



## Research article

# Enhancing the stability of lutein emulsions with a water-soluble antioxidant and a oil-soluble antioxidant

Yujiao Wang, Xiangxiang Zhang, Manlin Yan, Quanyu Zhao \*

*School of Pharmaceutical Science, Nanjing Tech University, 30 Puzhu South Road, Nanjing 211816, People's Republic of China*

## ARTICLE INFO

**Keywords:**

Emulsion  
Lutein  
Stability  
Tea polyphenols  
Propyl gallate  
Low energy method

## ABSTRACT

Lutein is critical for protecting the eye against light damage. The low solubility and high sensitivity of lutein to environmental stresses prevent its further application. The hypothesis is that the combination of one water-soluble antioxidant and one oil-soluble antioxidant will be beneficial to improve the stability of lutein emulsions. A low-energy method was performed to prepare lutein emulsions. The combination of a lipid-soluble antioxidant (propyl gallate or ethylenediaminetetraacetic acid) and a water-soluble antioxidant (tea polyphenol or ascorbic acid) were investigated for improving the lutein retention rates. It was shown that the highest lutein retention rate was achieved by using propyl gallate and tea polyphenol, 92.57%, at Day 7. It was proven that the lutein retention rates of emulsions with propyl gallate and tea polyphenol were 89.8%, 73.5% and 55.2% at 4 °C, 25 °C and 37 °C, respectively, at Day 28. The current study is helpful to prepare for the further application of lutein emulsions for ocular delivery.

## 1. Introduction

Lutein is an important pigment for human beings [1]. In particular, lutein and zeaxanthin are the major carotenoids found in the human retina and crystals [2]. The human body cannot produce it and can only be supplemented by external intake from foods or others [3]. Lutein has strong antioxidation activity and can eliminate the damage of oxygen free radicals caused by ultraviolet rays to skin as photoprotectors [4]. Lutein is also used as an anti-inflammatory agent. Lutein is mainly from plants and can be extracted from marigold flowers [5] or microalgae [6,7]. The solubility of lutein in water is very low. In addition, lutein is sensitive to temperature [8], light and pH [9]. This is a very large limit for further application. Therefore, it is necessary to find a suitable carrier that can protect lutein from various physical and chemical factors and maintain good stability, improve the dispersion of lutein in aqueous solution, and increase the utilization efficiency of lutein in the human body. Oil-in-water emulsions are a distinguished delivery system in the field of foods and pharmaceuticals [10]. Therefore, the oil-in-water emulsion is a good choice for the further application of lutein.

The critical parameters for the preparation of emulsions mainly include the average particle size, physical and chemical stabilities, and biological activities. The stabilities of particle size and lutein in emulsions are related to encapsulation modes [11], preparation methods, components of oil and emulsifier [12,13], temperature, pH and other conditions in preparation, and kinds and concentrations of antioxidants [14]. Antioxidants can improve the physical and chemical stability of bioactive products in emulsions. Many antioxidants have been widely used in many emulsions, including ascorbic acid (ASC) [15,16], ethylenediaminetetraacetic acid (EDTA) [15–17], propyl gallate (PG) [18], tea polyphenol (TP) [19], resveratrol [20], tocopherol [17,21], cellulose nanofibrils [22],

\* Corresponding author.

E-mail address: [zhaogy@njtech.edu.cn](mailto:zhaogy@njtech.edu.cn) (Q. Zhao).

and vitamin E [9,23]. Antioxidants will significantly improve the chemical stability of lutein during long-term storage.

In general, only one antioxidant is adopted in an emulsion preparation. There are few studies using a combination of antioxidants in emulsions. Mixtures of 1-*o*-galloylglycerol and rosmarinic acid and 1-*o*-galloylglycerol and tocopherols were applied as antioxidants in oil-in-water emulsions [18], in which the performances using two antioxidants were similar to a single one. It is complex to optimize the combination of antioxidants. The hypothesis is that the combination of one water-soluble antioxidant and one oil-soluble antioxidant will be beneficial for improving the stability of lutein emulsions. In this study, several emulsions of lutein were prepared by a low-energy method. The possible effects of four antioxidants were evaluated. The combinations of two antioxidants were investigated to improve the stability.

## 2. Materials and methods

### 2.1. Chemicals

Lutein was purchased from Bide Pharmaceutical Co. (Shanghai, China). Tween 80 and medium chain triglycerides (MCT) were obtained from Shanghai Macklin Co. (China). EDTA, PG and ASC were purchased from Lingfeng Chemical Agent Co. (Shanghai, China). TP was purchased from Saen Chemical Technology Co. (Shanghai, China).

### 2.2. Preparation of emulsions by the low-energy method

The preparation of emulsions includes the low-energy method [12,24], solvent evaporation method [25], ultrasonic method [26], electrostatic complexation [27], high pressure homogenization method [28,29] and others. The emulsions in this study were prepared by a low-energy method. First, about 1.5 mg lutein was dissolved in 1 g oil to prepare the oil phase, and the oil phase and emulsifier were mixed according to a certain proportion. Then, ultrapure water was added to the mixture at a certain dripping speed (0.2 mL/min) and stirred for 5–10 min until the gel state of the system was formed. Next, the speed was increased, and a large amount of ultrapure water was added and stirred for 20 min to make the system pass the gel phase quickly, forming an emulsion. Lutein was added to the oil to prepare the emulsion. The final concentration of lutein in the emulsion was approximately 0.01% (w/w). The emulsion formation with antioxidants was carried out similar to that without antioxidant mentioned above. ASC and TP were added to water, and EDTA and PG were added to oil before the preparation of emulsions.

### 2.3. Effect of the ratio of surfactant and oil

The effects of the ratios of emulsifier and oil on the properties of the lutein emulsion were investigated. The ratios of Tween 80 and MCT, 9:1, 8:2, 7:3 and 6:4 (w/w), were adopted when the contents of Tween 80 were 12–19%. Another ratio of Tween 80 and MCT, 1:9, was selected as a control when the content of Tween 80 was 5.44%. The prepared emulsions were stored at  $25 \pm 0.1$  °C for 7 days. The particle size distributions and zeta potentials of emulsions were analyzed by a Zeta Sizer Nano Series based on the standard protocol suggested by the manufacturer (NANO ZSE, Malvern Instruments Limited, Worcestershire, UK). Lutein retention rates (%) were determined at Days 0, 3 and 7. The lutein in the emulsion was extracted by dichloromethane and methanol (1:1) first. Next, the lutein content was analyzed by a reversed-phase C-18 column (4.6 × 250 mm, 5 μm particle size) in high-performance liquid chromatography. The mobile phase was 67.5% methanol: 22.5% dichloromethane: 9.5% acetonitrile: 0.5% water (v/v). The other operation conditions were shown in a previous study [7].

The lutein retention rate,  $E_r$ , was calculated by Eq. (1) below [30].

$$E_r(\%) = \frac{C_{\text{lutein},t}}{C_{\text{lutein},0}} \times 100 \quad (1)$$

where  $C_{\text{lutein},0}$  is the initial lutein concentration in the emulsion at Day 0 (mg/L) and  $C_{\text{lutein},t}$  is the lutein concentration in the emulsion at Day  $t$  (mg/L).

### 2.4. Effect of antioxidants on lutein retention rate

When the ratio of emulsifier and oil was 6:4 (w/w), a lutein emulsion was prepared by a low-energy method. The effects of four antioxidants, PG, TP, ASC and EDTA, on the lutein retention rate were evaluated. Three concentrations of these antioxidants, 0.001%, 0.005% and 0.01% (w/w), were added to the emulsions. Lutein emulsion without antioxidant was used as a control. These emulsions were stored for 7 days at  $25 \pm 0.1$  °C. The particle size distributions and lutein content of lutein emulsions were measured at Days 0, 3 and 7.

After the investigation of a single antioxidant on the lutein retention rate, the effects of the combination of two antioxidants (TP + PG, TP + EDTA, TP + ASC, PG + ASC, EDTA + ASC, EDTA + PG) were evaluated. It should be mentioned that the addition concentration of PG was 0.01% (w/w), TP was 0.01% (w/w), EDTA was 0.005% (w/w) and ASC was 0.01% (w/w). Then, emulsions with two antioxidants were prepared, and the ratio of Tween 80 to MCT was 6:4. The control was lutein emulsion without any antioxidants. These emulsions were stored for 7 days at  $25 \pm 0.1$  °C. The particle size distributions and lutein content of lutein emulsions were also measured at Days 0, 3 and 7.

The emulsion with TP + PG was stained with phosphotungstic acid (PTA) [31], and a transmission electron microscope was taken by a JEM-1200EX (Jeol electronics, Japan) at 100 Kv.

## 2.5. Long-term stability test

The emulsions were prepared with 0.01% PG and 0.01% TP. The control was an emulsion without any antioxidant. The ratio of Tween 80 to MCT was 6:4. These emulsions were stored in the dark for 28 days at  $4 \pm 0.1$ ,  $25 \pm 0.1$  and  $37 \pm 0.1$  °C. The lutein retention rates and average particle sizes were measured at Days 0, 3, 7, 21 and 28.

## 2.6. ABTS free radical scavenging activities

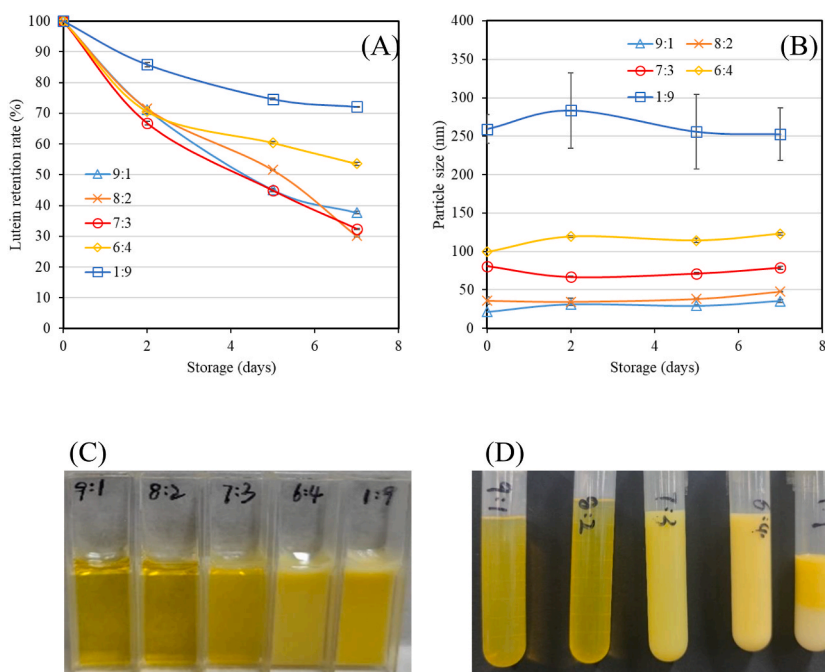
The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) assay is a typical colorimetric assay for the evaluation of antioxidant capability [32]. ABTS free radical scavenging activities were determined based on the procedure proposed in previous studies [33,34]. In the preliminary experiments, the ABTS free radical scavenging activities of the emulsion without PG and TP and the conventional emulsion without PG and TP were very low. Therefore, the ABTS free radical scavenging activities of these two emulsions were determined at Day 2 just after the preparation. The related activities of emulsions with PG and TP were determined at Day 24. The different concentrations of emulsions were collected for the ABTS assay. The antioxidative activities of emulsions with PG and TP and without lutein and without PG, TP and lutein were also measured at Day 2. The added emulsions were the same as those with lutein groups. The ABTS free radical scavenging activities were calculated by Eq. (2) [32].

$$\text{ABTS free radical scavenging activity(\%)} = \left(1 - \frac{A_{\text{emulsion}}}{A_{\text{control}}}\right) \times 100 \quad (2)$$

where  $A_{\text{emulsion}}$  and  $A_{\text{control}}$  are the absorbance of the emulsion and control at 734 nm, respectively. Control is the water without emulsion.

## 2.7. Statistical analysis

All of the experiments were performed with three replicates. Statistical analysis (ANOVA) was conducted by Excel (Microsoft, USA). Differences were considered significant if p was less than 0.05.



**Fig. 1.** Effects of the ratio of surfactant (Tween 80) and oil (MCT) on lutein retention rate (A) and particle size (B) of lutein emulsions made by the low-energy method. The experimental data are shown as the mean  $\pm$  SD ( $n = 3$ ). Photographs of emulsions at Day 5 (C) and Day 9 (D) when Tween 80: MCT was 9:1, 8:2, 7:3, 6:4 and 1:9, respectively.

### 3. Results and discussion

#### 3.1. Effects of surfactant:oil

Surfactants and oil are major components in emulsions. In this study, Tween 80 and MCT were selected as surfactant and oil, respectively. The ratio of Tween 80 and MCT is critical for emulsion formation, particle size distribution and lutein retention rate. As shown in Fig. 1, the lutein retention rates declined from Day 0 to Day 7. The patterns of ratios of surfactant and oil, 9:1, 8:2 and 7:3, were similar. At Day 7, the lutein retention rate was  $53.55 \pm 0.44\%$  when the ratio of Tween 80 and MCT was 6:4. Its average particle diameter was approximately 123.0 nm. Its lutein retention rate was significantly higher than those of ratios of 9:1, 8:2 and 7:3. The lutein retention rate was  $72.04 \pm 0.06\%$  when the ratio of Tween 80 and MCT was 1:9 (Fig. 1A). The related particle diameter of the conventional emulsion was  $252.2 \pm 34.47$  nm (Fig. 1B). It was indicated that the lutein retention rate was also related to the particle diameter of the emulsion [25,27]. The bioaccessibility of lutein in microparticles was higher than that in emulsions, but the particle diameters of both emulsions and microparticles were more than 1208 nm [27]. In another study, emulsions with small particle sizes had higher cellular uptake capability than conventional emulsions with large particle sizes. When the emulsions were stored for approximately 5 days, all of them were stable (Fig. 1C). On Day 9, the emulsion with a ratio of Tween 80 and MCT of 1:9 was shown as two layers (Fig. 1D). Although it could be dispersed after mixing, it was not stable during long-term storage. Finally, the ratio of Tween 80 and MCT, 6:4, was selected. The specific formulation of the lutein nanoemulsion prepared by the low-energy method is as follows: the oil phase ratio is 10.14% (w/w), the emulsifier ratio is 15.12% (w/w), and the water phase ratio is 74.74% (w/w).

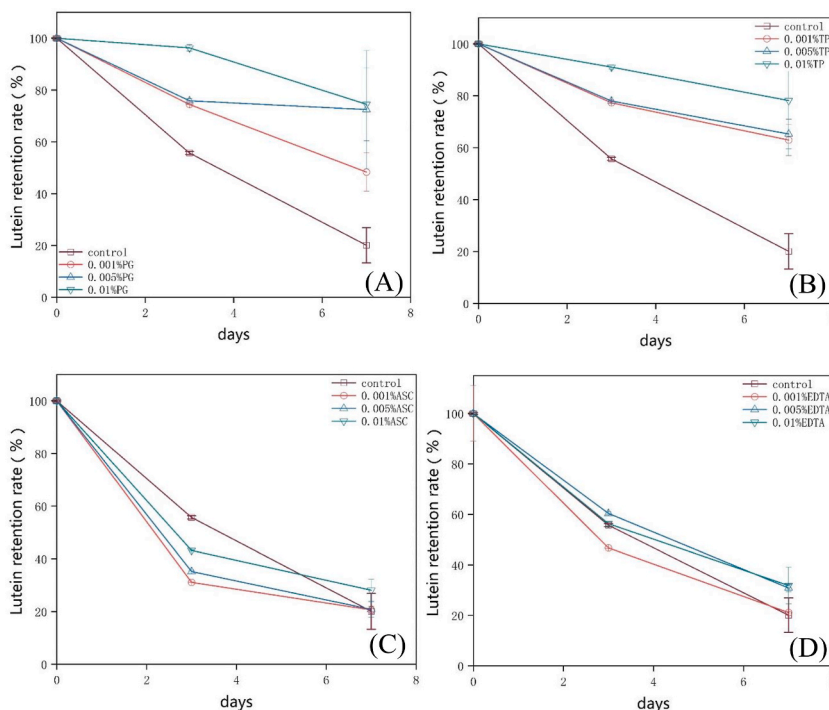
#### 3.2. Effects of a single antioxidant

The effects of the four antioxidants on the chemical stability of lutein were greatly changed. The lutein emulsions were stored at 25 °C for 7 days. The lutein retention rates of emulsions with PG and TP as antioxidants were higher than those of emulsions with ASC and EDTA as antioxidants, as shown in Fig. 2.

When PG was used as an antioxidant, the retention rate of lutein increased with increasing PG concentration at the beginning, but when the PG concentrations were 0.005% and 0.01%, the effect on the lutein retention rate was not significant. The retention rates of lutein in emulsions with 0.005% and 0.01% PG were approximately  $72.44 \pm 10.00$ – $74.47 \pm 7.22\%$  on the 7th day (Fig. 2A). The optimal concentration of PG was 0.01%.

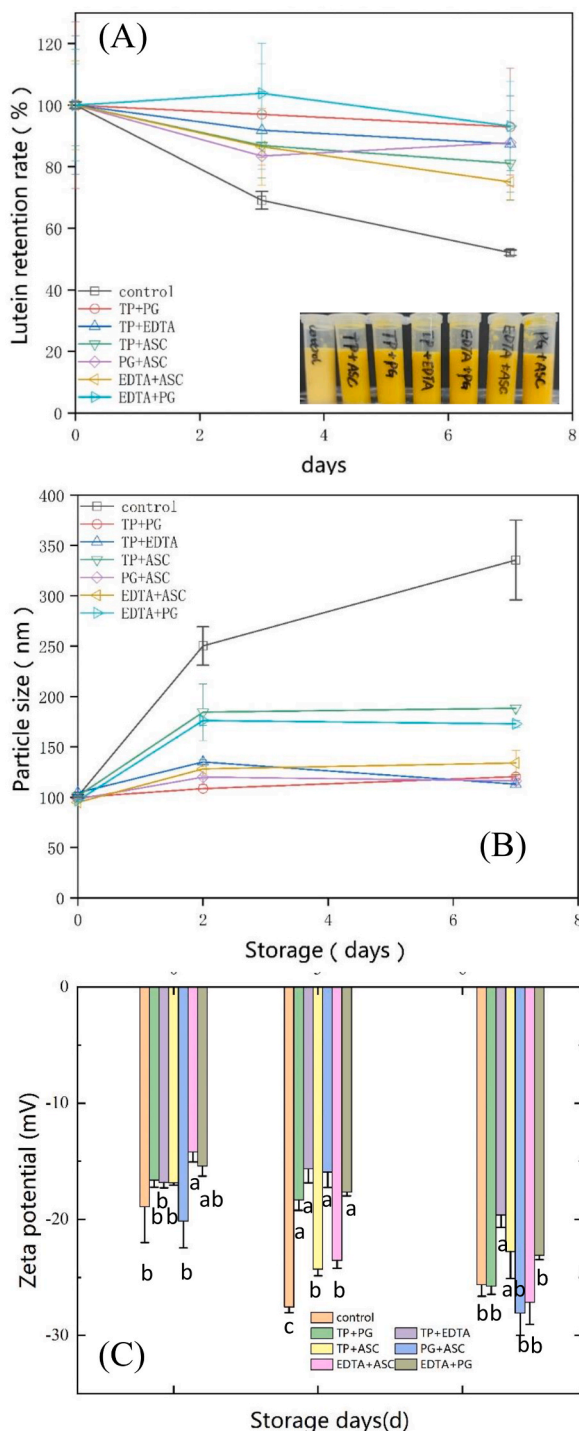
When tea polyphenols were used as antioxidants, the retention rate of lutein increased with increasing concentrations of tea polyphenols. The lutein retention rate of the emulsion with 0.01% tea polyphenols was  $78.16 \pm 10.11\%$  at Day 7 (Fig. 2B).

The protective effects of ascorbic acid and EDTA on the chemical stability of lutein were not obvious. The maximum lutein retention



**Fig. 2.** Effects of PG (A), TP (B), ASC (C) and EDTA (D) on lutein retention rates at 25 °C. The control is an emulsion prepared without any antioxidant. The experimental data are shown as the mean  $\pm$  SD (n = 3).

rates were only  $28.12 \pm 2.30$ – $31.8 \pm 4.21\%$  (Fig. 2C and D). When the concentration of ascorbic acid was 0.01%, the retention rate of lutein was similar to that of the control. In addition, there was no significant difference in the lutein retention rates in emulsions with 0.005% and 0.01% EDTA. The concentration of EDTA was 0.005% in the study below.



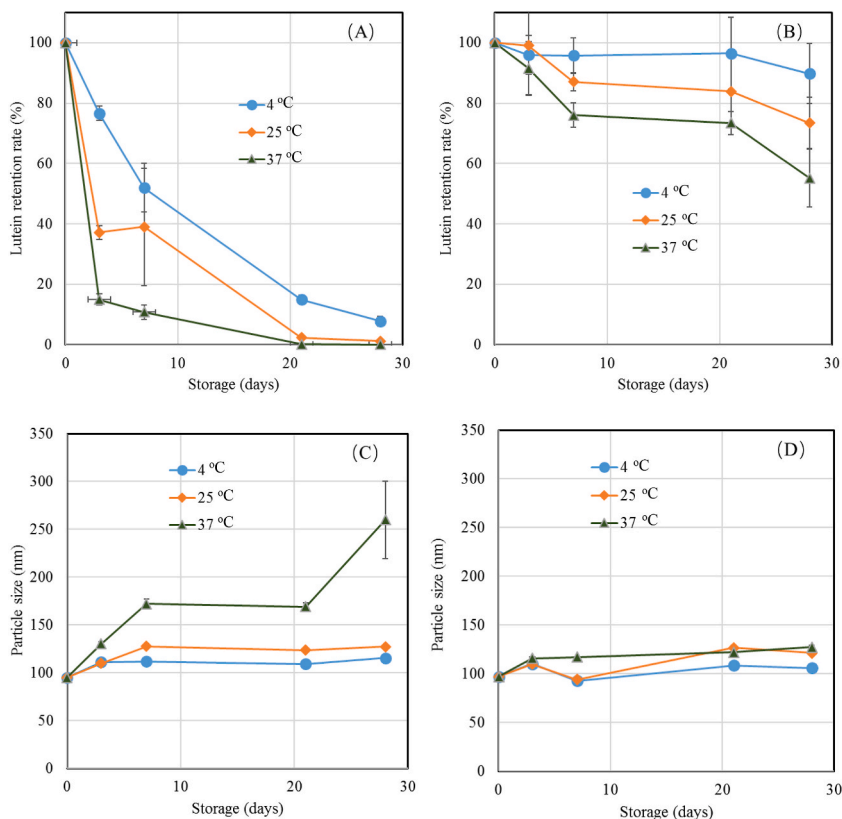
**Fig. 3.** Lutein retention rates (A), average particle size (B) and zeta potentials (C) of emulsions without any antioxidant (control) and different combinations of PG, TP, EDTA and ASC at 25 °C. The experimental data are shown as the mean ± SD (n = 3). Photographs of the emulsions were taken on Day 7. The superscripts, a, b and c, represent a significant difference (p < 0.05).

### 3.3. Effects of the combination of antioxidants

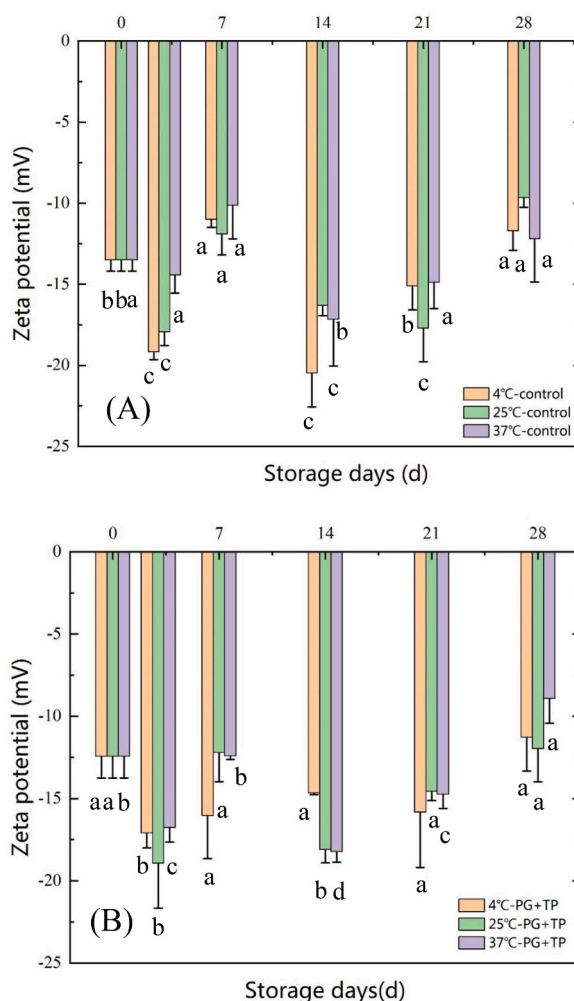
The results of the combination of the two antioxidants are shown in Fig. 3. The lutein retention rate of the emulsion with EDTA + ASC at Day 7 was  $76.69 \pm 15.77\%$  (Fig. 3A), which was significantly higher than those of emulsions with EDTA or ASC alone (Fig. 2C and D). However, it was similar to those of emulsions with 0.01% TP (Fig. 2A) or 0.01% PG (Fig. 2A). The addition of EDTA to emulsions with TP or PG (TP + EDTA or PG + EDTA) was beneficial to improve the chemical stability of lutein compared with emulsions with 0.01% TP or 0.01% PG. The combinations TP + PG and EDTA + PG had the highest lutein retention rates (>93%) compared with the control and other combinations of the two antioxidants. It should be mentioned that the average particle size of the emulsion with EDTA + PG increased from  $96.61 \pm 0.68$  nm (Day 0) to  $172.80 \pm 3.23$  nm (Day 7) (Fig. 3B). At the same time, that of the emulsion with TP + PG was slightly increased from  $99.80 \pm 0.64$  nm to  $120.40 \pm 2.26$  nm. It was proven that TP + PG had both physical and chemical stability compared with the control and other combinations. The zeta potentials are shown in Fig. 3C.

### 3.4. Long-term test

The physical and chemical stabilities of lutein emulsions without any antioxidants (control) and with TP + PG were investigated for 28 days. The results are shown in Fig. 4. If there were no antioxidants, the lutein in the control at 25 °C and 37 °C was almost absent at Day 28 (Fig. 4A). The color was fully white. At Day 3, the lutein retention rates of the control at 25 °C and 37 °C were  $37.17 \pm 2.21\%$  and  $15.00 \pm 1.91\%$ , respectively. It was proven that lutein was thermally sensitive. At 37 °C, the average particle size of the control increased from  $95.07 \pm 0.13$  nm to  $259.77 \pm 40.48$  nm (Fig. 4C). The final lutein retention rates of emulsions with TP + PG at 4, 25 and 37 °C were  $89.80 \pm 9.97\%$ ,  $73.50 \pm 8.53\%$  and  $55.17 \pm 9.59\%$ , respectively (Fig. 4B). The average particle sizes of emulsions with TP + PG under three temperatures were varied in a narrow range (Fig. 4D). The combined antioxidants TP + PG enhanced the physical and chemical stabilities of lutein emulsions. The prepared lutein emulsion had similar or better properties compared with other emulsions [35]. The zeta potentials are shown in Fig. 5. It was indicated that these emulsions were stable during the 21 days for emulsion with TP + PG (Fig. 5B) and control (Fig. 5A). TEM was taken when the emulsion was stored at 4 °C for more than 30 days. As shown in Fig. 6A and B, the emulsion is also stable.



**Fig. 4.** Lutein retention rates (A and B) and particle sizes (C and D) of emulsions without PG + TP (A and C) and with PG + TP (B and D). The experimental data are shown as the mean  $\pm$  SD ( $n = 3$ ).



**Fig. 5.** Zeta potentials of the control (A) and emulsions with PG + TP (B) at 4, 25 and 37 °C for 28 days. The superscripts, a, b, c and d represent a significant difference ( $p < 0.05$ ).

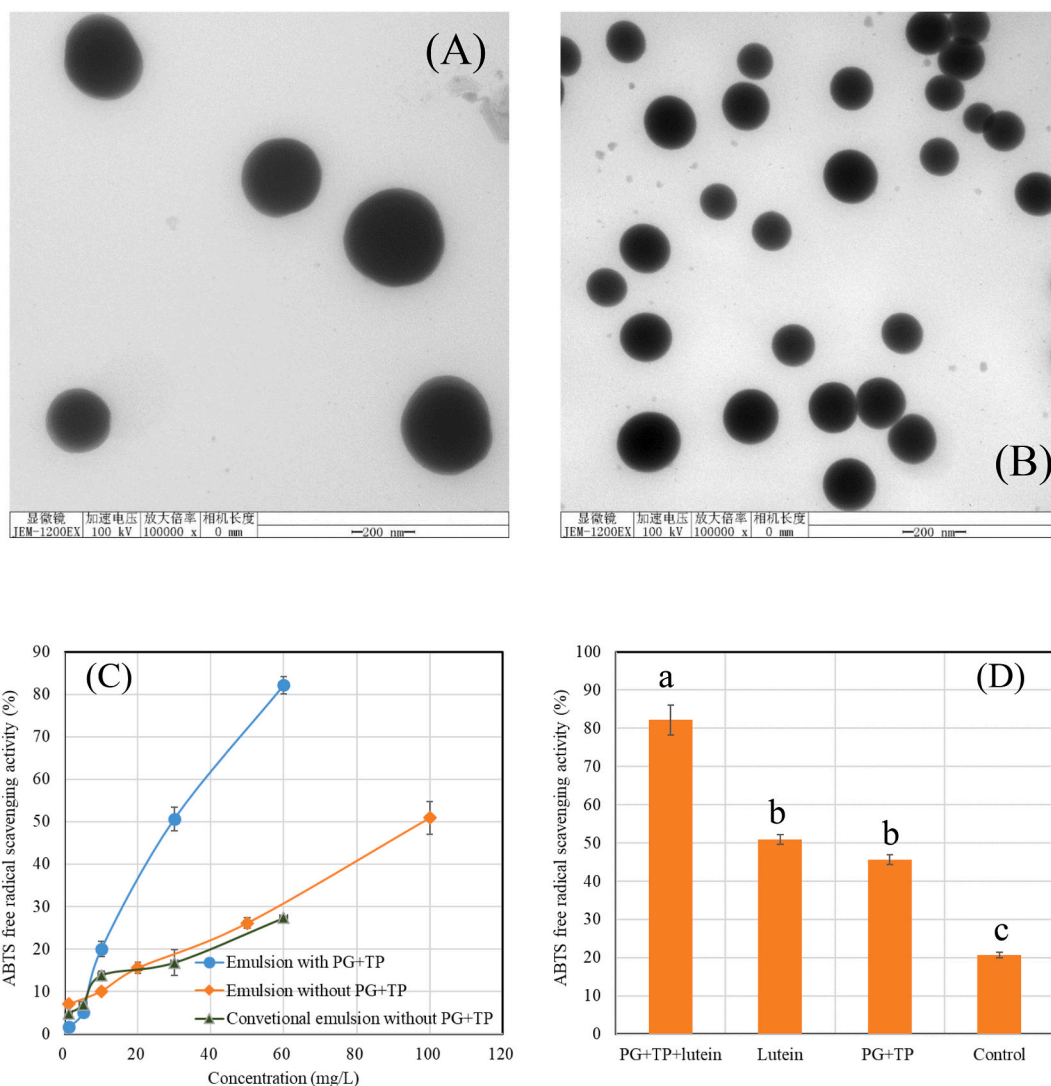
### 3.5. ABTS free radical scavenging activities of emulsions

The ABTS free radical scavenging activities of the lutein emulsion without PG and TP (surfactant:oil = 6:4) and the conventional emulsion without PG and TP (surfactant:oil = 1:9) were similar when the emulsion concentration was lower than 60 mg/L, as shown in Fig. 6C. It should be mentioned that both emulsions of lutein were stored for 2 days. This result indicated that particle size is not a dominant factor for ABTS activity. The ABTS free radical scavenging activities of the 60 mg/L lutein emulsion with PG and TP were more than 80% (Fig. 6C). It was stored for more than 20 days. It was proven that TP + PG improved the ABTS activity. In general, the antioxidative capability of the emulsion will be decreased after long-term storage. The antioxidative activity of emulsions depends on lutein and antioxidants. Compared with the control (without lutein and any antioxidants), the ABTS free radical scavenging activity of the lutein emulsion without antioxidants was significantly higher (Fig. 6D). The effect of lutein on antioxidative activity was similar to that of PG + TP.

### 3.6. Discussion

As shown above, neither EDTA nor ASC were good antioxidants to enhance the chemical stability of lutein when they were used as single antioxidants shown Fig. 3. However, the combination of EDTA and ASC increased the lutein retention rate more than twofold, from 28.12 to 21.83% to approximately  $75.00 \pm 0.63\%$ . EDTA is lipid soluble and ASC water soluble. One possible reason is that EDTA and ASC remove oxygen from oil and water, respectively. The combination of TP and PG has the best performance. TP is water soluble, and SP is lipid soluble. The possible mechanism is also similar to that of EDTA + ASC. TP + PG protects both the lipid phase and water phase of the oil-in-water emulsion.

Although TP and PG were added to the conventional emulsion (Tween 80: MCT = 1:9), its ABTS free radical scavenging activity was



**Fig. 6.** TEM (A and B) and ABTS activities (C and D). TEM of lutein emulsions without TP + PG (A) and with TP + PG (B). TEMs were taken by JEM-1200EX under 100 kv. The scale bar is 200 nm ( $\times 100,000$ ). ABTS free radical scavenging activities of lutein emulsions with PG + TP (Day 24) and without PG + TP (Tween 80: MCT = 6: 4, Day 2) and conventional emulsions without PG + TP (Tween 80: MC = 1: 9, Day 2) (C). The antioxidative activities of lutein emulsion with TP + PG (Day 24), lutein emulsion without TP + PG (Day 2), emulsion with TP + PG and without lutein (Day 2), and control (emulsion without lutein, TP and PG, Day 2) (D). The experimental data are shown as the mean  $\pm$  SD ( $n = 3$ ). The superscripts, a, b and c, represent a significant difference ( $p < 0.05$ ).

less than that of the emulsion with a small particle size (Tween 80: MCT = 6:4). A similar result was also presented [25]. The small particle size of the emulsion is beneficial to its utilization. It is also recognized that nanoparticles that are too small may be toxic. Bioaccessibility and antioxidative capability rely on components of emulsion, particle size and antioxidants in emulsion, and other conditions. Many more studies should be carried out before the utilization of lutein emulsions.

The properties of lutein emulsion depend on the selection of oils and emulsifiers, and preparation method. Some information of emulsions, microparticles, nanocapsules of lutein are collected in Table 1. Compared with emulsion, microparticles had a larger particle size and the lutein retention rate was improved from 30.74% to 74.51% [27]. The particle size was more than 1000 nm and the application may be limited if the particle size was a limiting factor. The lutein retention rate of this study was lower than that of emulsion [35]. Besides the oil and emulsifier, the preparation method may be much more critical to improve the lutein stability. Compared with the emulsions without any antioxidants [21,36–38,39,40], the lutein retention rates of this study under three temperature conditions are similar or better than them. The current study focused on the usage of combination of binary antioxidants. If other preparation methods were performed and the oil and emulsifiers were optimized, the physicochemical properties may be improved. Especially, the lutein retention rates under 25 °C and 37 °C should be increased.



**Table 1**  
Comparison of the preparation conditions and properties of lutein emulsions.

Type	Oil	Emulsifier	Antioxidants	Preparation method	Particle size (nm)	Retention temperature (°C)	Storage time (day)	Retention rate (%)	Ref.
Emulsion	Corn oil	NaCas	–	High speed mixing and high pressure	231.8	37	7	85	[35]
Emulsion	MCT oil	Tween 20	–	Ultrasonic	610	4	46	62	[21]
Emulsion	MCT oil	Tween 20/ lecithin	–	Ultrasonic	540	4	46	90	[21]
Emulsion	MCT oil	NaCas	–	Microfluidizer	1208	37	22	30.74	[27]
Microparticles	MCT oil	NaCas + NaALG	–	Electrostatic complexation	1750	37	22	74.51	[27]
Emulsion	Corn oil	LAE	–	Sonication	357	4	15	72	[36]
						25		65	
						37		62	
		Tween 80	–	Sonication	428	4	15	86	[36]
						25		78	
						37		58	
		SDS	–	Sonication	289	4	15	50	[36]
						25		40	
						37		30	
Emulsion	Corn oil			High-pressure homogenization	234.01	4	30	90	[37]
Lipid-core nanocapsules	PCL- SM- MCT	Polysorbate 80	–	Low-energy method	191.9	4	60	36	[38]
Lutein nanodispersions	Acetone	Tween 80 + SDS	–	Solvent displacement	67	4	56	52.3	[41]
Lutein nanodispersions	Acetone	NaCas	–	Solvent displacement	113	4	56	94.3	[41]
Nanoemulsion	Corn oil	EA + WPI	Tocopherol	High pressure homogenization + solvent evaporation	68.8	5	28	80	[25]
						20		32	
						40		10	
Emulsion	Corn oil	EA + WPI	Tocopherol	High pressure homogenization	147.3	5	28	99	[25]
						20		60	
						40		40	
Emulsion	Coconut oil	Xanthan + PGA	–	High-speed shear	30–40	4	7	52.3	[39]
						25		56.8	
						40		54.5	
Emulsion	Coin oil	WPI	–	High-speed shear and nano homogenize	242.16	4	21	84	[40]
						25		73	
						37		45	
						50		35	
Emulsion	Coin oil	CA-WPI	–	High-speed shear and nano homogenize	264.63	4	21	87	[40]
						25		78	
						37		58	
						50		50	
Emulsion	Coin oil	CA–WPI- DEX	–	High-speed shear and nano homogenize	219.4	4	21	88	[40]
						25		80	
						37		62	
						50		55	
Emulsion	Coin oil	CA–WPI- DEX	Vitamin E	High-speed shear and nano homogenize	224.73	4	21	90	[40]
						25		85	
						37		70	
						50		65	
Emulsion	Coin oil	WPI-DEX	–	High-speed shear and nano homogenize	216.2	4	21	85	[40]
						25		75	
						37		48	
						50		40	
Emulsion	Coin oil	BSA-CA-DEX	Vitamin E	High-speed shear and nano homogenize	220	4	15	88	[9]
						37		58	
						50		46	
Nanoemulsion	MCT	WPI	–	High intensity ultrasound	203–211	4	28	94	[42]
						25		93	
						37		94	
Emulsion	MCT	Tween 80	TP + PG	Low energy	97.06	4	28	89.8	This study
						25		73.5	
						37		55.2	

Bovine serum albumin (BSA), chlorogenic acid (CA) and dextran (DEX), Ethyl acetate (EA), Ethyl lauroyl arginate (LAE), Medium-chain-triacylglycerol (MCT), Poly ( $\epsilon$ -caprolactone) (PCL), Propylene glycol alginate (PGA), Sodium alginate (NaALG), Sodium caseinate (NaCas), Sodium dodecyl sulfate (SDS), Sorbitan monostearate (SM), Whey protein isolate (WPI).

- Indicates no antioxidant was used to prepare for emulsion.

#### 4. Conclusions

In this study, oil-in-water emulsions of lutein were prepared by low energy method. In addition to the effects of surfactant:oil, the effects of four antioxidants, EDTA, ASC, PG and TP, and their binary combinations were evaluated on lutein retention rates and physical stabilities. It was indicated that the combination of PG and TP was much more effective for improving the lutein retention rate at 4–37 °C. A large particle size (>250 nm) was beneficial for enhancing the lutein retention rate, but the ABTS free radical scavenging activity was lower than that of emulsions with PG and TP, in which the particle size was 97–120 nm.

#### Author contribution statement

Yujiao Wang: Performed the experiments; Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Quanyu Zhao: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xiangxiang Zhang, Manlin Yan: Wrote the paper.

#### Data availability statement

Data will be made available on request.

#### Additional information

No additional information is available for this paper.

#### Credit author statement

Y.J. Wang: Performed the experiments; Y.J. Wang and Q.Y. Zhao: Conceived and designed the experiments, and Analyzed and interpreted the data; Y.J. Wang, X.X. Zhang, M.L. Yan and Q.Y. Zhao: Wrote the paper.

#### Declaration of competing 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.

#### Acknowledgements

The authors acknowledge the supports received from National Natural Science Foundation of China (32270379) and Postgraduate Research & Practice In-novation Program of Jiangsu Province (KYCX21\_1208).

#### References

- [1] E. Agron, J. Mares, T.E. Clemons, A. Swaroop, E.Y. Chew, T.D.L. Keenan, Dietary nutrient intake and progression to late age-related macular degeneration in the age-related eye disease studies 1 and 2, *Ophthalmology* 128 (2021) 425–442, <https://doi.org/10.1016/j.ophtha.2020.08.018>.
- [2] D. Gazzolo, S. Picone, A. Gaiero, M. Bellettato, G. Montrone, F. Riccobene, G. Lista, G. Pellegrini, Early pediatric benefit of lutein for maturing eyes and brain - an overview, *Nutrients* 13 (2021) 3239, <https://doi.org/10.3390/nu13093239>.
- [3] M.S. Razavi, P. Ebrahimnejad, Y. Fatahi, A. D'Emanuele, R. Dinarvand, Recent developments of nanostructures for the ocular delivery of natural compounds, *Front. Chem.* 10 (2022), 850757, <https://doi.org/10.3389/fchem.2022.850757>.
- [4] B. Demmig-Adams, M. Lopez-Pozo, J.J. Stewart, W.W. Adams 3rd, Zeaxanthin and lutein: photoprotectors, anti-inflammatories, and brain food, *Molecules* 25 (2020) 3607, <https://doi.org/10.3390/molecules25163607>.
- [5] J.H. Lin, D.J. Lee, J.S. Chang, Lutein production from biomass: marigold flowers versus microalgae, *Bioresour. Technol.* 184 (2015) 421–428, <https://doi.org/10.1016/j.biortech.2014.09.099>.
- [6] D. Li, Y. Yuan, D. Cheng, Q. Zhao, Effect of light quality on growth rate, carbohydrate accumulation, fatty acid profile and lutein biosynthesis of *Chlorella* sp, *AE10, Bioresour. Technol.* 291 (2019), 121783, <https://doi.org/10.1016/j.biortech.2019.121783>.
- [7] Y. Zhu, X. Li, Y. Wang, L. Ren, Q. Zhao, Lutein extraction by imidazolium-based ionic liquid-water mixture from dried and fresh *Chlorella* sp, *Algal Res.* 60 (2021), 102528, <https://doi.org/10.1016/j.algal.2021.102528>.
- [8] C.E. Gumus, G. Davidov-Pardo, D.J. McClements, Lutein-enriched emulsion-based delivery systems: impact of Maillard conjugation on physicochemical stability and gastrointestinal fate, *Food Hydrocolloids* 60 (2016) 38–49, <https://doi.org/10.1016/j.foodhyd.2016.03.021>.
- [9] Y. Yan, Q. Zhu, C. Diao, J. Wang, Z. Wu, H. Wang, Enhanced physicochemical stability of lutein-enriched emulsions by polyphenol-protein-polysaccharide conjugates and fat-soluble antioxidant, *Food Hydrocolloids* 101 (2020), 105447, <https://doi.org/10.1016/j.foodhyd.2019.105447>.
- [10] T.J. Ashaolu, Nanoemulsions for health, food, and cosmetics: a review, *Environ. Chem. Lett.* 19 (2021) 3381–3395, <https://doi.org/10.1007/s10311-021-01216-9>.
- [11] B.M. Steiner, D.J. McClements, G. Davidov-Pardo, Encapsulation systems for lutein: a review, *Trends Food Sci. Technol.* 82 (2018) 71–81, <https://doi.org/10.1016/j.tifs.2018.10.003>.
- [12] Y. Chang, D.J. McClements, Optimization of orange oil nanoemulsion formation by isothermal low-energy methods: influence of the oil phase, surfactant, and temperature, *J. Agric. Food Chem.* 62 (2014) 2306–2312, <https://doi.org/10.1021/jf500160y>.
- [13] Z. Gombac, I.G. Osojnik Crnivec, M. Skrt, K. Istenic, A. Knez Knafelj, I. Pravst, N. Poklar Ulrih, Stabilisation of lutein and lutein esters with polyoxyethylene sorbitan monooleate, medium-chain triglyceride oil and lecithin, *Foods* 10 (2021) 500, <https://doi.org/10.3390/foods10030500>.

- [14] M. Liu, F. Wang, C. Pu, W. Tang, Q. Sun, Nanoencapsulation of lutein within lipid-based delivery systems: characterization and comparison of zein peptide stabilized nano-emulsion, solid lipid nanoparticle, and nano-structured lipid carrier, *Food Chem.* 358 (2021), 129840, <https://doi.org/10.1016/j.foodchem.2021.129840>.
- [15] M. Chaijan, K. Srirattanaachot, M. Nisoa, L.-Z. Cheong, W. Panpipat, Role of antioxidants on physicochemical properties and in vitro bioaccessibility of  $\beta$ -carotene loaded nanoemulsion under thermal and cold plasma discharge accelerated tests, *Food Chem.* 339 (2021), 128157, <https://doi.org/10.1016/j.foodchem.2020.128157>.
- [16] F. Weigel, J. Weiss, E.A. Decker, D.J. McClements, Lutein-enriched emulsion-based delivery systems: influence of emulsifiers and antioxidants on physical and chemical stability, *Food Chem.* 242 (2018) 395–403, <https://doi.org/10.1016/j.foodchem.2017.09.060>.
- [17] D. Xu, Z. Aihemaiti, Y. Cao, C. Teng, X. Li, Physicochemical stability, microrheological properties and microstructure of lutein emulsions stabilized by multilayer membranes consisting of whey protein isolate, flaxseed gum and chitosan, *Food Chem.* 202 (2016) 156–164, <https://doi.org/10.1016/j.foodchem.2016.01.052>.
- [18] J.R. Hyatt, S. Zhang, C.C. Akoh, Comparison of antioxidant activities of selected phenolic compounds in O/W emulsions and bulk oil, *Food Chem.* 349 (2021), 129037, <https://doi.org/10.1016/j.foodchem.2021.129037>.
- [19] L. Zhang, F. Zhang, Z. Fan, B. Liu, C. Liu, X. Meng, DHA and EPA nanoemulsions prepared by the low-energy emulsification method: process factors influencing droplet size and physicochemical stability, *Food Res. Int.* 121 (2019) 359–366, <https://doi.org/10.1016/j.foodres.2019.03.059>.
- [20] B.M. Steiner, V. Shukla, D.J. McClements, Y.O. Li, M. Sancho-Madriz, G. Davidov-Pardo, Encapsulation of lutein in nanoemulsions stabilized by resveratrol and Maillard conjugates, *J. Food Sci.* 84 (2019) 2421–2431, <https://doi.org/10.1111/1750-3841.14751>.
- [21] K. Frede, A. Henze, M. Khalil, S. Baldermann, F.J. Schweigert, H. Rawel, Stability and cellular uptake of lutein-loaded emulsions, *J. Funct. Foods* 8 (2014) 118–127, <https://doi.org/10.1016/j.jff.2014.03.011>.
- [22] J.C. Courtenay, Y. Jin, J. Schmitt, K.M.Z. Hossain, N. Mahmoudi, K.J. Edler, J.L. Scott, Salt-responsive pickering emulsions stabilized by functionalized cellulose nanofibrils, *Langmuir* 37 (2021) 6864–6873, <https://doi.org/10.1021/acs.langmuir.0c03306>.
- [23] D. Ye, L. Shen, Y. Sun, D. Zhang, X. Tan, P. Jing, M. Zhang, Q. Tian, Formulation and evaluation of an alpha-linolenic acid and vitamin E succinate microemulsion with low surfactant content and free of co-surfactant for use as a nutritional supplement, *Food Chem.* 364 (2021), 130433, <https://doi.org/10.1016/j.foodchem.2021.130433>.
- [24] Ö. Coban, S. Yildirim, T. Bakir, Alpha-lipoic acid and cyanocobalamin co-loaded nanoemulsions: development, characterization, and evaluation of stability, *J. Pharm. Innov.* 17 (2021) 510–520, <https://doi.org/10.1007/s12247-020-09531-4>.
- [25] A. Teo, S.J. Lee, K.K.T. Goh, F.M. Wolber, Kinetic stability and cellular uptake of lutein in WPI-stabilised nanoemulsions and emulsions prepared by emulsification and solvent evaporation method, *Food Chem.* 221 (2017) 1269–1276, <https://doi.org/10.1016/j.foodchem.2016.11.030>.
- [26] B. Niu, P. Shao, P. Sun, Ultrasound-assisted emulsion electrosprayed particles for the stabilization of  $\beta$ -carotene and its nutritional supplement potential, *Food Hydrocolloids* 102 (2020), 105634, <https://doi.org/10.1016/j.foodhyd.2019.105634>.
- [27] J. Hao, J. Xu, W. Zhang, X. Li, D. Liang, D. Xu, Y. Cao, B. Sun, The improvement of the physicochemical properties and bioaccessibility of lutein microparticles by electrostatic complexation, *Food Hydrocolloids* 125 (2022), 107381, <https://doi.org/10.1016/j.foodhyd.2021.107381>.
- [28] S. Shah, B. Bhandari, M. Soniwal, J. Chavda, Lutein-loaded solid lipid nanoparticles for ocular delivery: statistical optimization and ex vivo evaluation, *J. Pharma. Innov.* 17 (2021) 584–598, <https://doi.org/10.1007/s12247-021-09537-6>.
- [29] K.S. Yadav, K. Kale, High pressure homogenizer in pharmaceuticals: understanding its critical processing parameters and applications, *J. Pharma. Innov.* 15 (2019) 690–701, <https://doi.org/10.1007/s12247-019-09413-4>.
- [30] X.-Y. Qv, Z.-P. Zeng, J.-G. Jiang, Preparation of lutein microencapsulation by complex coacervation method and its physicochemical properties and stability, *Food Hydrocolloids* 25 (2011) 1596–1603, <https://doi.org/10.1016/j.foodhyd.2011.01.006>.
- [31] M. Yadav, N. Schiavone, A. Guzman-Aranguez, F. Giansanti, L. Papucci, M.J. Perez de Lara, M. Singh, I.P. Kaur, Atorvastatin-loaded solid lipid nanoparticles as eye drops: proposed treatment option for age-related macular degeneration (AMD), *Drug Deliv. Transl. Res.* 10 (2020) 919–944, <https://doi.org/10.1007/s13346-020-00733-4>.
- [32] L. Du, Y. Shen, X. Zhang, W. Prinyawiwatkul, Z. Xu, Antioxidant-rich phytochemicals in miracle berry (*Synsepalum dulcificum*) and antioxidant activity of its extracts, *Food Chem.* 153 (2014) 279–284, <https://doi.org/10.1016/j.foodchem.2013.12.072>.
- [33] Y. Guan, J. Wu, Q. Zhong, Eugenol improves physical and chemical stabilities of nanoemulsions loaded with  $\beta$ -carotene, *Food Chem.* 194 (2016) 787–796, <https://doi.org/10.1016/j.foodchem.2015.08.097>.
- [34] M. Ospina, K. Montana-Oviedo, Á. Diaz-Duque, H. Toloza-Daza, C.-E. Narvaez-Cuenca, Utilization of fruit pomace, overripe fruit, and bush pruning residues from Andes berry (*Rubus glaucus* Benth) as antioxidants in an oil in water emulsion, *Food Chem.* 281 (2019) 114–123, <https://doi.org/10.1016/j.foodchem.2018.12.087>.
- [35] G. Davidov-Pardo, C.E. Gumus, D.J. McClements, Lutein-enriched emulsion-based delivery systems: influence of pH and temperature on physical and chemical stability, *Food Chem.* 196 (2016) 821–827.
- [36] S.-J. Jeong, S. Kim, E. Echeverria-Jaramillo, W.-S. Shin, Effect of the emulsifier type on the physicochemical stability and in vitro digestibility of a lutein/zeaxanthin-enriched emulsion, *Food Sci. Biotechnol.* 30 (2021) 1509–1518, <https://doi.org/10.1007/s10068-021-00987-9>.
- [37] J. Li, R. Guo, H. Hu, X. Wu, L. Ai, Y. Wu, Preparation optimisation and storage stability of nanoemulsion-based lutein delivery systems, *J. Microencapsul.* 35 (2018) 570–583, <https://doi.org/10.1080/02652048.2018.1559245>.
- [38] A.A. Santana Brum, P.P. dos Santos, M.M. da Silva, K. Paese, S.S. Guterres, T.M. Haas Costa, A.R. Pohlmann, A. Jablonski, S.H. Flores, A.d.O. Rios, Lutein-loaded lipid-core nanocapsules: physicochemical characterization and stability evaluation, *Colloids Surf., A* 522 (2017) 477–484, <https://doi.org/10.1016/j.colsurfa.2017.03.041>.
- [39] L. Wang, Y. Li, D. Xiang, W. Zhang, X. Bai, Stability of lutein in O/W emulsion prepared using xanthan and propylene glycol alginate, *Int. J. Biol. Macromol.* 152 (2020) 371–379, <https://doi.org/10.1016/j.ijbiomac.2020.02.162>.
- [40] H. Wang, Y. Yan, X. Feng, Z. Wu, Y. Guo, H. Li, Q. Zhu, Improved physicochemical stability of emulsions enriched in lutein by a combination of chlorogenic acid–whey protein isolate–dextran and vitamin E, *J. Food Sci.* 85 (2020) 3323–3332, <https://doi.org/10.1111/1750-3841.15417>.
- [41] T.B. Tan, N.S. Yusoff, F. Abas, H. Mirhosseini, I.A. Nehdi, C.P. Tan, Stability evaluation of lutein nanodispersions prepared via solvent displacement method: the effect of emulsifiers with different stabilizing mechanisms, *Food Chem.* 205 (2016) 155–162, <https://doi.org/10.1016/j.foodchem.2016.03.008>.
- [42] C. Zhao, X. Shen, M. Guo, Stability of lutein encapsulated whey protein nano-emulsion during storage, *PLoS One* 13 (2018), e0192511, <https://doi.org/10.1371/journal.pone.0192511>.