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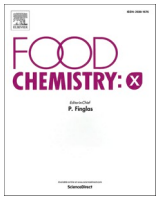

Fabrication, characterization and application of Pickering emulsion gels stabilized by defatted grape seed powder

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ABSTRACT

The feasibility of defatted grape seed powder (DGSP) stabilizing Pickering emulsion gels as butter substitute was investigated. The Pickering emulsion gel was constructed using DGSP through high-speed homogenization, and the effects of particle concentration (c) and oil-phase (Medium chain triglyceride) volume fraction (φ) on its structure and properties were investigated. Its application as a butter substitute was also evaluated. The results showed that DGSP had various particle shapes, a wide particle size distribution (3–130 μm), and a three-phase contact angle of 128.9 ± 2.3°. The O/W Pickering emulsion gels with $\varphi \geq 60\%$ could be obtained at $c \geq 2\%$. The droplet diameter was negatively correlated with c and positively correlated with φ, while the gel strength was positively related to c and φ. The resulting emulsion gel demonstrated solid-like viscoelastic behavior and pseudoplasticity, and had the potential to serve as a butter substitute. The results can promote the application of grape seeds in food.

1. Introduction

Grape is a widely cultivated and popular fruit in the world. According to statistics released by the International Organization of Vine and Wine, about 79.41 million tons of grapes were harvested worldwide in 2022. A large portion of these are processed into wine. In the wine-making process, >0.2 kg of grape residue is produced per kilogram of crushed grapes, of which grape seeds account for about 25% (Duba & Fiori, 2015). Grape seed contains about 40% fiber, 20% lipids, 11% protein, 7% water, and 3% minerals. A large number of studies have shown that grape seeds are nutritious and contain a large number of functional components, such as polyphenols, unsaturated fatty acids, and vitamins, which have high nutritional and medical value. These bioactive ingredients play an important role in anti-tumor, remission of toxicity caused by chemotherapy and radiotherapy, prevention of cardiovascular and neurodegenerative diseases, and oral health (Sochorova et al., 2020).

Therefore, the development of high-value products using grape seeds as raw materials will not only contribute to the recycling of grape seeds, but also promote the sustainable development of the wine and juice industry. The researchers' efforts in this area have been going on all the time. At present, the successful products developed based on grape seeds mainly include grape seed proanthocyanidin extract, grape seed oil and grape seed powder. Most of these products are sold in the market in the form of capsules and tablets and are favored by consumers (Martin, Grao-Cruces, Millan-Linares, & Montserrat-de la Paz, 2020). In addition, the addition of grape seed powder to flour has been a subject of interest for many researchers (Oprea, Popa, Apostol, & Gaceu, 2022). To sum up, the multi-level and multi-means development of grape seed products can not only achieve good economic benefits, reduce the grape processing cost, but also reduce pollution and obtain significant social benefits.

Pickering emulsion is an emulsion system constructed by solid particles instead of surfactants. It has a broad application prospect in biomedical materials, solid fat substitutes, nutraceutical delivery and so on (Sarkar, Ye, & Singh, 2017). Compared with the traditional emulsion, the advantages of Pickering emulsion are as follows: irreversible interfacial adsorption and excellent anti-coalescence stability. In the past two decades, there have been many reports on stabilizing Pickering emulsions with inorganic particles or organic synthetic polymers as emulsifiers. However, for the food and pharmaceutical fields, substances from food materials are expected to be superior to the above particles in terms of biocompatibility, cost, degradability and consumer acceptance (Harman, Patel, Guldin, & Davies, 2019). Therefore, the emerging research trend is to utilize natural biopolymer particles, which can not only be used as effective Pickering stabilizers but also find applications in food and pharmaceutical products.

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In our previous research, we found that proanthocyanidins, derived from grapes, had the capacity to stabilize Pickering emulsion gels (Geng et al., 2023). In addition, grape seed proanthocyanidin extract had been proved to possess high binding affinity for various types of proteins, including saliva protein, digestive enzymes, soy protein isolate, gelatin, wheat protein, rice protein and milk protein (Girard, Bean, Tilley, Adrianos, & Awika, 2018; Zou et al., 2019). The nutritional and health components encapsulated in the complexes usually have higher bioavailability and biological activity. The highly stable emulsions with biological activity had been successfully constructed by using pea protein, wheat gliadin, Zein and grape seed proanthocyanidin extract (Dai et al., 2020; Zhou, Yan, Yin, Tang, & Yang, 2018).

In summary, grape seed powder is rich in proteins and proanthocyanidins and has potential Pickering emulsifying ability, but related studies are still not reported. At present, there have been reports on the direct construction of Pickering emulsion based on black rice and purple sweet potato (Lu, Huang, Xiao, & Wang, 2022; Ye et al., 2024). Therefore, in this study, an attempt was made to construct a Pickering emulsion gel based on defatted grape seed powder (DGSP), and the effects of particle concentration (c) and oil-phase volume fraction (φ) on the micro-structure and properties of the emulsion gel were explored. Additionally, the feasibility of the resulting emulsion gels as a substitute for butter in cake making was evaluated. The results can promote the comprehensive utilization of grape seeds and provide reference for the development of food-grade Pickering emulsions.

2. Materials and method

2.1. Chemicals

The dried grape seeds were purchased from a local market. Medium chain triglyceride (MCT) was the product of Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Nile Blue A and Nile Red were from Sigma-Aldrich (St. Louis, MO, USA). Sunflower seed oil was purchased from Yihai Kerry Jindongyu Grain and Oil Food Co., Ltd. The ultrapure water from a Genpure UV/UF water system (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used.

2.2. Preparation of DGSP

The dried grape seeds (500 g) were crushed with a grinder, soaked in 5 L n-hexane and stirred for 3 h to remove fat. This operation was repeated until n-hexane was clarified, and the treated grape seed powder was placed in a cool place until n-hexane was completely volatilized. After passing the 100-mesh screen, the sample was weighed and preserved in a dryer at room temperature. The yield of DGSP was 46.7 ± 1.2% ($n = 3$). The moisture, crude fiber, crude lipid, and crude protein contents of DGSP were 6.08 ± 0.34%, 34.70 ± 0.41%, 2.25 ± 0.18%, 20.00 ± 0.30%, respectively.

2.3. Determination of particle size of DGSP

A Malvern 2000 laser particle size analyzer (Worcestershire, UK) was used to determine the size distribution of DGSP in water. The refractive index and absorption index of DGSP were 1.760 and 0.1, respectively, and the refractive index of water was 1.330 (Geng et al., 2023).

2.4. Contact angle measurement

The contact angle of DGSP was determined by a Theta Lite optical contact angle meter (Biolin Scientific, Stockholm, Sweden) based on our previous report (Geng et al., 2021). First, DGSP was pressed into a cylinder (height 3 mm, diameter 2 cm). Then, the cylinder was placed in a quartz vessel with a side length of 5 cm, poured into 30 mL of MCT, and a high-precision pipette was used to drop 6 μL of water onto the cylinder. The shape change of the droplet within 15 s was recorded. The contact angle was calculated by OneAttension software based on Young-Laplace equation.

2.5. Morphological observation of DGSP

The morphological observation was carried out according to the method of Geng et al. (2021) using a Sigma 300 field emission scanning electron microscope (Carl Zeiss, Oberkochen, Germany) at 500× magnification. The appropriate amount of DGSP were evenly fixed by adhesive tape on the sample table and treated with gold spray. Then, its morphology was recorded at the 5.0 kV acceleration potential in a low vacuum.

2.6. Preparation of Pickering emulsion

In order to investigate the effects of DGSP amount (c = 0.5%, 1.0%, 2.0%, 3.0%, 4.0%, φ = 50% and 70%) and oil phase volume fraction (φ = 40%, 50%, 60%, 70%, 80%, c = 2% and 4%) on the formation of Pickering emulsion gel, DGSP, MCT and water were mixed and homogenized at 15000 rpm for 3 min with a T18 digital ULTRA-TURRAX® disperser (IKA, Staufen, Germany). The φ and c were calculated with reference to the following formula, respectively:

$$\varphi = \frac{V_1}{V_1 + V_2} \times \% \quad (1)$$

$$c = \frac{m}{V_1 + V_2} \times \% \quad (2)$$

where V_1 (mL) is the volume of MCT, V_2 (mL) is the volume of water, and m (g) is the weight of DGSP.

2.7. Confocal laser scanning microscopy (CLSM) observation of emulsion

The CLSM observation of emulsion was carried out according to the method of Geng et al. (2023). First, DGSP was dyed with Nile blue A (0.1 wt%, in aqueous solution) while MCT was stained with Nile red (0.01 wt %, in propylene glycol). Then, the representative emulsions (φ = 70%, c = 4%; φ = 80%, c = 4.0%) were prepared according to section 2.6. Then, their microstructure was observed by a LSM780 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany). The distribution of DGSP and MCT was observed by laser excitation at 488 nm and 633 nm. The resulting images were processed with Zen 3.0 software.

2.8. Determination of droplet size of emulsion

The effects of particle concentration (c = 2%, 3%, 4%, φ = 70%, w/v) and oil phase volume fraction (φ = 60%, 70%, 80%, c = 4%) on the droplet size of emulsion were measured by a BT-9300H particle size analyzer (Bettersize Instruments Ltd., Dandong, China) at 25 °C. Distilled water was used as a dispersant, and the refractive index was lower than 2% before measurement, and the measurement began when the sample was added until the refractive index was between 15% and 16% (Geng et al., 2023).

2.9. Determination of rheological properties of emulsion gel

A HAAKE MARS III rheometer (Thermo Fisher Scientific Inc., Waltham, USA) with a P35TiL parallel plate probe was used to evaluate the rheological properties of emulsion gel. The gap between the probe and the sample table was set at 1 mm. Then, the oscillation amplitude sweep (f = 1 Hz, τ = 1–100 Pa), oscillation frequency sweep (τ = 5 Pa, f = 0.1–100 Hz) and shear rate sweep (τ = 5 Pa, γ = 0.1–20 s^{-1}) were performed according to a previous report (Geng et al., 2021).

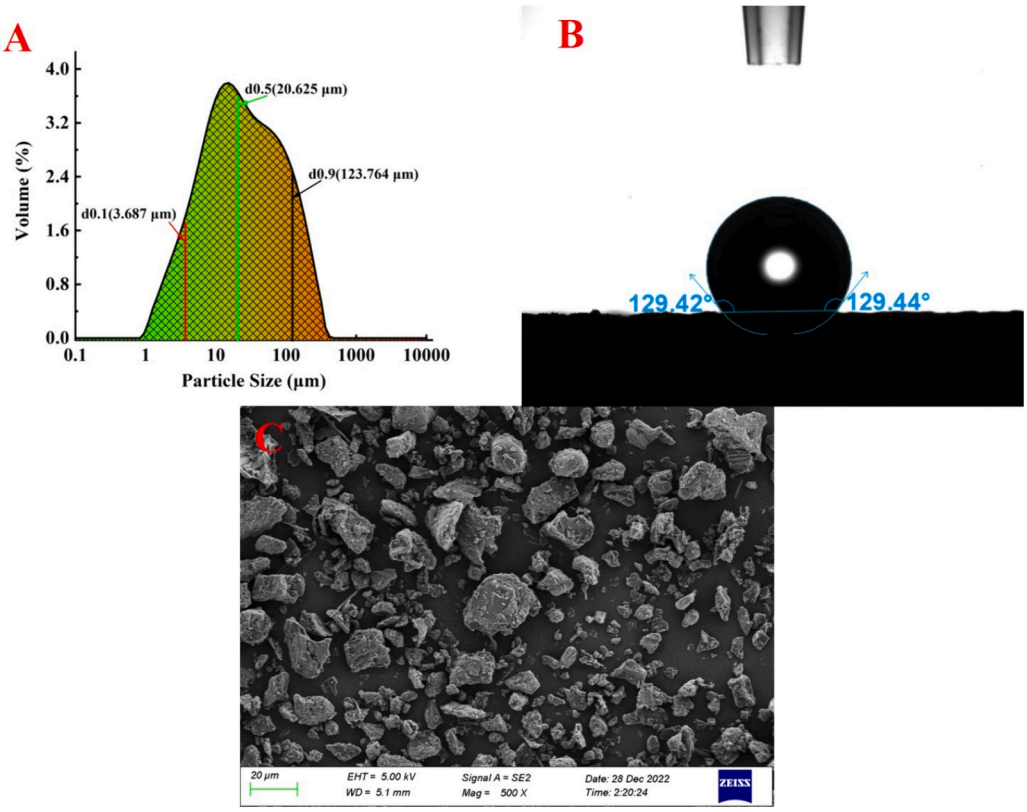

Fig. 1. Size distribution (A), three-phase contact angle (B), and morphological characteristics (C) of DGSP.

2.10. Determination the strength of emulsion gel

The gel strength of emulsion gel was determined according to the report of Fontes-Candia, Ström, Lopez-Sanchez, López-Rubio, and Martínez-Sanz (2020). The measurement was performed on a texture analyzer equipped with a P/0.5 cylindrical Delrin probe (TA-XT Plus, Stable Micro System Ltd., Surry, UK) in GMIA Gelation mode. During the test, the descending speed of the probe, the triggering force and the descending distance after triggering were set to 1.0 mm/s, 3.0 g and 4 mm respectively. The data were treated by Exponent 6.1 software.

2.11. Preparation of the cake

The Pickering emulsion gel with $c = 4\%$ and $\varphi = 70\%$ was constructed at 25 °C to replace butter to prepare cakes with different butter substitution percentage (W_{bs}). During the preparation process of emulsion gel, MCT was replaced with sunflower seed oil. The pound cake was prepared by using the formula in **Table S1**. All weighed ingredients were mixed and stirred with an eggbeater for 7 min. The cake paste was immediately put into the baking mold (15 × 8 × 7 cm) and baked for 40 min at 175 °C in a steam oven. Then, the cake was cooled at room temperature (23 ± 2 °C) for 2 h for follow-up analysis. The cakes with the W_{bs} values of 0, 20, 40, 60, 80 and 100% were recorded as Cake-H0, Cake-H20, Cake-H40, Cake-H60, Cake-H80 and Cake-H100, respectively.

2.12. Evaluation of cake quality

The volume of the cake was determined by using the rapeseed displacement method, and the mass of the cake was weighed, thus the density and specific volume of the cake were calculated (Sang et al., 2018). The rate of weight loss was recorded by comparing the weight of the cake batter before and after baking. The color was evaluated with a CR-400 colorimeter (Konica Minolta Holdings, Osaka, Japan) based on L^*, a^* and b^* color scale.

2.13. Measurement of cake structure

The cake was cut into 2 cm × 2 cm × 2 cm cubes, and its texture was determined in TPA mode using a texture analyzer with a P/36R probe. The pre-test speed, test speed and post-test speed were 2.0, 1.0 and 2.0 mm/s respectively, the triggering force was 5.0 g, and the compression strain was 50%. In addition, the cake was also cut into 2 cm thick slices, and its internal structure was analyzed by an imaging system (CC.300.06, Calibre Control International Ltd., Warrington, UK).

2.14. Statistical analysis

All measurements were repeated three times in parallel and data were statistically analyzed using the SPSS 18.0 software (SPSS Inc., Chicago, Illinois, USA) and expressed as mean ± standard deviation (SD), then the Duncan's test was applied to make statistical comparisons with a confidence level of 95%.

3. Results and discussion

3.1. Particle size of DGSP

The size of Pickering particles is the key to determine the droplet size and the appearance, rheology, stability and sensory perception of the emulsion. It has been reported that the particle size should be much smaller than the droplet size of Pickering emulsion (at least one order of magnitude), otherwise the particles will not be adsorbed at the oil-water interface to stabilize the emulsion. However, there are occasional reports suggesting that the droplet size of Pickering emulsions is smaller than the size of the original particles. This is attributed to particle size reduction caused by homogenization during the emulsification process (Aguilera-Miguel et al., 2018). Proper wettability and particle size make

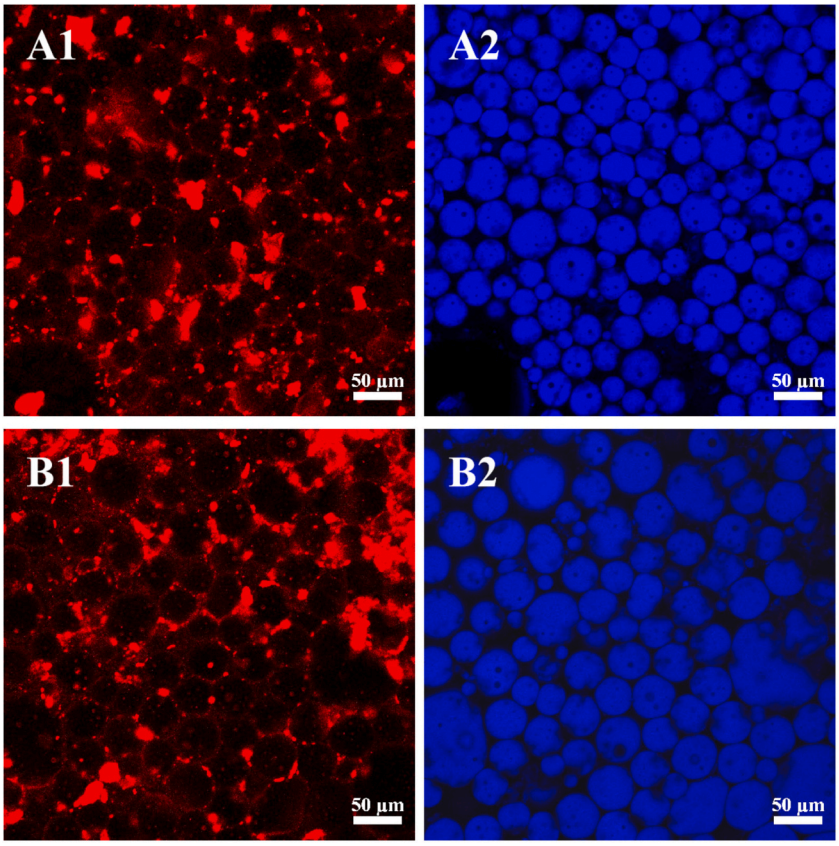

Fig. 2. CLSM image of Pickering emulsions stabilized by DGSP (A: $\varphi = 70\%$, $c = 4\%$; B: $\varphi = 80\%$, $c = 4\%$; DGSP stained by Nile Blue A appeared red while the oil phase treated with Nile Red exhibited blue regions). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the particles irreversibly adsorbed on the oil-water interface, forming a steric hindrance and preventing the accumulation of dispersed phases. At present, the main attempts to reduce the particle size without changing the chemical composition are mechanical cutting methods, such as ball milling, low temperature grinding, wet grinding and high pressure homogenizer.

As shown in Fig. 1A, the original particle size of DGSP was widely distributed, in which $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ were 3.687 μm, 20.625 μm and 123.764 μm, respectively. Some studies have shown that when there are a large number of small particles in the system, the finest particles are preferentially adsorbed at the interface (Liu & Tang, 2016). Moreover, compared with large particles, small particles can form smaller droplets and have superior stability. In addition, because of the low diffusivity, the adsorption kinetics of larger particles is slow and the filling efficiency at the interface is low. Tavernier, Wijaya, Van der Meeren, Dewettinck, and Patel (2016) studied the arrangement of fat crystals of different sizes at the oil-water interface, and found that the interface layer maintained by smaller particles was denser. However, the synergistic effects of different particle shapes and sizes should be considered, such as the complementary rigidity and flexibility of particles, and small particles can fill the gaps formed between large particles.

3.2. Wettability of DGSP

The wettability of particles is considered by some researchers to be the decisive attribute to control the formation and stability of Pickering emulsion, because it is closely related to the distribution and position of particles at the oil-water interface, and then determine whether it can exist at the interface to maintain the dynamic equilibrium of the body phase (Xiao, Li, & Huang, 2016a). Only particles with appropriate wettability can be used as stabilizers for Pickering emulsion. The three-phase contact angle (θ) is a semi-quantitative index to evaluate the wettability of particle surface. It is generally believed that the particles with contact angle $<90^\circ$ show strong hydrophilicity and those with contact angle $>90^\circ$ exhibit strong hydrophobicity. For colloidal particles, if θ falls between 30° and 150°, the desorption energy will be several orders of magnitude larger than the thermal energy of Brownian motion, which means that their adsorption process is irreversible. It is essentially different from the dynamic equilibrium process between the interfacial phase and bulk phase of the emulsion stabilized by traditional emulsifiers (Xiao, Li, & Huang, 2016b). On the other hand, emulsions stabilized by particles that are too hydrophilic or hydrophobic have larger emulsion droplets, are extremely unstable, and are prone to coalescence, making it difficult to form Pickering emulsions. The three-phase contact angle between DGSP and MCT-H_2O was $128.9 \pm 2.3^\circ$ (Fig. 1B). This means that it is more hydrophobic and has the potential to stabilize Pickering emulsion. At present, the emulsion based on food-derived particles was gradually reported, such as Tartary buckwheat bran powder (Zhang, Geng, Shi, Ma, & Liu, 2022).

3.3. Morphology of DGSP

The shape of particles is also an important factors affecting the stability of Pickering emulsion, the overall rheological properties and appearance of the emulsion, because it determines the arrangement of particles at the interface. The morphology of these particles should be highly concerned. The most commonly used Pickering emulsifier is spherical. In addition, the reported particle morphologies include ellipsoid, polygonal, rod -like, needle-like, filiform, roughly, nanogel, irregular and so on. Extensive work has explored the performance of various forms of solid particles on the formation of Pickering emulsion. Wei, Cheng, and Huang (2019) found that yolk transferrin fiber with

Table 1
Effect of c and φ on the droplet size of emulsion gels.

φ (%)	c (%)	D_3 (μm)	D_{10} (μm)	D_{25} (μm)	D_{50} (μm)	D_{75} (μm)	D_{90} (μm)
	2	39.45 ± 0.31^{j}	45.94 ± 1.35^{i}	54.44 ± 1.54^{g}	67.42 ± 1.75^{e}	84.30 ± 2.03^{b}	101.21 ± 2.60^{a}
70	3	24.35 ± 4.31^{l}	34.42 ± 2.29^{k}	42.95 ± 1.96^{ij}	53.48 ± 2.23^{gh}	65.68 ± 2.92^{e}	77.83 ± 3.98^{c}
	4	19.26 ± 0.13^{m}	31.02 ± 0.80^{k}	40.03 ± 0.98^{j}	50.50 ± 1.16^{h}	62.14 ± 1.39^{f}	73.86 ± 1.37^{d}
c (%)	φ (%)	D_3 (μm)	D_{10} (μm)	D_{25} (μm)	D_{50} (μm)	D_{75} (μm)	D_{90} (μm)
	60	17.11 ± 0.05^{p}	26.84 ± 0.57^{n}	35.01 ± 0.72^{l}	44.29 ± 0.78^{i}	53.85 ± 0.66^{f}	62.56 ± 0.84^{d}
4	70	19.26 ± 0.13^{o}	31.02 ± 0.80^{m}	40.03 ± 0.98^{j}	50.50 ± 1.16^{g}	62.14 ± 1.39^{d}	73.86 ± 1.37^{b}
	80	27.63 ± 0.36^{n}	37.40 ± 0.97^{k}	46.28 ± 1.26^{h}	58.03 ± 1.75^{e}	71.76 ± 2.29^{c}	85.16 ± 2.03^{a}

Annotation: D_n represents the proportion of droplet diameter distribution in the first n%.

high aspect ratio could be used to stabilize Pickering emulsion. Our previous study indicated that dihydromyricetin with high aspect ratio of rod-like particles could also stabilize excellent Pickering emulsion gel (Geng et al., 2021). It was reported that the emulsifying efficiency of rod-like cellulose nanocrystals was higher than that of ellipsoidal cellulose nanocrystals (Li et al., 2018). In Fig. 1C, it can be observed that DGSP is a mixture, showing a variety of shapes, including irregular ellipsoid, rod-like, polygonal, irregular shape. These forms may make them support each other at the interface, and there may be a mixed synergistic effect, which has the potential to give Pickering emulsion

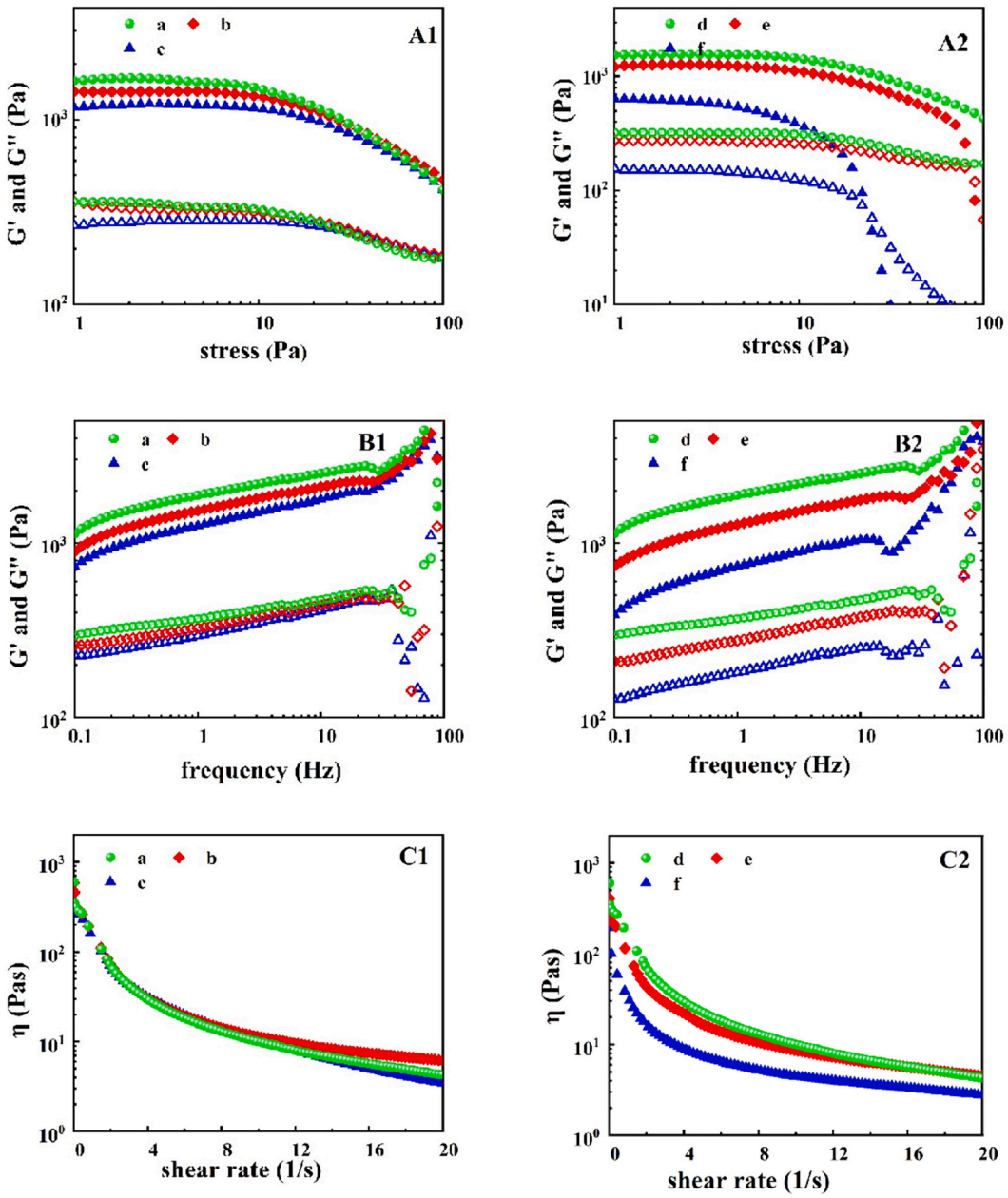

Fig. 3. Effect of c and φ on the rheological properties of emulsion gel (Stress sweep: A1 and A2; Frequency sweep: B1 and B2; Shear rate sweep: C1 and C2; a, b and c: emulsion gels developed at φ = 80%, c = 4, 3, and 2%; d, e and f: emulsion gels developed at c = 4%, φ = 80, 70, and 60%).

higher stability. However, whether DGSP can exert emulsifying role still needs further in-depth investigation.

3.4. Formation of emulsion gel

The effects of c and φ on the formation of Pickering emulsion gel were exhibited in **Fig. S1**. When c was increased from 0.5% to 4.0% (φ = 50%), the emulsion showed an obvious stratification after incubation for 24 h (**Figure S1A1**), indicating that it was difficult to form a stable Pickering emulsion under these conditions. When φ was set at 70% (**Figure S1B1**), a slight delamination phenomenon appeared at c = 0.5%, and the delamination phenomenon disappeared at c = 1.0%. It could be concluded that a stable Pickering emulsion was gradually formed with the increase of c. In **Figure S1B2**, the sample tended to form emulsion gel at c = 2–4%, and the apparent stability of emulsion gel was improved when c = 3–4%. The delamination phenomenon of the sample also gradually disappeared when φ increased from 40% to 80% at c = 2.0% (**Figure S1C1**). Besides, when φ = 70% and 80%, the emulsion gel was formed (**Figure S1C2**). When c was further increased to 4%, the emulsion gel was more easily formed (**Figure S1D1 and S1D2**). Therefore, a high φ value or a high c value was beneficial to promote the formation of Pickering emulsion gel. The same trend was found in studies about the Pickering emulsions stabilized by dihydromyricetin/lysozyme mixtures and dihydromyricetin/high-amylose corn starch composite particles (Geng et al., 2021; Geng et al., 2022).

3.5. CLSM analysis of emulsion gel

According to the difference between dispersed phase and continuous phase, the emulsion can be divided into two types: O/W type and W/O type. Multilayer emulsions can be obtained by combining different types of emulsifiers with suitable processing technology. The type of emulsion has a great influence on its application scene. For example, O/W or W/O emulsions can be used to protect and transfer oil-soluble and water-soluble nutraceuticals, respectively (Aditya et al., 2015). Although it has been reported that the contact angle tends to form W/O emulsion when the contact angle is >90°, a large number of attempts in food particles have confirmed that this conclusion is not applicable to some extent (Hong, Zhao, Liu, & Li, 2023). In Fig. 2, DGSP could form a dense network, separate oil phase from aqueous phase, and stabilize O/W Pickering emulsion. In addition, the adsorption types of particles at the interface could also be observed. There are two common types, one is that the particles are adsorbed only at the interface of one droplet, and the other is that the particles are adsorbed at the interface of two droplets at the same time. CLSM analysis indicated that DGSP particles primarily exhibited accumulation at the interfaces of two droplets.

3.6. Droplet size of emulsion gel

Microscopic characteristics play an important role in the study of materials, and droplet size is one of the microscopic characteristics. Lv et al. (2024) found that the emulsion gel had higher gel strength and water retention capacity in the system with smaller droplet size. Under the same processing conditions, the droplet size of Pickering emulsion is closely related to the particle type, the particle concentration and the oil volume fraction. Chen et al. (2018) adjusted the droplet size and viscosity of the emulsion by changing the concentration of octenyl succinic anhydride (OSA) modified cellulose nanocrystals. Yang et al. (2018) used starch nanocrystals produced by acid hydrolysis to prepare stable emulsions, and found that the Pickering high internal phase emulsion with higher nanocrystal concentration had stronger hardness and smaller droplet size, and when the oil phase volume fraction ascended, the droplet size of the emulsion stabilized by OSA modified gliadin nanoparticles increased. In Table 1, the droplet diameter decreased with the addition of DGSP, and the increasing φ also led to the increase of droplet diameter. A similar pattern was found in the dihydromyricetin-stabilized emulsions (Geng et al., 2021). However, the droplet size of emulsion stabilized by proanthocyanidins declined with the increasing φ (Geng et al., 2023). In general, an increase in the concentration of Pickering emulsifier particles tends to result in a decrease in droplet diameter, but the effect of φ may depend on the preference of these particles for oil phase and aqueous phase.

3.7. Rheological properties of emulsion gel

Understanding the rheological properties of Pickering emulsion is essential for related product processing. The rheological properties of Pickering emulsions is generally characterized by stress sweep, frequency sweep, and shear rate sweep. The particles on the interface of the emulsion form an interface film, which endows emulsion with elastic characteristics. The storage modulus G' is caused by the compression of emulsion droplets and interfacial elasticity. The former allows interfacial energy storage through the deformation of the droplet interface, while the latter comes from the adhesion between solid particles adsorbed on the oil-water interface (Xiong et al., 2018).

As shown in Fig. 3A, the storage modulus (G') and loss modulus (G") did not change obviously when the applied stress was within the critical value, and the emulsion exhibited the solid-like viscoelastic behavior of typical emulsion gel (G' > G"). After a period of platform, G' and G" decreased sharply with the increasing shear stress, indicating that the structure of the emulsion gel had been greatly damaged, and both G' and G" ascended with the increase of c and φ. In addition, the change of φ had a greater influence on the rheological properties of the emulsion gel, and the emulsion with low φ was more likely to be destroyed. The results of frequency sweep (Fig. 3B) were similar to those of stress sweep. In the shear rate sweep test (Fig. 3C), the viscosity of different emulsion gels decreased with the increasing shear rate until close to 0, confirming a typical pseudo-plastic behaviors (Song et al., 2015). At the same time, the increase of c had little effect on the shear viscosity, while the change of φ enhanced the viscosity. This phenomenon is mainly attributed to the

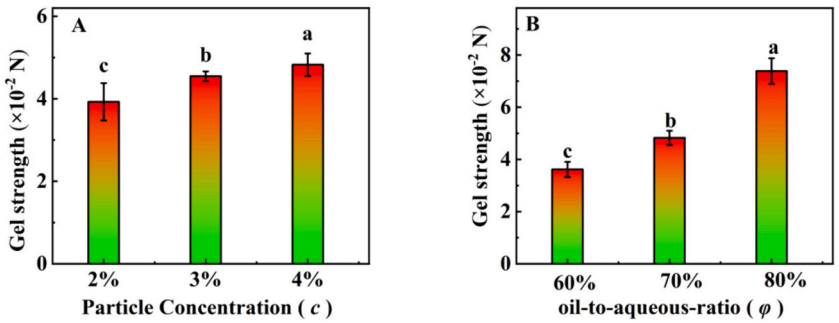

Fig. 4. Effect of c and φ on the gel strength of emulsion gels (A: emulsion gels developed at φ = 70%, c = 2, 3, and 4%; B: emulsion gels developed at c = 4%, φ = 60, 70, and 80%).

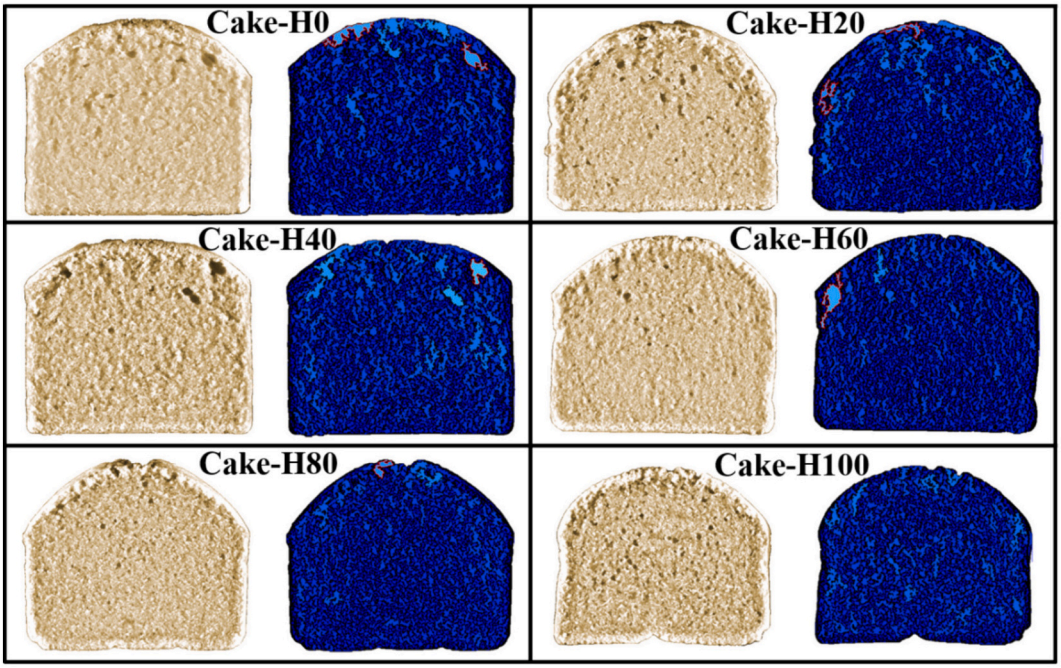

Fig. 5. Effect of W_{bs} on C-CELL images of cake slices.

fact that the oil phase itself has a certain viscosity and the particles have a preference for the oil phase, so they interact more closely.

3.8. Gel strength of emulsion gel

Emulsion gel is a kind of soft solid-like materials, which is widely used in pharmaceutical, cosmetic and food industry. Its gel strength is closely related to processing properties and application fields. Dickinson and Chen (1999) suggested that the interaction between protein matrix and oil droplets (disulfide bond, hydrophobic interaction and hydrogen bonding) was the key factor to determine the gel strength, and higher oil phase volume fraction and smaller particle size could enhance the filling effect. In addition, the gel strength of polyphenol cross-linked protein particles could be adjusted by changing temperature, pH and ion concentration (Zhu, Chen, McClements, Zou, & Liu, 2018). Oral tests showed that the higher the strength of the gel, the higher the hardness and the higher the degree of mastication. In another simulated digestion test, the expansion rate of the gel was affected by the gel strength. For the harder gel, only a small number of oil droplets were exposed to the gel surface, and attacked by lipase, while most of the oil droplets remained in the gel network were not affected by digestive juice (Guo, Bellissimo, & Rousseau, 2017). Therefore, it is possible to control the digestion of lipids in the intestine by designing emulsion gel structures with different gel strength to regulate the decomposition behavior of the gel in the digestive tract. In this study, it was observed that there was a significant positive correlation between the c and φ values of emulsion and its gel strength (Fig. 4). With the increase of c or φ, the interaction between particles and oil and water became stronger, and the density and stability of the interface film formed at the interface were also enhanced, which resulted in the increase of gel strength (Fontes-Candia et al., 2020).

3.9. Evaluation of cake quality

3.9.1. Appearance of cake

Gutierrez-Luna, Astiasaran, and Ansorena (2022) proposed that cake was a complex oil-in-water emulsion, which was composed of egg-sugar-water-fat mixture as continuous phase and air as dispersed phase. **Table S2** demonstrates the effect of W_{bc} on the density, specific volume, weight loss and color of the cake, which are closely related to the quality and sensory properties of the cake and play an important role in consumers' preference (Fernandes & Salas-Mellado, 2017). With the increase of W_{bs}, the cake density became higher, but the specific volume did not change obviously. When $W_{bs} \geq 60\%$, the specific volume decreased significantly **(Table S2)**, which was due to the interruption of protein network caused by emulsion gel. Zhang, Liu, Feng, Ren, and Wang (2023) also reported that the interaction between lipids and proteins could affect the volume, structure and hardness of bread. Moreover, with the increase of W_{bs}, the weight loss also ascended gradually. When $W_{bs} = 100\%$, the weight loss reached the highest ($4.874 \pm 0.152\%$), which was related to the evaporation of water in the emulsion gel during baking. The higher the W_{bs} value, the more water was introduced initially, and the greater the loss rate of water (**Table S2**). In terms of cake color, its L^* and b^* values decreased significantly with the increase of W_{bs} ($p < 0.05$), while a^* value increased significantly ($p < 0.05$). Moreover, the L^* component represents the luminance value, the a^* component represents the value from green to red, and the b^* component represents the value from blue to yellow. Compared with Cake-H0, the cake color gradually darkened, and changed from yellow to red. Martínez-Girón, Figueroa-Molano, and Ordóñez-Santos (2017) also observed a similar phenomenon when studying cakes added with peach palm (*Bactris gasipaes*) peel flour.

3.9.2. Texture properties of cake

Baked products are complex systems in which the existence and interaction of different components may be beneficial or disadvantageous to the final characteristics of the product. Hardness and chewiness can determine the sensory shelf life, while fat is an ingredient that has a positive effect on the texture of the product and can keep bread and cake soft for a longer time (Fernandes & Salas-Mellado, 2017). Therefore, the effect of W_{bs} on the texture properties of cake must be closely evaluated. In **Table S3**, when $W_{bs} \leq 60\%$, the hardness of the cake had no significant change. When W_{bs} continued to increase, the hardness, gumminess and chewiness of the cake increased, which was attributed to the fact that the cake lost too much water during baking and the butter in the cake was replaced by emulsion gel. In addition, the springiness and resilience of the cake did not change significantly. The results indicated that the cakes with $W_{bs} \leq 60\%$ had similar texture characteristics to the

control cake.

3.9.3. Internal structure of cake

C-CELL image analysis can reflect the difference in the internal structure of cake products. Fig. 5 and **Table S4** exhibited the effect of W_{bs} on the C-CELL images and parameters of cake slices. The specific volume of cake was the main index to measure the expansion degree of cake, which was proportional to the slice area. The cell structure inside the cake results from the formation and growth of pores during baking, and porous cakes are usually softer (Paraskevopoulou, Donsouzi, Nikiforidis, & Kiosseoglou, 2015). With the increase of W_{bs}, the cell number, cell density and cell diameter showed a complex fluctuation trend, and the fluctuation was the largest when the W_{bs} reached 100%. In the test, the change trend of the brightness of cake was the same as that of the L^* value, which were closely related to W_{bs}. Based on the texture and internal structure analysis, it could be inferred that with the addition of emulsion gel, the internal structure of the cake was first soft and delicate, and then due to the excessive addition of DGSP and the lack of butter, the cake gradually became hard and rough.

4. Conclusion

DGSP was a mixture of irregular ellipsoidal, rod-like, and polygonal particles with a size distribution ranging from 3 to 130 μm ($d_{0.5}$ = 20.625 μm). Its three-phase contact angle was 128.9° ± 2.3°. These particle characteristics endowed DGSP with Pickering emulsifying ability. DGSP could stabilize the O/W Pickering emulsion gels with $\varphi \geq$ 60% at $c \geq$ 2%. The droplet size, gel strength, and rheological properties of emulsion gels were also closely related to c and φ. The Pickering emulsion gel with c = 4% and φ = 70% could be used as a fat substitute to maintain the normal texture and internal structure of the cake when $W_{bs} \leq$ 60%. Our results can enrich the Pickering emulsion theory, and promote the application of grape seeds in food.

CRediT authorship contribution statement

Sheng Geng: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yuxiang Wang:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Benguo Liu:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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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Appendix A. Supplementary data

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