

Developing edible *oleogels* structure prepared with emulsion-template approach based on soluble biopolymer complex

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ARTICLE INFO

Keywords:

Basil seed gum
Rheological properties
Structured edible oil
Xanthan gum

ABSTRACT

The utilization of edible oleogels as oil structures has been proven to comprise a suitable alternative to fat. In this research, whey protein concentrate (WPC) oleogel structure was designed and improved by the creation of a soluble complex of WPC-basil seed gum (BSG) and WPC-xanthan gum (XG) at different concentrations (0.2, 0.4, and 0.6 % w/w). The results showed increasing the hydrocolloids had positive effects on oleogel characteristics, which can preserve oil well in the microstructure network of oleogel. Additionally, the incorporation of hydrocolloids promoted stability of oleogels against stress and heat. Therefore, the centrifuge stability of WPC oleogel was 26 and increase to ~98 and 100 % in 0.6XG:WPC and 0.6BSG:WPC oleogels, respectively. The evaluation of the rheological properties revealed the predominant elastic behavior of the oleogels. Overall, the addition of either XG or BSG into WPC-based oleogel improved its physicochemical and mechanical characteristics. Moreover, oleogels prepared using 0.6 % BSG-5 % WPC exhibited the best properties.

1. Introduction

Although the use of solid fats in foods improves technological and sensory characteristics, it may adversely affect human health (Romagny et al., 2017), colon, diabetes, cancer, and cardiovascular diseases (De Souza et al., 2015). In recent years, people have become more concerned about their dietary health, and their diet has shifted toward the production and consumption of reduced-fat foods. Therefore, the scientific community has actively researched the development of product formulations using fat substitutes with applications in the food industry.

Oleogels are self-standing systems that can be applied to create low fat without changing the chemical composition (Patel & Dewettinck, 2016). They are capable of trapping edible liquid oil into a tridimensional network showing different features of solid fat, such as spread ability, and increasing firmness, being used in food products like cookies or creams as a substitute for trans fats, and in the encapsulation and control release of bioactive components (Li et al., 2021; O'Sullivan et al., 2016). There are different ways to generate oleogelation and oil structuring, including direct dispersion, structured biphasic systems, oil

sorption, and indirect method (emulsion-template) (Patel & Dewettinck, 2016). The studies of the use of oleogel formation through the emulsion-template are an indirect method to prepare structured oil. This method includes a multi-step process to entrapped oil in a stable structure: (i) formation and stabilization of oil-in-water emulsion, (ii) hardening of the adsorbed surface layer through cross-linking or complex formation, (iii) removing the water phase from the emulsion by a drier system, and (iv) shearing gently the dried solids to obtain a desirable oleogel (Romoscanu & Mezzenga, 2006).

In order to obtain an oleogel with a strong structure, it is necessary to focus on the initial interaction of the biopolymers, followed by the structure of the emulsion. Therefore, the utilization of suitable biopolymers may enable the production of oleogels with suitable properties. Oil-in-water emulsions are the basis of many industrial processes, although the emulsions are thermodynamically unstable systems (McClements & Jafari, 2018). The soft-solid materials called ‘emulsion gels’ consist of the aggregated emulsion droplets in a network (emulsion particulate gels). Emulsion gels possess both emulsion and gel properties. The oil droplets are protected by a gel network and increase their

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<https://doi.org/10.1016/j.fochx.2024.101917>

Received 2 July 2024; Received in revised form 20 September 2024; Accepted 20 October 2024

Available online 22 October 2024

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stability in the emulsion gel structure (Lin et al., 2021). Therefore, many food products can be classified as emulsion gels, which including a wide range of traditional and industrial food products such as cheese, ice cream, processed meat, spreads, desserts, and yogurt (Farjami & Madadlou, 2019).

Proteins have traditionally been used as biopolymers in the production of emulsion gels, capable of stabilizing oils in water dispersion due to the presence of amino and carboxyl moieties, which increase surface activity (Isnaini et al., 2021). More specifically, whey proteins are widely used in the food industry due to their availability, nutritional, and functional properties, such as emulsifying and gelling agent, thickening, and water-binding capacity (Wang et al., 2023). Thereby, whey proteins are located on the emulsion droplet surface creating an electrostatic barrier against flocculation and coalescence of the disperse phase, stabilizing these complex systems accordingly (Ye & Singh, 2006). Proteins can create a strong interface layer in the emulsion system that following that a strong network of oleogel will be formed. The use of protein combined with polysaccharides increase the structure strength in the colloidal systems due to the synergistic effect. (Wijaya et al., 2017). Polysaccharides do not have the proper function to create strong bonds with oil due to not be sufficient molecular structure, while they work well in aqueous solvents. Therefore, the simultaneous use of protein and polysaccharide results in a cross-linked structure at the oil-water interfaces (Romoscanu & Mezzenga, 2006). The functional properties of the protein-polysaccharide interaction depend not only on the molecular properties of each polymer, but also on the nature of the interactions between them (Sarraf et al., 2021). Hydrocolloids are often used as gelling agents to achieve the desired textural properties in food formulations. Basil seed gum (BSG) is a native hydrocolloid used in the food industry and is extracted from the plant so-called *Ocimum basilicum* L., whose seeds are covered with mucilage. The mucilage obtained from the basil plant has been widely used as a stabilizer in the formulation of pharmaceutical suspensions and surfactant-free emulsions (Razavi & Naji-Tabasi, 2017).

Xanthan gum (XG) is another hydrocolloid produced by *Xanthomonas campestris* (Murad et al., 2019). The xanthan gum solution exhibits a pseudoplastic behavior and its viscosity decreases with increasing shear rate. In the last decades, this hydrocolloid has drawn the attention of the food industry due to its interesting properties, including its high water solubility, stability over a wide range of pH, and temperature, gelling and stabilizing properties (Murad et al., 2019).

In this study, oleogels were prepared by the indirect method using continuous aqueous phase emulsions as templates over a wide range of XG and BSG concentrations, and the effects of oleogelator concentration on the gel formation and mechanical strength were evaluated. The structural, mechanical, and physicochemical properties of the oleogels prepared with hydrocolloids from different sources were also compared. One of them is XG, a common gum with low cost and wide availability, and the other one is BSG, a new gum, which although it has mass production in different areas, its use is up to now limited. The comparison of different gums may help to find a suitable alternative to common gums and a further understanding of the self-assembled structures of oleogels that would allow its utilization as an alternative to fat. Finally, the prepared oleogels were extensively characterized through rheological and textural tests, oil binding capacity experiments, confocal microscopy, and XRD analysis.

2. Materials and Methods

2.1. Chemicals and reagents

Basil seeds were obtained from an herbal market in Mashhad (Iran). BSG was subsequently extracted according to the procedure described elsewhere (Sarraf et al., 2023). XG (Sigma, Germany) and WPC containing 70 % protein were purchased from Westland (New Zealand). Hydrochloric acid (HCl), sodium hydroxide (NaOH), calcium chloride

(CaCl₂), sodium azide (NaN₃), sodium thiosulfate (Na₂S₂O₃), and potassium iodide (KI) were supplied from Merck Co. (Germany). Commercial sunflower oil (Ladan, Iran) was used for the preparation of the oleogels. Purified deionized water was used for the solution preparation. All chemical products were of analytical grade.

2.2. Preparation of oleogel by emulsion-template method

Stock solutions (WPC 5 % and hydrocolloids ranged between 0.2, 0.4, and 0.6 % wt in deionized water) were stirred for 2 h and they were left overnight for complete hydration in the refrigerator ($\approx 5^{\circ}\text{C}$). Sodium azide (0.02 % w/w) was added to prevent bacterial growth.

The emulsion gels were prepared using BSG:WPC and XG:WPC ratios of 1:1 while adjusting pH to 6 by HCl and NaOH 0.1 N (Normality). The mixtures were placed in a heating circulator (Julabo, EH 19) at 85°C for 30 min and cooled immediately. In the next step, 40 % sunflower oil was added to gum:WPC mixtures and pre-homogenized with an ultraturrax (IKA, TG25) at 10,000 rpm for 2 min and then homogenized using a high-pressure homogenizer device (HL1.2, HST, Germany), at 20 MPa for 150 s at adjusted pH = 6. Finally, 10 mM CaCl₂ (as cross linker) was added to the emulsions and subsequently stirred (Sarraf et al., 2021).

Next, emulsion gels were freeze-dried (FDU-8606, OPERON, South Korea) applying a vacuum of 10^{-4} Torr, at $-50 \pm 5^{\circ}\text{C}$ for 72 h (Patel et al., 2015) to remove the water from the samples. To obtain the desired structure of the oleogel, the sample was briefly sheared with a mechanical homogenizer with the lowest stirring speed (RZR 2102 control, Heidolph, Germany) for 30 s.

2.3. Microstructure studies

The Confocal Scanning Laser Microscopy technique (CONFOCAL LEICA STELLARIS 8 STED, Leica Microsystems, Germany) was used to evaluate the microstructure of the formulated oleogels. During the preparation of oleogels, Fast Green reagent (0.2 g/L in water) and Nile Red dye (0.1 g/L in water) were used to stain the water and oil phases, respectively (Zembyla et al., 2018). The oleogels were placed on a laboratory-made well slide and a cover slip with 0.17 mm thickness. The coverslip was then sealed with lacquer to prevent the sample from drying out. Care was taken to ensure that there were no trapped air gaps or bubbles between the mixture and the coverslip. The mixtures were equilibrated for 5 min before testing. The emulsions were scanned with a glycerol immersion objective lens, 93 $\times$ at 25°C . Fluorescence was excited from the sample with lasers at 619 and 555 nm. Images were acquired and processed using Leica Application Suite X (LAS X) software. Each image was 1024 $\times$ 1024 pixels.

2.4. Colorimetric analysis

The colors of the oleogels were measured using a computerized system with five fluorescent lamps (Opplé, 8 W, model: MX396-Y82; 60 cm in length) with a color index (Ra) close to 95 %. The system consists of a digital camera (Canon EOS 1000D, Taiwan) with a lens focal length of 35 mm for color analysis and 45 mm, an image capture box a wooden box, 45 cm above the sample and at an angle of 45° with sample plane to give a uniform light. Colorimetric images were recorded in TIFF format. Upon placing the samples on a plate, color analysis was performed on three indications a* (red to green from +a* to -a*), b* (yellow to blue from +b* to -b*), and L* (darkness to brightness from 0 to 100). Data analysis was performed using Image J software. The parameters L*, a* and b* of the prepared images (resolution of 300 dpi) with bmp format were evaluated by the Image J software.

2.5. XRD analysis

The measurements of the X-ray diffraction patterns of the oleogels were evaluated according to Meng et al., 2018 with modifications based

on the conditions of the equipment. XRD analysis was performed with an X-ray diffractometer (GNR Explorer, Italy) equipped with a Cu source ($\lambda = 1.5458 \text{ \AA}$) at 30 mA and 40 kV. The different angles were set at 2θ ($5\text{--}30^\circ$) with a step size of 0.03° (0.03 s) and the disc thickness ~ 0.55 mm with detector scintillator at room temperature (Meng et al., 2018).

2.6. Characterization of oleogels

2.6.1. Stability of oleogels by centrifugation tests

The stability of the oleogels was assessed by centrifugation (Heidoph, Germany) at 6000 rpm for 30 min.

The thermal stability of the oleogels was measured by heating the samples at 80°C for 30 min in a water bath before centrifugation.

$$ES (\%) = \left(\frac{e_v}{t_v} \right) \times 100 [2]$$

where e_v is the oleogel volume after centrifugation and t_v is the total volume of the oleogel (Naji-Tabasi et al., 2020).

2.6.2. Storage stability test

Oleogels were placed in glass tubes at room temperature and the stability was determined every week for a month (Almeida & Bahia, 2006). The storage stability was calculated according to Eq. 2.

2.6.3. Oxidative stability

The oxidative value (PV) of the formulated oleogels was done according to Park et al. (2018) method. 1 g of the oleogel heated was mixed with 6 mL of the acetic acid/chloroform solution (3:2, w/w). then 100 μL of saturated KI was added and stirred for 1 min. After that, 6 mL of deionized water was added and shaken on a hot plate for 20 s, followed by 0.6 mL of 1 % starch indicator was added and titration with $\text{Na}_2\text{S}_2\text{O}_3$ or Sodium thiosulfate (0.01 N). Volumes of required for the titration were recorded. Peroxide index was evaluated as mEq O_2/kg on the 1st, 7th, 15th, and 30th day of storage at refrigerator temperature (4°C). Peroxide value indicates the amount of primary products formed during oxidation (Park et al., 2018).

$$PV = \frac{(O - B) \times N \times 1000}{W} \quad (1)$$

where O and B are the titration volume of oleogel and blank (Carrying out the test process in the absence of an oleogel), respectively, W is the weight of oleogel and N is the normality of the sodium thiosulfate solution.

2.7. Rheological measurement

The rheological properties of the oleogels were performed using a Physica MCR301 controlled-stress rheometer (Anton Paar, Germany), equipped with a 25 mm serrated parallel plate and an electric and convection chamber (H-ETD 400). Firstly, stress sweep tests were conducted at a constant frequency of 1 Hz, at both 25°C and 80°C , within the shear stress range from 0.01 to 1000 Pa, with a gap of 1 mm, in order to identify the linear viscoelasticity range (LVR). Therefore, an appropriate shear stress inside the LVR was selected subsequently applied in the successive frequency sweep experiments. The oleogels were placed on the rheometer plate and were allowed to relax for 5 min before the testing. The viscoelastic measurements were carried out at 25°C and 80°C and the reported results are the average of at least three replicates.

2.7.1. Frequency sweep

The frequency sweep tests were performed at a constant stress inside the LVR applying a frequency ranging from 3×10^{-2} up to $10^2 \text{ rad}\cdot\text{s}^{-1}$ to reveal frequency dependence of the oleogels at a constant temperature (25°C and 80°C). The storage modulus (G'), loss modulus (G''), loss tangent ($\tan \delta$), and complex viscosity (η^*) were measured.

2.7.2. Steady-state shear test

Flow properties of the prepared oleogels were also evaluated by means of flow sweep tests at 25°C and 80°C and within the shear rate range from 10^{-2} – 10^2 s^{-1} (25 mm diameter serrated plate-to-plate, 1 mm gap).

2.8. Texture analysis

The textural properties of the oleogels were measured by a texture analyzer (Stable Micro System, TA-Xt Plus, England) applying back extrusion method. The specifications of the process included: a flat (disc-like) probe with 35 mm diameter at a depth of 10 mm with a rate of $1 \text{ mm}\cdot\text{s}^{-1}$ at 25°C (Patel et al., 2015). From these experiments, hardness, adhesiveness, consistency, and apparent elastic modulus parameters were extracted and evaluated (Ahmed et al., 2005).

2.9. Statistical analysis

All measurements were analyzed as means $\pm$ SD using a completely randomized factorial design and the data were analyzed using SPSS (version 11.0) according to analysis of variance (ANOVA). Significant differences between means values were identified by Duncan's multiple range test ($P \leq 0.05$) and the curve was plotted using Excel 2013 and Origin Lab 2017 software.

3. Results and discussions

3.1. Microstructural features

In confocal images of oleogels, the oil, and protein were stained with Nile Red and Fast Green, respectively. According to the Fig. 1, systems prepared exhibited corrugated and layered surfaces with a number of pores (black spaces). This phenomenon was caused by the contraction occurring through the lyophilization process. The colloidal interactions resulted in a structure consisting of two separate phases of protein and liquid oil, apparently due to their thermodynamic incompatibility. The existence of two separated phases evinced the whey protein denaturation due to heating or tension, giving rise to the appearance of large whey protein aggregates.

Although oil droplets may begin to leave the network during the process of removing water from the continuous phase, all emulsion gels were stable during the drying process and no phase separation occurred. Indeed, the presence of an oleogel structure between the oil and water phases enabled the proper separation of the dispersed phase droplets. Therefore, the formation of a gel network through lyophilization fostered the emulsion stability of particle-stabilized gels against oil separation (Zhu et al., 2017). Therefore, regardless of the gums: WPC ratio, no significant difference was observed between the crystal morphology of oleogels.

As shown in Table 1, the color of the oleogel produced by WPC had the highest L^* value (64.15), and increasing percentage of gum decreased the L^* that indicated brightness index in oleogel's structure and resulted in the production of darker colors in the oleogels. The reduction in the L^* values is probably due to the intensity of the light as the porosity value decreases and the coherent structure increases with increasing percentage of gum concentrations. Thus, L^* parameter of 0.6 BSG:WPC and 0.6 XG:WPC was darker compared to WPC oleogel. This indicates that the color of the gums has a significant effect on the final color of the oleogels. The oleogels also appeared more opaque owing to the intense light scattering caused by the higher gums' content (Moradabbasi et al., 2022).

The results of a^* showed a steady increase and there was a significant difference between a^* value of the WPC oleogel (1.17) and that one ascribed to 0.6BSG:WPC oleogel (2.75). On the other hand, all oleogels had positive b^* values and there was a significant difference in b^* factor between the WPC and concentrations of 0.2 gum oleogels (0.2BSG:WPC

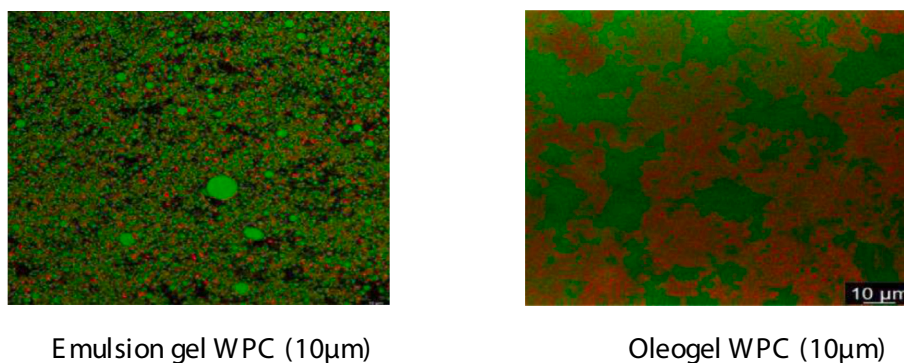


Fig. 1. Confocal images of oleogels stabilized by WPC compare with emulsion gel.

Table 1

Parameters L^* , a^* , b^* of oleogel stabilized by different concentrations of WPC, XG:WPC and BSG:WPC. * and *

Concentration (%)	Parameter		
	Color Analysis		
	L^*	a^*	b^*
WPC	64.15 ± 0.04^a	1.17 ± 0.03^e	6.61 ± 0.05^a
0.2 XG:WPC	57.11 ± 0.04^b	1.14 ± 0.05^f	4.10 ± 0.01^c
0.4 XG:WPC	47.02 ± 0.10^e	1.18 ± 0.05^e	5.33 ± 0.06^b
0.6 XG:WPC	46.84 ± 0.23^e	1.58 ± 0.05^c	5.73 ± 0.10^b
0.2 BSG:WPC	47.22 ± 0.30^e	1.46 ± 0.06^d	4.38 ± 0.01^c
0.4 BSG:WPC	48.67 ± 0.07^c	1.82 ± 0.04^b	5.61 ± 0.08^b
0.6 BSG:WPC	47.48 ± 0.09^d	2.75 ± 0.29^a	5.79 ± 0.43^b

* Values followed by the same letter in column are not significant difference ($P < 0.05$).

* Means $\pm$ standard deviation.

and 0.2XG:WPC) which had the highest and lowest b^* parameters, respectively. The highest level of yellowness was associated with the protein sample. By increasing of gum concentration to 0.2 % (w/w), the amount of yellowness decreased, which showed better coverage of oil in the presence of gums and the results showed a similar evaluation between both gums.

Higher a^* values might be attributed to the variation in moisture retention which results in light reflection (Rajkumar et al., 2016). On the other hand, Rather et al. (2021) reported that the higher increase in b^* values of control samples are related to its higher oxidation (Rather et al. 2021).

3.2. XRD analysis

XRD is a rapid analytical technique; even at low moisture content (Mohsin et al., 2018) such as oleogels, which was used to obtain the internal structure of the oleogels. All samples created a uniform structure with liquid oil binding without leakage.

According to Jaberi et al. (2020), the polysaccharide chains help to form a uniform structure that coated the oil droplets (Jaberi et al., 2020). Therefore, the elasticity of fat mainly depends on its internal network and microstructure (Qu et al., 2024). In analysis of obtained XRD patterns of oleogels WPC, XG:WPC and BSG:WPC in Fig. 2 were very similar each other and only the amount of diffraction intensity was different in the samples.

One of the parameters analyzed in this test is the d-spacing that can indicate the cell parameters and specific crystal types. Since the no crystal diffraction peak was found in among of oleogels, all samples exhibited the only a weak XRD peak, which were probably caused by a mixture of weakly crystalline and amorphous structures. Its amount at the crystal surface spacing $d = 4.45 \text{ \AA}$ was estimated that were wide and smooth, which may be created by the amorphous scattering of a large

amount of liquid oil trapped in the oleogel sample (Qu et al., 2024).

3.3. Oleogel stability

3.3.1. Stability by centrifuge tests

Oil binding strength is one of the most important factors that can be used to estimate the physical stability of oleogels and show the ability of the crystalline matrix to trap oil droplets (Marangoni 2012). The effect of XG and BSG concentrations on the oil binding capacity of the oleogel was evaluated using the centrifuge method at 25 and 80 °C and the results are shown in Table 2. It is obvious that the oil loss values of the samples decreased with the increase of gum concentration at 25 °C, so that, the oleogels with the highest gum concentrations (0.6BSG:WPC and 0.6XG:WPC) manifested enhanced stability due to their stronger network. Thus, the oleogel using only WPC showed the lowest values, highlighting the greater weakness of its structure.

Additionally, the stability of the structure was investigated at 80 °C, and the results showed that oleogel 0.6 BSG:WPC presented the highest structural stability against the thermal process, since this sample succeeded in maintaining its structure with no loss of oil. The stability of BSG:WPC oleogels was higher compared to XG:WPC in both thermal and non-thermal conditions. It shows the efficiency of oleogel containing BSG in structuring and entrapping oil droplets.

This condition can be related to two factors: on the one hand, it is related to the particle size of the initial emulsion gel for the preparation of oleogels. The volume average of the diameter of the particles or $D_{[4,3]}$ of emulsion gels were measured: WPC (17.80), 0.2BSG:WPC (2.05), 0.4BSG:WPC (11.50), 0.6BSG:WPC (9.22), 0.2XG:WPC (4.00), 0.4XG:WPC (8.77), and 0.6XG:WPC (5.26) μm (Sarraf et al., 2023). According to the particle size results a reduction from 9.22 to 5.26 μm (Sarraf et al., 2023), when 0.6 BSG:WPC is added. Thus, the higher concentration of polysaccharides increased the oleogel stability.

On the other hand, in the study that Sarraf et al. (2023) did on the primary emulsion gel (which use to prepare oleogels of this study), the ζ -potential values attributed to 0.6 BSG:WPC and 0.6 XG:WPC were measured -28.60 and -26.00 mV, respectively. The higher the ζ -potential of an emulsion gel, either positive or negative, the more likely the repulsive forces will exceed the attractive forces, thus ensuring the system stability (Sarraf et al., 2023).

The textural analysis and rheological properties of the oleogels also showed that the hardness and consistency and elastic properties of the oleogels had an upward trend and the network strength improved in the presence of the biopolymer complex compared to the WPC-based reference systems, which had an impact on the stability of the protein-polysaccharide-based oleogel. In the comparison between both polysaccharides, BSG showed more stability, so that no oil leakage was observed at 0.6BSG:WPC, neither at 25 °C nor at 80 °C.

Although the stiffness and strength of the gel decreased with temperature, reducing the oil storage capacity, the oil droplets within the oleogel structure were above 70 % with the addition of gum. This

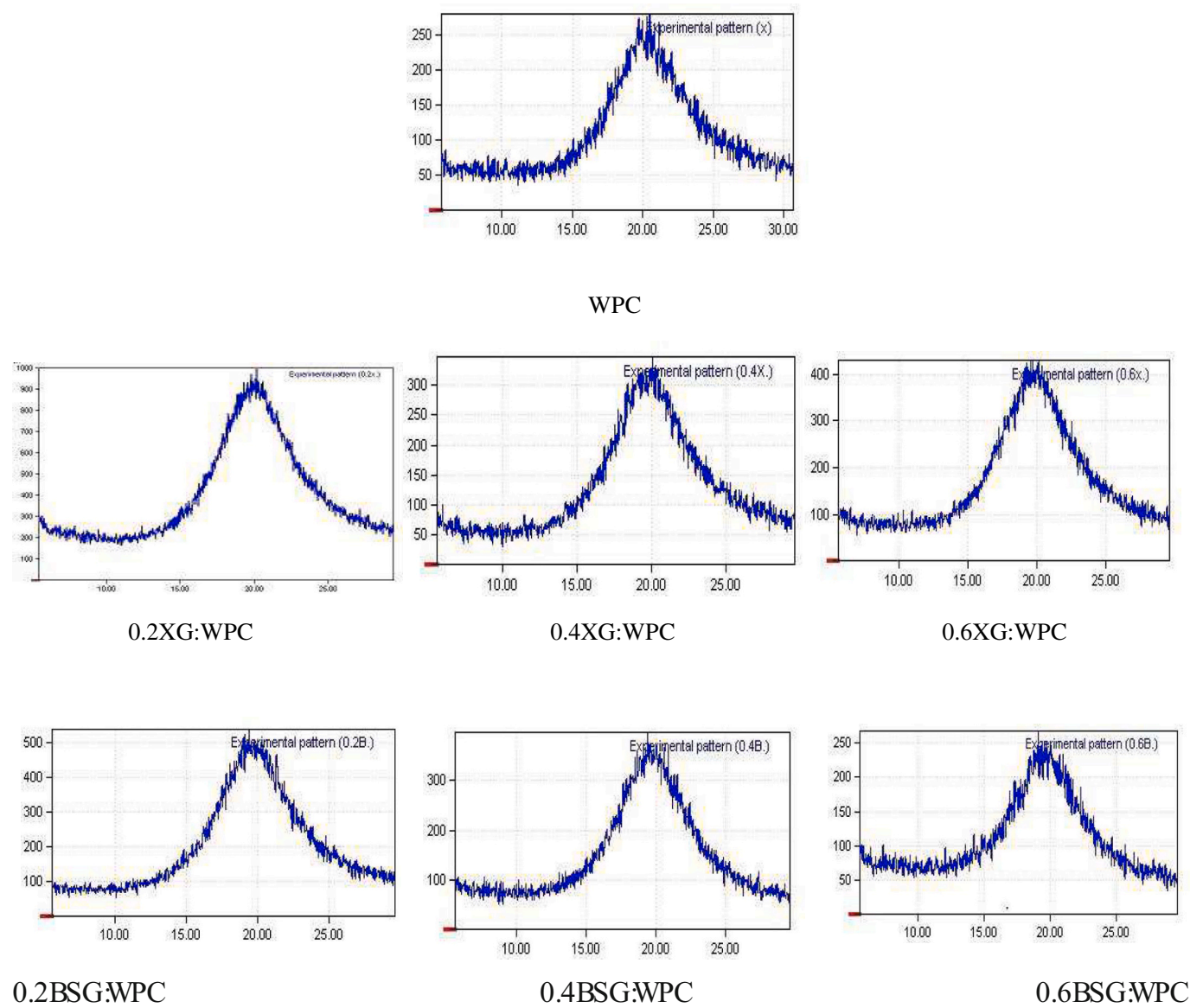


Fig. 2. XRD diffraction patterns of oleogels stabilized by WPC, XG:WPC and BSG:WPC.
*X-axis: 2θ (Degrees).
*Y-axis: Intensity (Arbitrary unit).

Table 2
Oil holding capacity of oleogel stabilized by different concentrations of WPC, XG:WPC and BSG:WPC.

Concentration (%)	Parameter	
	Stability by centrifuge (%)	
	25 °C	80 °C
WPC	26.10 ± 0.25 ^d	19.23 ± 3.05 ^d
0.2 XG:WPC	75.35 ± 3.79 ^c	69.75 ± 3.00 ^c
0.4 XG:WPC	80.92 ± 3.08 ^{bc}	74.10 ± 5.48 ^c
0.6 XG:WPC	97.80 ± 2.22 ^a	88.63 ± 1.98 ^b
0.2 BSG:WPC	77.00 ± 5.29 ^c	71.00 ± 2.00 ^c
0.4 BSG:WPC	85.70 ± 4.04 ^b	75.50 ± 2.25 ^c
0.6 BSG:WPC	100.00 ± 0.10 ^a	100.00 ± 0.10 ^a

*Values followed by the same letter in column are not significant difference (P < 0.05).
*Means ± standard deviation.

indicates that the oleogel structure increases stability against

temperature and stress. Some studies confirm the efficiency of the oleogel structure in trapping oil (Abdollahi, 2016; Fayaz et al., 2017). Abdollahi et al. (2020) reported that XG increased the viscosity, density, and hardness of the network, as well as the oil binding capacity by more than 92 %. However, it did not affect the moisture absorption ability. On their part, Meng et al. (2018) stated that among the oleogels containing carboxymethyl cellulose and xanthan, sodium alginate, arabic guar, flax seed, and bean seed, those containing XG in their formulation showed the lowest oil loss (Meng et al., 2018).

3.3.2. Storage stability test

One of the most important properties used to determine the physical stability of oleogels is storage stability. It illustrates the ability of crystalline networks to trap oil droplets (Marangoni, 2012). The weekly examination of the oleogels revealed that the stability of the oleogels were the same during one month of storage at 25 °C and did not show any difference at the end of 4th week. Consequently the samples studied developed stable matrices with the presence of polysaccharides which created a stronger texture with higher consistency in the oleogels,

preventing the oil leakage (Giacomozzi et al., 2021) Kamali et al., 2019). After one month, the appearance of an oily surface in the system containing 0.2 % gum:WPC manifested its structural instability. The change may be related to the higher particle size of the emulsion gel prepared with WPC (17.80 μm) (Sarraf et al., 2023) which indicates the effect of hydrocolloid content on the stability of the oleogels. Therefore, the control (WPC) and 0.2 XG:WPC and 0.2 BSG:WPC had the lowest ability to maintain the structure, which is related to the characteristics of their primary emulsion gels such as droplet size, stability, and hardness. (Sarraf et al., 2023). On the other hand, no change was observed in oleogels containing 0.4 and 0.6 gum:WPC over one month of storage. Habibi et al. (2022) prepared a combination of oleogel and hydrogel (K-carrageenan and WPC) and demonstrated that the highest rheological properties, hardness, and oil storage capacity were related to gels containing smaller particles. Moreover, it was reported that the oil droplets were surrounded by the biopolymer layer, hence preventing their coalescence. Velez-Erazo et al. (2020) confirmed that the prepared oleogel structure was stable for four weeks and could be used as a fat substitute in food. Furthermore, Espert et al. (2022) highlighted the superior structural properties of oleogels containing XG in comparison to other polysaccharides, since XG favored the oil droplets' entrapping. This is probably due to the high capacity of XG to form hydrogen bonds and hydrophobic interactions, providing improved stability to the oleogel (Pan et al., 2021). These studies showed that both BSG and XG, increase the stability of oleogels during storage.

In addition to this, Kim et al. (2021) reported that the stability of mayonnaise increased when raising the BSG content, identifying the highest stability with a 0.8 % BSG content in the sample. It was also reported that the presence of SUPER-BSG (part of the BSG fraction with molecular weight 1045 kDa) in BSG increased its emulsifying performance. The reason for this is related to the increase in uronic acid, flexibility, and also the expansion of the hydrophobic part, which improves the stability of the emulsion (Kim et al., 2021).

3.3.3. Oxidative values during storage

Oxidation is one of the most important phenomena affecting the oils quality. The amount of initial oxidation products (hydroperoxides) was measured by evaluating the peroxide index every week. Previous studies have shown that the oleogel network can delay oxidation while fostering oil stability (Abdollahi et al., 2020).

The results showed that there was no significant change in PV during the first three weeks of storage. However, the amount of PV was 0.24 and 0.23 mEq $\text{O}_2\cdot\text{kg}^{-1}$ for the oleogels with WPC and 0.2 XG:WPC in the 4th week, respectively (Fig. 3). To this respect, Lim et al. (2017)

reported that the oleogelator made a significant impact on the peroxide index evolution due to the likely limited oil mobility that has been theoretically observed in other studies. Additionally, Giacomozzi et al., 2021 reported that the structural features of oleogels can delay the oxidation reactions, so that their crystalline network may act as a barrier that obstructs the entry of oxygen at the reaction sites.

Because of this, stability of proposed XG and/or BSG-based edible oleogels favored at room temperature, under refrigeration and centrifugation and oleogelation process did not promote the degradation of their components that can be valuable in commercial production.

3.4. Rheological properties

3.4.1. Frequency sweeps

Viscoelastic properties evaluation in viscoelastic linear range can identify three types of systems: dilute solutions, concentrated solutions and gels (Kutz, 2019). As shown in Fig. 4, at 25 °C the G' values of the oleogels, which is indicative of the strength of the gel, were higher than G'' , indicating their predominant elastic behavior within the whole frequency range evaluated. The values of G' in all oleogels containing 0.4 and 0.6 % gum: WPC was almost 10^5 Pa, which was observed to be quite constant during frequency changes, except for the 0.2 % oleogels, and also WPC ($\sim 10^4$ Pa). Regarding G' modulus, all samples showed a stable trend with increasing frequency, at 25 and 80 °C, which confirmed a strong gel structure.

As can be inferred from Table 3, the storage modulus, loss modulus, complex viscosity, and loss tangent were examined at 1 Hz and 25 and 80 °C. WPC oleogel exhibited the lowest G' values (10,870 Pa), while the highest value was obtained by 0.6 XG:WPC (145,701 Pa) and 0.6 BSG:WPC (233,928 Pa). The highest G'' was also associated with the 0.6 % gum oleogels and the lowest value was attributed to the oleogel prepared using only WPC.

Overall, the sample with 0.6 % BSG:WPC had the highest G' and G'' and the lowest loss tangent of all the oleogels (Table 3). According to the loss tangent ($\tan \delta$), which shows the ratio of lost energy to stored energy in each cycle, all the data remained below 1, indicating the elastic behavior of the samples. The loss tangent values decrease by increasing the gum concentration. Consequently, the incorporation of higher concentrations of hydrocolloids provides oleogels with stronger elastic behavior (Tanislav et al., 2022).

Furthermore, the complex viscosity (η^*) of oleogels containing 0.6 % of gums was higher compared to samples containing less gum (Table 3). More specifically, η^* value of WPC oleogel was 11,226 Pa.s, which increased to 234,057 and 145,902 Pa.s for samples 0.6 BSG:WPC and 0.6

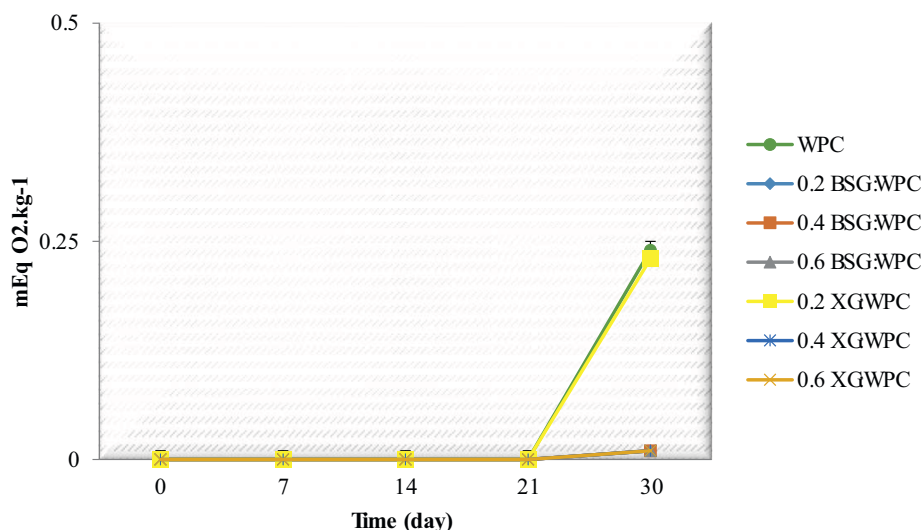


Fig. 3. Peroxide index of oleogels stabilized by different concentrations of WPC, XG:WPC and BSG:WPC during storage.

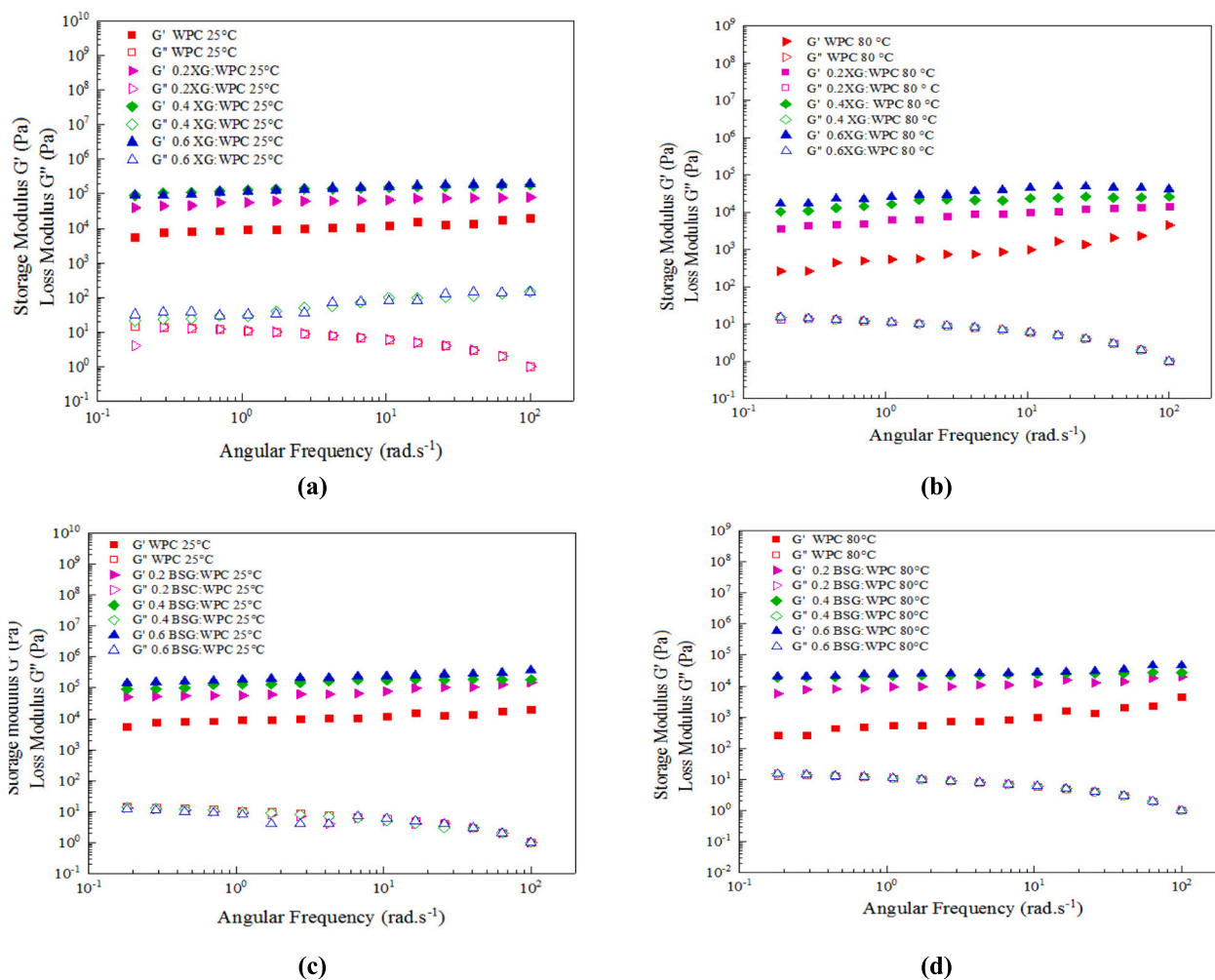


Fig. 4. Frequency sweep of oleogels stabilized by WPC (a-d), XG:WPC (a and b) and BSG:WPC (c and d) at 25 (left) and 80 (right) °C.

Table 3

Viscoelastic parameters of oleogels XG: WPC and BSG:WPC (G' , G'' , $\tan\delta$ and η^*) at 25 and 80 °C.

Oleogel (%)	Temp (°C)	G' (Pa)	G'' (Pa)	η^* (Pa.s)	$\tan\delta$
WPC	25	10,870.77	2801.87	11,226.00	0.25
	80	834.29	390.88	146.71	0.47
0.2 XG:WPC	25	64,242.69	7061.24	21,508.15	0.11
	80	9016.26	3036.48	1514.94	0.34
0.4 XG:WPC	25	144,269.77	6900.17	144,434.68	0.048
	80	20,753.59	18,179.31	4393.28	0.88
0.6 XG:WPC	25	145,701.72	7644.52	145,902.12	0.052
	80	39,021.66	42,560.17	9194.47	0.92
0.2 BSG:WPC	25	65,153.39	3010.06	65,222.89	0.046
	80	10,915	6423.63	4232.32	0.59
0.4 BSG:WPC	25	174,107.64	6089.24	174,214.09	0.035
	80	23,317.18	7228.46	11,877.99	0.31
0.6 BSG:WPC	25	233,928.57	7754.55	234,057.06	0.033
	80	26,172.09	74,228.47	12,533.014	0.35

XG:WPC, respectively. Based on these results, BSG-based systems presented a relatively higher elastic response when compared to XG-based oleogels, which shows more stable network formed by 0.6 % BSG. Hosseini-Parvar et al. (2015) reported that increasing the concentration of gums increased the chain interactions (such as BSG), leading to the formation of a denser network structure. Therefore, the concentration of gums creates a more elastic structure in the oleogel that has been attributed to the formation of the BSG and XG network throughout the protein matrix. According to Liu et al. (2008) the gel formed by pectin

links with other materials comprised the formulation of denser structures characterized by higher storage moduli. Moreover, owing to the high G' values, as well as the insignificant reliance of the viscoelastic moduli on frequency demonstrated the strength of the formulated oleogels (Espert et al., 2022).

All evaluated parameters including G' , G'' , η^* and $\tan\delta$ of oleogels were also investigated at 80 °C. By examining these characteristics results, similar results to obtain at 25 °C were observed (Table 3). However, the purpose of analysing the viscoelastic properties in two temperature ranges (25 and 80 °C) was to investigate the tolerance of oleogels against thermal changes. Although the trend of the results was the same in both temperatures, the viscoelastic moduli of the oleogels presented an inverse relationship with temperature, attaining lower moduli values (G' , G'') when raising temperature to 80 °C, so that the oleogel containing WPC had a G' of 10,870 and 834 (Pa) at 25 and 80 °C, respectively. Also $\tan\delta$ of 0.6 BSG:WPC oleogel declined from 0.35 and 0.03 with increasing the temperature which shows the temperature dependence on the elastic properties of samples.

3.4.2. Steady-state flow curve tests

The effect of shear rate on the viscosity of the oleogels at 25 and 80 °C was also investigated. In all cases, a non-newtonian shear-thinning response was observed, in which the apparent viscosity decreased with shear rate (Fig. 5).

At the beginning of the process at 25 °C, the steady state viscosity was about 10^6 Pa.s and the viscosity was less than 10 Pa.s at a shear rate of 10^3 s⁻¹. In general, a similar oleogels viscosity reliance on the gum

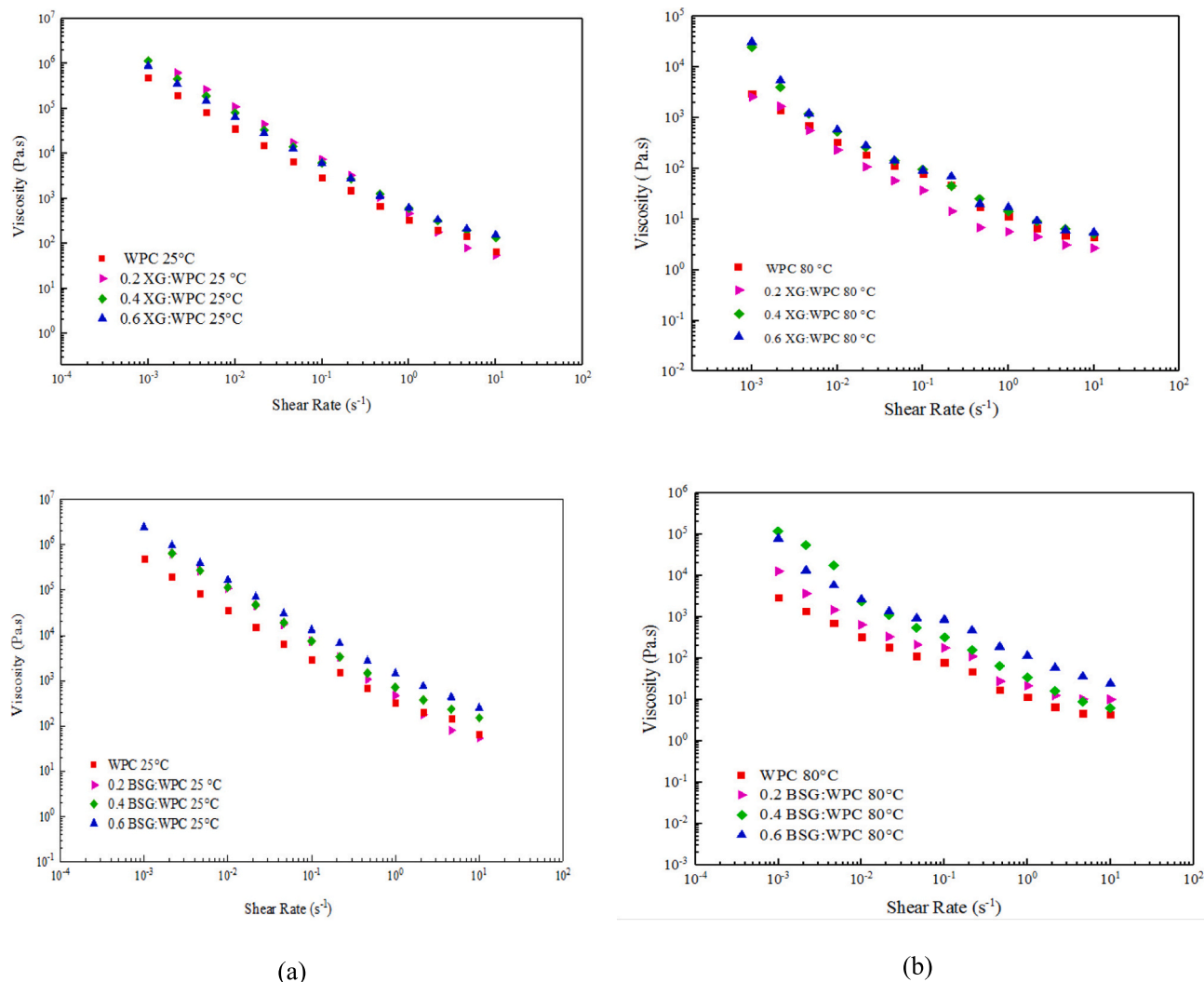


Fig. 5. Steady-state flow curves of oleogels stabilized by WPC, XG:WPC and BSG:WPC at 25 °C (a) 80 °C (b).

concentration was observed when raising the temperature to 80 °C, although the apparent viscosity values are lower than those achieved at 25 °C. Thus, the results illustrated that heat have a negative sign on viscosity and stability of structure and the temperature increase had a significant weakening effect on their structure.

3.5. Texture analysis

Table 4 shows the effect of different concentrations of hydrocolloids on the textural properties of the oleogels. Thus hardness reflects the strength of the gel structure under compression. The oleogels containing 0.6 XG:WPC (1837 g) and 0.6 BSG:WPC (3284 g) had the highest values of hardness, and also the stiffness parameter. It was found that oleogels prepared with higher BSG and XG concentrations resulted in better network structure and more trapped oil droplets in comparison with samples with lower concentrations (Meng et al., 2018).

Another important parameter is adhesiveness, which is the negative force area of the first surface characteristic and introduces the flow capability or liquid state of the material. While the results of the oleogels containing maximum hydrocolloid percentages showed superior adhesiveness, the WPC oleogel had the lowest hardness (197.63 g) and adhesion (−944.66 g.sec). Naji-Tabasi et al., 2020 obtained similar results for textured oleogels.

Furthermore, with regards to the consistency parameter, according to the results, oleogels prepared from BSG exhibited stronger networks,

Table 4

Textural properties of oleogels stabilized by XG:WPC and BSG:WPC.

Oleogel (%)	Parameter			
	Hardness (g)	Adhesiveness (g.sec)	Consistency (g.sec)	Apparent elastic modulus (g. sec ^{−1})
WPC	197.6 ± 8.6 ^f	−944.7 ± 10.1 ^d	1451.3 ± 31.1 ^e	19.6 ± 0.8 ^f
0.2 XG: WPC	1018.1 ± 17.9 ^b	−3550.7 ± 13.0 ^c	6831.8 ± 48.3 ^c	107.8 ± 10.0 ^d
0.4 XG: WPC	1714.1 ± 15.2 ^d	−2856.5 ± 19.0 ^{cd}	11,587.2 ± 51.1 ^b	175.6 ± 7.5 ^b
0.6 XG: WPC	1837.8 ± 16.1 ^b	−4831.1 ± 15.3 ^b	11,414.1 ± 31.1 ^b	183.6 ± 17.2 ^b
0.2 BSG: WPC	661.0 ± 14.6 ^e	−3080.0 ± 10.0 ^{cd}	4671.2 ± 34.6 ^{cd}	66.0 ± 1.3 ^e
0.4 BSG: WPC	1395.2 ± 20.1 ^c	−3695.2 ± 13.2 ^c	9170.5 ± 33.1 ^{bc}	141.0 ± 1.5 ^c
0.6 BSG: WPC	3284.7 ± 16.2 ^a	−7133.6 ± 16.5 ^a	18,026.4 ± 40.0 ^a	351.4 ± 11.2 ^a

*Values followed by the same letter in column are not significant difference (P < 0.05).

*Means ± standard deviation.

reporting consistency values of 18,026 g·sec, when adding 0.6 % BSG: WPC, against the 11,414 g·sec reported by the system with the same proportion of XG: WPC (0.6 XG:WPC). According to previous studies (Sarraf et al., 2023), although WPC may impart smoother texture to the emulsions, in terms of hardness and adhesiveness, the incorporation of hydrocolloids steadily improved a stronger texture. Therefore, it appears that the presence of hydrocolloids in the emulsion gel structure causes more bonds between the particles and electrostatic interactions, forming a stronger gel, and creating a thicker stabilizing layer around the particles, showing that the hardness of the texture depends on the concentration of biopolymers. The amount of zeta potential was driven toward a negative charge with raising polysaccharides concentration that is because of increasing the number of carboxyl groups on the polymer chains. So, the repulsive forces exceed the attractive forces with the high zeta potential of emulsion gel, resulting in a relatively stable system (Lu & Gao, 2010; Sarraf et al., 2021). Thus, the formation of more favorable oleogels is expected to increase with the gums content. Jaber et al., 2020 reported that at different concentrations of XG aerogels, the higher amount resulted in more hardness and a more appropriate network. The xanthan molecule has a cellulosic backbone, with side chains wrapping around it to make it stiff. This structure helps emulsions to have high stability after lyophilization and to form an oleogel (Jaber et al., 2020; Naji, Razavi, & Karazhiyan, 2013).

The apparent elasticity modulus indicates the stiffness of the oleogels and the value increased with the hydrocolloid concentration. The changes in the spatial structure are caused by BSG, which is capable of maintaining the structure and form a suitable gel, so it creates a denser structure with smaller spaces, increasing the hardness as well as the stiffness of the gel.

4. Conclusions

Oleogels are one of the latest systems to be introduced into the food industry due to the increasing demand for oil replacements. This paper investigated the interaction of WPC with different percentages of BSG and XG in the formation of oleogels. The results showed the concentration of hydrocolloid influenced on the oleogel stability, as well as their textural and rheological properties. Therefore, higher gum content improved texture hardness, stability over time, and resistance against stress, and oxidation. The oleogels with a more stable structure, whose dispersed oil droplets were uniformly covered with a polymer layer, thus preventing their coalescence. In comparison between two oleogels containing 0.6 %BSG and XG, the sample containing 0.6 % BSG resulted in the production of a stronger and optimized oleogel composition in terms of texture and elastic behavior. The viscoelastic properties showed the predominant elastic behavior of the formulated oleogels. On the other hand, although the gum concentration played an important role in improving the physico-chemical properties of oleogels, the temperature increase had a significant weakening effect on their structure. Finally, the interaction of WPC solution with BSG yielded more stable oleogels with superior elastic properties, when compared to XG-based systems.

Funding

The authors received no financial support for the research.

Consent for publication

All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Mozhdeh Sarraf: Writing – original draft, Methodology, Investigation, Formal analysis. **Sara Naji-Tabasi:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Adel Beig-**

Babaei: Writing – review & editing, Validation, Supervision, Methodology. **José Enrique Moros:** Writing – review & editing, Validation, Supervision, Methodology. **Maria Carmen Sánchez Carrillo:** Writing – review & editing, Validation, Methodology. **Adrián Tenorio-Alfonso:** Writing – review & editing, Validation, Investigation.

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.

Acknowledgments

The authors are thankful to the laboratories of the Research Institute of Food Science and Technology and Universidad de Huelva for their support to conduct the research work.

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