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An investigation on pickering nano-emulsions stabilized by dihydromyricetin/high-amylose corn starch composite particles: Preparation conditions and carrier properties

Sheng Geng [a], Yuan Yuan [a], Xinying Jiang [a], Ruhua Zhang [a], Hanjun Ma [a], Guizhao Liang [b,**], Benguo Liu [a,*]

[a] School of Food Science, Henan Institute of Science and Technology, Xinxiang, 453003, China
[b] Key Laboratory of Biorheological Science and Technology, Ministry of Education, Bioengineering College, Chongqing University, Chongqing, 400044, China

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ABSTRACT

With dihydromyricetin (DMY)/high-amylose corn starch (HCS) composite particles as the emulsifier, Pickering nano-emulsions were fabricated by combining high-speed shearing and high-pressure homogenization. The effect of particle properties and processing conditions on the formation and physicochemical properties of the Pickering nano-emulsions was then investigated systematically. The results showed that the DMY content of the composite particles, the oil phase volume fraction of the emulsion, and the homogenization conditions had obvious effects on the droplet size of the emulsion, where appropriate DMY content in the composite particles (5–20%) contributed to the formation of stable Pickering nano-emulsions. The oil phase of the obtained emulsions exhibited good stability during high-temperature storage, and their β-carotene protecting performance against UV irradiation was superior to the emulsion stabilized by Tween 20. The *in vitro* simulated digestion analysis indicated that the nano-emulsions developed by the composite particles could enhance the bioaccessibility of β-carotene and inhibit starch hydrolysis.

1. Introduction

Emulsion systems with an average droplet diameter of 50–500 nm can be defined as nano-emulsions (Anton et al., 2008), which have been mainly used in the fields of nutraceutical delivery (Shakeel et al., 2014), biological diagnosis (Patel et al., 2013), and disease treatment (Patel et al., 2013). The effectiveness of nano-emulsions for the encapsulation and delivery of nutraceuticals has been recognized. To a certain extent, they can improve the stability, permeability, and release of functional components in the gastrointestinal tract, thus promoting absorption (Ali et al., 2016). However, traditional synthetic surfactants usually cause intestinal irritation and poor hemolysis (Rayner et al., 2014), which can accumulate in the human body for a long period, resulting in liver injury (Jimenez-Escobar et al., 2020).

Food-grade Pickering nano-emulsions constructed with food-derived solid particles as an emulsifier are a new development in the application of Pickering emulsions in the fields of food and medicine (Dieng et al., 2020; Geng et al., 2022; Niu et al., 2020). The composite particles based on proteins, polysaccharides and polyphenols have been successfully used in the construction of Pickering emulsions (Tavernier et al., 2016). Food-grade Pickering nano-emulsions not only have high safety but also offer the advantages of an excellent absorption rate of nano-emulsions and good stability of Pickering emulsions, making them suitable for the transport of nutraceuticals (Shanmugam et al., 2022). Currently, most studies have focused on traditional Pickering emulsions by ultrasound or high-speed shearing (Shao et al., 2017; Taha et al., 2020; Tan et al., 2018). However, Pickering nano-emulsion is different from traditional Pickering emulsion, and its emulsifying effect is not only related to the properties of the emulsifier, but also closely related to the high-speed homogenization conditions used. And there are few studies on the effects of processing conditions on the formation and properties of Pickering nano-emulsion stabilized by composite particles; thus, related studies need to be conducted.

Dihydromyricetin (DMY) is a type of natural multifunctional active metabolite. Its main source plant, *Ampelopsis grossedentata*, was listed as a new food resource by the Chinese government in 2013, and its safety

* Corresponding author.
** Corresponding author.
E-mail addresses: gzliang@cqu.edu.cn (G. Liang), liubenguo@hist.edu.cn, zzgclbg@126.com (B. Liu).

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has been widely accepted (Liu et al., 2019). High-amylose corn starch (HCS) has been widely used in the food industry. In a previous study, we found that DMY/HCS composite particles could stabilize Pickering emulsion gels by high-speed shearing (Geng et al., 2021). The DMY/HCS composite particles have the advantages of simple preparation, low cost, and an outstanding emulsifying capacity, offering great application prospects in the food industry. In this study, high-speed shearing and high-pressure homogenization were combined to construct Pickering nano-emulsions using DMY/HCS composite particles. The effects of the composite particles (addition amount, w; DMY content, c), oil phase volume fraction (φ), and homogenization conditions (homogenization pressure, p; the number of homogenization cycles, n; and homogenization temperature, T) on the formation of the nano-emulsion were systematically investigated. The ability of the prepared Pickering nano-emulsion to delay oil oxidation and enhance the stability and bioaccessibility of β-carotene was also evaluated. The obtained results may not only enrich the Pickering emulsifying theory but also promote the development and application of new Pickering nano-emulsions.

2. Materials and methods

2.1. Chemicals

Medium-chain triglyceride (MCT), HCS (amylose content ≥72%), and DMY (purity ≥98%) were produced by Yuanye Biotechnology Co. Ltd. (Shanghai, China). Porcine pepsin (≥500 U/mg), α-amylase (50 U/mg), mucin (Type II), trypsin (8 × USP), and bile salt were the products of Sigma-Aldrich Chemical, Inc. (St. Louis, MO, USA). Tween 20 and β-carotene (purity ≥96%) were purchased from Aladdin (Shanghai, China). A glucose kit was obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Ultrapure water from a Thermo Genpure UV/UF water system (Waltham, MA, USA) was used in this work. All other chemicals were of analytical grade.

2.2. Preparation of the nano-emulsion stabilized by the DMY/HCS composite particles

The preparation of the DMY/HCS composite particles was performed according to our previous report (Geng et al., 2021). The HCS (4.0–5.0 g) and 200 mL of ultrapure water were mixed, stirred at 1000 rpm, and heated at 80 °C for 20 min. Then, a certain amount of DMY (0–1.0 g) was added to the mixture. The obtained mixture was stirred at 80 °C for 2 h. After freeze-drying, composite particles with DMY content of 0%–20% (w/w) were obtained. The composite particles, which were developed with the designated DMY content (c = 0, 2.5%, 5%, 10%, and 20%), were added to water and mixed with MCT at the designed addition amounts (w = 0.5%, 1.0%, and 1.5%) and oil phase volume fractions (φ = 5%, 10%). The mixture was sheared at 15,000 rpm for 5 min by an IKA Ultra-Turrax T18 disperser (Staufen, Germany) to obtain the primary emulsion. This was further homogenized at a specified homogenization pressure (p = 100 bar, 200 bar, 300 bar, 600 bar, and 900 bar), the number of homogenization cycles (n = 3, 6), and homogenization temperature (T = 4 and 30 °C) using an ATS AH-BASIC-10L/H high-pressure homogenizer (Toronto, Canada) (Li et al., 2022; Liu et al., 2022). The obtained nano-emulsions were used for the following analysis.

2.3. Measurement of emulsion droplet size

The distribution of emulsion droplet size was determined at 25 °C based on a previously published method (Liu et al., 2022). The 10 μL sample solution was diluted to 10 mL with ultrapure water, and its droplet size was analyzed by a Zetasizer Nano-ZS particle analyzer (Malvern Instruments Ltd., Worcestershire, UK).

2.4. Evaluation of emulsion stability

The nano-emulsions with c = 0–20%, w = 1%, and φ = 5% were prepared under the homogenization conditions of p = 300 bar, n = 3, and T = 4 °C. Then, they were sealed in glass bottles and stored at 4 °C and 30 °C for 7 days, during which the changes in droplet size were continuously recorded (Liu et al., 2022).

2.5. Determination of stability of oil in emulsion

According to the methods described in section 2.2, sunflower oil was used to replace MCT to construct the nano-emulsion, and the obtained emulsion was stored at 50 °C. At specified times, 200 μL of emulsion and 1.5 mL of isooctane/2-propanol solvent (3:1, v/v) were swirled, and then centrifuged at 2000 g for 5 min. The upper organic solvent phase (200 μL) was added to 2.8 mL of methanol/1-butanol solvent (2:1, v/v), and then 15 μL of NH_4SCN (3.94 M) and 15 μL of Fe^{2+} solution (a mixture of 0.144 M $FeSO_4$ and 0.132 M $BaCl_2$) were added (Geng et al., 2022). After 20 min, the absorbance of the mixture at 510 nm was read, and the corresponding peroxide content was calculated based on the standard curve. For comparison, the emulsion stabilized by Tween 20 (1%) was used as the control.

2.6. Evaluation of the β-carotene protective effect of the emulsion

Using β-carotene containing 2.0 mg/mL MCT as the oil phase, the nano-emulsion was prepared according to the methods described in section 2.2. The obtained emulsion was placed about 15 cm under an ultraviolet lamp (Power, 6 W) and stored at 30 °C. The emulsion sample (1 mL) was removed at regular intervals, mixed with 10 mL of ethanol/hexane solvent (2:3, v/v), and kept in the dark for 10 min. Then, the absorbance of the supernatant at 450 nm was read. Compared to the standard curve of β-carotene, the β-carotene content was determined, and the protective effect of the nano-emulsion could be evaluated by the β-carotene retention rate. The emulsion stabilized by 1% Tween 20 with the same β-carotene content was used as the control (Liu et al., 2022).

2.7. Simulated digestion method of the nano-emulsion

2.7.1. Preparation of the simulated digestion mediums

According to previous reports (Minekus et al., 2014; Perez-Burillo et al., 2021), the simulated saliva, gastric fluids, and intestinal fluids were prepared as follows.

For the simulated saliva fluid (SSF), KH_2PO_4 (0.5 M, 3.7 mL), $NaHCO_3$ (1.0 M, 6.8 mL), $MgCl_2$ $(H_2O)_6$ (0.15 M, 0.5 mL), $(NH_4)_2CO_3$ (0.5 M, 0.06 mL), and KCl (0.5 M, 15.1 mL) were blended and diluted to 400 mL with water.

For the simulated gastric fluid (SGF), KH_2PO_4 (0.5 M, 0.9 mL), $(NH_4)_2CO_3$ (0.5 M, 0.5 mL), $NaHCO_3$ (1.0 M, 12.5 mL), NaCl (2.0 M, 11.8 mL), $MgCl_2$ $(H_2O)_6$ (0.15 M, 0.4 mL), and KCl (0.5 M, 6.9 mL) were blended and diluted to 400 mL with water.

For the simulated intestinal fluid (SIF), KH_2PO_4 (0.5 M, 0.8 mL), $NaHCO_3$ (1.0 M, 42.5 mL), NaCl (2.0 M, 9.6 mL), $MgCl_2$ $(H_2O)_6$ (0.15 M, 1.1 mL), $(NH_4)_2CO_3$ (0.5 M, 0.5 mL), and KCl (0.5 M, 6.8 mL) were blended and diluted to 400 mL with water.

2.7.2. Simulated digestion program

The emulsion went through simulated oral digestion, simulated gastric digestion, and simulated intestinal digestion in turn.

For simulated oral digestion, 5.0 mL of emulsion, 3.5 mL of SSF, 0.5 mL of α-amylase solution (1500 U/mL), 25 μL of $CaCl_2$ (0.3 M), and 975 μL of ultrapure water were fully mixed in a 150 mL conical bottle. The mixture was then quickly adjusted to pH 7.0 and oscillated in a water bath at 37 °C for 2 min.

For simulated gastric digestion, 10.0 mL of the oral digestion samples, 7.5 mL of SGF, 1.6 mL of porcine pepsin (25000 U/mL, mucin

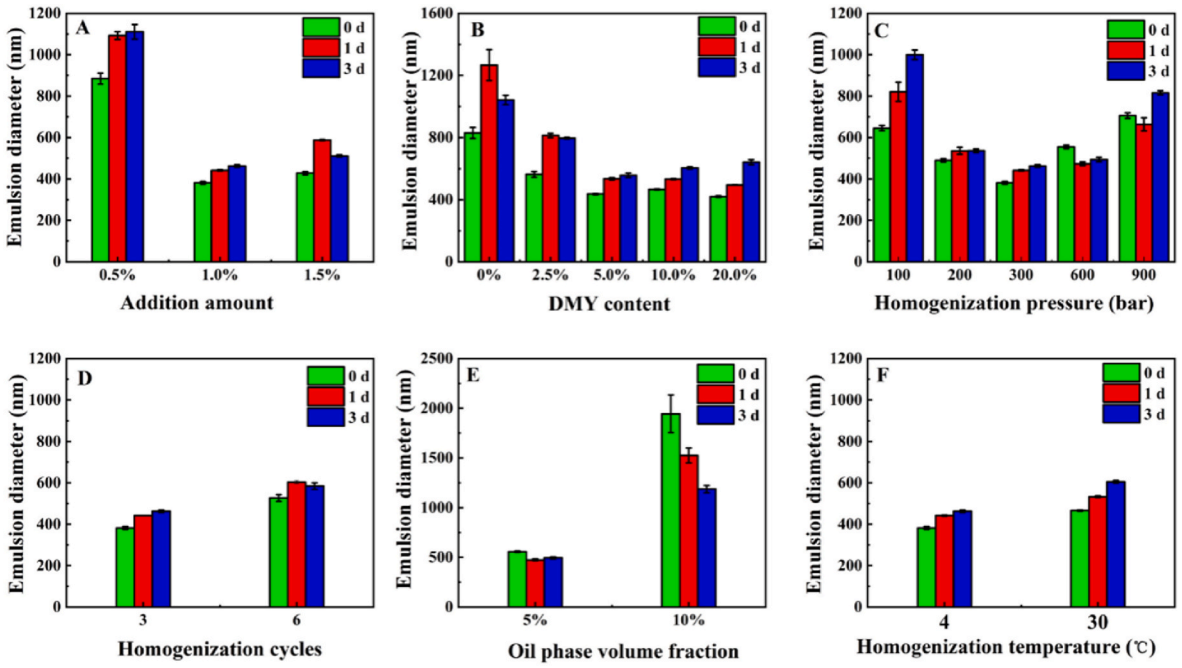

Fig. 1. Effect of addition amount (w), DMY content (c), homogenization pressure (p), the number of homogenization cycles (n), oil phase volume fraction (&phiv) and homogenization temperature (T) on the median diameter of the emulsion (A: samples developed at $w = 0.5\%$, 1.0%, 1.5%, $c = 10\%$, $p = 300$ bar, $n = 3$, $\varphi = 5\%$, and $T = 4$ °C; B: samples developed at $c = 0$–20%, $w = 1.0\%$, $p = 300$ bar, $n = 3$, $\varphi = 5\%$, and $T = 4$ °C; C: samples developed at $p = 100$–900 bar, $w = 1.0\%$, $c = 10\%$, $n = 3$, $\varphi = 5\%$, and $T = 4$ °C; D: samples developed at $n = 3$ and 6, $c = 10\%$, $w = 1.0\%$, $p = 300$ bar, $\varphi = 5\%$, and $T = 4$ °C; E: samples developed at $\varphi = 5\%$ and 10%, $w = 1.0\%$, $c = 10\%$, $n = 3$, $p = 600$ bar, $\varphi = 5\%$ and $T = 30$ °C; and F: samples developed at $T = 4$ °C and 30 °C, $w = 0.5\%$, 1.0%, 1.5%, $c = 10\%$, $p = 300$ bar, $n = 3$, and $\varphi = 5\%$).

concentration: 1.5 mg/mL), 5.0 μL of $CaCl_2$ (0.3 M), 0.2 mL of HCl (1.0 M), and 0.695 μL of ultrapure water were mixed. Then, the mixture was quickly adjusted to pH 2.0 and oscillated in a water bath at 37 °C for 2 h.

For simulated intestinal digestion, 20.0 mL of the stomach digestion sample, 11.0 mL of SIF, 5.0 mL of trypsin solution (200 U/mL), 2.5 mL of bile salt (0.684 mg/mL), 40 μL of $CaCl_2$ (0.3 M), and 1.31 mL of ultrapure water were mixed. The mixture was then quickly adjusted to pH 7.0 and oscillated in a water bath at 37 °C for 2 h.

2.7.3. Evaluation of β-carotene bioaccessibility and glucose concentration

The instability of β-carotene leads to its degradation and loss of biological activity during prolonged digestion, making it difficult to be absorbed and used effectively by the intestine. The bioaccessibility of β-carotene is considered to be the fraction of the food matrix or supplement that is added to the mixed micelles and readily absorbed in the intestinal tract (Li et al., 2020). The obtained intestinal digested sample was centrifuged. Then, 5.0 mL of the supernatant was added to 30.0 mL of ethanol-n-hexane mixed solvent (v/v = 2:3). After stratification, the β-carotene content was determined according to the methods described in section *2.6*. The bioaccessibility of β-carotene was calculated based on the following equation (Li et al., 2020):

$$\beta - \text{Carotene bioaccessility} = \frac{C_s}{C_0} \times 100\%$$

where C_0 and C_s denote the initial and reserved β-carotene amounts, respectively.

The glucose concentration of the intestinal digestion sample was measured by using a glucose kit based on the glucose oxidase method.

2.8. Statistical analysis

The tests were conducted in triplicate, and the results were expressed as mean ± SD. The statistical comparison was based on Duncan's test with a confidence level of 95%.

3. Results and discussion

3.1. Formation of nano-emulsions

Nano-emulsions are mainly prepared by the high-energy method or low-energy method (Komaiko and McClements, 2016). The high-energy method mainly uses high-pressure homogenizers, micro-fluidizers, and ultrasonic instruments to generate extra energy to change the size of the particles and emulsions (Wang et al., 2021). Unlike the high-energy method, the spontaneous or low-energy emulsifying method has little demand for external energy, with the energy needed for the emulsifying process coming from the internal energy of the unbalanced mixture system. In this study, high-speed shearing and high-pressure homogenization were used to systematically investigate the effects of particle characteristics (c), emulsion composition (w and φ), and the homogenization conditions (p, n, and T) on the median diameter of the emulsion droplet.

In Fig. 1A, the droplet size of the emulsion developed at $w = 0.5\%$ was obviously higher than the emulsions developed at $w = 1.0\%$ and 1.5%. This was because the lower the droplet size of the emulsion, the larger the oil-water interface and the higher the required amount of emulsifying particles. However, too many particles would cause flocculation or sedimentation, especially because the starch particles had a certain viscosity. In this study, the emulsion obtained at $w = 1.0\%$ had the smallest droplet size, which was below 500 nm during the 3-day storage period; therefore, it was a nano-emulsion. As shown in Fig. 1B, when $c = 0\%$, HCS had some emulsifying performance, but the droplet size of the obtained emulsion was in the submicron range, making it unstable and causing the droplet size to vary greatly during the storage process. However, the composite particles with $c = 5$–20% could stabilize the nano-emulsions with a median droplet diameter of approximately 350 nm and remain stable for 3 days, which fully confirmed that DMY could improve the emulsifying capacity of HCS. As p increased, the emulsion droplet size initially decreased and then increased (Fig. 1C),

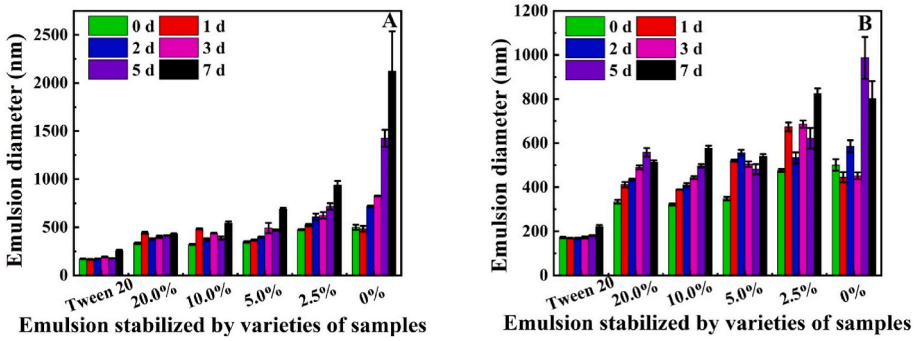

Fig. 2. Effect of DMY content in the composite particles on the droplet size of the emulsion at 4 °C (A) and 30 °C (B) with the emulsion stabilized by Tween 20 as the control (w = 1.0%, p = 300 bar, n = 3, φ = 5%, and T = 4 °C).

because an appropriate increase in p facilitated the dispersion of the oil droplets, while excessive pressure would destroy the emulsion structure (McClements and Jafari, 2018). When p was 300 bar, the droplet size showed little increase within 3 days, all on the nanometer level. This indicated that this pressure value was appropriate. Thompson et al. also found that the droplet size was larger with low treatment pressure while increasing the treatment pressure reduced the droplet size, and when the treatment pressure was increased to a certain pressure, there was little change in the droplet size (Thompson et al., 2017). Fig. 1D shows that n also had a significant effect on droplet size, and with an excessive number of homogenization cycles, the droplet size of the emulsion increased significantly. Due to the matching effect between p and n, inappropriate combinations could lead to over-processing, resulting in re-coalescence, which increased the droplet size and polymer dispersion index (Páez-Hernández et al., 2019; Santos et al., 2019). The increase in φ led to an increase in droplet size. Thus, the droplet size of the emulsion with φ = 10% did not reach the nanoscale, and this emulsion was unstable during storage (Fig. 1E). Dieng et al. (2020) also found that the droplet size of the emulsion was proportional to φ (10–90%) when using n-dodecane as the oil phase for high-pressure homogenization. A high homogenization temperature could cause an increase in the volume of the oil droplets; thus, the droplet size of the emulsion obtained at 30 °C was superior to the one obtained at 4 °C (Fig. 1F).

In summary, w, c, φ, p, n, and T could all affect the formation and structure of the emulsion. The presence of DMY (c ≥ 5%) could significantly enhance the emulsifying property of HCS. Under suitable conditions (w = 1%, φ = 5%, p = 300 bar, n = 3, and T = 4 °C), stable nano-emulsions could be obtained. Some studies have shown that the diameter of the nanoparticles required to stabilize Pickering nano-emulsions was about 5–10 times smaller than the droplet diameter, with speculation that the composite particle size could be about 30–100 nm (The droplet diameter is about 300–500 nm) (Thompson et al., 2017). Due to their smaller size, the nanoparticles had a greater ability to penetrate the intercellular spaces of tissues and organs. According to the Ostwald-Freundlich equation, when the particle size of a drug was reduced to nanometer size, the saturated solubility of the drug could be enhanced (Liu et al., 2020). Therefore, the obtained nano-emulsion could potentially improve the uptake and utilization of DMY by organisms.

3.2. Stability of nano-emulsions

Based on section *3.1*, the effect of DMY content in the composite particles on the droplet size of the emulsion stored at 4 °C and 30 °C was investigated (Fig. 2). During the 7-day storage period, the emulsion prepared at c = 0% was the most unstable and the droplet size changed the most, followed by the emulsion prepared at c = 2.5%. These could encounter flocculation or gravity sedimentation, resulting in rapid instability (Dieng et al., 2020). The emulsifying capacity of HCS itself was not sufficient to maintain the Pickering emulsion, and only when it was combined with a suitable amount of DMY (c ≥ 5%) could stable nano-emulsions form. The droplet curvatures in the emulsions stabilized by low-molecular-weight emulsifiers (e.g., Tween, Span, and lecithin) were greater (less steric hindrance), and their droplet sizes were smaller than those of emulsions constructed by natural polymer emulsifiers (e.g., caseinate and β-lactoglobulin) (Bouyer et al., 2012; Karbstein and Schubert, 1995). Solid particles were not as capable of reducing surface tension as the surfactants; thus, the droplet size of the Pickering emulsions was higher than that of the emulsions stabilized by surfactants (Vignati et al., 2003). In this study, the droplet size of the emulsion with Tween 20 was the smallest and remained stable within 7 days. When c = 5.0–20.0%, the emulsions stabilized by the composite particles also

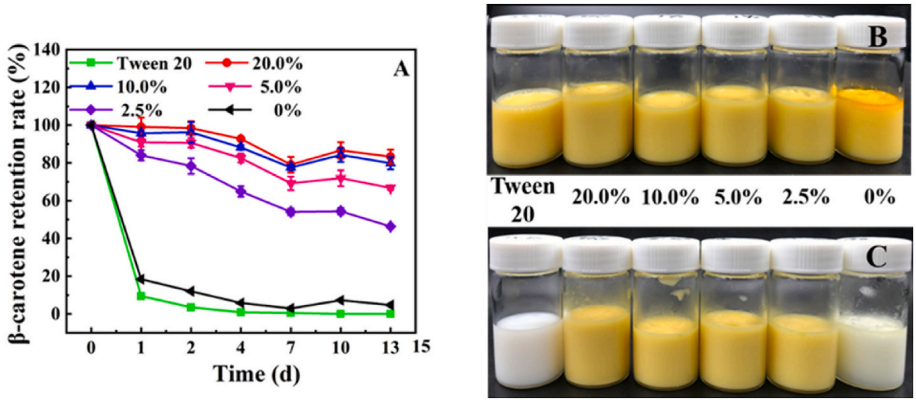

Fig. 3. Effect of DMY content in the composite particles on the production of peroxides in the emulsion at 50 °C with the emulsion stabilized by Tween 20 as the control (w = 1.0%, p = 300 bar, n = 3, φ = 5%, and T = 4 °C).

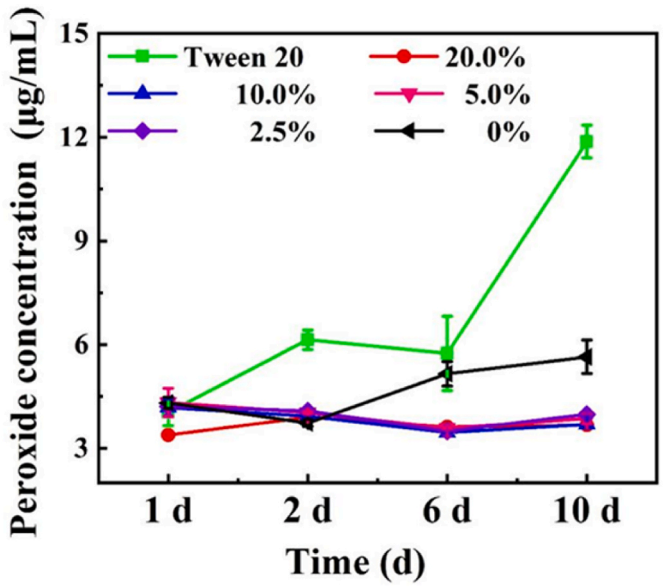

Fig. 4. Effect of DMY content in the composite particle (c = 0–20%) on the β-carotene retention rate (A) and appearance (B: 0 d; C: 6 d) of the emulsions with the emulsion stabilized by Tween 20 as the control (w = 1.0%, p = 300 bar, n = 3, φ = 5%, and T = 4 °C).

exhibited good stability at 4 °C and 30 °C. The previous report confirmed that nano-emulsions had more difficulty coalescing than traditional emulsions (Sheth et al., 2020). This was possibly because hydrodynamic lubrication caused by film drainage, which was caused by two small droplets close to each other, was not sufficient to deform the nano-droplets and increase the interfacial areas in contact. Coalescence of nano-emulsions occurred much more slowly than traditional emulsions (Baldessari and Leal, 2006).

3.3. Antioxidant activity of nano-emulsions

The specific surface area of the nano-emulsion was enormous, and the lipids in the oil droplets were in full contact with the oxidants at the oil-water interface, which could be easily oxidized. To determine whether the composite particles could delay the oxidation of the lipids, the effect of DMY content in the composite particles on the peroxide content of the emulsion stored at 50 °C was investigated. It is generally accepted that 1 day of storage in a 60 °C oven is equivalent to 1 month of conventional storage (Kochhar, 1993). To effectively monitor the ability of the nano-emulsion to protect the oil and prevent the emulsion from rapid delamination due to heating, the storage temperature was set to 50 °C in this study. As shown in Fig. 3, although Tween 20 could stabilize the nano-emulsion, the obtained emulsion was easier to delaminate and the peroxide content increased most rapidly during heating. When HCS (c = 0%) was used as the emulsifier, the peroxide content of the nano-emulsion increased gradually with the progression storage; however, its increase was much lower than the sample with Tween 20. This was possibly due to the shell-like structure formed by the starch particles outside the oil droplets, which could effectively block contact between the oxidizer and the oil. However, the peroxide content of the nano-emulsion stabilized by the composite particles (c = 2.5–20%) did not increase significantly during 10 days of storage, and the DMY content of the composite particles had no difference in the antioxidant activity of the emulsion. This was possibly because DMY had a strong antioxidant capacity, and when its concentration exceeded a certain threshold, the antioxidant effect remained stable for a time (Geng et al., 2016; Liu et al., 2019). Similarly, Hu et al. (2022) demonstrated that mesoporous silica nanoparticles loaded with EGCG exhibited a dual stabilization effect on Pickering emulsion. Lipid oxidation is one of the main factors affecting the shelf life of foods, and oxidative derivatives usually have a negative impact on the appearance, flavor and nutritional value of foods. Emulsions with excellent antioxidant properties have an important advantage when transporting unstable nutraceuticals.

3.4. β-Carotene protective capacity of nano-emulsions

β-Carotene, as one of the carotenoids, is an orange fat-soluble compound and one of the most common vitamin A supplements that can significantly improve human vision. However, it is unstable under oxygen, heat, and light, limiting its use in food. In this study, the effect of DMY content in the composite particles on the β-carotene protective capacity of nano-emulsions was evaluated (Fig. 4A). Emulsions stabilized by Tween 20 and HCS (c = 0%) showed the smallest protection effect on β-carotene under UV irradiation. β-Carotene floated soon after the preparation of the emulsion stabilized by HCS (Fig. 4B), which meant that HCS failed to form a rigid film and effectively encapsulate β-carotene. Under UV irradiation for 1 day, only about 20% of the β-carotene remained in the emulsion stabilized by Tween 20, and about 10% of the β-carotene remained in the emulsion developed by HCS. On the 6th day, the above emulsions were almost bleached (Fig. 4C). The nano-emulsions based on the DMY/HCS composite particles exhibited an outstanding protective effect of β-carotene, and with the increase in c, the β-carotene retention rate increased gradually; however, the protective effect of the emulsions developed at c = 10% and 20% was similar. On the 13th day, the β-carotene retention rate was about 90%. Our results coincided with a previous report, which showed that the β-carotene retention rate of a Pickering emulsion stabilized by a chitosan hydrochloride-carboxymethyl starch (CHC-CMS) nanogel was superior to a Tween 80-stabilized traditional emulsion and raw oil (Li et al., 2020). The protection mechanism of the composite particles could be attributed to the following points. (1) DMY has antioxidant and UV-absorbing abilities, which can remove oxidants in water and absorb UV radiation (Liu et al., 2019). (2) The composite particles are arranged to form a physical barrier at the oil-water interface, which can greatly reduce the contact of β-carotene with the oxidants and UV, and effectively prevent β-carotene from oxidization or decomposition (Zhou et al., 2018). In this study, the rigid network formed by the composite particles was less likely to pass through UV than Tween 20, thus protecting the β-carotene of the nano-emulsion from irradiation. We observed that the smaller the droplet diameter, the more stable the droplet, and the more pronounced the β-carotene protection. It should be noted that the type of oil used in emulsion construction and the introduction of bioactive components possibly significantly affected the microstructure and physicochemical properties of the Pickering emulsion. It has been reported that the stability of a corn oil-based Pickering emulsion was generally inferior to that of an MCT-based Pickering emulsion, and the addition of β-carotene had a negative effect on the formation and stability of the corn oil-based Pickering emulsion (Tan et al., 2021).

3.5. In vitro simulated digestion of nano-emulsions

Nano-emulsions have been widely used to encapsulate low water-soluble nutraceuticals to improve their bioavailability, with emulsifiers playing an important role in the construction of stable nano-emulsions. Complex physical, chemical, and biological changes can take place in the process of gastrointestinal digestion, resulting in varying degrees of structural changes, thus affecting the biological functions of nano-emulsions. The dietary intake of β-carotene can effectively regulate vitamin A deficiency and night blindness. Numerous studies have shown that β-carotene had positive effects on embryonic development, immunity improvement, and the prevention and treatment of cardiovascular diseases (Donhowe and Kong, 2014). However, directly ingested β-carotene will be susceptible to the gastrointestinal environment, leading to degradation and a great reduction in its

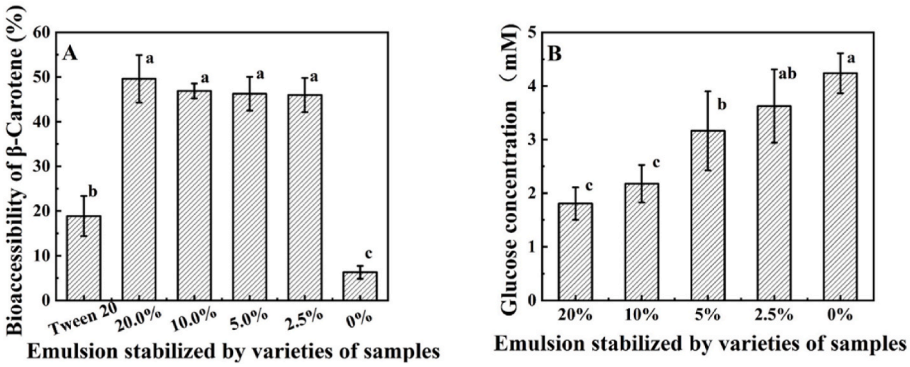

Fig. 5. *In vitro* simulated digestion results of the β-carotene-loaded emulsions developed by the composite particles (c = 0–20%) and Tween 20 (A: bioavailability of β-carotene; B: glucose concentration).

biological activity. Foods containing nano-emulsions can effectively improve their metabolism, absorption, and bioavailability in the body. The *in vitro* simulation of gastrointestinal digestion has been widely used in many areas of nutritional science, as *in vivo* experiments are often expensive and resource intensive. Minekus et al. proposed a generic, standardized, and practical method for static digestion, including a series of parameters for oral, gastric, and small intestinal digestion. Based on physiological conditions, this method could be applied to a variety of situations and modified to meet further specific needs (Minekus et al., 2014; Perez-Burillo et al., 2021). It has also been used in the simulated digestion of Pickering emulsions (Li et al., 2020). In this study, the results of *in vitro* simulated digestion of β-carotene-loaded nano-emulsions stabilized by the composite particles (c = 0–20%) and Tween 20 were investigated (Fig. 5). The nano-emulsion stabilized by the composite particles had higher β-carotene bioaccessibility that those stabilized by Tween 20 and HCS (Fig. 5A). The nano-emulsions stabilized by the composite particles allowed for the more efficient transport of β-carotene. Although the composite particles with c = 2.5% could not be built as a stable nano-emulsion, due to the protective effect of DMY on β-carotene, β-carotene could still reach the intestine at a high percentage. The high permeability of nano-emulsions was possibly more favorable for the absorption of β-carotene, and the release process was more controllable. DMY could inhibit the activity of digestive enzymes and reduce the degree of emulsion destruction, as well as inhibit oxidation and other unfavorable reactions during digestion, thus increasing the transport of β-carotene. In addition, Pickering nano-emulsions have been reported to be highly resistant to pH in gastric juices, reducing the early release of drugs (Dieng et al., 2020). In a study on chitosan hydrochloride/carboxymethyl starch (CHC-CMS) nanogels as stabilizers, the bioaccessibility of β-carotene in the Pickering emulsion was higher than that of β-carotene in crude oil (Li et al., 2020). In previous studies, our group found that DMY significantly inhibited α-glucosidase, which could be used as a new hypoglycemic nutraceutical (Geng et al., 2016). Inspired by this, we investigated the hydrolysis of HCS in simulated digestion. As shown in Fig. 5B, with an increase in DMY content in the composite particles, the glucose content in the simulated intestinal digestive fluid declined significantly, indicating that the composite particles not only possessed a Pickering emulsifying capacity, but also lowered blood glucose.

4. Conclusions

Under homogenization conditions (p = 300 bar, n = 3, and T = 4 °C), the DMY/HCS composite particles with c = 5–20% could construct a stable Pickering nano-emulsion with φ = 5% with an appropriate addition amount (w ≥ 1%). The oil phase of the obtained nano-emulsion had good stability in the process of high-temperature storage, and its protective effect on β-carotene from UV irradiation was superior to the nano-emulsion stabilized with Tween 20. The *in vitro* simulated digestion test also confirmed that the nano-emulsion stabilized by the composite particles could significantly enhance the bioaccessibility of β-carotene and retard the hydrolysis of starch. Our results indicate that the Pickering nano-emulsion prepared by the DMY/HCS composite particles has good carrier properties and can be used in functional food.

CRediT authorship contribution statement

Sheng Geng: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, preparation, Writing – review & editing, Visualization, Funding acquisition. **Yuan Yuan:** Methodology, Investigation, Data curation, Visualization. **Xinying Jiang:** Methodology, Data curation, Visualization. **Ruhua Zhang:** Validation, Formal analysis. **Hanjun Ma:** Validation, Resources, Funding acquisition. **Guizhao Liang:** Software, Validation, Formal analysis, Supervision. **Benguo Liu:** Conceptualization, Methodology, Software, Investigation, Resources, Writing – original draft, preparation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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