Food Innovation and Advances

ARTICLE

Open Access

https://doi.org/10.48130/fia-0026-0025

Food Innovation and Advances **2026**, 5(2): 315–328

From extraction to encapsulation: optimizing the processing strategy of soybean oil bodies for enhanced thermal and photochemical stability of lutein

Yao Yang[1,2], Rao Fu[1,2], Boran Yang[1,2], Jiayi Zuo[1,2], Yuanying Ni[1,2], Xin Wen[1,2*] and Mo Li[1,2*]

[1] *College of Food Science and Nutritional Engineering, China Agricultural University, No. 17 Qinghua East Road, Beijing 100083, China*
[2] *National Engineering Research Center for Fruits and Vegetables Processing, No. 17 Qinghua East Road, Beijing 100083, China*
* Correspondence: xin.wen@cau.edu.cn (Wen X); limo0125@cau.edu.cn (Li M)

Abstract

Lutein, a bioactive compound with limited stability under thermal and light exposure, faces challenges in food applications, which is the core research problem to be solved. Soybean (*Glycine max*) oil bodies (SOBs), natural oil-in-water emulsions, are a promising delivery platform for hydrophobic compounds like lutein. This study optimized the encapsulation and stabilization of lutein using SOBs extracted at two pH levels, namely crude oil bodies (COB, pH 7) and purified oil bodies (POB, pH 11), via three techniques: stirring, homogenization, and ultrasonication. The key research results are as follows. All samples had an encapsulation efficiency (EE) > 60%, with homogenized COB with a 0.05% concentration of lutein achieving the highest (94.14%). COB had higher loading efficiency (LE) than POB (extraneous proteins promoted lutein binding), and ultrasonication/stirring yielded higher LE than homogenization. All emulsions exhibited unimodal particle size distributions (350–520 nm) and negative zeta potentials. Ultrasonication enhanced physical stability, and lutein did not affect viscosity or fluidity. POB emulsions better protected lutein (retention > 50% after heating at 90 °C for 12 h). Homogenization reduced lutein retention through membrane damage. These findings validate SOBs as lutein carriers, clarify the effects of SOBs' composition and encapsulation methods on delivery performance, and provide insights for optimizing hydrophobic bioactive encapsulation in functional products.

Citation: Yang Y, Fu R, Yang B, Zuo J, Ni Y, et al. 2026. From extraction to encapsulation: optimizing the processing strategy of soybean oil bodies for enhanced thermal and photochemical stability of lutein. *Food Innovation and Advances* 5(2): 315–328 https://doi.org/10.48130/fia-0026-0025

Introduction

Oil bodies (OBs) are spherical organelles that store lipids in plants and have a natural oil-in-water structure. They are mainly found in seeds and nuts, and comprise a triacylglycerol (TAG)-rich 'core' surrounded by a 'shell' of phospholipids and embedded proteins[1]. The unique binding of phospholipids and proteins via electrostatic, hydrophobic, and van der Waals forces forms an amphiphilic structure in OBs and gives them flexibility and compressibility, which enhances their physicochemical stability against external stresses[2]. As natural and sustainable carriers, OBs exhibit excellent biocompatibility and biodegradability while eliminating the need for additional emulsifiers. These properties align with the global trend towards 'clean labeling' in the food industry, making OBs a research hotspot in the field of bioactive compound delivery systems. Owning to these advantageous structural features, OBs are considered to be excellent candidates for natural delivery systems, and soybean (*Glycine max*), as a vital grain crop and important oil source, offers a particularly promising platform for such applications. Under neutral pH conditions, soybean oil bodies (SOBs) appear as vesicles with diameters ranging from 250 to 700 nm. Compared with peanut (*Arachis hypogaea*) and sunflower (*Helianthus annuus*) seed OBs, SOBs exhibit significantly higher protein-to-lipid ratios, which may facilitate the encapsulation of hydrophobic bioactive substances. Furthermore, their average particle size is notably smaller, giving SOBs greater outstanding physicochemical stability[3].

In recent years, the direct extraction and utilization of OBs from oilseeds have emerged as more sustainable and environmentally friendly alternative to conventional oil refining. Aqueous extraction, in particular, is widely adopted because of its safety and minimal environmental impact[4]. This method yields crude oil bodies (COBs), which contain substantial amounts of endogenous non-OB proteins. The removal of the extraneous proteins can be achieved by pH adjustment, resulting in purified oil bodies (POBs). The distinct protein profiles of COBs and POBs lead to differences in their interfacial composition, emulsifying properties, antioxidant activity, and stability[5]. Our previous studies found that POBs from soybean exhibited better stability than COBs under various pH and ionic strength conditions caused by differences in their membrane composition[5,6]. However, current research on SOBs as delivery carriers primarily focuses on a single extraction pH. There is a lack of systematic comparisons and analyses of the differences in the encapsulation effect and stability of bioactive compounds between SOBs with different interfacial compositions (COBs vs POBs).

The amphiphilic nature of OBs' membranes enables them to act as effective delivery systems for enhancing the antioxidant activity, bioavailability, and gastrointestinal fate of bioactive compounds. Previous studies have demonstrated that the OB membrane is permeable to hydrophobic molecules, allowing them to accumulate in the interface or inner core of the OBs[7]. In the context of clean labeling formulations, as natural pre-emulsified oil-in-water emulsions, SOBs may provide an alternative to the synthetic emulsions currently utilized in emulsion-based applications[8]. Numerous studies have successfully encapsulated bioactive components by various methods, such as stirring, ultrasonication and homogenization. Acevedo et al. encapsulated astaxanthin into rapeseed (*Brassica rapa*) OBs using a stirring method (200 r/min, 5.3 h). This process achieved a microencapsulation efficiency of up to 99% and significantly improved the retention rate of astaxanthin under both light and dark conditions[9]. Zhang et al. extracted flaxseed

(*Linum usitatissimum*) OBs to encapsulate β-carotene, achieving an encapsulation efficiency of 96.88% under optimized ultrasound conditions (250 W, 7 min), with a retention rate of 84.06% after 30 d of storage at 4 °C[10]. Chen et al. encapsulated lutein esters with rice (*Oryza sativa*) bran OBs by homogenization (50 MPa, three cycles), and found that the particle size of rice bran OBs decreased from 2 μm to 264.48 nm, with the encapsulation efficiency of lutein esters reaching 94%[11]. Nonetheless, no single encapsulation technique is universally applicable to all hydrophobic components, and systematic comparisons among these techniques or strategies for optimizing encapsulation protocols remain limited. In addition, the effect of extraction-induced variations in OBs' interfacial membrane composition on the encapsulation efficiency and stability of bioactive substances has not been thoroughly investigated.

Lutein is a lipophilic carotenoid containing hydroxyl groups. It processes strong antioxidant activity and has been associated with health benefits such as tumor suppression, immune enhancement, and UV (ultraviolet) protection[12]. Therefore, it is widely used in food, health products, and cosmetics[13]. However, the conjugated double bonds in lutein make it susceptible to oxidation, light, and heat, resulting in poor stability and limited application[14]. To address these limitations, various microencapsulation and separation technologies have been extensively investigated, such as spray drying[15,16] and colloidal gas aphron (CGA) separation with Tween-20 surfactant[17]. However, despite these advances, there remains a persistent demand for more biocompatible and natural delivery vectors[18].

Given their natural structure and physicochemical properties, SOBs are promising carriers for enhancing the stability of lutein. Previous studies have primarily focused on either the encapsulation process or the carrier properties of OBs individually, though systematic investigations into the synergistic effects of extraction pH-induced variations in interfacial composition and of encapsulation procedures on lutein's stability are still limited. The present study provides an in-depth examination of this issue, aiming to establish a theoretical foundation for optimizing the processing of SOB-based delivery systems. This study aimed to encapsulate lutein into both COBs and POBs derived from soybean using three different treatments (stirring, homogenization, and ultrasonication). The physicochemical properties and stability of the resulting emulsions were compared. Furthermore, the encapsulation efficiency and the stability of the encapsulated lutein under heat and light stress were evaluated. Overall, this research aims to provide theoretical and technical support for the development of innovative SOB-based delivery systems for lipophilic bioactive components.

Materials and methods

Materials

Soybeans were purchased from Harbin Hongxing Agricultural Development Co., Ltd. (Bayan County, Heilongjiang, China). Lutein (purity ≥ 80%) was purchased from Yuanye Bio-Technology (Shanghai, China). All chemicals were of analytical grade and purchased from China National Pharmaceutical Group Corporation (Beijing, China).

Preparation of oil bodies

Extraction of COBs and POBs

COBs and POBs were extracted according to the previous studies with some modifications[19].

Extraction of COBs: Soybeans were soaked in deionized water at a ratio of 1:5 (g/mL) at 4 °C for 18 h. Then the soybeans were blended at the maximum setting of the blender (HR2095, Philips, Hong Kong) for 120 s, and the filtrate was obtained after filtration through three layers of gauze, with the pH adjusted to 7 with a NaOH solution (0.1 M). The filtrate was centrifuged at 11,000 rpm and 4 °C for 30 min (Avanti JXN-30, Beckman Coulter, Indiana, USA), and the upper cream was separated. Deionized water was added to dissolve the cream at a ratio of 1:10 (g/mL), then the pH was adjusted to 7, followed by centrifugation (11,000 rpm, 30 min). After repeating the washing process twice, the upper cream was referred to as the COBs.

Extraction of POBs: Soybeans were soaked in deionized water at a ratio of 1:5 (g/mL) at 4 °C for 18 h. Then the soybeans were blended at the maximum setting of the blender (HR2095, Philips, Hong Kong) for 120 s, and the filtrate was obtained after filtration through three layers of gauze, with the pH adjusted to 11 with a NaOH solution (0.1 M). The filtrate was centrifuged at 11,000 rpm and 4 °C for 30 min, and the upper cream was separated. Deionized water was added to dissolve the cream at a ratio of 1:10 (g/mL), then the pH was adjusted to 11, followed by centrifugation (11,000 rpm, 30 min). After repeating the washing process twice, the upper cream was referred to as the POBs.

Analysis of composition

The water content of the COBs and POBs was determined with a moisture analyzer (DSH-50A-1, Shanghai Huyueming Scientific Instruments Co., Ltd., China). The drying temperature was set at 105 °C, and the samples were continuously dried until the moisture content no longer changed.

The oil content of the COBs and POBs was determined using a Soxhlet device (SE206, ALVA, China). The dried OBs were sealed in a piece of cut filter paper (degreased), then the filter paper was placed into a Soxhlet extractor (60 °C, 5 h), with petroleum ether as the extraction reagent. After the extraction was completed, the mixture was transferred into a distillation flask for rotary evaporation (R-100, BUCHI, Switzerland) until no further weight loss was observed.

The protein content of the COBs and POBs was determined by the Kjeldahl method with a semiautomatic device (KN520, ALVA, China). Dried OBs were accurately weighed into a digestion tube, followed by the addition of a high-efficiency catalytic tablet (comprising potassium nitrate and copper sulfate) and concentrated sulfuric acid. Kjeldahl digestion was then conducted. After the tube had cooled to room temperature, nitrogen content was determined with an automatic Kjeldahl nitrogen analyzer. For this, the sample was titrated with a standard hydrochloric acid solution until the solution turned grayish green.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

The protein composition of the COBs and POBs was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Fu et al.[20] with some modifications. Fresh COBs and POBs were separately diluted with deionized water. Aliquots of 30 μL from each sample were mixed with 10 μL of a protein loading buffer containing β-mercaptoethanol, heated at 95 °C for 5 min, cooled, and then centrifuged. A 5% stacking gel and a 15% separating gel were used for electrophoresis. For this, 10 μL of the supernatant from each sample was loaded onto the gel. Electrophoresis was performed at a constant voltage of 80 V until the bromophenol blue front entered the separating gel, after which the voltage was adjusted to 110 V and electrophoresis continued until the bromophenol blue reached the bottom of the glass plate. After

electrophoresis, the gel was stained with a rapid staining solution and destained until the electrophoresis bands were clear with a clean background. The gel was then imaged using a gel imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Preparation of lutein-loaded OBs emulsions

Encapsulation of lutein

A flowchart of lutein encapsulation by OB emulsions is shown in Fig 1. Lutein was dissolved in absolute ethanol at concentrations of 0.05% and 0.5% (w/v), followed by 20 s of ultrasonication. COBs or POBs were quantified by adjusting the water content to 40%, and then diluted with deionized water to form an emulsion with a 10% mass fraction of OBs by stirring at 750 rpm for 30 min. A lutein–ethanol solution was added dropwise to the emulsion under continuous stirring at room temperature for 30 min. The concentration of ethanol in the final solutions was lower than 2 vol%. The resulting product was defined as the stirring treatment group (COB-S or POB-S). To produce homogenized COBs and POBs (COB-H and POB-H, respectively) COB-S or POB-S samples were subjected to homogenization at 30 MPa for thre cycles in a high-pressure homogenizer (Sihuan Qihang Technology Co., Ltd., Beijing). To produce ultrasonicated COBs and POBs (COB-U and POB-U, respectively), COB-S or POB-S samples were subjected to ultrasonication (200 W, 4 min, 4 s on, 2 s off) in an ultrasonic processor (SCIENTZ-IID, Ningbo, China). Sodium azide (as an antimicrobial agent) was added in all lutein-loaded OB emulsions at a concentration of 0.02% (w/w). The final emulsions were stored in a refrigerator at 4 °C.

Encapsulation efficiency and loading efficiency

Extraction of free lutein (C_1): The lutein-loaded OB emulsion was centrifuged at 13,000 rpm for 30 min and separated into the supernatant and cream layers. The supernatant was then mixed with an extraction solution consisting of methanol, ethyl acetate, and petroleum ether (1:1:1, v/v/v), each containing 0.1 g/L BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), followed by vigorous vortex mixing (1,000 rpm, 30 s). The mixture was treated using an ultrasonic disruptor (100 W, 1 min, with cycles of 2 s on, 2 s off). Subsequently, it was centrifuged at 3,000 × *g* for 3 min, and the supernatant was collected[21]. The extraction process was repeated until the supernatant became colorless. All supernatants were combined and dried by nitrogen blowing.

Extraction of total lutein (C_0): The extraction solution (methanol/ethyl acetate/petroleum ether at a volume ratio of 1:1:1, containing 0.1 g/L each of BHA and BHT) was added directly to the emulsion. The mixture was vortexed (1,000 rpm, 30 s) thoroughly, and subsequently subjected to the ultrasonication treatment (100 W, 1 min, with cycles of 2 s on, 2 s off). After ultrasonication, the sample was centrifuged at 3,000 × *g* for 3 min, and the supernatant was collected. The extraction procedure was repeated until the supernatant became colorless. The combined supernatants were then dried by a flow of nitrogen.

Determination of lutein content: The residue in the brown glass vial after nitrogen drying was dissolved in absolute ethanol. The lutein content was determined using a UV spectrophotometer (UV 1600 PC spectrophotometer, VWR, Shanghai) at a wavelength of 445 nm. The lutein standards were determined by the abovementioned process to obtain a standard curve ($y = 14.426x + 0.0915$, $R^2 = 0.9999$), which was used to calculate the content of lutein in different samples.

The encapsulation efficiency (EE) and loading efficiency (LE) were calculated using the following formulas

$$EE\ (\%) = \frac{C_0 \times V_0 - C_1 \times V_1}{C_0 \times V_0} \times 100\%$$

$$LE\ (\%) = \frac{C_0}{C} \times 100\%$$

where, C denotes the initial concentration of lutein added to the emulsion (mM), C_0 denotes the concentration of total lutein in the emulsion (mM), C_1 denotes the concentration of lutein in the supernatant after centrifugation (mM), V_0 denotes the initial volume of the emulsion (mL), and V_1 denotes the volume of the supernatant after centrifugation (mL).

Characterization of lutein-loaded OBs emulsions

Particle size and zeta potential measurements

The particle size, PDI (polydispersity index), and zeta potential of all samples were measured by a dynamic light scattering apparatus (DLS Zetasizer NanoZS, Malvern Instruments Ltd, UK) according to the method of Iwanaga et al.[22] SDS (sodium dodecyl sulfate), an anionic surfactant, was used. The emulsion was mixed with a 10% SDS solution at a 1:9 volume ratio, where low-concentration SDS was used to disrupt noncovalent interactions between proteins in the emulsion and reduce the system's flocculation. The refractive

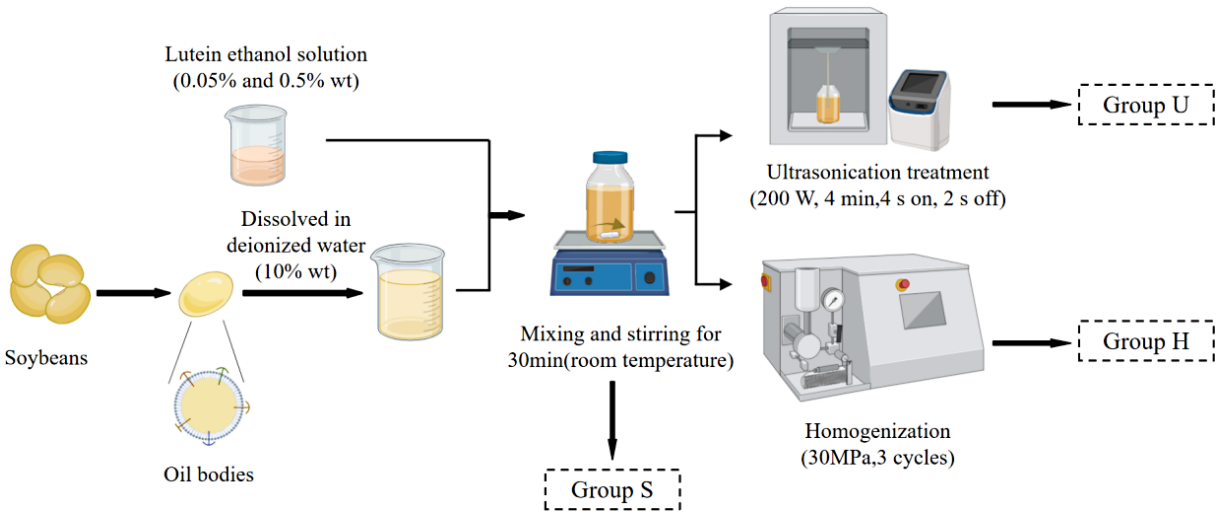

Fig. 1 Flowchart of lutein encapsulation by OB emulsions.

index of the emulsion was set to 1.47, and the refractive index of the solvent dispersion medium was set to 1.33. The experimental temperature was maintained at 25 °C.

Rheological properties

The apparent viscosity of all samples was characterized using a DHR-2 rotational rheometer (TA Instruments, UK) following the methodology described by Fu et al.[20] with a parallel plate (40 mm diameter). The flow ramp mode was selected, and the viscosity–shear rate curves were determined under the following conditions: Measurement temperature, 25 °C; equilibrium time, 30 s; gap size, 1,050 μm; shear rate range, 1–100 s^{-1}; measurement time, 60 s.

Confocal laser scanning microscopy

The microstructure of all samples was observed with a laser scanning microscope (AXR NSPARC, Nikon, Japan) with a 100× oil-immersion objective lens according to the method of Yang et al.[23]. The emulsion was mixed with deionized water at a 1:1 ratio and shaken well. Dyes were prepared by dissolving Rhodamine B (0.1 wt%, excitation wavelength = 633 nm) and Nile red (0.1 wt%, excitation wavelength = 488 nm) in isopropanol. Next, 1 mL of the diluted emulsion was mixed with 10 μL of the Rhodamine B solution or 10 μL of the Nile red solution, followed by thorough vortexing. A 10-μL aliquot of the stained emulsion was pipetted onto a glass slide, and a coverslip was carefully placed to avoid bubble formation. The slide was inverted and mounted on the stage for observation.

Physical stability of lutein-loaded OBs emulsions

The LUMiFuge full-function stability analyzer (L.U.M. GmbH, Germany) was used to determine the temporal and spatial changes in the light transmittance of OB emulsions during accelerated centrifugation[24]. For this, 0.4 mL of the emulsion was accurately added to the calibration line of the LUMiFuge centrifuge tube, with the temperature set at 25 °C, a centrifugal force of 3,000 rpm, an interval of 10 s, and 360 cycles for measurement.

Thermal stability of lutein in OBs emulsions

The method of determining thermal stability proposed by Zhu et al.[25] was used with slight modifications. Fresh COB and POB emulsions loaded with a high concentration of lutein were heated at 60, 75, and 90°C for 12 h. Samples were taken out at 0, 2, 4, 6, 8, 10, and 12 h and rapidly cooled to room temperature. The lutein retention rate, particle size, and zeta potential were determined using the aforementioned methods, and a thermodynamic analysis of degradation was performed.

$$\text{Retention rate}\ (\%) = \frac{C}{C_0} \times 100\%$$

$$\text{Ln}\left(\frac{C}{C_0}\right) = -kt$$

$$t_{1/2} = (Ln2)/k$$

$$\text{Ln}\ k = \text{Ln}\ A - E_a/RT$$

where, C_0 denotes the initial concentration of lutein in the emulsion (mM), and C denotes the concentration of lutein in the emulsion after thermal treatment (mM).

Photochemical stability of lutein in OB emulsions

The method of determining photochemical stability proposed by Chen et al.[11] was used with slight modifications. Freshly prepared lutein emulsion samples were irradiated under dark conditions (B) and with a 10-W UV (320–400 nm) lamp for 15 d. To ensure uniform light intensity for each sample, the sample bottles were placed parallel to the lamp at a distance of approximately 15 cm. Samples were taken on 0, 1, 3, 5, 7, 9, 11, 13, and 15 d. After sampling, observations of appearance, determination of color, and measurement of lutein retention rate were conducted.

Statistical analyses

All measurements were carried out in triplicate, and the data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed with IBM SPSS Statistics 27.0 software (IBM Corporation, Armonk, NY, USA), and analysis of variance (ANOVA) was conducted by Duncan's test to compare the significant differences among the results. All values were plotted with Origin 64 software (Origin Lab Corporation, Northampton, MA, USA). A p-value < 0.05 was considered to be statistically significant.

Results and discussion

Main composition of COBs and POBs

The moisture, protein and oil content of the COBs and POBs are presented in Supplementary Table S1. COBs and POBs contained approximately 40% moisture. The oil content of COBs and POBs reached 87.01% and 92.82% (dry basis), respectively, which closely align with the oil content range of approximately 88%–93% reported in previous studies[8,19]. The protein content of the COBs and POBs was 6.89% and 3.94%, respctively. This difference might be attributed to the removal of extraneous proteins from the OBs to prepare POBs in the alkaline environment (pH 11), compared with COBs. The alkaline condition (pH 11) could induce conformational changes in the extraneous proteins (such as P34 and glycinin) bound to the surface of the COBs, disrupting the hydrophobic interactions and hydrogen bonds between these proteins and the OBs' interface[26,27].

SDS-PAGE

As shown in Fig 2, SDS-PAGE analyses revealed that the protein profiles of COBs and POBs were widespread, with their molecular weights spanning from 15 to 95 kDa. In the protein profiles of COBs, the bands corresponding to 14, 18, 22, 24 and 34 kDa were the most intense. This finding was partly consistent with prior research, in which the most prominent bands were situated within the 16–24 and 34 kDa molecular weight regions[19]. Notably, the bands at 34 kDa, which were present in COBs, appeared noticeably fainter in POBs, similar to P34. This protein has long been recognized as one

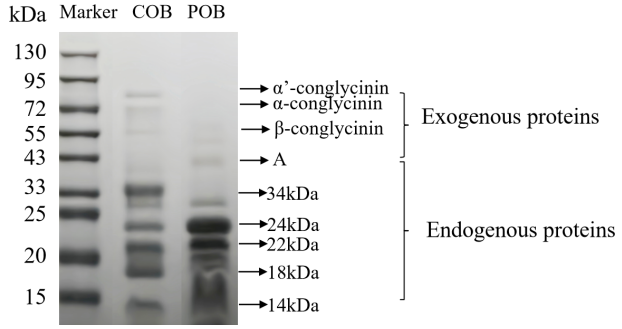

Fig. 2 The protein composition of COBs and POBs determined by SDS-PAGE.

of major allergenic proteins in soybeans[28]. Furthermore, other high-molecular-weight proteins, potentially identified as glycinin and conglycinin (α, α', β, γ) in a previous study[29], were also removed from the surface of OBs through alkali treatment when preparing POBs. This result aligns with the findings discussed in section 'Main composition of COBs and POBs'. A relatively faint band appeared at approximately 94 kDa, which corresponds to lipoxygenase[19]. The band of lipoxygenase in COBs was clearer, indicating that their lipoxygenase content was higher than that in POBs. Consequently, the protein profiles of POBs were dominated by the 22 and 24 kDa bands, which were deemed to represent the intrinsic oleosins in SOBs[30].

Encapsulation efficiency and LE of lutein in OBs emulsions

The EE and LE are critical metrics for evaluating the potential of SOBs as the delivery system for lutein. As shown in Table 1, when the concentration of lutein was at a low level (0.05% w/v), the EE of all COB treatment groups was above 90%, and the highest value of 94.14% was obtained after homogenization with 0.05% lutein (COB-H-0.05). When the concentration of lutein was increased to 0.5% (w/v), there was a significant decrease in the EE for all COB treatment groups ($p < 0.05$), ranging from approximately 60% to 80%. A similar change was observed in the POB samples as the concentration of lutein increased from 0.05% (w/v) to 0.5% (w/v), which was consistent with the findings reported by Vardar et al.[31]. They found that the EE of 0.01 wt% curcumin encapsulated into 10 wt% rapeseed OBs was 100% ± 0.00%, whereas the EE of 0.1 wt% curcumin was 61.57% ± 0.16%. Collectively, these results indicated that both COBs and POBs have a limited capacity for encapsulating lutein. When the lutein concentration was low, the binding sites were unsaturated, allowing most of the lutein to stably bind to the OBs' interface or core, resulting in a high EE. However, as the concentration increased, the binding sites might become saturated, and the excessive lutein could not effectively bind to the OBs and instead dissolved in the aqueous phase, leading to a significant decrease in EE. In both COBs and POBs with a low concentration of lutein, homogenization resulted in the highest EE of lutein among the three treatment methods, whereas the EE values for the stirring and ultrasonication treatments showed no significant difference ($p > 0.05$). In the groups with a high lutein concentration, the three treatments showed significant differences ($p < 0.05$) in the EE of lutein, following the orders COB-H-0.5 > COB-S-0.5 > COB-U-0.5 and POB-S-0.5 > POB-H-0.5 > POB-U-0.5. The ultrasonication treatment resulted in the lowest EE in both COBs and POBs. This is likely because a proportion of the lutein was only weakly associated with the OBs' interface, causing it to readily dissociate during the extraction step of the EE analysis procedures. Compared with the stirred groups, homogenization exerted different effects in COBs and POBs, which might be related to the content and composition of the interfacial proteins in OBs. Homogenization enabled COBs to effectively form a denser and more stable interfacial protein membrane, facilitating better lutein encapsulation[32]. Conversely, in POBs, the lack of extraneous proteins may render the native oleosin–phospholipid monolayer more susceptible to damage induced by homogenization, resulting in structural collapse and a decrease in EE.

Table 1. The encapsulation efficiency and loading efficiency of lutein-loaded OBs emulsions with different treatments.

Treatment group	Encapsulation efficiency (%)	Loading efficiency (%)
COB-S-0.05	91.11 ± 0.86b	92.15 ± 7.47a
COB-H-0.05	94.14 ± 0.36a	66.73 ± 4.08de
COB-U-0.05	92.86 ± 0.81ab	83.28 ± 3.53abc
COB-S-0.5	72.70 ± 3.63e	86.29 ± 6.67ab
COB-H-0.5	77.30 ± 0.55d	74.67 ± 3.15cd
COB-U-0.5	63.15 ± 2.45g	86.92 ± 5.78ab
POB-S-0.05	90.36 ± 0.43bc	90.42 ± 4.77ab
POB-H-0.05	92.04 ± 0.43ab	82.22 ± 3.58bc
POB-U-0.05	87.93 ± 0.98c	89.81 ± 1.88ab
POB-S-0.5	78.43 ± 1.54d	65.90 ± 4.22de
POB-H-0.5	68.41 ± 1.86f	62.55 ± 6.71e
POB-U-0.5	63.28 ± 1.70g	68.05 ± 4.36de

Notes: S, stirring; h, homogenization; U, ultrasonication. Error bars indicate the standard deviation from triplicate tests. Different letters indicate significant differences ($p < 0.05$) in different treatment groups. 0.05 and 0.5 represent the concentrations of the lutein–ethanol solution.

The LE primarily evaluates the actual incorporation effectiveness of lutein, whereas the EE mainly assesses the stability of encapsulation. At low lutein concentrations, there was no significant difference ($p > 0.05$) in LE between the COB-S and POB-S emulsions. However, after homogenization, the LE decreased in both COBs and POBs, which is opposite to the trend observed for EE. These results indicated that although homogenization was effective at stabilizing lutein by encapsulation, it had a limited impact on enhancing loading capacity. The effect of the ultrasonication treatment on the LE of both COBs and POBs was similar to that of the stirring treatment, indicating that ultrasonication was a gentler processing method than homogenization. At high lutein concentrations, the LE of all COB groups was higher than that of the POB groups. This phenomenon is most likely attributed to the difference in interfacial protein composition between COBs and POBs. COBs retain substantial amounts of extraneous non-OB proteins (e.g., the 34-kDa P34 protein, glycinin, and conglycinin) on its interfacial membrane. In contrast, POBs effectively remove these extraneous proteins, leaving only intrinsic oleosins (22 kDa and 24 kDa) at the interface. At high lutein concentrations, the binding capacity of the OBs' core (TAGs) becomes saturated, and the extraneous proteins in COBs provide additional binding sites for excess lutein through hydrophobic interactions and other noncovalent bonds[33]. This enables COBs to accommodate more lutein, thereby achieving a higher LE.

Particle size, PDI and Zeta potential of lutein-loaded OBs emulsions

As shown in Fig. 3a, b, the particle sizes of all lutein-loaded OB emulsions were below 500 nm, indicating the successful formation of a stable nanoemulsion system that is suitable for lutein delivery. Overall, COB emulsions exhibited trends of changes in particle that were consistent with those of POB emulsions across all treatments (stirring, homogenization, and ultrasonication) and under the addition of low or high concentrations of lutein. Stirring did not significantly alter the particle size compared with the control, although both homogenization and ultrasonication significantly reduced the particle size relative to the control ($p < 0.05$), with ultrasonication showing a more pronounced effect. Lin et al.[32] also found that ultrasonication and homogenization could reduce the particle size of emulsions. This may be attributed to the breakup of primary droplets and the formation of smaller fat particles caused by the intense mechanical dispersion and disruption abilities of ultrasonication and homogenization[34]. The addition of a high concentration of lutein after homogenization, as well as both low and high concentrations after stirring and ultrasonication, resulted in a slight increase in particle size ($p < 0.05$). This suggested that the incorporation of lutein may lead to a slight thickening of the OBs' interface. The PDI is a crucial parameter for characterizing the degree of droplet aggregation and stability[35]. As shown in

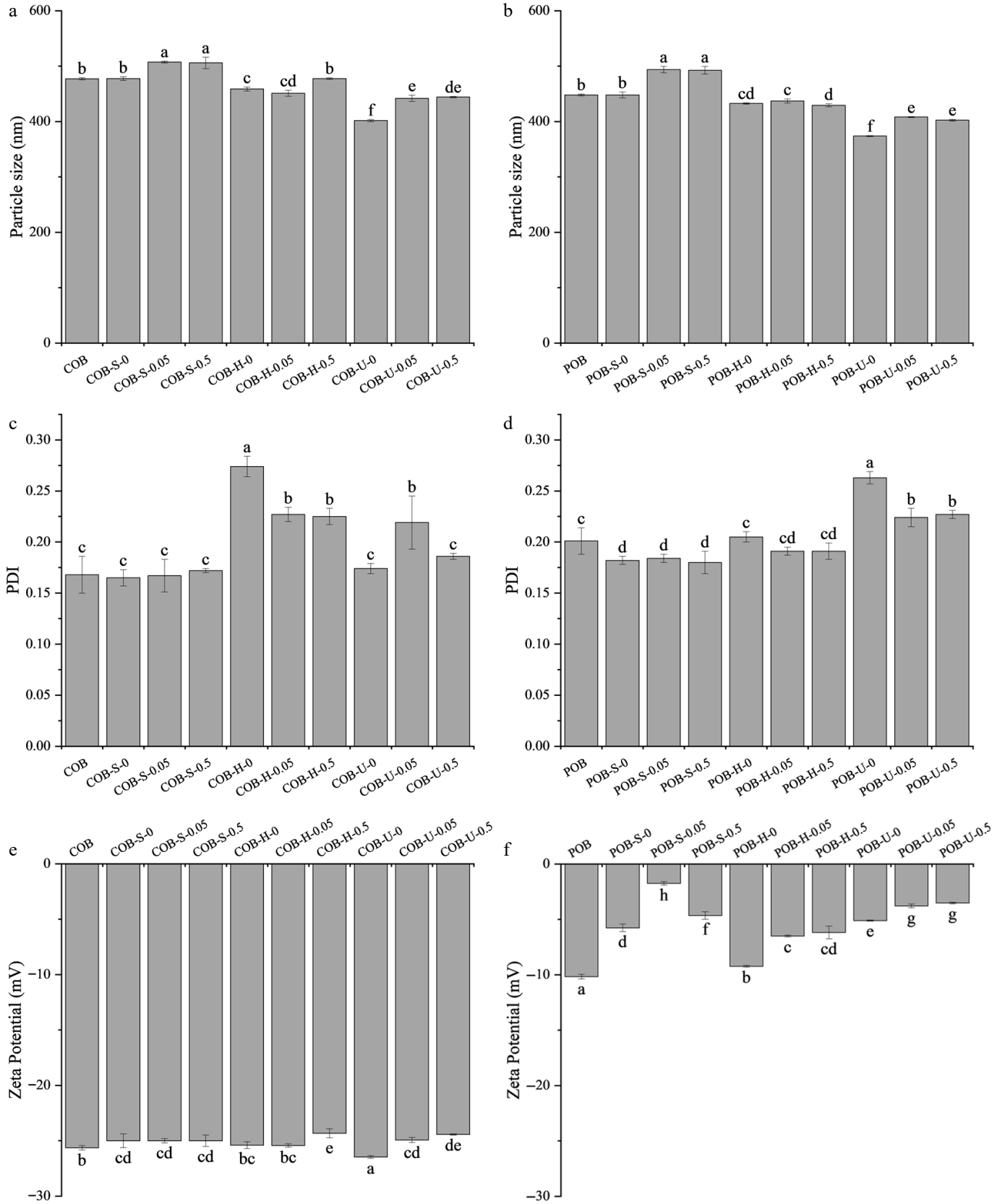

Fig. 3 The zeta potential, particle size, and PDI of emulsions in different treatment groups. (a) The particle size of COB emulsions. (b) The particle size of POB emulsions. (c) The PDI of COB emulsions. (d) The PDI of POB emulsions. (e) The zeta potential of COB emulsions. (f) The zeta potential of POB emulsions.

Fig. 3c, d, the PDI of all the emulsions remained below 0.3, indicating that monomodal size distributions were preserved across all treatments (stirring, homogenization, ultrasonication) and upon the incorporation of lutein at both low and high concentrations. Specifically, a significant increase was observed in COB emulsions after homogenization, and in POB emulsions after ultrasonication. This broadening of the size distribution was likely attributable to the intense mechanical forces of homogenization and ultrasonication, which could generate a heterogeneous mixture of original droplets and newly formed smaller particles[36,37].

The zeta potential, a key indicator of an emulsion's stability, reflects the electrostatic repulsion between droplets; a higher absolute value generally signifies greater stability[38]. As shown in Fig. 3e, f, all emulsions exhibited negative zeta potentials, which was attributed to the presence of numerous negatively charged polar groups (e.g., phosphate groups and protein carboxyl groups) on the interfacial membranes of SOBs. This observation is consistent with the findings reported by Ding et al.[39] In COB emulsions, stirring slightly reduced the absolute zeta potential, although adding lutein at either concentration showed no significant effect. Homogenization alone did not induce a significant change, but the subsequent addition of a high lutein concentration led to a marked decrease ($p < 0.05$). Ultrasonication significantly increased the absolute value compared with the control, yet this increase was reversed upon the addition of lutein at both low and high concentrations ($p < 0.05$). For POB emulsions, all three physical treatments (stirring, homogenization, and ultrasonication) resulted in a slight decrease in the absolute zeta potential. Furthermore, the incorporation of lutein, regardless of the concentration, caused a significant reduction ($p < 0.05$). Moreover, the absolute zeta potential of POBs was lower than that of COBs, which might be caused by the alteration in the surface protein components of SOBs. In particular, the extraneous proteins were removed from POBs' surfaces, whereas those bound to the surface of COBs provided an additional negative charge[40]. Additionally, a noticeable reduction in the absolute zeta potential was observed in several lutein-loaded groups (COB-S, COB-H, POB-S, POB-H, and POB-U) compared with their lutein-free counterparts. We hypothesize that the encapsulation of hydrophobic lutein molecules into the phospholipid–protein interface of OBs may partially displace native charged groups. This displacement would reduce the net charge density at the oil–water interface, thereby explaining the observed decrease in zeta potential[41].

Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) images (Fig. 4) revealed the distribution of lipids and proteins in COB and POB emulsions after different treatments. Owing to the small molecular size of lutein, variations in its content were unable to be discriminated in CLSM observations. Consequently, a lutein concentration of 0.5% was adopted in this experiment. Compared with the stirring group, the ultrasonication and homogenization groups exhibited reduced lipid droplet sizes, and both treatments completely eliminated the large initial aggregates. Notably, it was observed that COB emulsions contained more protein than POB emulsions (Fig. 4b). This result corresponds to the results for particle size, PDI and component contents.

Apparent viscosity of lutein-loaded OBs emulsions

Apparent viscosity is an important parameter for characterizing the flow behavior of non-Newtonian fluids. As shown in Fig. 5a, b, the apparent viscosity of all emulsions was below 0.05 Pa·s, indicating that the OB emulsions still maintained low viscosity and good fluidity after loading lutein. In all cases, it was observed that the apparent viscosity gradually decreased as the shear rate increased from 0.1 to 10 s^{-1}, exhibiting a typical shear-thinning behavior. Similar results were observed in previous studies on OBs[11,42]. This property is beneficial for the industrial production of emulsions, and it helps to reduce viscosity during shear processing to adapt to the production process while maintaining the system's stability during static storage, thereby enhancing overall practicality and shelf life[43]. All samples exhibited a consistent trend of change in in viscosity with the shear rate, suggesting that the encapsulation of lutein did not affect the fluidity of the emulsions. This implies good compatibility between lutein and OB emulsions. Furthermore, the treatment methods (stirring, ultrasonication, and homogenization) did not influence the viscosity and fluidity of the emulsions.

Physical stability

Through centrifugal acceleration experiments conducted using the LUMi-Sizer, the dynamic changes in the light transmittance of emulsions during the centrifugal separation of OBs were continuously measured. A lower instability index indicated greater physical stability of the emulsion. As shown in Fig. 5c, d, in both COB and POB emulsions, the ultrasonication groups exhibited significantly lower instability indices compared with the stirring and homogenization groups ($p < 0.05$), indicating that ultrasonication improved the emulsions' physical stability. This might be attributed to the reduction in the particle size of the emulsion caused by the ultrasonication treatment, which produced a more uniform system[44]. It may also be related to the fact that the ultrasonication treatment could promote the rearrangement of proteins and phospholipids at the OBs' interface, forming a denser and more robust interfacial film. This film effectively prevents droplet coalescence during centrifugation, which could effectively prevent the aggregation and fusion of adjacent OB particles caused by collision during the centrifugation process[45]. Although homogenization also reduced the particle size of the emulsions, the localized high temperature induced during the homogenization process may lead to lipid oxidation and the generation of free radicals, thereby compromising the stability of the emulsion system[46]. Figure 5e, f reveals that the instability indices increased over time, suggesting a decline in stability across all groups. In the absence of lutein, untreated COB and POB emulsions exhibited the most rapid increase in the instability index, surpassing all treatment groups, with the order of control > stirring > homogenization > ultrasonication. This also demonstrated a clear enhancement in stability by the treatments, which aligned with the particle size results (Fig. 2c, d). Furthermore, compared with the lutein-free emulsions, both the COB and POB emulsions loaded with lutein exhibited a slower rise in the instability index, with the high-concentration group showing a markedly slower rate. This suggested that lutein loading also enhanced the physical stability of both COB and POB emulsions. Although the encapsulation of lutein reduced the absolute value of zeta potential of SOB emulsions (Fig. 3e, f), it formed a more robust composite interface membrane through hydrophobic interaction with the OB membrane[47], which significantly reduced the instability index of the emulsion, and the improvement in stability offset the influence of charge reduction.

Thermal stability

It is reported that temperature is a crucial parameter for inducing the degradation of lutein and the instability of an emulsion[48]. This is because heating in the presence of oxygen and metals can lead to the formation of various free radical species that are transformed

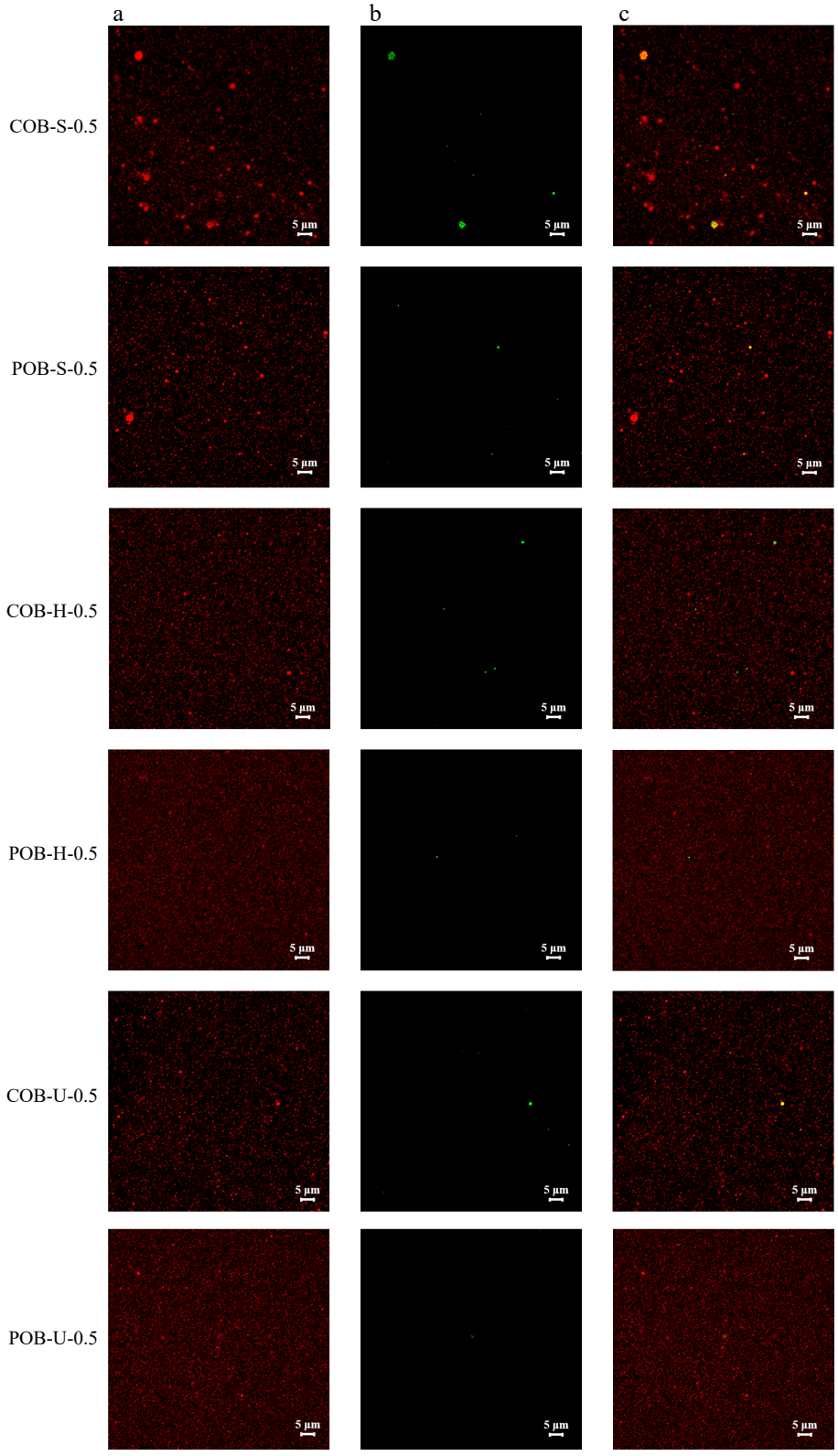

Fig. 4 The CLSM images of COB and POB emulsions stained with (a) Nile red and (b) Rhodamine B, and (c) overlay of Panels (a) and (b). Notes: S: Stirring, H: Homogenization, U: Ultrasonication.

into peroxyl radicals, which can undergo propagation reactions with lutein[49]. It was evaluated whether there were any differences in the thermal stability of lutein emulsions prepared with soybean COBs or POBs, which had different interfacial compositions of proteins and phospholipids.

Lutein degradation

As presented in Fig. 6, the lutein content in all samples showed a decreasing trend over time, and the overall retention rate decreased as the temperature increased from 60 to 90 °C. After 12 h of heating, the lutein retention rates ranged between 55% and 75% at 60 °C,

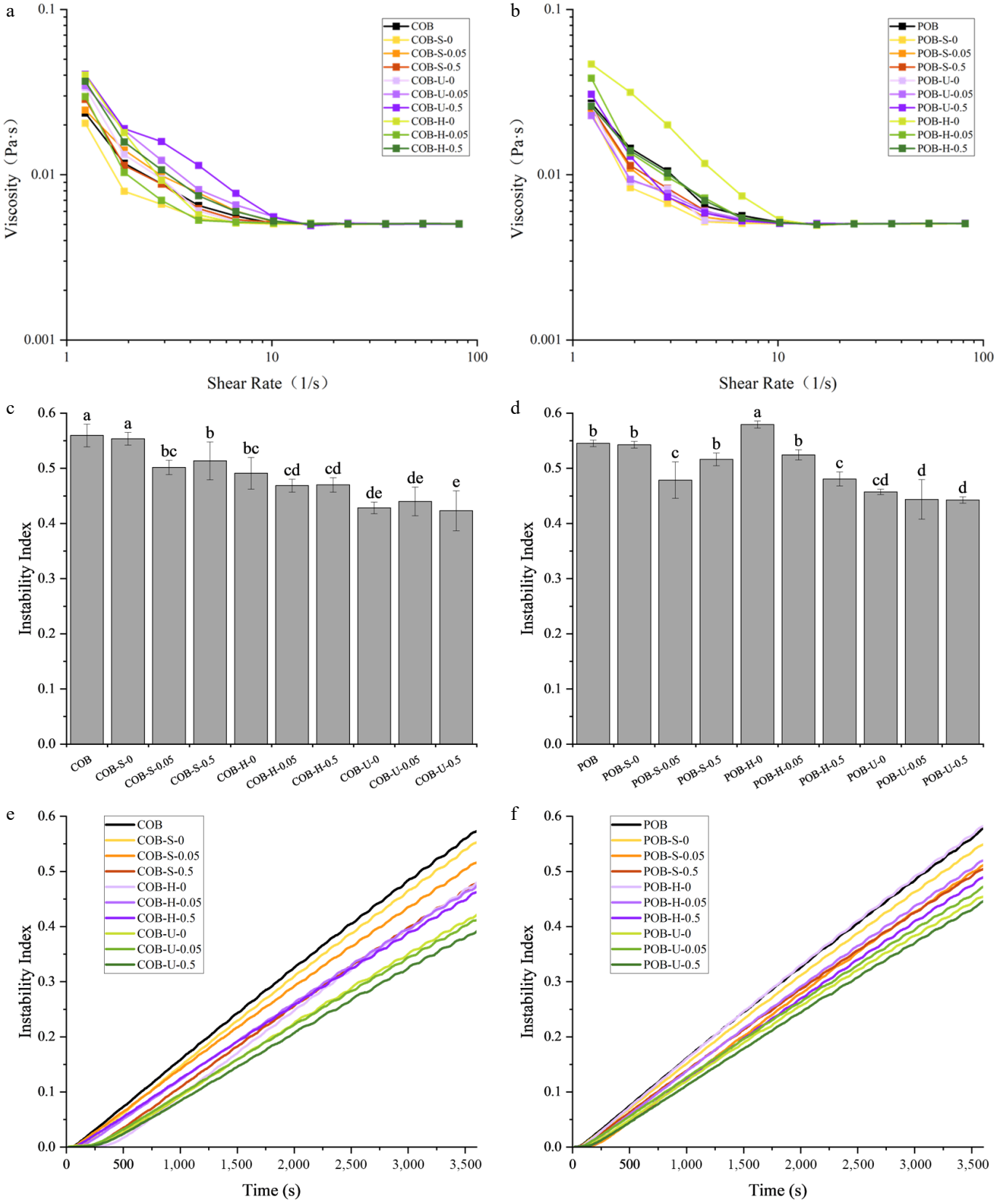

Fig. 5 The physical stability and viscosity of emulsions in different treatment groups. (a) The viscosity of COB emulsions. (b) The viscosity of POB emulsions. (c) The instability index of COBs (LUMi-Sizer). (d) The instability index of POBs (LUMi-Sizer). (e) The instability index over time of COBs (LUMi-Sizer). (f) The instability index over time of POBs (LUMi-Sizer).

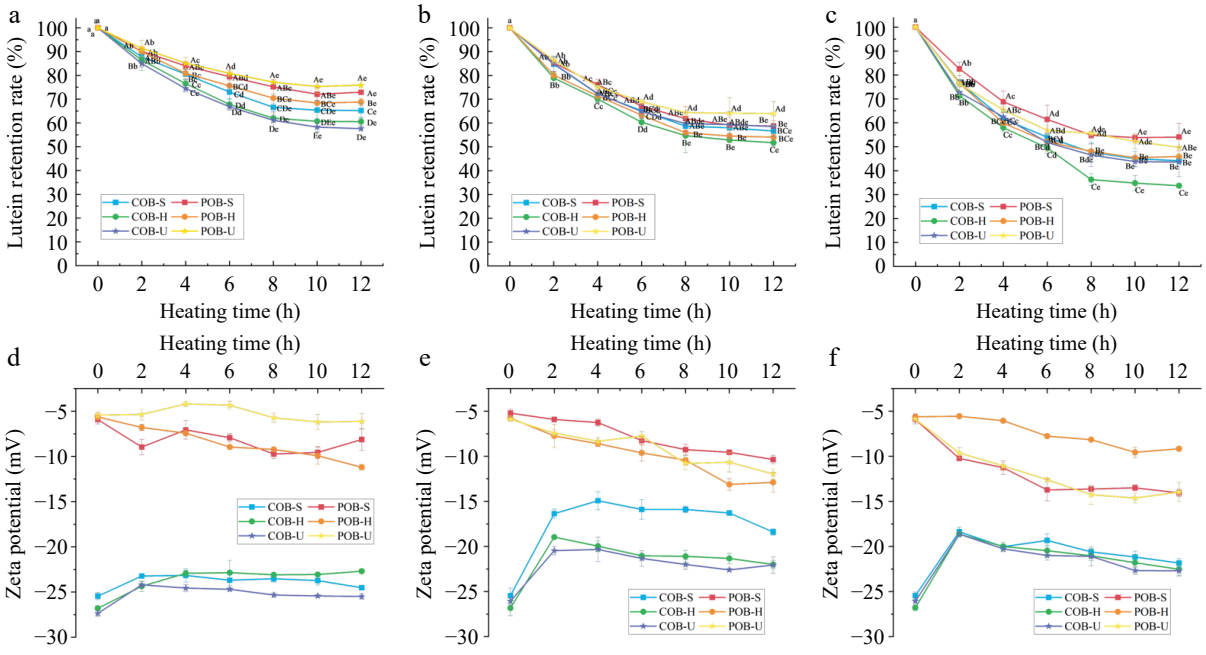

Fig. 6 The lutein retention rate of emulsions (the concentration of lutein–ethanol was 0.5%) in different treatment groups during heating: (a) 60 °C, (b) 75 °C, and (c) 90 °C. The zeta potential of emulsions in different treatment groups during heating: (d) 60 °C, (e) 75 °C, (f) 90 °C.

50%–65% at 75 °C, and 30%–50% at 90 °C. In comparison with previous studies, where lutein emulsions prepared with whey protein isolate and sodium alginate showed only about 30.67% retention after 30 min at 90 °C[50], both the COB and POB emulsions in our study exhibited better lutein retention under similar storage conditions. This suggests that the native oleosin-embedded membrane of OBs potentially provided superior stability and protection for lutein compared with the synthetically assembled interfaces in artificial emulsions.

Across all temperatures and encapsulation methods, POB emulsions exhibited significantly higher lutein retention than COB emulsions ($p < 0.05$), indicating better protective capacity. This may be attributed to the POBs' interfacial membrane, which is composed of a compact oleosin–phospholipid monolayer (without extraneous protein interference) and acts as a more effective barrier against oxygen and heat. This physical barrier isolates lutein from oxidative stressors, reducing its degradation[51]. In contrast, COB emulsions retain inherent impurities such as lipoxygenase (LOX) and probable thiol protease (P34), which were stored in protein storage vacuoles and associated with the surface of SOBs. P34 leads to the partial hydrolysis of 24-kDa oleosin through its proteolytic activity and high cleavage site specificity. This hydrolysis was a critical factor enabling LOX's exposure to substrates in SOBs[27]. The residual LOX catalyzed the oxidation of TAGs, resulting in the formation of peroxides (PV) and thiobarbituric acid-reactive substances (TBARS), consequently accelerating the oxidative degradation of lutein[52]. A previous study has reported that similar TAG oxidation behavior was observed in COB emulsions of soybean oil under thermal treatment at 70 and 90 °C. In comparison, POB emulsions exhibited markedly higher oxidative stability under the same thermal treatment[53]. Therefore, this may be the main reason why a low level of lutein degradation was observed in POB emulsions.

Regarding encapsulation methods, homogenization consistently led to the lowest lutein retention in both COB and POB emulsions. For COB emulsions, stirring led to higher retention of lutein than homogenization and ultrasonication at 60 °C, whereas ultrasonication surpassed homogenization at 75 °C. At 90 °C, both stirring and ultrasonication outperformed homogenization. Similarly, in POB emulsions, stirring and ultrasonication resulted in significantly higher retention than homogenization at all temperatures ($p < 0.05$). This may be attributed to the intense shear, impact, and cavitation effects generated during homogenization process, which may result in an interfacial film that is thinner, structurally looser, and less dense, leading to structural collapse and increased exposure of lutein to oxidative stressors[54,55]. This accelerates the degradation of lutein under thermal treatment conditions. Another possible explanation is that homogenization promoted the rupture of OBs at the interface and the subsequent rearrangement of their components, which caused the hydrophobic entrance cavity of LOX to penetrate the oleosin–phospholipid membrane and to enter the TAG matrix, thereby accelerating oxidation. In contrast, stirring and ultrasonication, as mild processing treatments, better preserve the integrity of the native oleosin–phospholipid barrier.

As shown in Supplementary Table S2, kinetic analysis confirmed that lutein degradation in all samples followed the first-order kinetics ($R^2 > 0.97$). The degradation rate constant (k) increased with temperature, consistent with previous reports[25]. COB emulsions showed higher k values and shorter half-lives ($t_{1/2}$) than POB emulsions, indicating the lower thermal stability of lutein in COBs. Moreover, POB emulsions had higher activation energy (Ea), implying that more energy is required to initiate the degradation of lutein, which also suggests better ambient storage stability. In both COB and POB emulsions, the stirred and ultrasonicated groups exhibited higher Ea values than the homogenized groups, further corroborating the enhanced thermal stability provided by these mild treatments. In summary, POB emulsions offered improved lutein protection, especially those prepared by stirring or ultrasonication.

Physical stability after heating

The evolution of particle size and PDI are shown in Supplementary Table S3. Neither the COB nor POB emulsions exhibited significant changes in particle size across all treatment groups compared with the control. Specifically, the mean particle sizes ranged across approximately 460–495 nm for COBs and 420–440 nm

for POBs, depending on the emulsification method. These results indicate that prolonged heating at 60–90 °C did not cause substantial structural disruption or droplet aggregation in either emulsion type. Consistent with previous findings on SOBs[6], thermal treatment had minimal impact on the colloidal stability of COB and POB emulsions, with no marked fluctuation in droplet size. Moreover, all samples maintained a PDI below 0.2, reflecting a uniform droplet size distribution without heat-induced differentiation or graded aggregation. This behavior aligns with the reported thermal stability of walnut (*Juglans regia*) and peanut OB emulsions, which also preserve a unimodal distribution after heating[56].

The changes in zeta potential over 12 h of heating at different temperatures are shown in Fig 6. For COB emulsions, at 60 °C, the absolute zeta potential decreased slightly within the first 2 h to around 25 mV and remained stable thereafter. At 75 and 90 °C, a more pronounced initial decrease occurred (from −26 to −20 mV), followed by a slight increase during the remaining heating period. This trend may be attributed to competing interfacial phenomena: The oxidation of phospholipids and proteins initially reduces the surface charge by degrading electronegative groups, such as phosphate groups[57]. Simultaneously, charged lipid oxidation products (e.g., carboxyl-containing aldehydes and ketones) adsorb onto the droplets' surfaces, partially restoring the charge[58]. Additionally, the initial decline may reflect the thermal denaturation of extraneous proteins, whereas the subsequent recovery suggests interfacial rearrangement or readsorption of components over time[59,60]. In contrast, POB emulsions exhibited superior interfacial stability. At 60 °C, changes in zeta potential were negligible (within 5 mV). At 75 °C, all POB groups showed a significant increase ($p < 0.05$) from −5 to −12 mV. After 12 h at 90 °C, the absolute zeta potential of POB-S and POB-U increased by 10 mV, whereas that of POB-H increased by 5 mV. This is likely caused by the heat-induced rearrangement of the interfacial membrane. The high temperature might have triggered a conformational reorientation of oleosins, which exposed the negatively charged phosphate groups of phospholipids that were previously buried[61]. Consequently, the surface charge density increased. Additionally, compared with COBs, the absence of aggregated extraneous proteins in POBs eliminated potential charge shielding, allowing for a more pronounced negative charge under heating conditions[62].

Photochemical stability

Lutein degradation

Owing to the photosensitivity of lutein, it can be rapidly degraded into α-Ionone, β-Ionone, dihydroactinidiolide, and β-damascenone[63], reducing its biological activity and limiting its application. Therefore, the photochemical stability of lutein was evaluated by exposing OB emulsions to UV radiation; those kept in dark conditions were set as a control group. As shown in Fig. 7, the lutein retention rate decreased over the 15-day storage period in all samples, with a more pronounced decline observed in COB emulsions than in POB emulsions ($p < 0.05$). Under dark conditions, POB emulsions maintained a lutein retention rate above 80% on Day 15, with no significant differences among the three processing methods. In contrast, COB emulsions showed markedly lower retention, where stirring and ultrasonication (~40%) outperforming homogenization (below 30%). Under UV conditions, lutein degradation accelerated significantly ($p < 0.05$). POB emulsions retained approximately 70% of lutein by Day 15, again with no notable intertreatment differences, whereas all COB groups fell below 30%. It was worth noting that and acceleration in lutein degradation was observed between Days 3 and 7, particularly in COB emulsions, which could be attributed to their inherently unstable interface and residual pro-oxidants such as LOX. These factors collectively initiate and propagate an autocatalytic oxidation cascade. Over time, cumulative oxidative damage—facilitated by COB's porous interfacial structure, particularly in homogenized samples—enables enhanced oxygen penetration and promotes interactions among pro-oxidants, TAG, and encapsulated lutein. In contrast, POB emulsions, characterized by a cleaner composition and a more compact, intact interfacial membrane, could effectively delayed the onset of rapid oxidation by acting as a superior barrier against oxygen influx. As a result, although some acceleration of degradation still occurred in POB emulsions, it was both delayed and less severe, leading to consistently higher lutein retention throughout storage. Another possible reason may be related to the fluidity of the native protein–phospholipid membrane of POB emulsions. It is hypothesized that during storage, some lutein might migrate into the interior of the OBs, thereby enhancing the protective effect[64].

Color stability

The visual appearance and color of emulsions are critical for food applications, as these attributes directly influence consumer acceptance. Supplementary Fig S1 illustrates the color changes in lutein-loaded OB emulsions over 15 d of storage under dark and UV conditions, using stirring, homogenization, and ultrasonication as the encapsulation methods. On Day 15, a noticeable fade in color was observed in the UV-treated groups compared with those stored in the dark, reflecting greater lutein degradation under UV exposure. Furthermore, COB emulsions exhibited visibly lighter

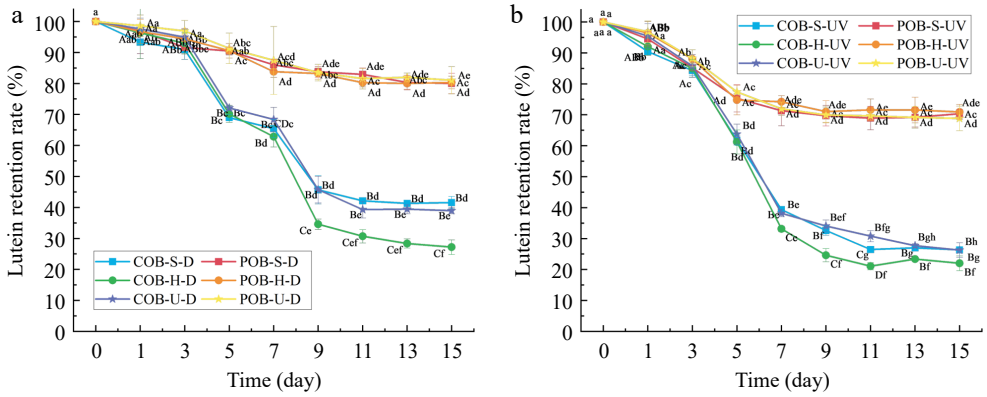

Fig. 7 The lutein retention rate of OB emulsions (the concentration of lutein–ethanol was 0.5%) with different treatments during light exposure. (a) Dark; (b) UV light.

coloration from Day 5 onward, indicating the faster degradation rate of lutein relative to POB emulsions.

Quantitative color parameters (L^*, a^*, b^*, and ΔE) were analyzed to monitor color changes during storage, where L^* indicates lightness, a^* represents the red–green axis, b^* reflects yellow–blue intensity, and ΔE describes the total color difference compared with Day 0. As summarized in Supplementary Fig. S2, UV irradiation significantly reduced the b^* values in all samples by Day 5 compared with dark storage ($p < 0.05$), indicating a visible fading of yellow color associated with lutein's degradation. Lutein is the main source of the yellow color in emulsions, and the b^* value directly reflects its content. UV irradiation accelerates the degradation of lutein, leading to a decrease in the lutein concentration, yellow's intensity, andthe b^* value. POBs could effectively protect lutein against degradation, resulting in a smaller reduction in the b^* value in the POB group compared with the COB group. No consistent trends were observed in L^* or a^* values. ΔE values increased over time under both storage conditions but were consistently higher in UV-exposed samples. Furthermore, all POB groups exhibited lower ΔE values than the COB groups, confirming that POB encapsulation more effectively preserved the lutein content and color stability under UV stress.

Conclusions

This study systematically investigated the encapsulation and stabilization of lutein using SOBs extracted via the aqueous method at two distinct pH levels (pH 7 for COBs and pH 11 for POBs), with three different encapsulation techniques (stirring, homogenization, and ultrasonication) used to load the lutein. Lutein was successfully encapsulated into COB and POB by all three methods. The EE exceeded 60% in all groups, with the highest value observed in the COB homogenization group (94.14%). The LE of COBs was higher than that of POBs, likely because of the presence of extraneous proteins binding to additional lutein. Among the processing methods, ultrasonication and stirring resulted in higher LE than homogenization. All emulsions exhibited a unimodal particle size distribution and negative zeta potentials. The ultrasonication and homogenization groups showed smaller particle sizes compared with the stirring group. In terms of physical stability, the ultrasonication group was more stable than the homogenization and stirring groups, and the incorporation of lutein enhanced the emulsion's stability during accelerated centrifugation without affecting viscosity or fluidity. Finally, thermal and photochemical stability evaluations revealed that POB emulsions provided better protection for lutein than COB emulsions, whereas homogenization significantly reduced lutein retention compared with stirring and ultrasonication. Collectively, these findings confirm the feasibility of using SOB emulsions as carriers for lutein encapsulation, clarify the regulatory effects of OBs' composition and encapsulation methods on the delivery of lutein, and expand the potential applications of soybean-based delivery systems in the food industry.

In future research, it will be necessary to further explore the molecular interaction mechanism between lutein and the membrane proteins and phospholipids of SOBs, investigate the stability of lutein-loaded SOB emulsions in practical food systems under different processing and environmental conditions, and optimize the preparation process's parameters, so as to provide a theoretical basis and technical support for the industrial scale-up of applying SOBs as a lutein delivery system and promote the development of the related functional food industry.

Author contributions

The authors confirm their contributions to the paper as follows: writing – review and editing, writing – original draft, visualization, methodology, formal analysis: Yang Y; methodology: Fu R; writing – review and editing: Yang B; visualization: Zuo J; supervision, conceptualization: Ni Y, Wen X; conceptualization, writing – review and editing, supervision, methodology: Li M. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed in the current study are available from the corresponding author on reasonable request.

Acknowledgement

This research is supported by the National Key Research and Development Program (2024YFD2101303) and the Major International Joint Research Project of the National Natural Science Foundation of China (No. 32020103015).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary information accompanies this paper online at: https://doi.org/10.48130/fia-0026-0025.

Dates

Received 19 December 2025; Revised 3 April 2026; Accepted 20 April 2026; Published online 30 June 2026

References

[1] Tzen J, Cao Y, Laurent P, Ratnayake C, Huang A. 1993. Lipids, proteins, and structure of seed oil bodies from diverse species. *Plant Physiology* 101(1):267–276

[2] Nikiforidis CV. 2019. Structure and functions of oleosomes (oil bodies). *Advances in Colloid and Interface Science* 274:102039

[3] Zhou X, Han W, Li D, Cui C, Jiang L, et al. 2018. Differences in the structure and properties of seed oil bodies from diverse oilseed crops. *Food Science* 39:133–139

[4] Shi Z, Li K, Meng Z. 2024. Recent trends in oleosomes: extraction methods, structural characterization, and novel applications. *Trends in Food Science & Technology* 151:104621

[5] Wei S, Huang J, Zhang L, Sun Q, Sun X, et al. 2020. Physicochemical properties and stabilities of crude and purified oil bodies extracted from high oleic peanuts. *European Journal of Lipid Science and Technology* 122(4):1900183

[6] Liu Z, Mei Y, Peng Y, Fu R, Qin C, et al. 2022. Effect of exogenous proteins on soybean oil body stability. *Food Science* 43:1–9

[7] Vardar US, Bitter JH, Nikiforidis CV. 2024. The mechanism of encapsulating curcumin into oleosomes (Lipid Droplets). *Colloids and Surfaces B: Biointerfaces* 236:113819

[8] Zaaboul F, Zhao Q, Xu Y, Liu Y. 2022. Soybean oil bodies: a review on composition, properties, food applications, and future research aspects. *Food Hydrocolloids* 124:107296

[9] Acevedo F, Rubilar M, Jofré I, Villarroel M, Navarrete P, et al. 2014. Oil bodies as a potential microencapsulation carrier for astaxanthin stabilisation and safe delivery. *Journal of Microencapsulation* 31(5):488–500

[10] Zhang S, Chen H, Geng F, Xie B, Sun Z, et al. 2023. Solvent-free encapsulation of β-carotene in natural flaxseed oil bodies induced via tepidity-physical field treatment: Formation, characteristic and stability. *Food Hydrocolloids* 144:108913

[11] Chen W, Li X, Zhang W, Alouk I, Wang Y, et al. 2024. Physicochemical properties, photostability, and digestive characteristics of natural emulsion system fabricated by recombinant rice bran oil bodies for lutein ester delivery. *Food Hydrocolloids* 156:110270

[12] Mitra S, Rauf A, Tareq AM, Jahan S, Bin Emran T, et al. 2021. Potential health benefits of carotenoid lutein: an updated review. *Food and Chemical Toxicology* 154:112328

[13] Gholipour-Varnami K, Mohamadnia S, Tavakoli O, Ali Faramarzi M. 2025. A review on the biological activities of key carotenoids: Structures, sources, market, economical features, and stability. *Food Bioscience* 68:106529

[14] Jv DJ, Ji TH, Xu Z, Li A, Chen ZY. 2023. The remarkable enhancement of photo-stability and antioxidant protection of lutein coupled with carbon-dot. *Food Chemistry* 405:134551

[15] Gül LB. 2025. Royal jelly microencapsulation with a maltodextrin/gum Arabic binary blend by spray drying: process optimization and characterization of microcapsules. *Italian Journal of Food Science* 37(4):403–421

[16] Castillo-Barzola A, Paisig HL, Faieta M, Jordán-Suárez O, Porras-Sosa E, et al. 2025. Effect of microencapsulation by spray drying on the protein content of *Spirulina* (*Arthrospira platensis*). *Italian Journal of Food Science* 37(2):423–439

[17] Maisarah Tuan Putra TN, Omar NN, Khairi Zainol M, Taghavi E, Mohd Isa NS, et al. 2025. Enchanted stability of butterfly pea flower extract through colloidal gas aphrons (CGA) separation method using Tween-20 surfactant. *Quality Assurance and Safety of Crops & Foods* 17(2):37–57

[18] Rehman A, Tong Q, Jafari SM, Assadpour E, Shehzad Q, et al. 2020. Carotenoid-loaded nanocarriers: a comprehensive review. *Advances in Colloid and Interface Science* 275:102048

[19] Zhao L, Chen Y, Yan Z, Kong X, Hua Y. 2016. Physicochemical and rheological properties and oxidative stability of oil bodies recovered from soybean aqueous extract at different pHs. *Food Hydrocolloids* 61:685–694

[20] Fu R, Qin C, Peng Y, Ni Y, Li J, et al. 2025. Unlocking flaxseed oleosomes (oil bodies) potential: optimal extraction and functional insights through enzymolysis or dehulling treatment. *Food Research International* 204:115955

[21] Hempel J, Fischer A, Fischer M, Högel J, Bosy-Westphal A, et al. 2017. Effect of aggregation form on bioavailability of Zeaxanthin in humans: a randomised cross-over study. *British Journal of Nutrition* 118(9):698–706

[22] Iwanaga D, Gray DA, Fisk ID, Decker EA, Weiss J, et al. 2007. Extraction and characterization of oil bodies from soy beans: a natural source of pre-emulsified soybean oil. *Journal of Agricultural and Food Chemistry* 55(21):8711–8716

[23] Yang J, Zhu B, Lu K, Dou J, Ning Y, et al. 2023. Construction and characterization of Pickering emulsions stabilized by soy protein hydrolysate microgel particles and quercetin-loaded performance *in vitro* digestion. *Food Research International* 169:112844

[24] Hao J, Xu J, Zhang W, Li X, Liang D, et al. 2022. The improvement of the physicochemical properties and bioaccessibility of lutein microparticles by electrostatic complexation. *Food Hydrocolloids* 125:107381

[25] Zhu J, Wang H, Miao L, Chen N, Zhang Q, et al. 2023. Curcumin-loaded oil body emulsions prepared by an ultrasonic and pH-driven method: fundamental properties, stability, and digestion characteristics. *Ultrasonics Sonochemistry* 101:106711

[26] Gao Y, Zhou L, Yao F, Chen F. 2021. Effects of pH on the composition and physical stability of peanut oil bodies from aqueous enzymatic extraction. *Journal of Chemistry* 2021(1):2441385

[27] Zhao L, Chen Y, Chen Y, Kong X, Hua Y. 2016. Effects of pH on protein components of extracted oil bodies from diverse plant seeds and endogenous protease-induced oleosin hydrolysis. *Food Chemistry* 200:125–133

[28] Herman EM, Melroy DL, Buckhout TJ. 1990. Apparent processing of a soybean oil body protein accompanies the onset of oil mobilization. *Plant Physiology* 94(1):341–349

[29] Zhao L, Chen Y, Cao Y, Kong X, Hua Y. 2013. The integral and extrinsic bioactive proteins in the aqueous extracted soybean oil bodies. *Journal of Agricultural and Food Chemistry* 61(40):9727–9733

[30] Hao J, Zhang F, Decker EA, Sun B, Xu D. 2024. Study on the interfacial properties of rice bran oil bodies affecting their physicochemical properties, stability and adsorption behavior at the oil-water interface for the design of precisely emulsified systems. *Food Hydrocolloids* 148:109449

[31] Vardar US, Konings G, Yang J, Sagis LMC, Bitter JH, et al. 2025. Modifying the interfacial dynamics of oleosome (lipid droplet) membrane using curcumin. *Journal of Colloid and Interface Science* 678:1077–1086

[32] Lin Z, Zhou P, Deng Y, Liu G, Li P, et al. 2025. Impact of homogenization methods on the interfacial protein composition and stability of peanut oil body emulsion with sodium caseinate and maltodextrin. *LWT* 215:117286

[33] Yang H, Li L, Xie C, He M, Guo Z, et al. 2022. Characteristics and structure of a soy protein isolate–lutein nano complex produced via high-pressure homogenization. *Journal of the Science of Food and Agriculture* 102(12):5411–5421

[34] Zhang H, Li Y, Jin Z, Xue C, Wei Z. 2025. Effect of ultrasonication and homogenization on electrostatically self-assembled whey protein isolate/pectin particles: EGCG encapsulation, stability, and controlled release properties. *Food Hydrocolloids* 166:111264

[35] Deng XH, Ni XX, Han JH, Yao WH, Fang YJ, et al. 2023. High-intensity ultrasound modified the functional properties of *Neosalanx taihuensis* myofibrillar protein and improved its emulsion stability. *Ultrasonics Sonochemistry* 97:106458

[36] de Souza Soares L, Milião GL, Tonole B, de Souza GB, de Fátima Ferreira Soares N, et al. 2019. Chitosan dispersed in aqueous solutions of acetic, glycolic, propionic or lactic acid as a thickener/stabilizer agent of O/W emulsions produced by ultrasonic homogenization. *Ultrasonics Sonochemistry* 59:104754

[37] Song JF, Li DJ, Pang HL, Liu CQ. 2015. Effect of ultrasonic waves on the stability of all-trans lutein and its degradation kinetics. *Ultrasonics Sonochemistry* 27:602–608

[38] Jia X, Ma P, Taylor KS, Tarwa K, Mao Y, et al. 2023. Development of stable Pickering emulsions with TEMPO-oxidized chitin nanocrystals for encapsulation of quercetin. *Foods* 12(2):367

[39] Ding J, Dong Y, Huang G, Zhang Y, Jiang L, et al. 2021. Fabrication and characterization of β-carotene emulsions stabilized by soy oleosin and lecithin mixtures with a composition mimicking natural soy oleosomes. *Food & Function* 12(21):10875–10886

[40] Matsumura Y, Sirison J, Ishi T, Matsumiya K. 2017. Soybean lipophilic proteins – origin and functional properties as affected by interaction with storage proteins. *Current Opinion in Colloid & Interface Science* 28:120–128

[41] Moore FG, Richmond GL. 2008. Integration or segregation: how do molecules behave at oil/water interfaces? *Accounts of Chemical Research* 41(6):739–748

[42] Han H, Zhao L, Liu X, Guo A, Li X. 2020. Effect of water bath-assisted water extraction on physical and chemical properties of soybean oil body emulsion. *Food Science & Nutrition* 8(12):6380–6391

[43] Dong B, Qin Z, Wang Y, Zhang J, Xu Z, et al. 2022. Investigating the rheology and stability of heavy crude oil-in-water emulsions using APG08 emulsifiers. *ACS Omega* 7(42):37736–37747

[44] Zhang X, Chen Y, Li R, Shi Y, Zhao Y, et al. 2024. Fabrication of pea protein isolate-stabilized oil-in-water emulsions with high freeze-thaw stability: effect of high intensity ultrasonic on emulsions and interfacial protein structure. *Food Hydrocolloids* 157:110484

[45] Jiang J, Song Z, Wang Q, Xu X, Liu Y, et al. 2019. Ultrasound-mediated interfacial protein adsorption and fat crystallization in cholesterol-reduced lard emulsion. *Ultrasonics Sonochemistry* 58:104641

[46] Li Y, Xiang D. 2019. Stability of oil-in-water emulsions performed by ultrasound power or high-pressure homogenization. *PLoS One* 14(3):e0213189

[47] Mantovani RA, Hamon P, Rousseau F, Tavares GM, Mercadante AZ, et al. 2020. Unraveling the molecular mechanisms underlying interactions between caseins and lutein. *Food Research International* 138:109781

[48] Teeranachaideekul V, Boribalnukul P, Morakul B, Junyaprasert VB. 2022. Influence of vegetable oils on *in vitro* performance of lutein-loaded lipid carriers for skin delivery: nanostructured lipid carriers vs. nanoemulsions. *Pharmaceutics* 14(10):2160

[49] Lim ASL, Griffin C, Roos YH. 2014. Stability and loss kinetics of lutein and β-carotene encapsulated in freeze-dried emulsions with layered interface and trehalose as glass former. *Food Research International* 62:403–409

[50] Niu J, You T, Wang X, Ya H, Liang S, et al. 2024. Study on stability and *in vitro* digestion property of Lutein Pickering emulsions encapsulated with whey protein isolate/sodium alginate/tea polyphenols. *Journal of Food Measurement and Characterization* 18(12):9736–9751

[51] Chen N, Wang Z, Zhu J, Ning Y, Jiang L, et al. 2025. Effect of extraction pH on the emulsion stability and surface protein structure of soybean oil body. *Food Chemistry* 473:143029

[52] Zhou X, Sun R, Zhao J, Liu Z, Wang M, et al. 2022. Enzymatic activity and stability of soybean oil body emulsions recovered under neutral and alkaline conditions: impacts of thermal treatments. *LWT* 153:112545

[53] Abdolahi Alkami P, Esmaeilzadeh Kenari R, Farahmandfar R, Azizkhani M. 2022. Investigation of the antioxidant effect of red quinoa (*Chenopodium formosanum* Koidz) carotenoid extracted on the oxidative stability of soybean oil. *Journal of Food Processing and Preservation* 46(3):e16406

[54] Chandel A, Xavier JR, Chauhan OP. 2025. Applications of high pressure homogenization in food industry for ensuring quality and safety. *Journal of Food Process Engineering* 48(4):e70105

[55] Gao F, Wang X, Han X, Zhang S, Wang T, et al. 2025. Unraveling the effect of high pressure homogenization treatment combined with polyphenols on the improvement of emulsion stability of rice bran oil bodies. *Food Hydrocolloids* 160:110871

[56] Wu L, Sun Y, Chen Y, Yan S, Xie F, et al. 2022. Effect of pasteurization on oxidative stability of oil body emulsions from various crops. *Food Science* 43:23–30

[57] Mosca M, Ceglie A, Ambrosone L. 2011. Effect of membrane composition on lipid oxidation in liposomes. *Chemistry and Physics of Lipids* 164(2):158–165

[58] Ghelichi S, Hajfathalian M, Yesiltas B, Sørensen AM, García-Moreno PJ, et al. 2023. Oxidation and oxidative stability in emulsions. *Comprehensive Reviews in Food Science and Food Safety* 22(3):1864–1901

[59] Keerati-u-rai M, Corredig M. 2009. Heat-induced changes in oil-in-water emulsions stabilized with soy protein isolate. *Food Hydrocolloids* 23(8):2141–2148

[60] Sun F, Wang Q, Gao C, Xiao H, Yang N. 2023. Effect of extraction pH and post-extraction heat treatment on the composition and interfacial properties of peanut oil bodies. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 656:130351

[61] Wijesundera C, Boiteau T, Xu X, Shen Z, Watkins P, et al. 2013. Stabilization of fish oil-in-water emulsions with oleosin extracted from canola meal. *Journal of Food Science* 78(9):C1340–C1347

[62] Faraji H, McClements DJ, Decker EA. 2004. Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. *Journal of Agricultural and Food Chemistry* 52(14):4558–4564

[63] Nonier MF, De Gaulejac NV, Vivas N, Vitry C. 2004. Characterization of carotenoids and their degradation products in oak wood. Incidence on the flavour of wood. *Comptes Rendus Chimie* 7(6–7):689–698

[64] Wisniewska-Becker A, Nawrocki G, Duda M, Subczynski WK. 2012. Structural aspects of the antioxidant activity of lutein in a model of photoreceptor membranes. *Acta Biochimica Polonica* 59(1):119–123