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Investigating Baneh (*Pistacia atlantica*) gum properties and applying its particles for stabilizing Pickering emulsions

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ABSTRACT

The purpose of this research was to investigate Baneh gum (BG) properties and prepare Pickering emulsion stabilized by BG particles at different concentrations (0.1, 0.3, 0.5, and 0.7 % (w/w)). Average size of the particles was 948 nm, and the SEM images confirmed the presence of the particles. Surface and interfacial tension of the BG particles were 48.39 and 15.36 (mN/m), respectively. Contact angle of water- and oil-BG particles was 99° and 42.68°, respectively, which can stabilize oil-in-water emulsions. Increment of the Pickering particles concentration decreased the size of the emulsion droplets and increased the emulsion stability ($p \leq 0.05$). The size of emulsion droplets was in the range of 1.65–1.76 μm and the highest zeta potential value was obtained by 0.7 % (w/w) BG particles (−30.02 mV). It can be concluded that increasing BG particles to 0.7 % resulted in creating the most stable emulsion.

Introduction

Emulsions stabilized by solid particles instead of surfactants are known as "Pickering emulsions" (PE). Pickering emulsions, as a result of the irreversible surface absorption of particles in the contact surface of two phases, have unique characteristics such as higher stability against oil droplet coalescence, and biodegradability, and are generally safer than conventional emulsions (Muiz, Klojdová, & Stathopoulos, 2023; Shah et al., 2016). In recent years, there has been an increasing research interest in the design of Pickering emulsions stabilized by solid colloidal particles (Naji-Tabasi, Mahdian, Arianfar, & Naji-Tabasi, 2021; Sarkar et al., 2018). The biocompatibility of Pickering emulsion stabilized by inorganic molecules and synthetic polymers is weak, which limits its application in food, medicine, and other fields. Renewable and environment-friendly organic nanoparticles can dominate these shortcomings and endow them broad application prospects in food, drug-sustained release, and cosmetics (Ding, Wu, Wang, Huang, & Cai, 2023). It is important to choose the appropriate type of nano/microparticles to obtain the specific type, property, and application of Pickering emulsions (Kou, Zhang, Ke, & Meng, 2023; Yang et al., 2017). Solid particles that are used to stabilize Pickering emulsions are polysaccharide, protein and fat particles (Kou et al., 2023; Shah et al., 2016). Baneh gum particles, which have nutritional properties, are used to prepare Pickering particles.

Wild pistachio (Baneh), with the scientific name "*Pistacia atlantica*", is one of the woody plants of arid and semi-arid regions of Iran. The height of its tree is between 2 and 7 m and grows in mountainy regions. Apart from its environmental values, also has wide medicinal and industrial applications (Esmaili, Mousavi Mirkala, Alijanpour, Hajjarian, & Ghanbari, 2022). The fruit, leaves and gum of Baneh have antibacterial, antifungal, cytotoxic, anticancer, anti-proliferative, antioxidant, antimutagenic, anti-inflammatory, antidiabetics, anti-hepatitis, anti-atherosclerosis, and anti-cholinesterase effects (Ghalavand, Esmaeili-Gouvarchin-Ghaleh, Mirzaei-Nodooshan, Vazifedost, & Mohammadi-Yeganeh, 2022; Mohammadi, Ehsani, & Bakhoda, 2019; Pasban-Aliabadi, Esmaeili-Mahani, Najafipour, Askari, & Jalalian, 2019). Parts of the main compounds of BG are α-pinene, terpenoids, and flavonoids, which prevent the growth and acid production of microorganisms involved in tooth decay (Mohammadi et al., 2019). Hydro-alcoholic extract of BG decreases hypercholesterolemia and increases the phagocytic ability of macrophages (Ghalavand et al., 2022). The BG composed of galactose, arabinose, xylose, rhamnose, and glucose. The most abundant amino acids of BG are histidine, glutamic acid, proline, serine, and aspartic acid (Mirahmadi, Mizani, Sadeghi, & Givianrad, 2019). The onset and melting temperature of BG are 80 °C and 240 °C, respectively. The loss of moisture and desorption of volatile essential oils

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occur at 80 °C (mass loss of 20 % to 25 %). Mirahmadi et al. (2019) reported that Beneh gum has a high decomposition temperature because of polysaccharide and polypeptide thermal decomposition (240 °C). The second stage of the decomposition happens at 200 to 300 °C (mass loss of 45 % to 53 %), and the main decomposition occurs above 240 to 360 °C (mass loss of 80 % to 92 %). Baneh gum has two glass transition temperatures (−34.08 –24.16 °C and 39.48–72.27 °C) (Mirahmadi et al., 2019).

In addition to the health properties of Baneh, it has received attention in the food industry, such as confectionary, dairy, and beverage industries, due to the creation of good taste and texture to the products. The unique properties of Baneh increase the need to use it in food products. It is very important to prepare food products considering the health needs of society, and it is necessary to know more about new natural gums to replace commercial sources. However, little attention has been paid to using Baneh gum in various industries, including the food industry.

The primary goal of this research was to fabricate bane gum particles. In the next step, structure and functional properties (surface and interfacial tension, wettability and Fourier transform infrared spectrum (FTIR)) of BG solid particles were determined. Then, Pickering emulsions were prepared at four concentrations of (w/w) BG solid particles (0.1, 0.3, 0.5, and 0.7 %), and the effect of different Pickering particles concentrations on emulsion properties (size distribution of droplets, viscosity, creaming index, centrifuging and heat stability) were investigated.

Materials and methods

Materials

Baneh gum was provided from Baneh forest trees in Kurdistan region. The Baneh gum was purified and its impurities were removed. BG was dried by freeze dryer and then milled. The BG powder was packed and stored in dried place for more investigations. Commercial sunflower oil was purchased from the market to prepare emulsions. All reagents were of laboratory grade. Deionized water was used to prepare all the solutions.

Analysis and characterization of BG properties

Physical properties

The samples were placed in an oven (BINDER, made in Germany) with a temperature of 105 °C to reach a constant weight. After leaving the oven, the samples were placed in a cold desiccator, and the moisture content was calculated based on the weight loss and reported as a percentage (AOAC, 2000).

In order to measure the amount of ash, 3.0 g of the milled sample was heated. Then it was put in a furnace (Excision, Germany) at 550 °C until the sample became completely white. Weight loss after cooling the sample in a desiccator was calculated and the amount of ash was reported based on percentage (AOAC, 2000).

Protein content was determined based on nitrogen content (N = 6.25) by Kjeldahl method.

Total carbohydrates

Total sugar was determined by phenol–sulfuric acid method, using D-glucose as standard at 490 nm using a spectrophotometer (model UV 2601, Ray Lee Company, China) (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).

Molecular weight

The molecular weight of BG was measured by Amini and Razavi procedure with some modification. In brief, the stock BG Solutions (0.003 g/ml) were prepared by dispersing freeze-dried BG powder in deionized water under constant mixing (1000 rpm) using a magnetic stirrer for 30 min at room temperature. Then, the stock solution was diluted to lower concentrations ranging (0.001 to 0.003 g/ml). Then, the molecular weight were measured using Zetasizer Nano ZS (Malvern Instruments, UK) based on static light scattering (25 °C) (Amini & Razavi, 2012).

FTIR spectroscopy of BG

The spectrum of the sample was measured by an infrared spectrometer (Avatar 370, Thermonicolt, USA) at wavelengths from 400 to 4000 cm^{-1} (Khalili & Huhtanen, 2002). The BG powder was mixed with potassium bromide to prepare.

Preparation of BG Pickering particles

The freeze-dried BG (1 % (w/v)) was poured in the mixture of water/ethanol (9:1) and dispersed, and then the pH of the suspension reduced to 6.0 by acid citric. The suspension was then immediately sonicated by ultrasound (VCX750, SONICS Company, England) at the amplitude of 40 % with 1 s pulse on and 3 s pulse off, for 1 min. Finally, BG particles were dried in a freeze dryer. In the end, BGPs were converted into powder using a miller and passed through a mesh.

Characteristics of BG particles

Particle size distribution and zeta potential

BG Particle size was determined using a Master sizer (Malvern, UK). The particles suspension was diluted with deionized water in 1 to 10 ratio at 25 °C. Zeta-sizer (CAD, France) was used to determine the zeta potential of particles (Le, Loveday, Singh, & Sarkar, 2020).

Morphology of BG particles

A scanning electron microscope (SEM) (ZEISS LEO 912 AB, Germany) was used to study the microstructure of the particles. For this purpose, a portion of the dried particles was transferred to an aluminum holder and coated with gold (SC7620, England) under the vacuum. The applied voltage of the device was 20 kV and the images were prepared at different magnifications.

Measurement of surface and interfacial tension of BG particles

Surface and interfacial tension of prepared solution made with 0.1 % (w/w) concentration of BG particles by the so-called ring method Nouy du method (using a data-physics DCAT 21 tensiometer) and at a temperature of 25 °C was measured. For this purpose, the applied force on the ring was measured as it was pulled up (Huang, Kakuda, & Cui, 2001).

Contact angle of BG particles

The particle powder was pressed into a thin tablet with a thickness of 2.0 mm and a diameter of 13 mm. Then, a drop of water and oil were slowly placed on the surface of the tablets. After reaching the equilibrium, the picture of the droplet was captured. The contact angle θ_o/w was obtained by the imaged droplet contour with the LaPlace-Young equation (Dai, Sun, Wei, Mao, & Gao, 2018).

Preparing BG solid particles Pickering emulsion

Different concentrations of BG particles (0.1, 0.3, 0.5, and 0.7 % (w/w)) were used as an aqueous phase to prepare emulsions. For preparation, Baneh gum Pickering emulsion (BGPE), sunflower oil (5.0 %) was slowly added to the suspension of solid particles under a homogenizer (Ultra Thorax T25, IKA, Germany) at a speed of 600 rpm. After the oil addition, homogenization was performed at 12,000 rpm for 3 min to obtain Baneh gum particles stabilized Pickering emulsion (BGPs PE).

Droplet mean diameter of BGMP-stabilized Pickering emulsion

To study the droplet characteristics, a Zetasizer made by Malvern

Company (England) was used. In another method for investigation of particle size, the images of emulsions were captured as the aforementioned procedure (1-6-2). Then, the threshold of gray-scale images was adjusted and particle size distribution was analyzed by Image-J software (National Institutes of Health, USA, version 1.45 s). Pixel values converted to distance units by a set scale icon of Image-J (Emil Kaya et al., 2019).

Zeta potential of BG Pickering emulsion droplets

A zeta sizer device (CAD, France) was used to determine the zeta potential of the Pickering particles. In order to perform the emulsion test, they were diluted with deionized water in a fixed ratio and then injected into the machine, and each sample was tested at least four times.

Microstructure of BG Pickering emulsion

The microstructure of the Pickering particle was studied by a digital light microscope (demobaL, Japan) at 400x magnification. The prepared emulsions were diluted in SDS solution (0.1 % w/v) in a ratio of 1 to 10 and then placed on a slide, and the samples were photographed.

Stability of BG Pickering emulsion

The Pickering emulsions were centrifuged (Hattich, Germany) to separate the upper oil layer (6000 rpm for 10 min). The volume of the separated part was measured, and the stability of the emulsion was calculated according to below equation (Manzocco et al., 2017):

$$\text{Emulsion stability} = \frac{\text{Emulsion volume}}{\text{Total volume}} \times 100 \tag{1}$$

Also, the stability of the emulsions was investigated during storage (4 weeks) through visual observation. In this method, the samples were transferred into glass test containers, and kept at room temperature. The emulsion stability was recorded during each week and calculated based on Eq. (1).

Thermal stability of BG Pickering emulsions

The emulsions were heated at 80 °C for 30 mins in a water-bath, and cooled to room temperature. The heated emulsions were centrifuged at 6000 rpm for 10 min (Hattich, Germany). The stability of the emulsion was calculated according to Eq. (1) (De Vries, Hendriks, Van Der Linden, & Scholten, 2015; Manzocco et al., 2017).

Rheological properties

Rheological properties of Pickering emulsions were measured in the shear rate range of 1–300 s^{-1} using ULA spindle rotary viscometer model LVDV III Ultra of Brookfield Company, USA (25 °C) (Razjoo, Azizkhani, & Esmailzadeh Kenari, 2021).

Statistical analysis

A completely randomized design was applied for statistical analysis. The data were analyzed by analysis of variance (ANOVA) and Duncan multiple range test ($P \leq 0.05$). Results were expressed by means ± standard deviation.

Results and discussion

Evaluation of BG properties

Physicochemical properties of BG

The moisture, ash, and protein content of BG were 0.97 ± 0.02 %, 5.02 ± 0.16 %, and 2.32 ± 0.12 %, respectively. In previous research, the values of moisture, ash, and protein of BG were reported to be16.03–19.26 %, 0.86–0.94 %, and 3.97–5.05 %, respectively. Studies conducted on the gums exuded from baneh trees are mainly composed of polysaccharides containing galactose, arabinose, rhamnose and uronic acids (Mirahmadi, Mizani, Sadeghi, & Givianrad, 2023; Rahimi, Abbasi, Sahari, & Azizi, 2013). BG was dried by freeze drier in this study, and the moisture content was very low. The total carbohydrate analysis of Baneh gum was about 38.93 ± 1.34 %. The amount of total carbohydrates in BG was reported to be around 39.38–45.56 %, which varies depending on the gender and species of the plant, which arabinose and galactose are the most abundant monosaccharides in BG (Mirahmadi et al., 2019).

The molecular weight of Baneh gum was 5440 ± 12.34 (Da). Beneh gum is a low molecular weight gum according to its low glass transition temperature. In this condition, the transition of glass to elasticity occurs quickly due to water absorption and can cause physical properties such as adhesion. Also, the intrinsic viscosity of BG is in the range of 17.24–20.03 $dL.g^{-1}$ based on its region (Mirahmadi et al., 2019).

FT-IR analysis of BG

The FTIR spectrum of BG is presented in Fig. 1. Baneh gum had peaks in 800–1200 cm^{-1} (Guo et al., 2011). It had absorbance at the wavelength of 990–1150 cm^{-1}, which is related to C—O and C—O—C stretching vibration. The observation of 1600–1630 cm^{-1} bands indicates the presence of free carboxylate groups (Naji-Tabasi, Razavi, Mohebbi, & Malaekeh-Nikouei, 2016). The results of the FTIR spectrum showed a band at 3000–2900 cm^{-1}, which was assigned to aliphatic C—H stretching due to the presence of galactose, arabinose, and rhamnose.

The results of FTIR spectra for BG gum samples in Mirahmadi, Mizani et al. (2019) also indicated a band at 3000–2900 cm^{-1} that was assigned to aliphatic C—H stretching related to the existence of galactose, arabinose and rhamnose (Mirahmadi et al., 2019). The peak at 1700 cm^{-1} was associated with carboxylic acid and ketone stretching (Mirahmadi et al., 2019).

O—H stretching absorption due to inter- and intramolecular hydrogen bonds created a wide region of absorption between 3000 and 3500 cm^{-1}. This region is presented several features, including free hydroxyl groups that occurred in the samples in the vapor phase and showed the O—H bond of carboxylic acid. Broad absorption band with strong intensity between 2800 and 13000 cm^{-1} (about 2916 cm^{-1}) referred to CH absorption, which includes CH, CH_2, and CH_3 stretching vibrations (Kang et al., 2011).

Characteristics of BG particles

Particle size and zeta potential

According to the results, the size of the BG particles was 948.15 ± 11.09 nm. The poly dispersion index (PDI) was 0.48 ± 0.12, which indicates a slightly heterogeneous dispersion. The particles had a zeta potential of –23.00 ± 3.00 mV. Aggregation and agglomeration of nanoparticles primarily depend on zeta potential and particle size. Zeta potential is a physical property exhibited by each particle in a suspension. Particles with high zeta potential will repel each other and the suspension will remain well dispersed and stable. If zeta potential is close to neutral, there will not be enough force to repel each other and the particles may agglomerate or aggregate and their surface area will decrease, resulting in less reactivity (Shahbazizadeh, Naji-Tabasi, & Shahidi-Noghabi, 2022).

SEM images (Fig. 2) confirmed the presence of BG particles and confirmed the size of nanoparticles obtained by DLS test. The overall appearance of the particles was similar, and the structure was spherical to elliptical, dense, smooth, and in some cases irregular in shape. Although in some images the particles were not completely smooth. Accumulation of particles and their enlargement due to connection were observed in some images, which resulted in a more irregular shape.

Surface and interfacial tension of particles

Hydrocolloids are mainly hydrophilic polymers, which do not show significant surface and interfacial activity. Although, some hydrocolloids have been found that migrate slowly between water–oil and

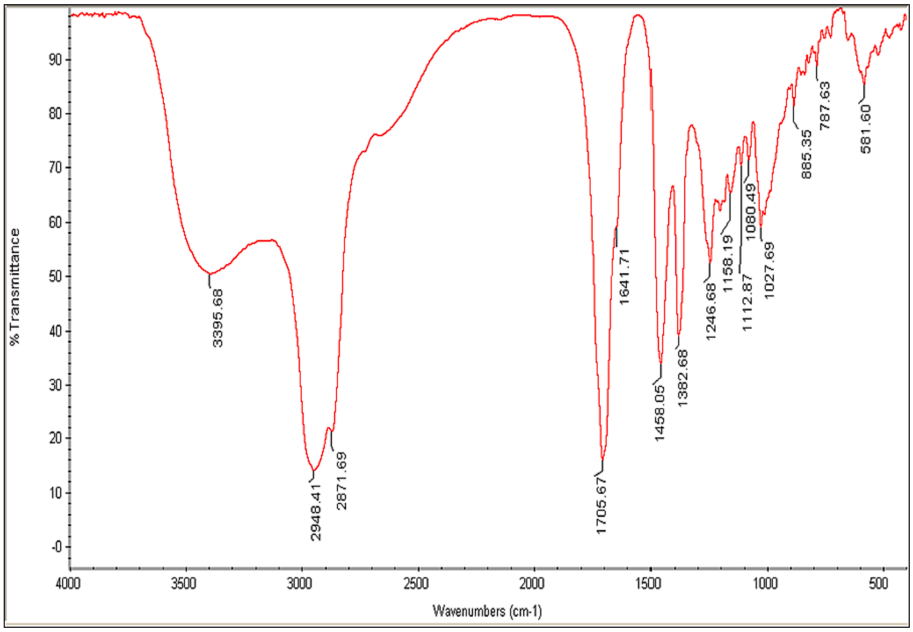

Fig. 1. FTIR spectrum of Baneh gum.

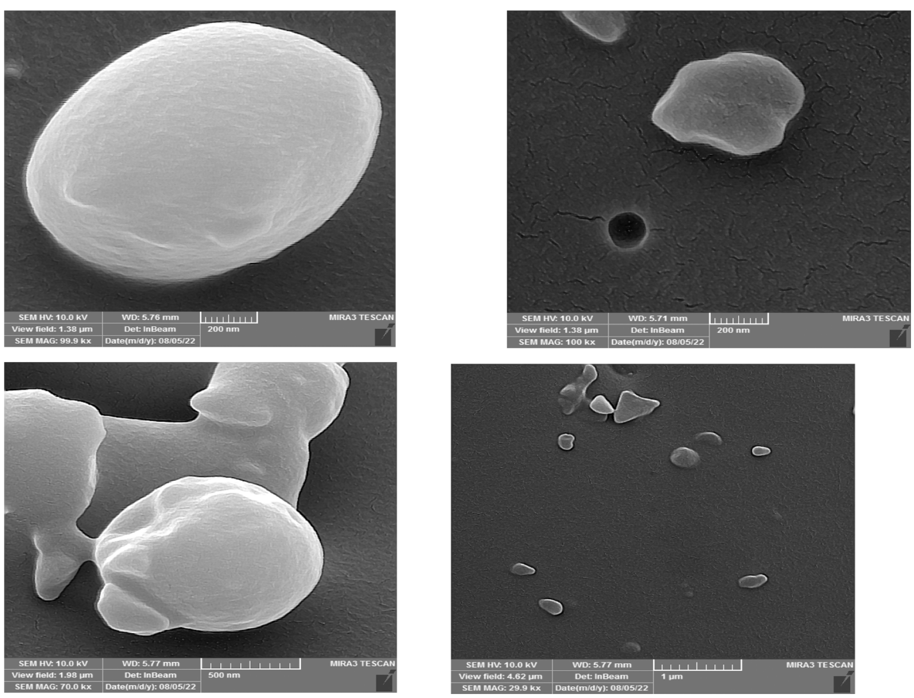

Fig. 2. Scanning Electron Microscopy (SEM) images of Baneh gum particles.

water–air surface and show surface and inter-surface activity (Osano, Matia-Merino, Hosseini-Parvar, Golding, & Goh, 2010). The surface and interfacial tension of the 0.7 % (w/w) BG particles suspension was 48.43 and 15.41 (mN/m^2), respectively (Fig. 3) was lower than water (71.78 mN.m^{-1}) (Gianino, 2006). The surface tension of 0.5 % fenugreek, pectin, guar, xanthan, gum Arabic, carrageenan, methylcellulose, and basil seed gum solutions are 50.3, 53.6, 55.2, 60.8, 46.9, 65, 52.9, and 57–64 mN.m^{-1}, respectively (Huang et al., 2001; Naji-Tabasi et al., 2016). The interfacial tension of BG is between 18.96 and 23.70 mN.m^{-1} based on gender and geographical regions. Therefore, the BG particles have more ability to reduce interfacial tension than BG. Mirahmadi et al. (2023) reported the interfacial tension of BG had a positive correlation with *trans*-carveol, p-cymen-7-ol, 1,8-cineole, myrcene, and tricyclene ($P < 0.01$), and comphene, glutamine, and α-terpinolene ($P < 0.05$). But limonene, terpinen-4-ol bornyl acetate, cysteine, longifolene, and alanine ($P < 0.01$) and α-thujene, Phenylalanine, and sabinene ($P < 0.05$) contents display significant negative correlation with interfacial tension of BG (Mirahmadi et al., 2023). The presence of hydrophobic and hydrophilic side chains in the polymer structure creates surface activity. The amount of interfacial tension gives valuable information about the emulsifying properties of a compound and the stability of drops against intermingling and Ostwald enlargement. It can be concluded that the BGMPs had interfacial activity and it can prevent emulsion instability by interfacial effects. Different properties of

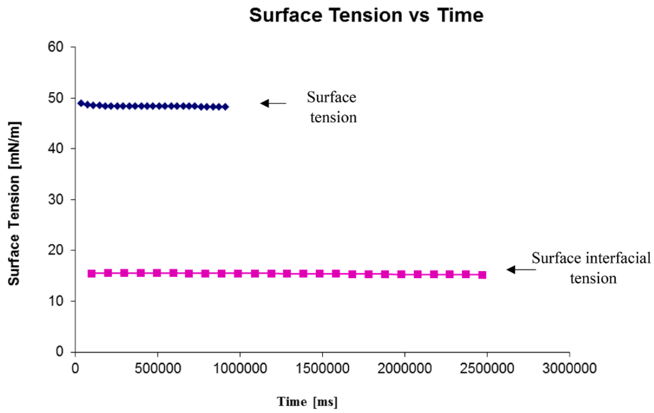

Fig. 3. Surface tension and surface interfacial of Baneh gum particles.

Pickering particles favor the formation of physical support that makes the emulsion system more stable The nanoparticles not only reduce interfacial tension to facilitate the formation of stable emulsion; their morphology and length-to-width ratio, also impart considerable deformability (Shi, Feng, Wang, & Adhikari, 2020).

Contact angle of BG particles

The three-phase contact angle (θ) at the contact point of three combinations of oil-solid-particles-water explains the degree of wettability of solid particles at the oil–water interface, which is generally expressed as the three-phase contact angle for water phase (water contact angle). Solid particles as an emulsifier create one or more layers on the droplets, which increase the steric hindrance between the droplets and increase the stability of the emulsion (Binks, Isa, & Tyowua, 2013). Pickering particles should be partially wet by both aqueous and oil phases, and the determination of contact angle is a reliable means to reflect the wettability of Pickering emulsifier (Wei & Huang, 2019). Most hydrophilic particles with a contact angle of less than 90° are immersed in the aqueous phase and form an oil-in-water emulsion. To obtain water-in-oil emulsions, hydrophobic particles with a contact angle greater than 90° should be used (Menon & Wasan, 1988). Generally, PEs cannot be formed when the three-phase contact angle is close to 0° or 180° (Shi et al., 2020). The contact angle of the solid particle is an important factor in determining the emulsion type. Accordingly, a contact angle of less than 90° indicates the hydrophilicity of the particles, which enhances the stability of the o/w Pickering emulsion (Zhou, Yu, Luo, Yang, & Yin, 2023).

Some researchers divide surfaces into three categories based on the size of the contact angle. If the contact angle is less than 30°, the water drop will thoroughly wet the surface, and the surface will be wettable. If the contact angle is between 30 and 89°, the surface is relatively wettable, and if the contact angle is more than 90°, the surface is considered hydrophobic (Shekarchizadeh & Akafian, 2022).

The water–particle contact angle on BG particles was 99°. The oil-particle contact angle was 42.68°, and these particles are relatively wettable and suitable for obtaining oil-in-water emulsions. BG has a hydrophilic nature due to its carboxyl and hydroxyl groups and is also lipophilic because it is an oleoresin gum (Jahangiri, Hamedi, & Ahari, 2022). The MPs of BG have the ability to stabilize oil-in-water emulsions. Therefore, BG particles were not completely soluble in water or oil. Particles are able to stabilize emulsions by providing a physical barrier to droplet coalescence. If the particles are neither soluble in oil nor in water they will accumulate (Linke & Drusch, 2018). Also, the stability of PEs is easily influenced by many factors, such as pH of the continuous phase, capillary pressure, shape, the presence of electrolytes, and concentration of the solid particles (Shi et al., 2020). The extent of this prevention depends on the density of particles accumulated in the interface and the energy required to separate the particles (Heidari Dalfard, Ziaiifar, Jafari, & Anton, 2022).

Effects of BG particle concentration on Pickering emulsion characteristics

PE droplet size

In this study, the effect of Baneh gum (BG) solid particle concentration on mean diameter and droplet-size distribution of Pickering emulsion was investigated by Dynamic Light Scattering (DLS) analysis, which is widely known for determining particle size and particle-size distribution, and an image-processing technique (Table.1). The particle size of BGPs using DLS analysis showed a size range of 1.76 ± 0.64 to 1.65 ± 0.05 μm. Increasing the BG concentration from 0.1 to 0.7 %, the particle size significantly decreased from 1.74 ± 0.12 μm to 1.67 ± 0.09 μm. When the concentration of particles was low, larger droplets were shaped as a low concentration of BG could not completely cover oil droplets. As a result, uncovered droplets start to connect and the average size of the droplets increases. If a sufficient concentration of BG is available, smaller and more stable particles will form. Sufficient BG particles formed a three-dimensional solid-like network in the continuous phase, which prevented the coalescence and formation of clots in the emulsion droplets (Xiong et al., 2022).

Poly dispersing index (PDI) indicates the Pickering particles distribution and their homogeneity. PDI values more than 0.5 indicated a wide dispersion of particle size. A PDI of zero indicates the homogeneity of the particle size distribution. Table 1 showed the particle distribution index was in the range of 0.70–0.85. An increase in the amount of BG particles led to increment of the particle distribution index. Although the size of the particles decreased by increasing the level of gum, the particle distribution index increased. More microparticles by applying by protecting and dispersing the pressure force, leads to non-uniform dispersion of the homogenization energy input to the Pickering emulsion and as a result, increases the dispersion of the Pickering emulsion particle size (Tiwari & Pathak, 2011). On the other hand, these particles may create a multilayer coating on some droplets and make the particle size distribution more heterogeneous (Das, Ng, & Tan, 2012; Pooja, Tunki, Kulhari, Reddy, & Sistla, 2016).

The mean diameter of BG oil droplet was also measured by image processing method (Table 1). The size of the droplets was in the range of 1.60–1.96 μm. The mean droplet diameter of PEs decreased significantly ($p < 0.05$) as the BGPs concentration increased (0.10–0.70 %). The results showed that with the increase in the percentage of Pickering particles in the emulsion, the droplet size of the emulsion decreased ($p < 0.05$), which was in line with the results of the device evaluation. The difference between the samples of 0.3 %, 0.5 % and 0.7 % BGMPs was not significant ($p > 0.05$). These observations were consistent with the results of the studies by Xiong et al. (2022). They used different levels of xanthan gum/lysozyme nanoparticles (0.2 to 0.5 %) as carrageenan-Based Pickering Emulsion Gels. They found that the particle size decreased with increasing concentration of nanoparticles (Xiong et al., 2022). Hedjazi and his colleagues (2018) evaluated the effect of cellulose nanoparticle concentration on the physicochemical properties of canthaxanthin Pickering emulsion droplet size. The diameter of the emulsion droplets increased from 246 nm to 404 nm with a decrease in the concentration of cellulose nanoparticles from 0.9 to 0.3 % w/w. The size of the droplets indicates a direct relationship between the low concentration and the interconnectedness of the droplets (Hedjazi, Razavi, Kordjazi, & Khodaiyan, 2019).

The emulsion droplet size distribution was between 0.42 and 0.61, and with increasing the percentage of Pickering particles, droplets with a narrower distribution were formed. According to the results, PE was the most stable at BGPs concentration above 0.5 %. The distribution of PE droplet size showed a single peak system at samples prepared with 0.5 % and 0.7 % BGPs, which was the monodisperse system. The PDI of BGMP emulsions was higher based on image-processing technique in compared with DLS analysis, which was consistent with the results of other researchers who had used these two methods (Emil Kaya et al.,

Table 1
Droplet mean diameter, size distribution, zeta potential, and viscosity of Pickering emulsion stabilized by different concentration Baneh gum (BG) particles.*

Sample	Dynamic Light Scattering (DLS) analysis			Image-Processing technique		viscosity (Pa.s)
	Droplet mean diameter (μm)	droplet size distributions	Zeta potential (mv)	Droplet mean diameter (μm)	Size distribution width	
0.1 %BG	1.74 ± 0.12^{b}	0.70 ± 0.02^{b}	-26.05 ± 1.20^{b}	1.96 ± 0.10^{a}	0.61 ± 0.03^{a}	1.14 ± 0.00^{b}
0.3 %BG	1.76 ± 0.64^{a}	0.72 ± 0.08^{b}	-27.14 ± 0.94^{ab}	1.78 ± 0.11^{b}	0.54 ± 0.04^{b}	1.16 ± 0.01^{a}
0.5 %BG	1.65 ± 0.05^{d}	0.83 ± 0.06^{a}	-29.23 ± 1.54^{ab}	1.72 ± 0.09^{b}	0.55 ± 0.05^{a}	1.16 ± 0.01^{a}
0.7 %BG	1.67 ± 0.09^{c}	0.85 ± 0.03^{a}	-30.02 ± 1.35^{a}	1.60 ± 0.10^{b}	0.42 ± 0.02^{b}	1.04 ± 0.01^{c}

* Different letters indicate significant differences between samples at $p < 0.05$ by Duncan test. Data were expressed as mean ± standard deviation.

2019). In the image processing method, fewer droplets are evaluated. Maybe, the presence of aggregated droplets increased the droplet size distribution.

Zeta potential of PE droplets

Zeta potential of droplet was in the range of -26.05 ± 1.20 to -30.02 ± 1.35 mV, which indicated the presence of the negative groups, mostly arranged on the surface of the bio-polymeric chains (Cardial et al., 2019). The amount of negative charge on the droplets significantly increased from -26.05 ± 1.20 mV in the concentration of 0.1 % to -30.02 ± 1.35 mV in the emulsion containing 0.7 % BG, which was due to –COO$^-$ and –OH$^-$ groups of anionic polysaccharides ($P \leq 0.05$). The increase in surface charge increases the stability of the emulsion due to the increase in the repulsive forces between the droplets, which reduces the risk of clumping and coalescence. The high zeta potential of droplets indicates the high stability of the Pickering emulsion against aggregation and clot formation (Cardial et al., 2019). Increasing the concentration of BG particles improved the zeta potential value, because more negative particles surround the oil droplets and there may be more layers of particles around the droplets.

Microstructure of Pickering emulsion

Microscopic images of oil-in-water BG MPs Pickering emulsions at concentrations of 0.1 to 0.7 % are presented in Fig. 4. The structure of Pickering emulsions depended on the concentration of BG MPs. At the concentration of 0.7 % BGMPs, there are clear gray droplets and no distinct color can be seen in the continuous phase, which indicates that all the BG MPs are adsorbed at the oil–water interface. As can be seen, the increase in droplet size was associated with a decrease in the amount of BG MPs. Also, with the increase in the concentration of MPs, the uniformity of the emulsion droplets increased.

In addition, it was observed that the average size of the droplets decreased in proportion to the increase in the concentration of MPs. Complete droplet coverage occurs when there are enough Pickering particles in the emulsion (Hedjazi et al., 2019). In a similar study, they investigated the effect of independent variables of nanoparticle concentration and oil content on the morphology and size of Pickering emulsion droplets stabilized by chitosan-tripolyphosphate nanoparticles.

They concluded that by increasing the chitosan-tripolyphosphate nanoparticles concentration from 0.45 to 0.75 %, the size and morphology uniformity of the emulsion droplets decreased and increased, respectively (Heidari Dalfard et al., 2022).

Emulsion stability of BGMPs Pickering emulsion

Storage stability assay. The emulsion stability during storage was evaluated as a function of BG MPs concentration (Table 2, Fig. 5). On the first week, all samples were completely stable and no alteration was observed. In the second and third weeks, instability was observed in all

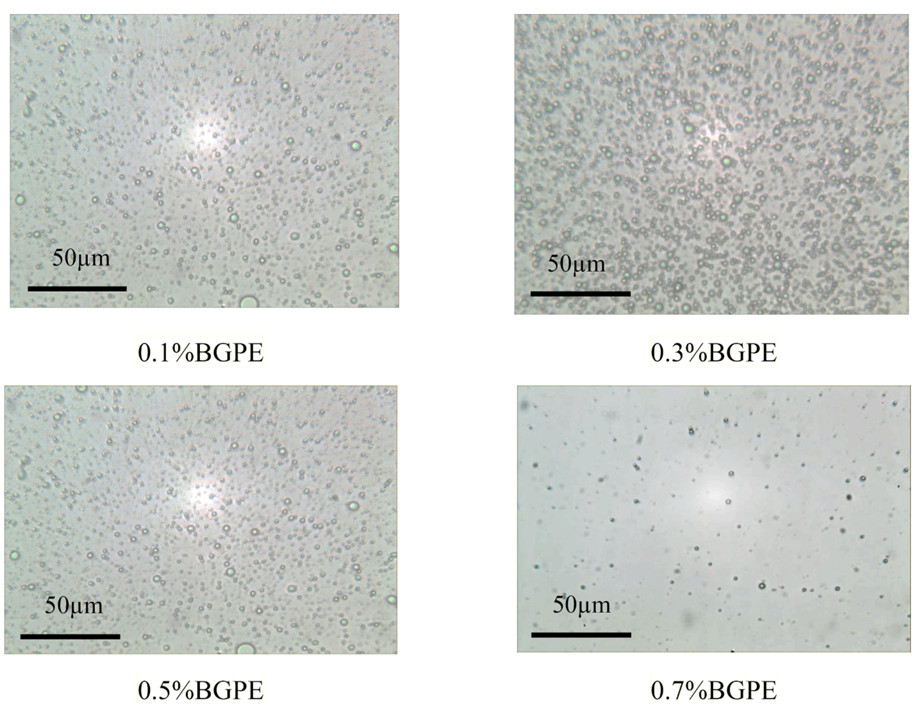

Fig. 4. Optical microscopy images of the oil-in-water Pickering emulsion stabilized by 0.1, 0.3, 0.5, and 0.7% (w/w) Baneh gum microparticles at 400× magnification. The oil/water phase ratio was 5:95.

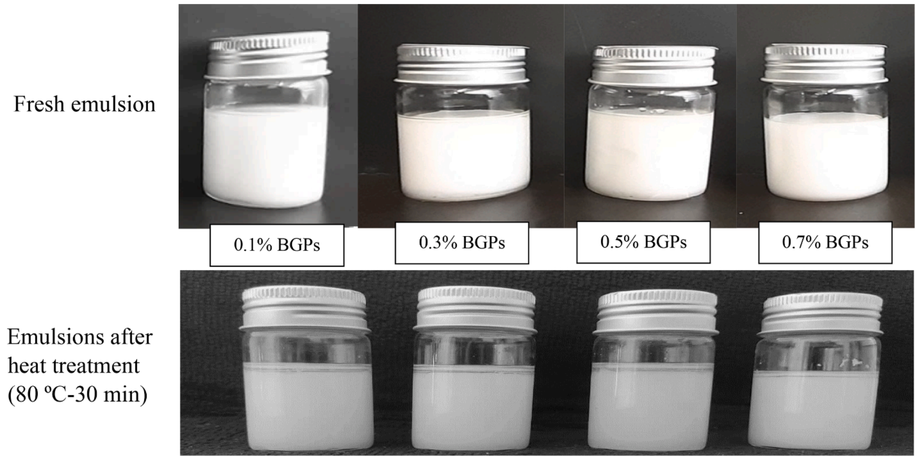

Fig. 5. The stability of Pickering emulsion stabilized with BGMPs after thermal treatment at 80 °C for 30 min.

samples. In the fourth week, the stability of Pickering emulsion samples containing 0.1, 0.3, 0.5, and 0.7 % of BG MPs was equal to 80.05, 91.33, 91.97, and 93.22 %, respectively. The effects of different concentrations of particles on the stability of Pickering emulsion were consistent with the centrifugation stability results (Table 2). The high stability of Pickering emulsion after one-month of storage at 25 °C indicated the irreversible absorption of BG particles on the surface of the droplets.

The highest stability value was related to the sample containing 0.7 % of BGMPs. The stability of the oil inside the formed network significantly increased with the increase in BGMPs concentration and the leakage rate decreased. The reason for this can be related to the formation of a stronger layer around the oil droplets at higher particle concentrations (Zou, van Baalen, Yang, & Scholten, 2018). The stability of 0.5 and 0.7 % BG emulsions increased due to the complete coverage of the droplet surface and the increment of electrical repulsion between the emulsion particles (Ghadermazi, KHOSROWSHAHI, AZIZI, & Tamjidi, 2019). In addition to the more absolute value of the zeta potential, the small size of the oil droplets also resulted in more emulsion stability (Naji-Tabasi et al., 2021). More stability against coalescence and flocculation was observed in the emulsions with small droplet sizes. Particle with smaller size provides a better cohesive barrier at the oil–water interface due to more efficient coverage (Hossain, Deeming, & Edler, 2021). Sample stabilized by 0.7 % (w/w) BG NPs with a −30.02 ± 1.35 mV zeta potential exhibited almost no phase separation when kept for over a month of storage (Tables 1 and 2).

Table 2
The stability of Pickering emulsion stabilized by Baneh gum (BG) particles against centrifugation, heating and during 4 weeks storage at 25 °C.

sample	1st week	2nd week	3rd week	4th week	Centrifuging stability (%)	Heating stability (%)
0.1 % BG	100 ± 0.00^{a}	90.80 ± 0.12^{c}	85.20 ± 0.10^{c}	84.60 ± 0.11^{c}	89.51 ± 0.16^{c}	80.05 ± 1.05^{b}
0.3 % BG	100 ± 0.00^{a}	95.89 ± 0.25^{b}	90.97 ± 0.07^{b}	87.00 ± 1.01^{b}	94.33 ± 0.55^{b}	91.33 ± 1.32^{a}
0.5 % BG	100 ± 0.00^{a}	96.63 ± 0.95^{a}	96.50 ± 0.70^{a}	94.51 ± 1.41^{a}	95.55 ± 1.15^{b}	91.97 ± 2.04^{a}
0.7 % BG	100 ± 0.00^{a}	98.58 ± 1.01^{a}	97.4 ± 0.09^{a}	96.43 ± 0.15^{a}	97.45 ± 1.03^{a}	93.22 ± 1.87^{a}

Different letters indicate significant differences between samples at $p < 0.05$ by Duncan test.
Data were expressed as mean ± standard deviation.

Centrifugation test. The effect of the concentration of BG particles on the stability of Pickering emulsion is presented in Table 2. The BG particles concentrations had a significant influence on stability of oil droplets ($p \leq 0.05$). Centrifugation stability of Pickering emulsion containing 0.1, 0.3, 0.5 and 0.7 % of BG particles was 89.51, 94.44, 95.55 and 97.45 %, respectively. These results showed a significant increase in centrifugation stability simultaneously with the increase in the concentration of BG particles in Pickering emulsion ($p \leq 0.05$). In the presence of a high concentration of gum, the stability increased, which is related to forming a thicker surface layer around the oil droplets by these MPs. The sample containing 0.7 % of BG MPs created the strongest layer around the oil droplets with higher mechanical strength (Naji-Tabasi, Mahdian, Arianfar, & Naji-Tabasi, 2020). The lowest stability was observed in the sample containing 0.1 % BG MPs ($p \leq 0.05$). Pickering particles containing 0.1 % and 0.3 % of gum had larger particle size (1.74 and 1.76 μm, respectively) than other samples. It seems that the size of the particles is effective on the stability of the emulsion and ultimately the oil absorption capacity. Also, more particles may help droplet bridging and provide more effective mechanical stability and contribute to gel-like rheology in Pickering emulsions.

Thermal stability of Pickering emulsions. With the increase in temperature, the droplets in the emulsion are easy to gather, leading to the deterioration of the emulsion stability (Ding et al., 2023). The effect of different concentrations of BG particles in Pickering emulsion against heat treatment (80 °C/30 min) is presented in Table 2. The highest and lowest thermal stability was related to the sample containing 0.7 and 0.1 % BG particles, respectively. Although there was no significant difference between the samples containing levels of 0.3, 0.5, and 0.7 % of BG MPs. The results showed that the BG Pickering emulion had high stability against thermal treatment and stability of Pickering emulsion increased significantly with the increase of BG particles concentration. Mirahmadi et al. (2019) reported that Beneh gum has a low glass transition temperature. In this condition, the transition of glass to elastic quickly occurs due to water absorption and can cause physical properties such as adhesion (Mirahmadi et al., 2019). This alteration creates an impermeable coating on the emulsion droplets after heating and causes high thermal stability of BG Pickering emulsion. This structure can improve the elasticity and water retention of the gel (Xiong et al., 2022). According to the results, it can infer that the increase in BGMPs concentration can improve the thermal stability of Pickering emulsion. The beginning of the decomposition temperature of BG is very high (240 °C) (Mirahmadiet al., 2019). Similar findings have been reported in the literature about Pickering emulsions stabilized by glidian/sodium caseinate nanoparticles and konjac glucomannan. Xu and collougose broaded the idea of the processing treatment of Pickering emulsions in food applications. They showed that Pickering emulsions stabilized by glidian/sodium caseinate nanoparticles and konjac glucomannan showed desirable viscoelasticity and particle size even after thermal treatment at 100 °C, and Pickering emulsions formed a gel-like network structure at 70 °C in presence of KGM (Xu et al., 2023). Du et al. (2023) reported that Pickering emulsions stabilized by 15 % (v/v) lentil protein isolate–arabic gum conjugate microgel particles was stable at temperatures between 4 and 85 °C (Du, Wang, Yang, & Chen, 2023).

Rheological properties of Pickering emulsion

The viscosity of Pickering emulsion containing BF particles was summarized in Table 1. The rheological behavior of all emulsions in the shear rate of 1–300 s^{-1} was Newtonian. The viscosity of Pickering emulsion samples containing 0.1, 0.3, 0.5, and 0.7 % of BG MPs were 1.14, 1.16, 1.16, and 1.04 Pa.s, respectively. The viscosity of the PEs was affected by the content of BGNPs. The viscosity significantly increased with the increment of the BG particle concentration from 0.1 to 0.3 and 0.5 % (w/w). Li et al. (2020) reported the intensity of droplet network structure grew with the increase in particle concentration and the viscosity of the Pickering emulsion increased (Li et al., 2020). It seems that reducing the size of the droplets because of the increase of BG particles concentration up to 0.5 % (w/w) led to the increase in the viscosity. Xiong et al. (2022) found that the consistency index increased with the increase in the concentration of Pickering particles, which is it attributed to the formation of a strong coating and surface layer (Xiong et al., 2022). However, this trend was not observed for 0.7 % PGPE, and the viscosity decreased significantly. The viscosity of Pickering emulsion depends on different factors such as contact angle, the ratio of bare droplet radius to solid nanoparticle radius, and the volume fraction of bare droplets (Pal, 2017). The Pickering emulsions rheology is not as well understood as that of traditional surfactant-stabilized emulsions (Pal, 2017).

Conclusion

The increasing attention to Pickering emulsion as encapsulation platforms results in more investigation to find appropriate Pickering partilce. The results showed that Baneh gum particles with anionic structure and surface tension properties could be applied as a stabilizer of Pickering emulsion. The concentration of BG had a significant effect on the size and distribution index of particles, zeta potential, and viscosity of emulsion. By increasing the concentration of BG paticle, the Pickering emulsion droplets were entirely covered and due to the increase in the repulsive forces between the droplets, the stability of the emulsion increased. In addition, the zeta potential showed high absolute values, which indicated the high stability of the Pickering emulsion against aggregation. The creaming index decreased with increasing microparticle concentration. Based on the results, 0.7 % BG Pickering emulsion was more stable against heat and centrifugation. Therefore, the solid particles of 0.7 % BG can be a good candidate for stabilizing the interface between oil and water in Pickering emulsion.

Ethics declarations

Ethics approval and consent to participate: Not applicable.
Consent to participate: Not applicable.

CRediT authorship contribution statement

Sara Naji-Tabasi: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **Monir-sadat Shakeri:** Writing – review & editing. **Atena Modiri-Dovom:** Investigation, Software. **Saeedeh Shahbazizadeh:** Software, Writing – original draft.

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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