 3 mL screw in the samples of glass bottles, in 25℃ at room temperature, outdoor place, taking picture as record every 7 days.

2.6.2. pH stability testing

Before preparation of the emulsion, methyl orange was in the water phase as a pH indicator, then placed good emulsion preparation in different pH (1, 2, 3, 4, 5) aqueous solution of hydrochloric acid, solution during the filming of a soak 6 h color and emulsion, record emulsion layer state.

2.7. *Lactobacillus casei* was counted

By plate count method to calculate the sample in the number of living bacterium, the specific method for the preparation of microbial load good w/o HIPEs five times diluted with sterile corn oil, will be diluted emulsion with a sterile saline solution containing the 0.5% of polysorbate 80 further dilution to the appropriate concentration, then, take the diluent 0.1 mL evenly coated in MRS agar plate. Then, the agar plates were placed upside down in a constant temperature incubator at 36 ± 1℃ and incubated for 48 h. The number of viable probiotic colonies in the loaded emulsion was calculated by counting the colonies grown on the agar plates. All the above operations were performed in an ultra-clean workbench.

2.8. Storage stability test

Placed microbial load emulsions in 4℃ refrigerator save, at the same time will not join the aseptic package of *Lactobacillus casei* PBS buffer (pH = 7.4), also put in 4℃ refrigerator save, take out the sample regularly, with live probiotic counting in ultra-clean workbench.

2.9. In vitro digestion simulation of water-in-oil high internal phase emulsion

2.9.1. The preparation of simulated gastric intestinal fluid

According to 2020 edition pharmacopoeia of the People's Republic of China [9], simulated gastric fluid (SGF) was prepared as follows: 16.4 mL dilute hydrochloric acid, adding water about 800 mL and pepsin 10 g, shake well, add water dilute to 1,000 mL, 0.22 μm membrane filter in addition to probiotic and quick simulated gastric fluid. Simulated intestinal fluid was prepared by taking potassium dihydrogen phosphate 6.8 g, water 500 mL solution, with a 0.1 mol/L sodium hydroxide solution to adjust pH value to 6.8, mixing two solutions, diluted to 1,000 mL with water, and then filtered through 0.22 μm filter membrane to remove probiotic.

2.9.2. The experimental process

To evaluate the gastroprotective capabilities of HIPEs for probiotics, an in vitro digestion experiment mimicking gastrointestinal conditions was conducted based on previous research methodologies [10]. Probiotic-loaded HIPEs (1 mL) were incubated in preheated sterile simulated gastric fluid (SGF) at 37℃ for 2 hours under agitation at 120 rpm/min to simulate gastric digestion. Subsequently, the emulsion was transferred to simulated intestinal fluid (SIF) and incubated under the same conditions to simulate intestinal digestion for an additional 2 hours. Plate counts were performed at three stages: Before digestion, after simulated gastric digestion, and after simulated intestinal digestion, to assess the protective efficacy of HIPEs against probiotics. All materials and

procedures were conducted under sterile conditions in a laminar flow hood.

2.10. Anti-tumor experiments in vivo

After one week of adaptive feeding, 4T1 cells in the logarithmic phase of growth were taken, digested, centrifuged and resuspended in PBS buffer for three times, followed by dilution to 2 × 10^6 cells/mL in PBS. The cell suspension was inoculated subcutaneously into the right mammary gland of mice in 200 μL per mouse. When the tumor volume reached 90–110 mm^3, 10 mice were randomly divided into two groups, the control group and the bacterial emulsion group, with 5 mice in each group. In order to ensure the effect of emulsion dosage volume by 200 μL to fill the stomach with medicine (such as control group by gastric volume normal saline). The mice were administrated every other day for a total of 5 times. The body weight of the mice was recorded every day, and the length and short diameter of the transplanted tumor of the mice were measured. The tumor volume and tumor inhibition rate were calculated according to equations (1) and (2), respectively.

$$V = \frac{1}{2} \times short\ diameter^2 \times long\ diameter \quad (1)$$

$$TGI = \frac{Tumor\ quality\ in\ control\ group\ - Tumor\ quality\ in\ the\ treatment\ group}{Tumor\ quality\ in\ control\ group} \times 100\% \quad (2)$$

2.11. Data analysis

All experiments are conducted at least three times, and according to these values to compute the mean and standard deviation. Statistical analysis software (SSPS) was used for data analysis. Analysis of variance and t-test were used to test for significant differences between trials ($P < 0.05$ indicated as *).

3. Results and discussion

3.1. Preparation and process screening of W/O HIPEs

By putting a polyglycerol castor alkyd resin (PGPR) and sodium alginate as emulsifier and stabilizer, thickener respectively, under the condition of high speed homogeneous, preparation of corn oil as continuous phase, sodium alginate solution as the dispersed phase of w/o HIPEs, in the process of preparing HIPEs, PGPR concentration, water phase ratio, homogenization speed, homogenization time and sodium alginate concentration can affect the stability and preparation effect of emulsion. A single factor experiment was designed to investigate the above factors and screen out the most influential factors on the stability and preparation effect of the emulsion.

3.1.1. Effect of PGPR concentration on the stability and preparation of the emulsion

Fixed at 1.5% of the concentration of alginate, water accounted for 75%, homogeneous speed 4000 r/min, the homogeneous time 45 s. The effects of PGPR concentration (0%, 2.5%, 5%) on the stability and preparation efficiency of the emulsion were investigated. The results are shown in **Figure 1** (The left shows the newly prepared finished appearance, and the right shows the appearance placed for 24 hours) and **Table 1**. Therefore, PGPR concentration of 5% was chosen.

Table 1. Results of PGPR dosage investigation

PGPR	0%	2.5%	5%
Appearance	Translucent emulsion	White cream	White cream
Separation	Yes	No	No

Figure 1. Appearance analysis of different concentrations of PGPR emulsions

3.1.2. Effect of water phase ratio on emulsion stability and preparation effect

The concentration of alginate was fixed at 1.5%, PGPR at 5%, homogenization speed at 4,000 r/min, and homogenization time at 45 s. Inspection water accounted for 60%, 65%, 75%, 85%, the stability of emulsion and preparation of effects. The results are shown in **Figure 2**, **Table 2**, choice of water phase accounted 75%.

Table 2. Results of water phase proportion investigation

Water phase	55%	65%	75%	85%
Appearance	White cream	White cream	White cream	White cream
Separation	No	No	No	Yes

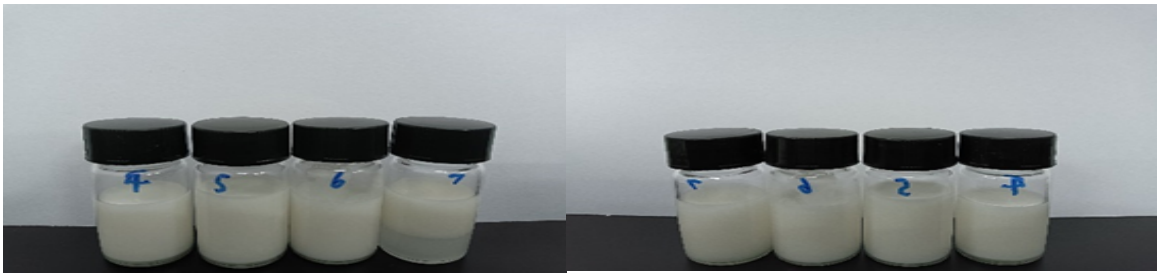


Figure 2. Appearance analysis diagram of emulsions with different aqueous phase proportions

3.1.3. Effect of homogenization speed on the stability and preparation effect of emulsion

Fixed at 1.5% of the concentration of alginate, PGPR concentration 5%, water accounted for 75%, homogeneous, and time is 45 s. Examine homogeneous speed 3,000, 4,000, 5,000 r/min effects on stability of emulsion and preparation. The results are shown in **Figure 3** and **Table 3**, and the homogenization speed was selected as 3,000 r/min.

Table 3. Results of homogeneous rotational speed investigation

Homogeneous speed	3,000 r/min	4,500 r/min	6,000 r/min
Appearance	White cream	White cream	White cream
Separation	No	No	No

Figure 3. Appearance analysis diagram of emulsions with different homogeneous speed

3.1.4. Homogeneous time effects on the stability of emulsion and preparation

Fixed at 1.5% of the concentration of alginate, PGPR concentration of 5%, water accounted for 75%, homogeneous, and the speed of 4,000 r/min. Homogeneous time 45 s, 30 s, 60 s effects on stability of emulsion and preparation. The results are shown in **Figure 4** and **Table 4**. The duration of homogenization was selected as 30s.

Table 4. The results of homogeneous time investigation

Homogeneous time	30 s	45 s	60 s
Appearance	White cream	White cream	White cream
Separation	No	No	No

Figure 4. Appearance analysis diagram of emulsions with different homogenization time

3.1.5. Effect of sodium alginate concentration on the stability and preparation of emulsion

The concentration of PGPR was fixed at 5%, the proportion of the water phase was 75%, the homogenization speed was 4,000 r/min, and the homogenization time was 45 s. The effects of 0%, 1%, 1.5% and 2% sodium alginate on emulsion stability and preparation efficiency were investigated. The results are shown in **Figure 5** and **Table 5**. According to the results of single factor experiment, the proportion of water phase, PGPR and sodium alginate concentration were the main factors affecting the stability and preparation effect of the emulsion. Preparation technology, therefore, choose water accounted for 75%, 5% concentration of PGPR, homogeneous speed 3,000 r/min, the homogeneous time 30 s. The optimal concentration of sodium alginate needs to be further determined by subsequent experiments.

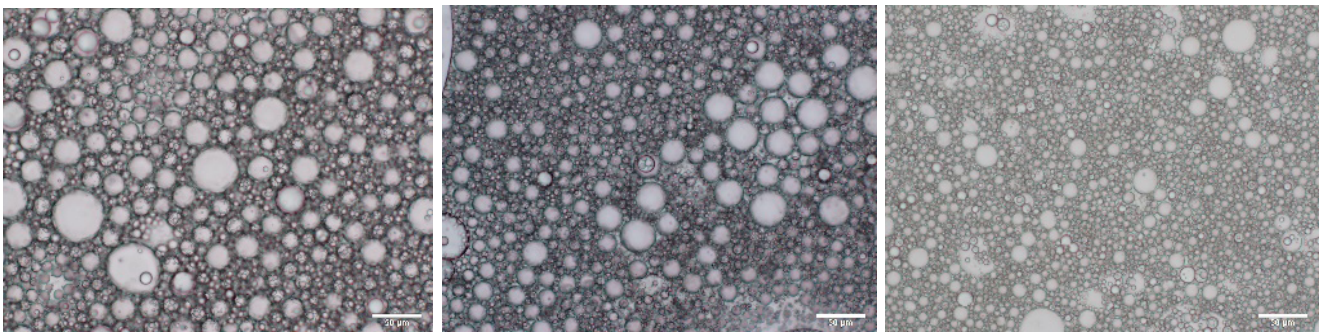
Table 5. Results of SA concentration investigation

SA	0%	1.0%	1.5%	2%
Appearance	Partial emulsification	White cream	White cream	White cream
Separation	Yes	No	No	No

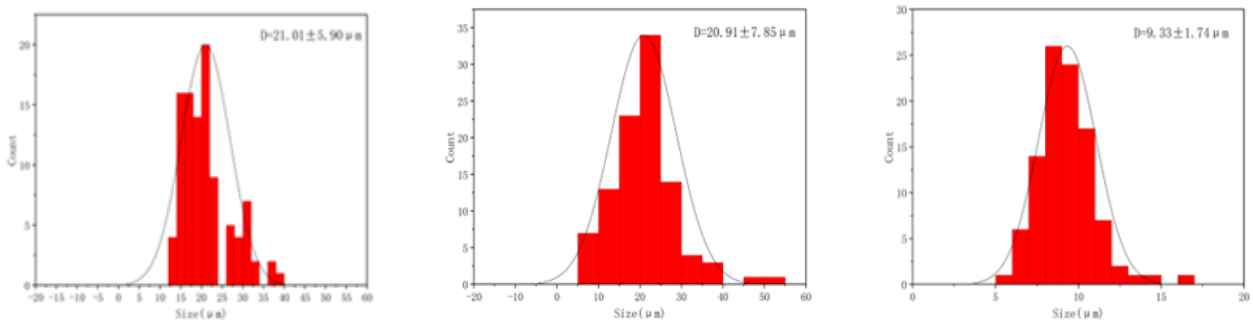
Figure 5. Appearance analysis of emulsions with different SA concentrations

3.2. HIPEs microstructure characterization

The preparation of sodium alginate concentration was 1%, 1.5%, and 2%, three different formulations of HIPEs, the HIPEs were characterized by the confocal microscope, three different formulations of emulsions can observe water droplets, and fit closely, showed the characteristics of the high internal phase emulsion, microstructure and particle size distribution as shown in **Figure 6**. Results showed that 1%, 1.5%, and 2% in three different formulations of HIPEs diameter were 21.01 ± 5.90 μm, 20.91 ± 7.85 μm and 9.33 ± 1.74 μm. When sodium alginate concentration increases, the emulsion droplet size decreases, and the droplet size is more uniform. The result may be due to the concentration of sodium alginate in droplets increasing, the viscosity increase of the emulsion system, system of dispersed phase is not easy to gather and condense to form larger droplets. At the same time, sodium alginate forms a three-dimensional network structure in the droplet, which can stabilize the droplet structure [11]. However, the higher the concentration, the denser the network structure inside the droplet, and the weaker the tendency of droplet aggregation, making the particle size decrease with the increase of sodium alginate concentration.



(A) 1% SA HIPEs (B) 1.5% SA HIPEs (C) 2% SA HIPEs



(D) 1% SA HIPEs size (E) 1.5% SA HIPEs size (F) 2% SA HIPEs size

Figure 6. Microstructure and particle size distribution of HIPEs with different SA concentrations

3.3. Evaluation of stability

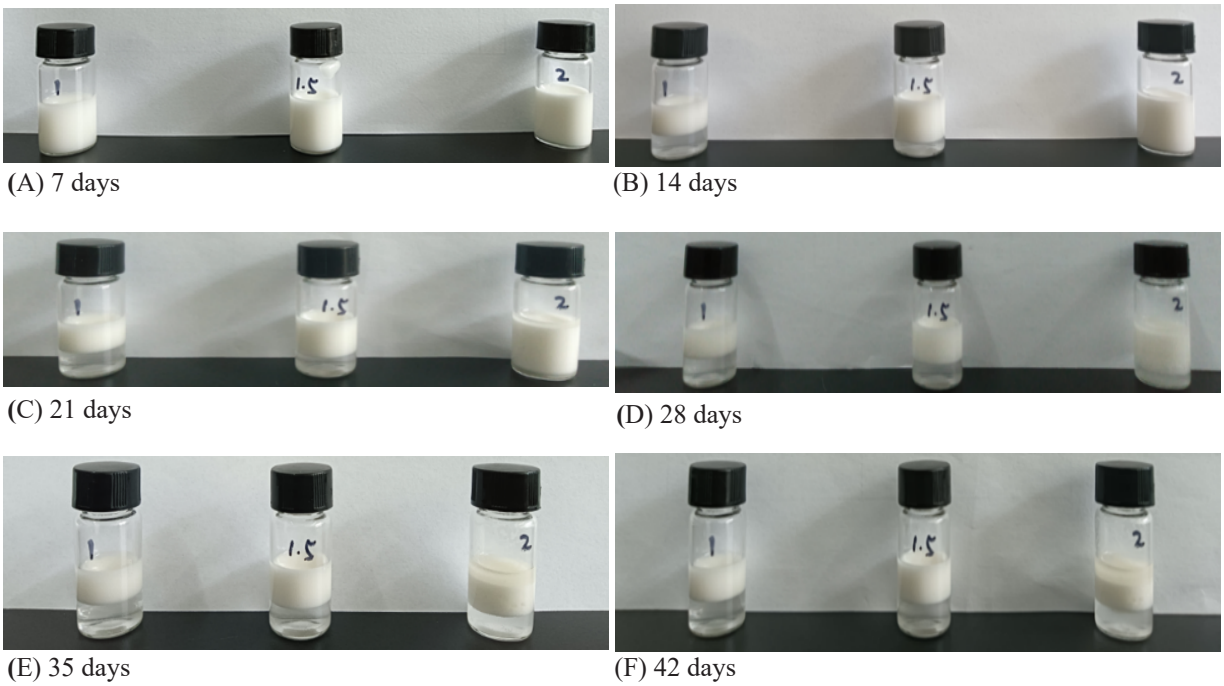
3.3.1. Stability of placement

To investigate the stability of HIPEs, 3 mL of three emulsions with SA concentrations of 1%, 1.5%, and 2% were placed in glass bottles with threaded mouths at room temperature, respectively, and observed every 7 days, and images were taken, as shown in **Figure 7**, according to the results from the 14th day, 1% and 1.5% concentration of SA HIPEs appear stratified, the former layer is more obvious than the latter, 2% concentration of SA HIPEs layered began from 21 days, with the increase of mixing time, emulsion layering is more obvious. The stability difference might be due to the following two reasons:

(1) Three HIPEs droplet sizes with the concentration of rising and falling, generally, larger droplet size

suggests HIPEs will with the passage of time is not stable, because of their small particle size has a larger surface area, which has a higher bulk density and stability [12].

(2) With the higher concentration of sodium alginate, the higher the concentration of sodium alginate in droplet formation of the gel network structure is more stable close [13], at the same time, the higher the layered system makes the dispersed phase viscosity is not easy to gather.



(A) 7 days (B) 14 days

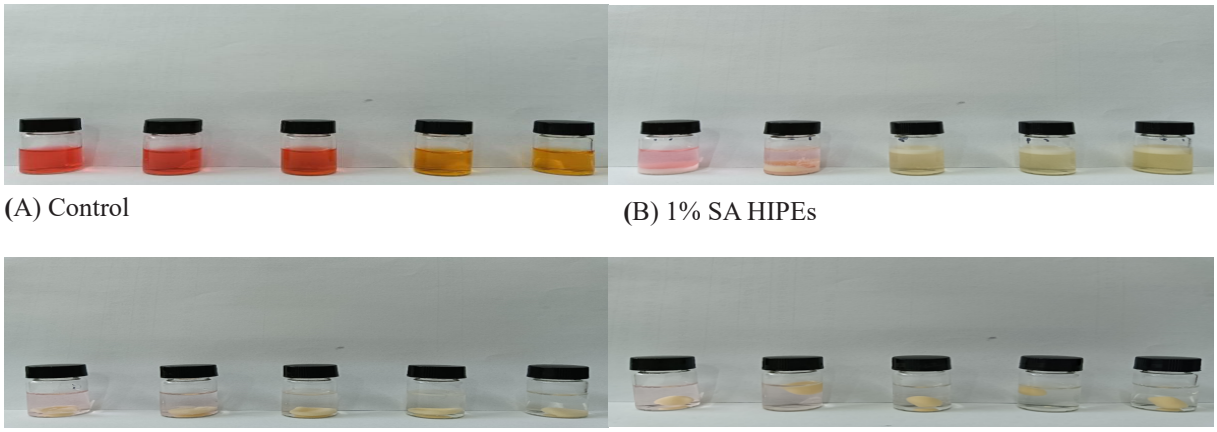
(C) 21 days (D) 28 days

(E) 35 days (F) 42 days

Figure 7. Results of HIPEs placement stability at different SA concentrations

3.3.2. Stability of pH

Probiotics are easy to be inactivated in acidic conditions because there is a high concentration of hydrogen ions leading to damage to the cell membrane. Therefore, the stability of HIPEs in hydrochloric acid under different pH conditions (1, 2, 3, 4, 5) was evaluated according to the reference scheme [14]. An acid-base indicator (methyl orange) was added to the aqueous phase. The stability of HIPEs to acid was evaluated and the results are shown in **Figure 8**. The results showed that the structure of HIPEs at 1% SA concentration in water was extremely unstable and could not maintain its morphology, and the aqueous phase containing methyl orange was largely transferred into hydrochloric acid. 1.5% and 2% concentrations of SA HIPEs in hydrochloric acid can maintain the stability of the basic form, color itself did not change. Compared with 2%, and 1.5% HIPEs there is still a small amount of water in the hydrochloric acid. The above results can be attributed to the difference in emulsion viscosity, the lower the viscosity, the more unstable the emulsion forms in hydrochloric acid, and the viscosity difference between the emulsions may be due to the positive correlation between the viscosity of the emulsion and the viscosity of the dispersed phase (aqueous phase). With the increase of the concentration of sodium alginate, the thickening effect becomes stronger and the viscosity of the emulsion increases.



(A) Control (B) 1% SA HIPEs

(C) 1.5% SA HIPEs (D) 2% SA HIPEs

Figure 8. Stability of emulsions under different pH conditions

3.3.3. Storage stability evaluation

The survival situation during the embedding process is necessary. If a large number of probiotics die during the preparation process, the subsequent protection is meaningless, and the quality of the embedding process is measured by the embedding rate. In addition, the survival of probiotics during storage with loaded HIPEs was of concern, so the number of viable probiotics was measured after 5 days of storage at 4℃ for both encapsulated and unencapsulated probiotics (**Figure 9**). As shown in **Figure 10**, the microbial load of the 2% concentration of SA HIPEs embedding rate was 90.97 ± 27.09% is significantly higher than 1.5% concentration of SA the embedding rate was 56.12 ± 11.03%, which may be due to the higher concentration of SA to form the more populated gel mesh structure, reduced the probiotics in the process of high-speed homogeneous mechanical damage. After 5 days storage at 4℃, the number of viable probiotics in 1.5% SA group increased from 8.06 ± 0.09 log CFU/mL to 7.60 ± 0.19 log CFU/mL. 2% of the number of living bacterium SA group from 8.30 ± 0.08 log CFU/mL – 7.79 ± 0.17 log CFU/mL. The number of viable probiotics in the unembedded group increased from 8.30 ± 0.12 log CFU/mL to 7.34 ± 0.07 log CFU/mL. The storage stability of embedded probiotics was better than that of unembedded probiotics, indicating that loading probiotics into HIPEs could increase the viability of probiotics, which may be due to the oil layer of W/O emulsion isolating the erosion of external water, oxygen, inorganic salts and other substances, thereby protecting the probiotics dispersed in the aqueous phase and improving the survival of probiotics.

$$Embedding\ rate = \frac{The\ actual\ number\ of\ viable\ bacterial\ colonies\ after\ embedding}{After\ the\ embedding\ theory\ of\ living\ bacterium\ colony\ number} \times 100\% \qquad (3)$$

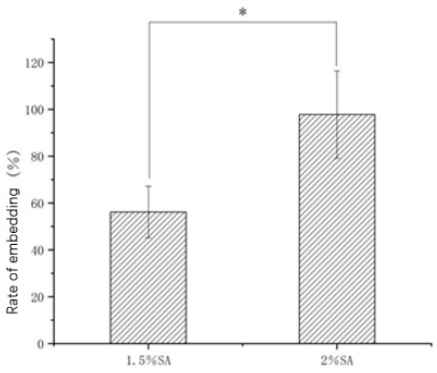


Figure 9. The embedding rate (n = 3)

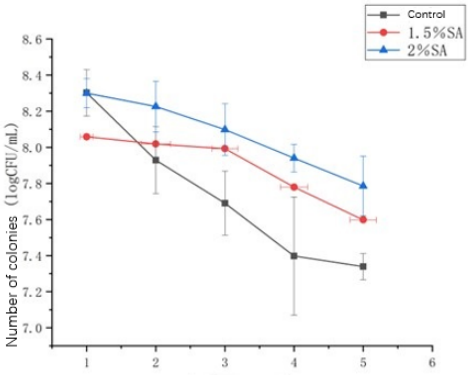


Figure 10. Count of viable probiotics stored at 4℃ (n = 3)

3.4. HIPEs in vitro simulated digestion experiments

Simulated digestion in vitro can directly reflect the protective effect of HIPEs on probiotics, which has a guiding significance for the digestion of HIPEs in vivo. The simulated digestion results in vitro are shown in **Figure 11**. After digestion with gastric fluid, the viable number of probiotics without emulsion protection increased from 8.32 ± 0.16 log CFU/mL to 0 CFU/mL. 1.5% and 2% after simulated gastric fluid to digest HIPEs SA concentration of 8.06 ± 0.09 log CFU/mL and 7.91 ± 0.25 log CFU/mL and 8.30 ± 0.08 log CFU/mL and 8.05 ± 0.16 log CFU/mL. After simulated intestinal fluid digestion, they were 7.43 ± 0.57 log CFU/mL and 7.76 ± 0.15log CFU/mL. The results showed that *L. casei* was difficult to survive in the strong acid environment of gastric fluid, and the protective effect of HIPEs of 2% SA was slightly better than that of 1.5% SA. According to the previous stability analysis, the HIPEs of 2% SA were more stable, so it could better isolate the damage of *L. casei* contained in it by strong acid, enzyme and inorganic salt.

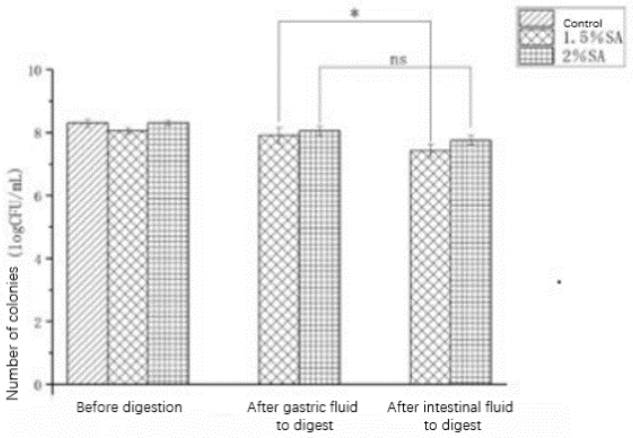


Figure 11. Probiotic count at different stages of simulated digestion in vitro (n = 3)

3.5. Probiotics HIPEs antitumor evaluation

Results as shown in **Figure 12** and shown in **Table 6**, compared with gastric normal saline control group, to fill the stomach take *Lactobacillus casei* HIPEs treatment group tumor volume growth, be suppressed, the 14th day control tumor size 1,565.85 mm^3, this could be due to *L. casei* engraftment in colon in mice, improve the intestinal flora environment [15,16], at the same time, *L. casei* showed an inhibitory effect on tumor growth. The tumor inhibition rate reached 33.78%, and the figure shows the volume change trend of the mice. There was no abnormal change in the body weight of the mice in the treatment group, which was attributed to the fact that there was no significant negative effect on the appetite and metabolism of the mice during the treatment with probiotic-loaded HIPEs.

Table 6. Tumor quality and inhibition rate in each group

Group	Tumor quality (g)	Tumor growth inhibition rate (TGI)
Control	0.57 ± 0.12	/
Probiotic	0.38 ± 0.20	33.78%

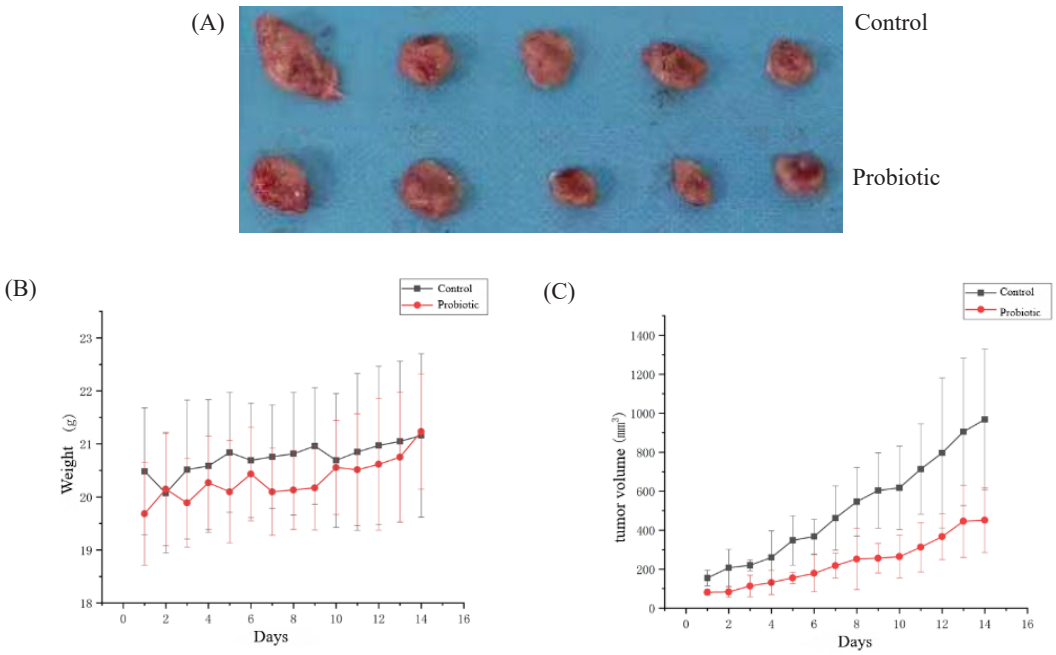


Figure 12. (A) Physical tumor images of control group and treatment group; (B) Weight change curve of mice in control group and treatment group; (C) Tumor volume change curve between treatment group and control group (n = 5)

4. Conclusion

In this study, a w/o HIPE was developed to encapsulate *L. casei*, focusing on preparation, stability, appearance, microstructure, and *in vitro* digestion. The optimized formulation included 5% polyglycerol polyricinoleate, 75% aqueous phase volume, homogenization at 3,000 rpm for 30 seconds, and 2% sodium alginate in the aqueous phase. Post *in vitro* digestion, the w/o HIPE maintained *L. casei* viability above 10^7 CFU/mL, demonstrating effective probiotic encapsulation and gastrointestinal protection. The emulsion's outer oil phase shielded probiotics from gastric acid and intestinal enzymes, highlighting its potential in food and medical applications. Additionally, the study explored w/o HIPE's potential in breast cancer therapy, showing a 33.78% tumor inhibition rate versus controls. Future research should investigate its antitumor mechanisms and synergies with chemotherapy to enhance *L. casei*'s therapeutic benefits.

Disclosure statement

The authors declare no conflict of interest.

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