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Fabrication and characterization of Pickering high internal phase emulsion stabilized by mung bean flour

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Abstract

Pickering high internal phase emulsion (HIPE) has a broad application prospect in the field of solid fat substitution and nutraceutical delivery, but the safe and available food-derived particle emulsifier is the bottleneck of its practical application. In this study, mung bean flour was used as a particle emulsifier to stabilize oil-in-water Pickering HIPEs. The results showed that the particle size of mung bean flour was suitable (median diameter, 26.41 μm) and had certain wettability. The volume fraction of oil phase (φ) and the concentration of mung bean flour (*c*) had significant effects on the formation of Pickering HIPEs. When $c \geq 1\%$, the Pickering HIPE of $\varphi = 80\%$ could be obtained. Mung bean flour not only played an emulsifying role at the oil/water interface, but also prevented the coalescence of oil droplets through its viscosity effect in the aqueous phase. With the increase of *c*, the droplet size of Pickering HIPEs decreased, the mechanical properties increased gradually, and the ability of HIPEs protecting β-carotene against ultraviolet irradiation was also enhanced.

Keywords: mung bean; Pickering emulsion; high internal phase emulsion; characterization; mechanical properties.

Practical Application: This work systematically studies preparation and characterization of Pickering HIPEs stabilized by mung bean flour. The results can not only provide a reference for the deep processing of mung bean, but also promote the green and efficient preparation of food grade Pickering HIPEs.

1 Introduction

Mung bean (*VignaradiateL.Wilczek*) is an annual herb of cowpea in Leguminosae, which is widely planted in Northeast Asia, Southeast Asia, South Asia, Middle East and other regions. It has the advantages of short growth period and strong stress resistance. In China, mung beans are widely used to make traditional foods such as cakes, sprouts, porridge, vermicelli, vermicelli and so on (Ganesan & Xu, 2018; Zheng et al., 2022). Mung bean starch rich in amylose is also used in the preparation of resistant starch and edible film (Lee et al., 2020). However, compared with wheat, corn and other major crops, the degree of development and utilization of mung bean is still not high, and its new food application needs to be carried out urgently.

Pickering emulsion is an emulsion stabilized by solid particles instead of surfactants. Compared with the traditional emulsion, Pickering emulsion has the lower cost, not easily affected by environmental factors such as pH value, salt ion concentration and temperature (Liu et al., 2021). Pickering emulsions with internal phase volume fraction exceeding 74% are called Pickering high internal phase emulsions (HIPEs) (Geng et al., 2022). Pickering HIPEs possess the properties similar to soft solids, have certain texture and rheological properties, high anti-condensation and Ostwald maturity, and can be used in the fields of solid fat substitution and nutraceutical delivery (Geng et al., 2021). In recent years, there are many studies on the preparation of Pickering HIPEs using inorganic particles or organic polymers as particle emulsifiers, but these particles have some shortcomings such as low biocompatibility and poor degradability, which limit their application in the food field, so food-derived particle emulsifiers have gradually become a research hotspot (Rodriguez & Binks, 2022). At present, the related research is mainly focused on protein, starch and other biopolymers. However, these natural polymers have strong hydrophilicity, in order to improve their emulsifying effect, they need acidolysis, esterification, complexation and other pre-treatment (Liu et al., 2022; Wang et al., 2022), which bring some problems, such as tedious processing, environmental pollution,potential toxicity and so on.

Cereal is an important source of human food, which is a natural complex composed of starch, protein and other components (Brasil et al., 2021; Brites et al., 2019), and is a potential Pickering emulsifier. Lu et al. (2022) found that red rice and black rice particles could not only stabilize Pickering emulsion, but also significantly improve the antioxidant properties of emulsions (Lu & Huang, 2020; Lu et al., 2022). Song et al. (2022) found that sorghum flour could stabilize the oil-in-water (O/W) Pickering emulsion gels with medium chain triglycerides (MCT) as the oil phase (Song et al., 2022). Inspired by this, the feasibility of mung bean flour stabilizing Pickering HIPEs was evaluated, and the effect of its concentration on the structure, mechanical properties and β-carotene protective ability of Pickering HIPEs were also investigated.

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2 Materials and methods

2.1 Preparation and composition determination of mung bean flour

Mung beans were crushed with a high-speed multi-function grinder. The contents of moisture, protein, starch, fat and ash in mung bean flour were determined according to GB 5009.3-2016, GB 5009.5-2016, GB/T 20194-2018, GB 5009.6-2016 and GB 5009.4-2016 (Zhang et al., 2021), respectively.

2.2 Determination of appearance and size of mung bean flour

The mung bean flour was glued to the test platform with double-sided tape, and then coated with gold film in vacuum. Under the accelerating voltage of 20 kV, the appearance of mung bean flour was observed by h an FEI Quanta 200 environmental scanning electron microscope (Hillsboro, OH, USA). At the same time, using distilled water as dispersion medium, the particle size distribution of mung bean flour was determined by a BETTER BT-9300H laser particle analyzer (Dandong, China).

2.3 Determination of interfacial tension of mung bean flour

The effect of mung bean flour on the interfacial tension of MCT/water was measured by a Theta Lite optical contact angle meter (Biolin Scientifific, Stockholm, Sweden). After adding excess mung bean flour to distilled water, the mixture was treated with ultrasound for 10 min, then stood still for 15 min. The supernatant was dropped into a colorimetric dish containing MCT by syringe to form the oil/water interface. Axisymmetric droplet shape analysis was used to calculate the interfacial tension with distilled water as control (Song et al., 2022).

2.4 Determination of contact angle of mung bean flour

The contact angle of mung bean flour was measured by a Theta Lite optical contact angle meter (Biolin Scientifific, Stockholm, Sweden). Using a tablet press, mung bean flour was pressed into a thin film under 20 MPa and placed in a colorimetric dish containing MCT, then a drop of water was extruded from the syringe and released onto the film surface, the drop image was automatically recorded, and the contact angle was calculated using the ellipse fitting method (Song et al., 2022).

2.5 Preparation method of Pickering emulsion

A certain amount of mung bean flour was dispersed in water as aqueous phase, MCT as oil phase, mixed at room temperature, 12000 r/min high speed shearing for 3 min, and Pickering emulsions with oil phase volume fraction (φ) of 50, 60, 70, 80, 90% (v/w) and emulsifier concentration (*c*) of 1.0, 3.0, 5.0, 7.0, 9.0% (w/v) were prepared. The gel formation was investigated by inverted bottle method for 24 h.

2.6 Microscopic determination of Pickering HIPEs

The Pickering HIPEs with φ = 80% and *c* = 5.0, 7.0, 9.0% were prepared. 20 μ L of the emulsion was placed on a glass slide, observed using a BH200P polarizing microscope (Shanghai Sunny Hengping Scientifific Instrument Co., Ltd., Shanghai, China). At the same time, with distilled water as dispersion medium, the particle size distribution of droplets was determined by a BETTER BT-9300H laser particle analyzer (Dandong, China).

2.7 Determination of gel strength of Pickering HIPEs

The Pickering HIPEs with φ = 80% and *c* = 5.0, 7.0, 9.0% were prepared, and their gel strength values were determined by using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, UK) based on GMIA Gelation mode. The probe was P/0.5, the pre-test speed was 1.5 mm/s, the mid-test speed was 1.0 mm/s, the post-test speed was 1.0 mm/s, and the trigger force was 2.0 g.

2.8 Determination of microrheological properties of Pickering HIPEs

The Pickering HIPEs with φ = 80% and *c* = 5.0, 7.0, 9.0% were prepared, and their microrheological behavior was determined using a Rheolaser LAB6 microrheometer (Formulation, France). The emulsion of 20 mL was added to the 25 mL test bottle. The test bottle was placed in the chamber of the instrument and monitored by CCD detector at 25 °C for 6 h. The data were recorded and processed by Rheosoft Master 1.4.0.0 software, and the results were expressed as elasticity index (EI) and macroscopic viscosity index (MVI) (Li et al., 2022).

2.9 Determination of the protective effect of Pickering HIPEs on β-carotene

The MCT containing 2.0 mg/mL β-carotene was used as the oil phase, the Pickering HIPEs with φ = 80% and *c* = 5.0, 7.0, 9.0% were prepared according to section *2.5*. The samples were placed under a ultraviolet lamp (Power, 6 W) at 30 °C. Every 24 h, 1 mL of the sample solution was mixed with 10 mL ethanol/n-hexane mixture (2:1, v/v), and centrifuge at 1000 rpm for 10 min. The absorbance of the supernatant at 450 nm was read, and the retention rate of β-carotene was calculated. MCT with the same β-carotene content was used as control (Li et al., 2022).

3 Results and discussion

3.1 Composition analysis of mung bean flour

Table 1 shows the chemical composition of mung bean flour. Although the starch content of mung bean was lower than that of rice and wheat, its protein content was as high as 22.79%, which was significantly superior to that of rice flour and wheat flour. The protein contains many hydrophilic or hydrophobic amino acid residues, which can be adsorbed on the oil-water interface to reduce the oil-water interfacial tension and play the role of emulsification, and the charged groups such as-NH^{3+},-COO^{-} can prevent droplets from gathering and improve the stability of the emulsion through electrostatic exclusion (Kim et al., 2020). In

Table 1. Chemical composition of mung bean flour.

Water (%)	Protein (%)	Starch (%)	Lipid (%)	Ash (%)
8.62 ± 0.03	22.79 ± 0.23	54.72 ± 0.53	0.84 ± 0.07	2.78 ± 0.06

addition, protein can not only be used as emulsifier alone, but also form composite colloidal particles with biological macromolecules such as starch, which can be used as Pickering emulsified particles (Kierulf et al., 2020). Therefore, mung bean flour had potential Pickering emulsifying ability because of its high protein content.

3.2 Particle appearance and size distribution of mung bean flour

The formation of Pickering emulsion is affected by many factors, such as the shape, size, wettability, concentration of solid particles, pH value and ionic strength of aqueous phase, properties and volume fraction of oil phase, etc. The particle size of emulsifier used to stabilize Pickering emulsion should be nanometer or micron. Therefore, we investigated the appearance and size distribution of mung bean flour particles. As shown in Figure 1A, the shape of mung bean flour particles was mostly ellipsoidal, with a small number of irregular small particles adhering to the surface; particle size analysis showed that the particle size is micron, and the median diameter is 26.41 μm (Figure 1B). The droplet size of starch-based Pickering emulsion gel was generally 1-100 μm, so the size of mung bean flour met the requirements and had the potential to stabilize Pickering emulsion.

3.3 Wettability of mung bean flour

Whether the particles can play Pickering emulsifying role is closely related to the wettability of the particles. The wettability allows the particles to gather spontaneously at the oil-water interface and stabilize the aggregation through volume exclusion and spatial hindrance. The wettability of particles can be judged by their ability reducing oil/water interfacial tension and contact angle. In Figure 2, compared with the control (25.43 ± 0.41 mN/m), mung bean flour could significantly reduce the interfacial tension of MCT/water (12.61 ± 0.25 mN/m). Although the main component of mung bean flour was starch, it still contained a certain amount of natural emulsifier-protein and lipid, which led to the reduction of oil/water interfacial tension. Previous studies have also shown that starch/protein composite or starch/lipid composite have the ability to emulsify Pickering (Marefati et al., 2018). When used as Pickering emulsifier, solid particles should meet the following requirements: I) particles should be partially wetted by continuous phase and dispersed phase, but should not be dissolved in either phase; II) particles should maintain appropriate partial wettability to obtain sufficient interfacial absorption efficiency. The three-phase contact angle can reflect the partial wetting effect of solid particles in oil and water phases. In this study, it was found that the contact angle of mung bean flour was 93.77°, close to the optimum contact angle of spherical particles (90°), indicating that mung bean flour had better wettability and could be used in the construction of Pickering emulsion.

3.4 Formation of Pickering HIPEs

In order to evaluate the feasibility of constructing Pickering HIPEs with mung bean flour, the effects of the amount of mung bean flour (*c*, 1-9%) and the volume fraction of oil phase (φ, 50-90%) on the formation of HIPEs were investigated. As shown in

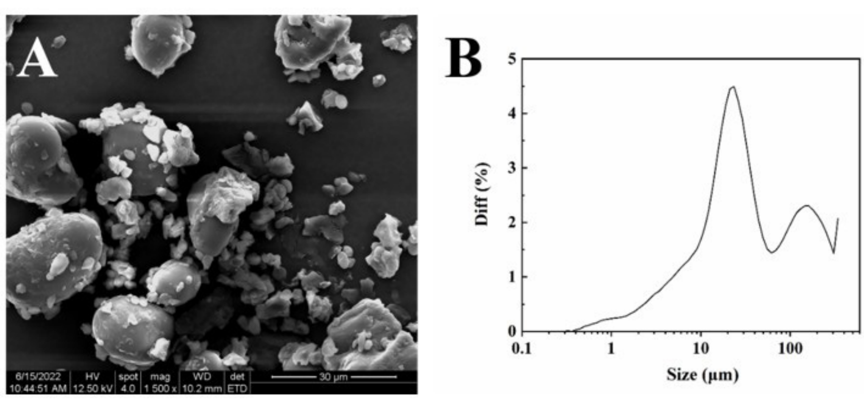

Figure 1. SEM image (A) and size distribution (B) of mung bean flour.

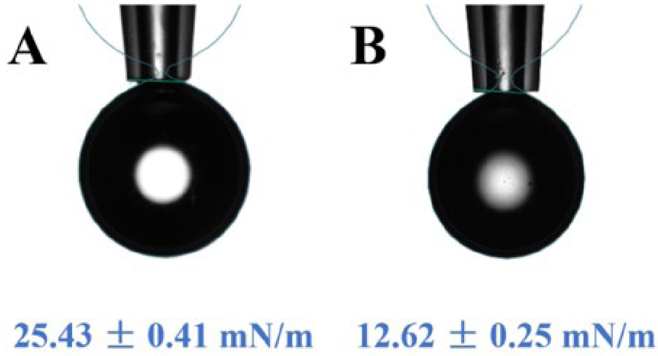

Figure 2. The MCT/water interfacial tension of mung bean flour (A: control; B: mung bean flour).

Figure 3, when φ = 50% and 90%, the Pickering emulsion was not stable in the *c* range of the experiment; when φ = 60 and 70%, the Pickering emulsion was stable at *c* = 7 and 9%; when φ = 80%, stable Pickering emulsion gel could be obtained in the experimental range, because the volume fraction of oil phase was more than 74%, it indicated that mung bean flour could stabilize Pickering HIPEs. In order to reveal the mechanism of mung bean flour stabilizing Pickering emulsion gel, the stable Pickering HIPEs of mung bean flour at φ = 80% c = 5, 7 and 9% were selected for subsequent study of microstructure, mechanical properties and β-carotene protective ability.

3.5 Microscopic analysis of Pickering HIPEs

A series of mung bean flour stabilized Pickering HIPEs (*c* = 5, 7 and 9%, φ = 70%) could be dispersed in water, but not in MCT, indicating that it is an O/W emulsion. Figure 4 shows the effect of the amount of mung bean flour (*c*) on the droplet size of the emulsion. With the increase of *c*, the emulsion droplet size decreased significantly, and Yan et al. (2019) also found that the emulsion droplet size decreased with the increase of emulsifier concentration. When the particle emulsifier concentration is low, the coverage of emulsifier on the surface of oil droplets is not enough to prevent droplets from merging, so the size of oil droplets is larger, and the coverage of emulsifier increases with the further increase of the amount of emulsifier. A dense interface particle layer can be formed outside the droplets, thus inhibiting the further merging of the droplets, so that the size of the oil droplets is smaller.

3.6 Gel strength analysis of Pickering HIPEs

The strength of emulsion gel not only reflects its structural tightness, but also is closely related to its processing properties and application fields. in this study, the effect of the amount of mung bean flour on the gel strength was investigated. When the concentration of mung bean flour was 5%, 7% and 9%, the gel strength values of Pickering HIPEs were 3.71 ± 0.21 g, 5.42 ± 0.16 g and 6.83 ± 0.10 g respectively. The gel strength was positively correlated with the concentration of mung bean flour, which is due to the increase of emulsifier concentration, the smaller emulsion droplets, and the closer accumulation of emulsion droplets, so the gel structure is more stable. Geng et al. (2021) found a similar phenomenon when studying dihydromyricetin stabilized Pickering emulsion gel (Geng et al., 2021).

3.7 Microrheological analysis of Pickering HIPEs

Microrheology is a viscoelastic measurement method based on diffusion spectrum, which can reflect the rheological behavior of samples without mechanical shear. In the process of measurement, the CCD probe tracks the Brownian motion of the particles in the solution and obtains the rheological parameters of the system. The EI and MVI indices obtained from microrheological analysis correspond to G' and G" in traditional mechanical rheology, respectively, which can reflect the elasticity and viscosity of the gel. Figure 5 demonstrates the effect of the amount of *c* on EI and MVI of Pickering HIPEs. In the process of determination, the EI and MVI values of all samples

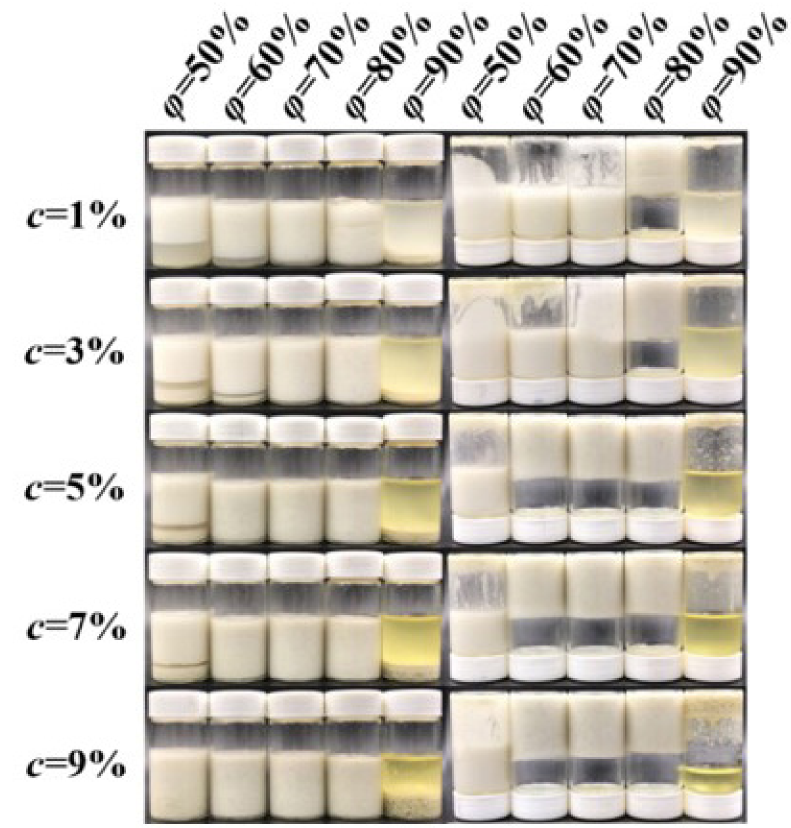

Figure 3. Formation of Pickering emulsions stabilized by mung bean flour.

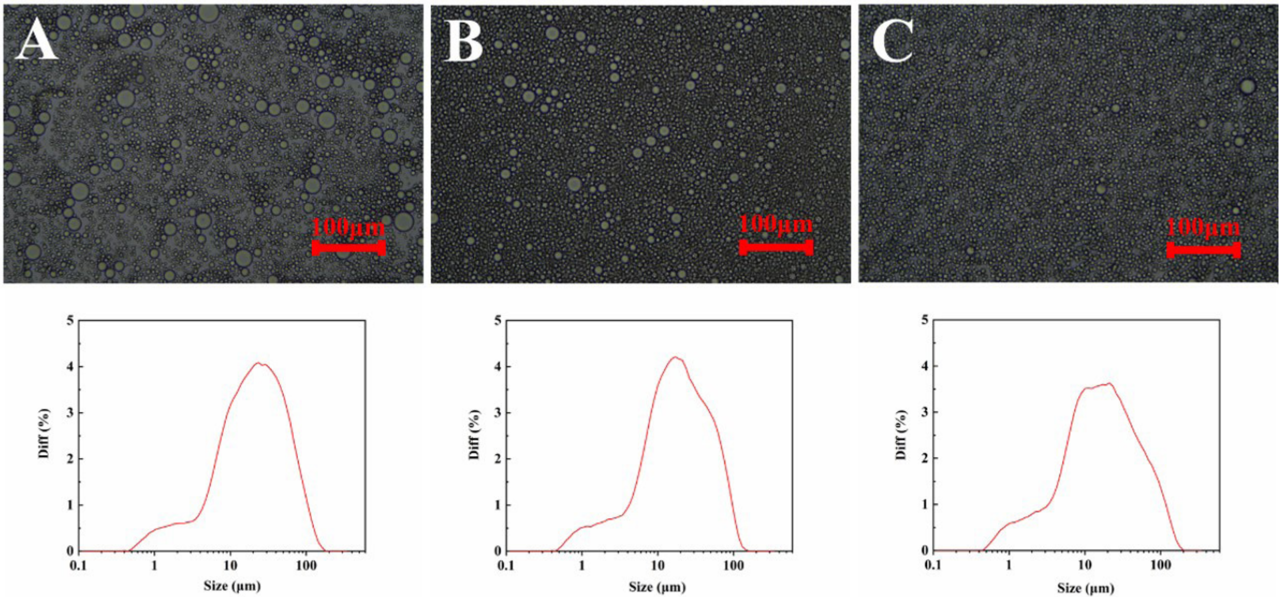

Figure 4. Microscopic observation of the Pickering HIPEs developed by mung bean flour (A: φ = 80%, c = 5%; B: φ = 80%, c = 7%; C: φ = 80%, c = 9%).

increased gradually with the extension of time, suggesting that mung bean flour not only played the emulsifying role at the oil-water interface, but also increased the stability of the emulsion through its viscosity effect in the water phase, and the viscosity effect became more significant with the increase of the amount of mung bean flour. At the same time, the EI and MVI values of the samples were positively correlated with the amount of mung bean flour. The EI fluctuates greatly at 3%, indicating that the gel was weak, which was consistent with the results of the gel strength experiment.

3.8 Protective effect of Pickering HIPEs on β-carotene

β-carotene is a common vitamin A supplement, which can improve human visual function, but its application is limited because of its unstable exposure to oxygen, heat and light. In this study, the protective effect of mung bean flour stabilized Pickering HIPEs on β-carotene was investigated. Figure 6 exhibits the effect of mung bean flour dosage on the β-carotene protection ability of Pickering HIPEs against ultraviolet irradiation. Compared with MCT, mung bean flour stabilized Pickering HIPEs had a significant protective effect on loaded β-carotene. After UV irradiation for 5 days, the retention rate of β-carotene in MCT was close to zero, while the retention rate of β-carotene in Pickering HIPEs stabilized by mung bean flour was still more than 95%. On the 9th day, the β-carotene retention rates of Pickering HIPEs with *c* = 5, 7 and 9 were similar, still more than 95%, and there was no significant difference. The β-carotene protective mechanism of Pickering HIPEs stabilized by mung bean flour may be as follows: (1) mung bean flour adsorbs on the oil-water interface to form a shell structure, which weakens the irradiation of ultraviolet light on β-carotene in MCT; (2) mung bean flour contains a certain amount of polyphenols (Zheng et al., 2020), which has ultraviolet absorption and helps to improve the protective effect of β-carotene.

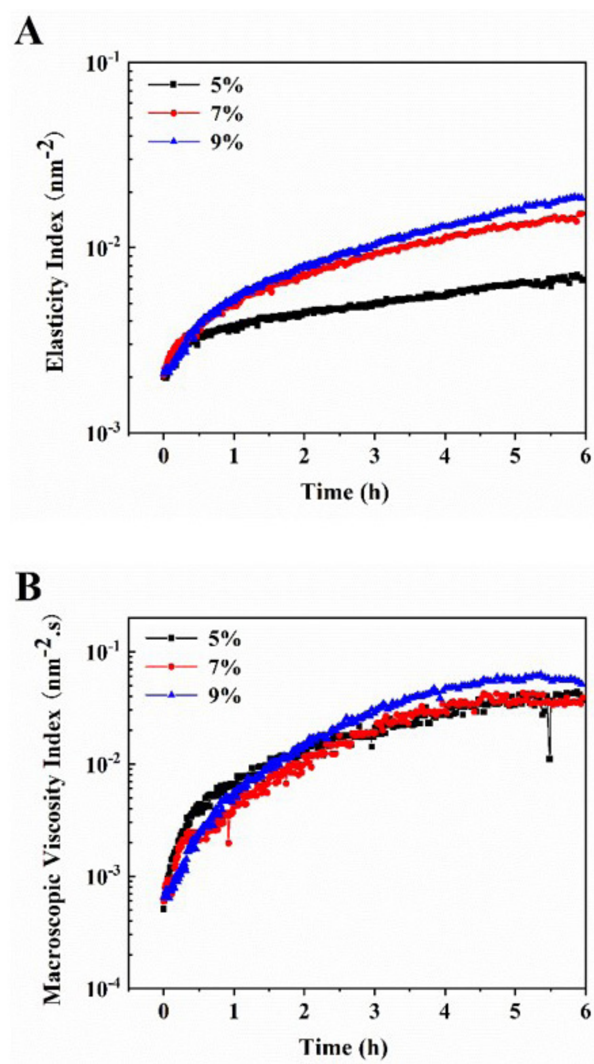

Figure 5. EI (A) and MVI (B) values of the Pickering HIPEs developed by mung bean flour at φ = 80%, *c* = 5, 7 and 9%.

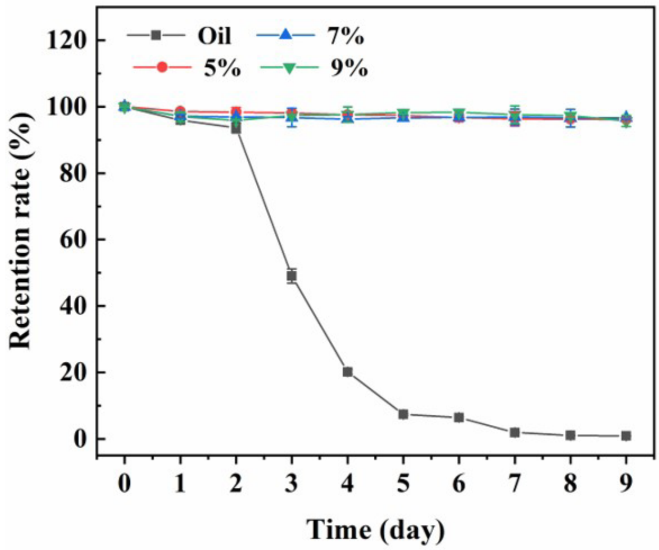

Figure 6. Light stabilities of β-carotene under UV irradiation in the oil and Pickering emulsion gels developed by Sorghum flour at φ = 80%, *c* = 5, 7 and 9%.

4 Conclusion

Mung bean flour had suitable particle size, good wettability, and could stabilize O/W Pickering HIPEs with oil phase volume fraction of 80%. It not only played an emulsifying role at the oil-water interface, but also prevented oil droplets from coalescing through its viscosity effect in the aqueous phase. With the increase of the concentration of mung bean flour, the size of emulsion droplets decreased, the mechanical properties of Pickering HIPEs gradually increased, and the β-carotene protective ability of Pickering HIPEs against ultraviolet irradiation also increased. The obtained results can not only provide a reference for the deep processing of mung bean, but also promote the green and efficient preparation of food grade Pickering HIPEs.

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