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Rheological and Functional Properties of Hydrocolloids from *Pereskia bleo* Leaves

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Abstract: The food industry has increased its interest in using natural and consumer-friendly ingredients to produce food products. In the case of hydrocolloids of natural origin, the materials are biodegradable and environmentally friendly. This study aimed to isolate hydrocolloids from *Pereskia bleo* leaves and evaluate their proximal composition, technological and rheological properties. High-carbohydrate *Pereskia bleo* with high water holding capacity and emulsifying stability were obtained. The samples showed a shear-thinning behavior adjusted to the Cross model ($R^2 > 0.93$) and a high dependence on temperature corroborating with the higher activation energy value (11.78 kJ/mol, $R^2 = 0.99$) as an indicator of a rapid change in viscosity and microstructure. The viscoelastic properties are shown with a storage modulus higher than the loss modulus, presenting a gel structure. The isolation of hydrocolloids from leaves is a major challenge for commercializing natural ingredients with technological properties. Therefore, this study suggests that these hydrocolloids from *Pereskia bleo* leaves can be good ingredients in microstructure and texturizing products, improving the stability as thickener agents.

Keywords: *Pereskia bleo*; leaves; hydrocolloids; rheological; technological properties



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1. Introduction

Pereskia bleo belongs to the *Cactaceae* family [1], employed for its medical properties to remedy hypertension, infections, diabetes, asthma, and other dietary benefits [2–4]. Recent studies have reported biological activities, including anticancer, antitumor, anti-inflammatory, antimicrobial, and antioxidant [5–8].

The development of natural hydrocolloids requires a thorough understanding of the rheological and physicochemical characteristics of natural chemicals [9]. Macromolecular assemblies of a water-soluble character play a key role in biological phenomena, where the thermodynamic hydrocolloid dispersions in foods have subsequent effects in texture, rheological and nutritional aspects. *Pereskia bleo* leaves are known to distribute mucilage content with technological properties such as water holding capacity, solubility [10], and emulsification [11] and are considered safe alternative sources for the development of industrial techno-functional ingredients in food.

Recent work has shown the current interest of people in natural products that improve health and quality of life, while the food, pharmaceutical, and chemical industries are looking for healthy ingredients that will improve the technological properties of products. Hydrocolloids as biological macromolecules are used as water retention agents, thickeners, emulsion stabilizers, binders, or suspending agents [12–15]. Therefore, knowing the rheological behavior of new hydrocolloid sources is important since they are applied to modify textural attributes [16–18]. Every biological macromolecule's rheological behavior

is unique, and this information is handy in many industrial applications. It should be considered for design and modeling purposes [9,19,20]. The nutritional and physiological aspects of *Pereskia bleo* leaves have been extensively reported. However, studies on the isolation of their hydrocolloid leaves are quite limited. Consequently, the objective of this study was to isolate hydrocolloids from *Pereskia bleo* leaves and study their proximal composition, technological, and rheological properties.

2. Materials and Methods

2.1. Chemicals

The *Pereskia bleo* leaves were recovered in the Sucre department (Colombia) and transported to the laboratory of complex fluid engineering and food rheology (IFCRA) at Cartagena University (Cartagena, Colombia). Ethanol (99.5% purity) and hexane were obtained from Panreac (Barcelona, Spain). NaOH, acetic acid, and phenolphthalein were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were analytical grade and were used as received.

2.2. Hydrocolloid Extraction

Hydrocolloid extractions were performed following the procedures described by Orgulloso-Bautista et al. [18] and Quintana et al. [16,17] with some modifications. A ratio of 1:15 *w/v* leaves: water was employed to solubilize compounds at pH 3 (adjusted with 1 N acetic acid) for 4 h at 80 °C. The mixture was centrifugated for 15 min at 4000 rpm to recollect the supernatant. After that, the hydrocolloid-based extracts precipitate by mixing the viscous solution with ethanol in a ratio of 1:1 *w/v* for 2 h, at 4.0 ± 0.5 °C. The precipitate was collected and lyophilized for 48 h. All experiments were done per triplicate.

2.3. Physicochemical and Proximal Analysis

The physicochemical and proximal composition of hydrocolloids was performed following the method described by the Association of Official Analytical Chemists-AOAC [21]. Titratable acidity determination (TTA) was analyzed according to method number 967.21; 2 g of samples were diluted in 50 mL using distilled water and mixed with 0.2 mL of phenolphthalein as an indicator and titrated with 0.1 N NaOH, the results were expressed as mg of citric acid/100 g of hydrocolloids. The pH values were determined according to method 981.12 using a digital pH meter (Model HANNA HI 9124).

Moisture was determined by dehydration of 10 g of samples for 4 h in an oven at 105 °C. Ash was determined by incineration of 10 g of samples at 550 °C until constant weight. The protein content was determined using the Kjeldahl method, the fat determination was done using hexane as a solvent in a Soxhlet apparatus for 4 h, and total carbohydrates were calculated per difference.

2.4. Functional Properties

2.4.1. Water Holding Capacity (WHC)

The WHC expresses the amount of water retained by the hydrocolloids. It was quantified by placing 0.5 g of sesame hydrocolloids in a centrifuge tube, adding 3 mL of water, shaking for one minute. The mixture was centrifuged at 3200 rpm at 24 °C for 30 min to measure the water volume not retained [21]. The %WHC was determined using Equation (1):

$$\%WHC = \frac{\text{mL held water}}{\text{g sample}} \times 100 \quad (1)$$

2.4.2. Foaming Capacity (FE) and Foam Stability (FS)

Foam capacity and stability were determined following the methods described by Jahanbin [22]. 1 g of sesame hydrocolloid was added to 100 mL of distilled water; then

strong agitation was applied for 5 min. %FE was calculated according to Equation (2) and %FS according to Equation (3):

$$\%FE = \frac{\text{mL after agitation} - \text{mL before agitation}}{\text{mL before agitation}} \times 100 \quad (2)$$

$$\%FS = \frac{\text{Foam volume (30 min)}}{\text{initial foam volume}} \times 100 \quad (3)$$

2.4.3. Emulsifying Ability (EA)

Emulsions were prepared by adding 6 mL of commercial oil in an aqueous distilled water solution (60 mL) with sesame hydrocolloids at 0.5, 0.25, and 0.1% *w/w*. Dispersions were mixed by magnetic stirring and subsequently homogenized for 1 min (Ultra Turrax T-25, IKA, Staufen, Germany). The %EA was calculated according to Equation (4):

$$\%EA = \frac{\text{mL emulsion}}{\text{mL final}} \times 100 \quad (4)$$

2.4.4. Emulsifying Stability (ES)

The stability of the emulsion was analyzed following the method described by Sciarini et al. [23]. The emulsions were heated in a water bath at 80 °C for 30 min and then centrifuged for 10 min. The %ES was calculated according to Equation (5):

$$\%ES = \frac{\text{mL final volume}}{\text{mL initial volume}} \times 100 \quad (5)$$

2.5. Rheological Analysis

The rheological characterization of hydrocolloids on a wet basis was carried out in a controlled stress rheometer (Modular Advanced Rheometer System Haake Mars 60, Thermo-Scientific, Dreieich, Germany), based on Quintana et al. [24] using a rough plate geometry of 35 mm in diameter and 1 mm in the gap to prevent wall slip effects. The temperature was fixed (using a Peltier system), and each sample was equilibrated at 600 s before the rheological test to ensure the same thermal and mechanical history.

Viscous flow tests were carried out at a steady-state, in a shear rate range between 0.001 and 1000 s⁻¹, analyzing the variation of apparent viscosity. Stress sweeps were performed at a frequency of 1 Hz, applying an ascending series of stress values from 0.001 to 1000 Pa to determine the linear viscoelasticity interval. Frequency sweeps were performed in a frequency range between 10⁻² and 10² rad·s⁻¹ to obtain the mechanical spectrum using a stress value within the linear viscoelastic range. All analyzes were performed at 10, 25, 40, and 80 °C per duplicate.

2.6. Statistical Analysis

The results were analyzed using the Statgraphics Centurion XVI (Statgraphics, Rockville, MD, USA). An ANOVA (unidirectional) test was applied to determine statistically significant differences (*p* < 0.05) between the samples submitted to the characterizations.

3. Results and Discussion

3.1. Hydrocolloids of *Pereskia bleo* Leaves

The proximal composition and technological properties of hydrocolloids from *Pereskia bleo* leaves are shown in Table 1. Hydrocolloids were found to be extracted using hot acid extraction with extraction yields of 13.83 ± 0.03%. The samples contain mainly carbohydrates (80.91 ± 0.54%), could contain mannose galactose, arabinose, and uronic acid [10] reported in their mucilage. As expected, the sample contains 13.68 ± 0.38% moisture, 3.3 ± 0.27% protein, 0.63 ± 0.03% fat, and 1.48 ± 0.05% ash. The results demonstrate that the leaves of *Pereskia bleo* are an excellent source of carbohydrate for the commercial production of hydrocolloids.

Table 1. Proximal composition and functional properties of hydrocolloids from *Pereskia bleo* leaves. WHC: water holding capacity, ES: emulsifying stability, FE: foaming capacity, and FS: Foam stability.

Parameter	Hydrocolloids
Moisture (%)	13.68 ± 0.38
Protein (%)	3.3 ± 0.27
Fat (%)	0.636 ± 0.03
Ash (%)	1.48 ± 0.05
Carbohydrate (%)	80.91 ± 0.54
mL/g WHC	3.62 ± 0.04
%ES 0.1	60.4 ± 0.13
%ES 0.25	68.6 ± 0.04
%ES 0.5	73.9 ± 0.07
%FE	5.47 ± 0.07
%FS	1%

Results are expressed as mean ± standard deviation.

The technological properties (Table 1) showing high values of water holding capacity WHC (3.62 ± 0.04 mL/g) can be ascribed to the small pores of the samples that retain water swiftly entrapped; the samples present a high value in comparison with the hydrocolloids from tamarillo (3.44 g water/g dry sample) [25] and gum from durian seed (1.45 g water/g dry sample) [26]. The WHC properties of hydrocolloids can improve food products' stability, avoid syneresis or loss of taste, and modify products' texture during processing and storage.

Emulsion ability (EA) is the ability to facilitate the solubilization of the dispersions of two immiscible liquids, and Emulsion Stability (ES) is the capacity to maintain an emulsion and its resistance to phase separation [27]. Hydrocolloids led to the formation of emulsions that improved with increasing hydrocolloids in emulsions; however, the ES evaluation showed that hydrocolloids were effective in stabilizing and maintaining oil/water emulsions with ES > 60% at a concentration of 0.1%, attributed to the high concentration of carbohydrates or the composition and molecular structural components, such as the content of polysaccharides and hydrophobic groups (methyl and acetyl groups) [28]. Hydrocolloids increase the viscosity of the samples, preventing drainage from occurring at the interface [29] and help stabilize the samples. Therefore, hydrocolloids from *Pereskia bleo* leaves have been suggested to help the food industry act as thickening and stabilizing agents, reducers, or syneresis retarders.

Foam is a colloidal system consisting of a gaseous phase dispersed in a continuous aqueous phase [30]. The foaming capacity FC and the stability FS were lower, associated with the lowest protein concentration, which can provide hydrophilic properties and have a relatively high surface hydrophobicity so that they can be efficiently absorbed in the air-water or oil-water interface, forming a continuous viscoelastic network that allows the emulsion to remain stable over time [31]. This behavior could have been because foams have a higher surface tension at the air-water interface, having considerably shorter lives than oil-in-water dispersions [32]. Generally, hydrocolloid carbohydrate was not considered an excellent foaming agent since it could not adsorb at the interface [33]. Therefore, this study suggests that these hydrocolloids from *Pereskia bleo* leaves can be good ingredients in the food industry.

3.2. Rheological Properties

3.2.1. Steady-State Shear Rate

The viscous flow behavior of the hydrocolloids of the *Pereskia bleo* leaves shows a decrease in viscosity with respect to the applied shear rate (Figure 1), presenting a non-Newtonian-type shear thinning. In these cases, the polymer molecule chains are arbitrarily positioned when the sample solutions are in quiescent conditions but are aligned in the same flow direction after shear forces. Hydrodynamic forces deform the aggregates, which eventually break down, resulting in reduced viscosity in shear-thinning fluids [34,35].

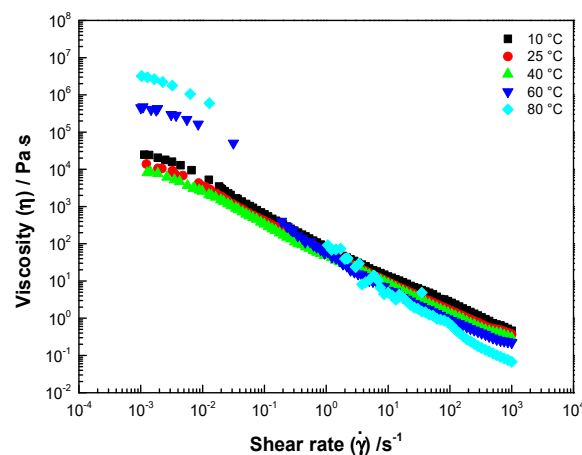


Figure 1. Viscous curves of hydrocolloids from *Pereskia bleo* leaves.

Food products’ texture and mouthfeel are organoleptic properties associated with the hydrocolloid viscosity, depending on their concentration and application temperature. The curves showed the highest viscosity in the samples at 60 and 80 °C and the lowest at 10, 25, and 40 °C, showing the potential use of samples as thickener agents. The steady shear flow behavior of hydrocolloids was described by the Cross model (Equation (6)):

$$\eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (C\dot{\gamma})^m} \tag{6}$$

where, η_{∞} is the infinite shear viscosity, η_0 is the zero-shear viscosity, C is the Cross time constant, and m is the Cross-rate constant. Adjustment parameters are shown in Table 2, presenting a high correlation coefficient value ($R^2 > 0.93$), the η_0 of samples between 10 and 40 °C showed a linear decrease, but when the temperature increased at 60 and 80 °C, an increase was observed denoting a change in molecular conformation of samples, η_{∞} decrease in function of temperature, C decrease in function of temperature, indicating that the critical shear rate to onset for shear-thinning decreases with temperature. Simultaneously, m values increase with the temperature, showing an increase in the degree of dependence of viscosity on the shear rate in the shear-thinning region.

Table 2. Adjustment parameters of Cross model to viscous curves of hydrocolloids from *Pereskia bleo* leave extracted at pH 3, at 10, 25, 40, 60 y 80 °C. η_0 : zero-shear viscosity, η_{∞} : infinite shear viscosity, m : Cross-rate constant, and C : known as the Cross time constant.

Temperature °C	η_0 Pa·s	η_{∞} Pa·s	C s	m	R^2
10	40,376.08 ^c	0.518 ^c	858.45 ^d	0.81 ^a	0.95
25	20,471.30 ^b	0.475 ^c	302.97 ^c	1.02 ^a	0.94
40	14,141.91 ^a	0.247 ^b	321.46 ^c	0.95 ^a	0.93
60	578,300.86 ^d	0.216 ^b	258.39 ^b	1.42 ^b	0.98
80	874,423.83 ^e	0.007 ^a	118.54 ^a	1.50 ^b	0.96

Significant differences in the samples were < 0.05 . The different letter within each column is significantly different at $p < 0.05$.

An increase in temperature decreases hydrocolloid viscosity due to the rise in the kinetic energy of molecules. The effect of temperature on hydrocolloid viscosity was explained by Arrhenius-type equations [36] (Equation (7)):

$$\eta_{\dot{\gamma}=10s^{-1}} = Ae^{\left(\frac{E_a}{RT}\right)} \tag{7}$$

where T (K) is the absolute temperature, R (8.314 J/mol K) is the universal molar gas constant, and E_a (kJ/mol) is the activation energy of viscous flow, reflecting the sensibility of viscosity to temperature changes and an indicator of the movement of molecules [35].

The activation energy of hydrocolloids was determined to plot $\eta_{\dot{\gamma}=10s^{-1}}$ in function of $1/T$ (Figure 2), obtaining a value of 11.78 kJ/mol ($R^2 = 0.99$), which was higher than E_a reported for nettle seed gum (17.99 kJ/mol) [37] and cress seed gum (9.23 kJ/mol) [38]. Higher EA values mean that the viscosity changes rapidly with temperature and the microstructure is more prone to changes than a strong interaction between polymer chains, being less prone to alteration during thermal processing [39].

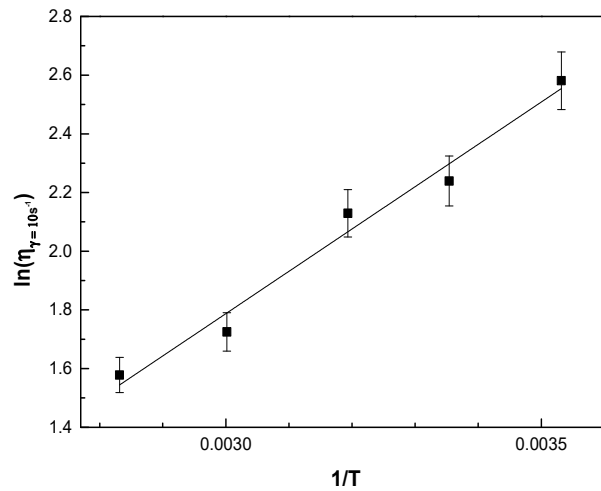


Figure 2. Viscosity at 10 s^{-1} (as function of the temperature of hydrocolloids from *Pereskia bleo* leaves.

3.2.2. Dynamic Rheological Properties

Stress sweep (Figure 3) was performed to determine the limits of the linear viscoelastic (LVE) range and the flow point, reflecting the changes in storage modulus (G') and loss modulus (G''), according to the increasing stress of hydrocolloid samples. For all samples, G' was higher than G'' over the entire LVE range, which indicated a viscoelastic gel behavior [40]. After LVE, G' and G'' values decreased with the increased stress showing the non-linear viscoelastic range.

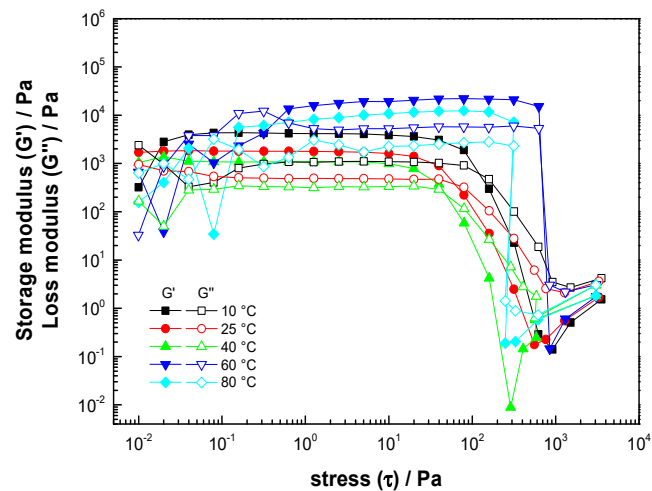


Figure 3. Stress sweep of hydrocolloids from *Pereskia bleo* leaves.

Table 3 shows the viscoelastic parameters G' , G'' , crossover and τ_C obtained stress sweep. There was a substantial increase in moduli values as the temperature increased, indicating stronger structures at higher temperatures related to intermolecular interactions

and entanglement. The yield stress (τ_C) was calculated as the limiting value of LVE in the stress state. τ_C increases with the temperature and can be regarded as the strength's weakening [41]. The flow point was calculated with the crossover point ($G' = G''$) provided information about the internal structure breakdown, resulting in the final flow [42]. An increase in temperature decreased the crossover when the higher crossover indicated more resistant gels against structural deformation related to the polymer chain association.

Table 3. The viscoelastic parameters were obtained with the stress sweep test of hydrocolloids from *Pereskia bleo* leaves.

Temperature °C	G' Pa	G'' Pa	τ_C Pa	$G' = G''$ Pa
10	4096.13 ^c	1120.57 ^c	21.62 ^a	1671 ^e
25	1694.32 ^b	448.22 ^b	23.14 ^a	425.4 ^d
40	1089.70 ^a	317.00 ^a	21.89 ^a	265.4 ^b
60	19,909.45 ^d	5989.25 ^e	79.31 ^b	399.4 ^c
80	11,644.49 ^e	2316.74 ^d	194.4 ^c	185.2 ^a

Significant differences in the samples were < 0.01 . The different letter within each column is significantly different at $p < 0.05$.

The viscoelastic properties of hydrocolloids influence the processing parameter and the quality of the final products [43]. These properties were determined using a frequency sweep test performed within the LVE range. The dynamic frequency sweep test was done in the linear viscoelastic (LVE) range to determine the frequency dependence of storage modulus (G'), loss modulus (G''), complex viscosity η^* , and loss tangent ($\text{Tan}\delta = G''/G'$) of samples.

In all samples present, G' was higher than G'' in the applied frequency range except at 80 °C when a crossover at low frequency was observed (Figure 4a). Therefore, the gel structure and viscoelastic behavior of hydrocolloids in the range of temperatures studied were stable. Similar results have been reported for hydrocolloids with high carbohydrate content, i.e., seed gum-xanthan blends [44] and amorphous Gelditsia depending on their concentration [45].

To analyze the viscoelastic properties of hydrocolloids at different temperatures, the values of G' , G'' , η^* , and $\text{Tan}\delta$ at 1 Hz in frequency sweep were summarized in Table 4. G' did not show a relation with the increased temperature; nevertheless, the samples at 80 °C present a 200% higher than 10 °C. The G'' , η^* , and $\text{Tan}\delta$ decrease from 10 to 60 °C and change at 80 °C, increasing for G'' and η^* , and decreasing for $\text{Tan}\delta$.

All samples show a linear decrease of η^* with increasing frequency (Figure 4b), shown to occur at permanent disentanglement of long-chain polymers. These results demonstrated a non-Newtonian shear thinning behavior, which may be due to the disappearing of the house of cards microstructure and planer alignment of the particle-matrix towards the flow direction under shear force [46,47].

According to the frequency sweep test, $\text{Tan}\delta$ (Figure 4c) was in the range of 0.19–0.26, indicating that the samples generate weak-gel networks by a tenuous association of rigid and ordered molecular structures in a solution similar to xanthan gum [48].

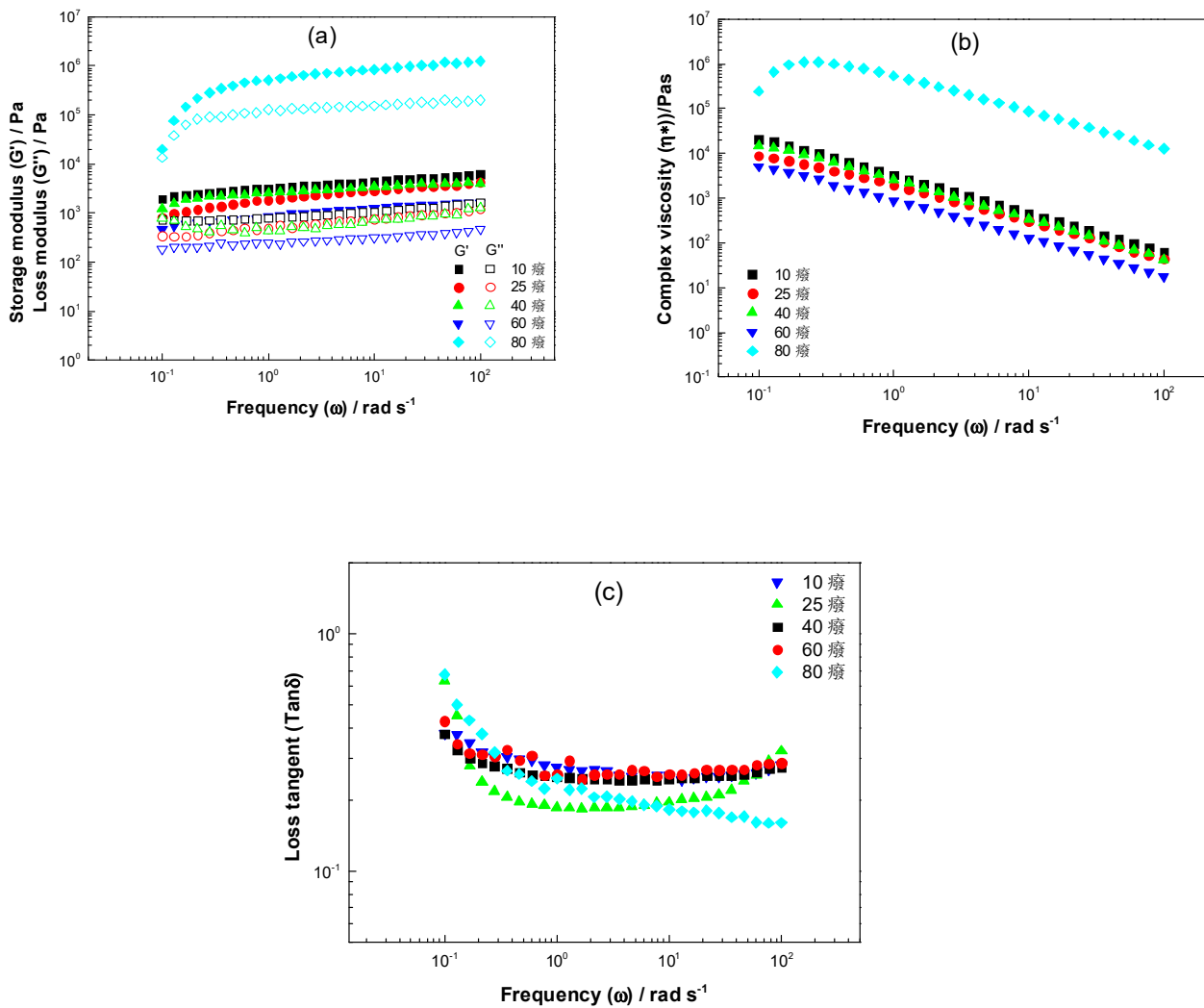


Figure 4. Viscoelastic parameters in function of the frequency of hydrocolloids from *Pereskia bleo* leaves. (a) storage G' and loss G'' modulus, (b) complex viscosity (η^*), and (c) $\text{Tan}\delta$.

Table 4. The viscoelastic properties of hydrocolloids from *Pereskia bleo* leaf in a frequency sweep at 1 Hz.

Temperature °C	G' Pa	G'' Pa	η^* Pa·s	$\text{Tan}\delta$
10	4052 ^c	989.8 ^d	695.8 ^d	0.24 ^b
25	2663 ^b	705.8 ^c	459.5 ^b	0.26 ^b
40	3193 ^c	593.7 ^b	541.8 ^c	0.24 ^b
60	1169 ^a	300.5 ^a	201.3 ^a	0.25 ^b
80	778,200 ^d	147,700 ^e	132,100 ^e	0.19 ^a

Significant differences in the samples were < 0.01 . The different letters within each column are significantly different at $p < 0.05$.

4. Conclusions

The results demonstrate that the leaves of *Pereskia bleo* are an excellent source of carbohydrates for the commercial production of hydrocolloids. Hydrocolloids have high water-holding capacities at WHC and were influential in stabilizing and maintaining oil/water emulsions. These properties are related to improving the technological properties of food products' stability, avoiding syneresis or loss of taste, and modifying the texture of products during processing and storage. Their emulsifying and stabilizing capacity helps produce and stabilize oil/water emulsions attributed to the molecular struc-

tural constituents. The samples' rheological properties indicate that hydrocolloids present a non-Newtonian viscous flow behavior type shear thinning, presenting a dependence of pseudoplasticity with the temperature. The viscoelastic properties show that the gel structure and the temperature range studied were stable, with the storage modulus being higher than the loss modulus in the frequency range studied. *Pereskia bleo* leaves of hydrocolloids increase the viscosity of samples to stabilize food products, acting as a thickening and stabilizing agent, reducer, or syneresis retarders. Therefore, this study suggests that these hydrocolloids from *Pereskia bleo* leaves can be good ingredients in emulsified and texturizing products such as sausages, mayonnaise, bread, dairy desserts, sauces and improve the stability as thickener agents.

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