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Research Paper / Araştırma Makalesi

Ultrasound-Assisted Extraction of Okra Mucilage: Rheological Properties of its Aqueous Solutions

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ABSTRACT

Using chemical and physicochemical techniques, we extracted the mucilaginous component of okra (*Abelmoschus esculentus* L.) by the ultrasound-assisted extraction method, then evaluated the resulting polysaccharide extract's rheological properties. Our investigation encompassed examining the flow behavior of polysaccharides extracted under different okra to distilled water ratios (1:10 and 1:30) and various polysaccharide concentrations (1, 2, 3 and 4%, w/v) over a temperature range of 10°C to 80°C. Employing the power law model, we derived parameters and found that okra polysaccharides displayed non-Newtonian pseudoplastic flow characteristics. The flow behavior index ranged from 0.234 to 0.947, with the consistency coefficient ranging from 4.37 to 244.50 mPa.s. Increasing temperature resulted in a decrease in both the consistency coefficient (K) and flow behavior index (n), while concentration elevation led to higher consistency coefficient values. However, the flow behavior index did not exhibit consistent trends with concentration variations. Three statistical parameters; correlation coefficient (R^2), root mean square error (RMSE) and chi-square (χ^2) were used to evaluate the fit of the power law model to the experimental data. Our study further explored temperature's impact on the apparent viscosities of okra polysaccharide samples and modeled the influence of temperature on the consistency index using the Arrhenius equation. Samples with solid-to-solvent ratios of 1:10 and 1:30 showed increasing activation energy with concentration rise, with the highest value recorded at 275.84 kJ/mol for the 1:10 ratio sample with a 4% concentration. In SEM images, okra polymers exhibit irregular, wavy, rough textured surface, and amorphous appearance. These findings hold promise for optimizing ultrasound extraction protocols and enhancing the industrial utilization of mucilages through their rheological properties.

Keywords: Okra mucilage, Rheological properties, Ultrasound, Extraction method

Bamya Müsilajının Ultrason Destekli Ekstraksiyonu: Sulu Çözeltilerinin Reolojik Özellikleri

ÖZ

Kimyasal ve fizikokimyasal teknikler kullanılarak, bamyanın müsilajinöz bileşeni ultrason desteği ile ekstrakte edilmiş, ardından elde edilen polisakkarit ekstraktının reolojik özellikleri değerlendirilmiştir. Araştırmamız, 10°C ile 80°C sıcaklık aralığında farklı bamya-damıtık su oranları (1:10 ve 1:30) ve çeşitli polisakkarit konsantrasyonları (%1, 2, 3 ve 4, w/v) altında ekstrakte edilen polisakkaritlerin akış davranışlarının incelenmesini kapsamaktadır. Güç yasası modelini kullanarak parametreler türetilmiş ve bamya polisakkaritlerinin Newtonyen olmayan psödoplastik akış özellikleri gösterdiğini bulunmuştur. Akış davranış indeksi 0,234 ile 0,947 arasında değişirken, kıvam katsayısı 4,37 ile 244,50 mPa.s arasında değişmiştir. Artan sıcaklık hem kıvam katsayısında (K) hem de akış davranış indeksinde (n) düşüşe neden olurken, konsantrasyon artışı daha yüksek kıvam katsayısı değerlerine yol açmıştır. Ancak, akış davranış indeksi konsantrasyon değişimleri ile tutarlı eğilimler sergilememiştir. Güç yasası modelinin deneysel verilere uyumunu değerlendirmek için üç istatistiksel parametre; korelasyon katsayısı (R^2), kök ortalama kare hatası (RMSE) ve ki-kare (χ^2) kullanılmıştır. Çalışmamızda ayrıca sıcaklığın bamya polisakkarit örneklerinin görünür viskoziteleri üzerindeki etkisi araştırılmış ve Arrhenius denklemi kullanılarak sıcaklığın kıvam indeksi üzerindeki etkisi modellenmiştir. Bamya-damıtık su oranı 1:10 ve 1:30 olan numuneler konsantrasyon artışıyla birlikte artan aktivasyon enerjisi göstermiş, en yüksek değer %4 konsantrasyona sahip 1:10 oranlı numune için 275,84 kJ/mol olarak kaydedilmiştir. SEM görüntülerinde, bamya polimerleri düzensiz, dalgalı, pürüzlü dokulu yüzey ve amorf görünüm sergilemektedir. Bu bulgular, ultrason ekstraksiyon protokollerinin optimize edilmesi ve reolojik özellikleri aracılığıyla müsilajların endüstriyel kullanımının artırılması için umut vaat etmektedir.

Anahtar Kelimeler: Bamya müsilajı, Reolojik özellikler, Ultrason, Ekstraksiyon yöntemleri

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a widely cultivated plant globally in tropical, subtropical, and temperate regions, including coastal areas bordering the Mediterranean Sea. This widespread cultivation is primarily due to the plant's significant economic and nutritional value [1]. It is often grown extensively in the Aegean part of our country and eaten fresh, frozen, or dried [2]. The viscous and mucilaginous consistency observed in extracts derived from okra is primarily ascribed to its polysaccharide composition, garnering significant attention in both food and non-food sectors for its inherent technological properties. Extensive examination of its various applications has been previously documented in scientific literature [3-5]. Early studies characterized the latter as acidic polysaccharides composed of galactose, rhamnose, and galacturonic acid, with reports indicating partial acetylation of these polysaccharides. Lengsfeld et al. [6], along with Deters et al. [7], identified the sugar components of okra polysaccharides to include rhamnose, galacturonic acid, galactose, glucose, and glucuronic acid. Okra mucilage, derived from the pods of okra plants, is a dense fluid composed of polysaccharides with random coil structures, including the aforementioned sugars [4, 8, 9]. Numerous studies have focused on investigating the rheological behavior of okra polysaccharide extracts in solution to better understand the relationship between their structure and function [10, 11].

Historically, conventional techniques including hydrodistillation, squeezing, cold pressing, maceration, and stirring-based extraction were employed for obtaining mucilage from plant origins. Nevertheless, these approaches were largely dependent on solvents, heat, and extended extraction durations, frequently resulting in the diminishment of antioxidant properties and overall phenolic content due to oxidation, hydrolysis, and ionization processes. Consequently, the exploration of more advanced alternative methods became essential [12]. Nonetheless, in recent years, there has been significant exploration into non-thermal methods as alternatives or supplements to traditional heat-based treatments. One such technique, sonication, utilizes ultrasonic waves for food processing without applying heat. This method relies on piezoelectric materials to convert electrical energy into mechanical energy, generating ultrasonic waves. When these waves pass through a liquid, they create cavitation bubbles due to fluctuating pressure. The collapse of these bubbles along the sound wave's path leads to localized areas of increased temperature and pressure. The energy transferred to the food during ultrasonic treatment can be characterized using terms such as ultrasonic power, intensity, acoustic energy density, or cavitational intensity [4, 13, 14].

The rheological properties of these extracts hold significant importance for prospective industrial utilization. Despite this, there has been a scarcity of research concerning the rheology of such systems when in solution [15, 16].

The significance of rheological properties lies in their crucial role in elucidating heat transfer phenomena and in the design, assessment, and simulation of continuous processes. The measurement of these properties offers valuable insights into the behavior and predictive outcomes related to processing, alterations in formulation, aging effects, and overall quality across diverse product categories[11]. Previous studies on rheology have demonstrated that okra mucilage exhibits characteristics of a structured fluid, displaying properties such as viscoelasticity, shear-thinning behavior, adhesion, stringiness, ductility, and cohesion [17]. In addition, the researchers stated that the extraction protocols used had distinct impacts on the rheological properties of okra mucilage, suggesting the potential for weak gel-like behavior in okra mucilage. Additionally, the viscosity of okra mucilage highlights the intimate connection between shear and extension directions. The extensional viscosity of okra mucilage significantly surpasses its shear viscosity, often by two to three orders of magnitude [9]. The mentioned rheological characteristics attributed to okra polysaccharides underscore their significant technical value for various applications, serving as viscosity enhancers, gelling agents, and thickeners and are widely used in food and medical fields [18].

Hence, the primary objective of the current investigation was to employ ultrasound extraction technology to procure okra polysaccharides under varying solid-to-solvent ratios, aiming to elucidate the solution behavior of these biopolymers across both dilute and concentrated states. These results provide a valuable data base for the potential utilization of okra mucilage obtained by ultrasound technology in various industries.

MATERIALS and METHODS

Materials

The soft and ripe okra of about 5 - 7 cm in length and 3 cm in diameter grown in Tire (Izmir, Türkiye) were obtained from a local market and immediately dried, ground and stored under suitable storage conditions. A solar dryer provided by Tartes Tarim in Izmir, Türkiye, was employed to dry the okra. Subsequently, the dried okra was pulverized into powder with particle sizes ranging from 400 to 500 µm using a hammer mill (Brook Crompton Controls, Wakefield, UK) and stored appropriately. The ethanol (96% ultra-pure) utilized for purifying polysaccharides was sourced from Tekkim Kimya (Bursa, Türkiye).

Methods

Ultrasound-Assisted Extraction Method

For ultrasound-assisted extraction of polysaccharides from okra, the extraction and purification steps used by Öncü Glaue et al. [4], 2023 were performed using Hielscher UP400S, 24 kHz, Germany, equipped with H14 probe (14 mm diameter; 90 mm height). In the extraction process, a power of 200 watts and an extraction time of 5 min, which were determined by preliminary trials, were used. To initiate the extraction procedure, samples were prepared from powdered okra using two distinct ratios of distilled water, namely 1:10 g/mL and 1:30 g/mL.

The extract was centrifuged at 2000× g for 15 minutes to separate the supernatant. The supernatant was combined and filtered through a fine-mesh cheesecloth before being concentrated in an evaporator at 70°C and 100 rpm, reducing its volume to approximately 1/3–1/4 of the original. The concentrated solution containing polysaccharides underwent precipitation and purification by adding six times its volume of ethanol, followed by centrifugation at 5000 g for 10 minutes to remove impurities. Finally, the samples were freeze-dried and stored at -20°C for preservation.

Rheological Measurements

The rheological properties of polysaccharide solutions obtained from ultrasound-assisted extracts with 1:10 and 1:30 okra/distilled water ratios and prepared at different concentrations (1, 2, 3 and 4%, w/v) were determined at different temperatures (10, 20, 40, 60 and 80°C) using a TA DHR3 (TA Instruments Inc., New Cattle, DE, USA) rheometer with concentric cylinder probe (diameter 27.99 mm, length 41.07 mm, measuring cup diameter 30 mm). The experiments were performed in two parallel runs and each run was performed with a new sample.

Apparent viscosity is a function of shear rate and in this study shear calculations (flow curves) were used to determine the flow behavior of polysaccharide solutions. The samples were completely dissolved by means of vortex and ultrasonic bath before measurement and then the temperature of the samples was adjusted to the desired temperatures using a circulating water bath. Shear stress, shear rate and viscosity values were noted for each speed. The experimental data were tested to find the most appropriate flow model based on the shear stress-shear rate data.

Flow Behavior

Shear stress (τ) and shear rate (γ) played important roles on okra polysaccharide extracts viscosity. Shear rate was systematically increased within the range of 0-100 s^{-1}. To characterize the flow behavior of polysaccharide solutions prepared at various concentrations, the two-parameter Power-Law model, extensively utilized in both theoretical analysis and practical engineering computations, was employed for testing purposes (Equation 1.) [19, 20].

$$\tau = K\gamma^n \tag{1}$$

where τ is the shear stress (Pa), γ is the shear rate (1/s). The constants K and n are the coefficient of consistency (Pa.s) and the flow behavior index, respectively.

In Equation 2, the logarithm of both sides is taken,

$$log\,\tau = log\,K + n\,log\,\gamma \tag{2}$$

The values of K and n are determined by logarithmic plots of shear stress (τ) versus shear rate (γ) [21].

Apparent Activation Energy

The impact of temperature on the apparent viscosities of okra polysaccharides was determined using an Arrhenius-type equation (3);

$$K = K_0 e^{\frac{-E_a}{R\left(\frac{1}{T_0}-\frac{1}{T}\right)}} \tag{3}$$

In the equation, K_0 represents the consistency factor in units of Pa.s, R stands for the universal gas constant in kJ/mol K, E_a denotes the apparent activation energy measured in kJ/mol, and T represents the polysaccharide temperature in Kelvin (K). For this analysis, a reference temperature (T_0) of 20°C (293.15 K) was assumed.

To linearize Equation 3, we can take the natural logarithm of both sides (Equation 4).

$$ln\,K = lnK_0 - \frac{E_a}{R}.\left(\frac{1}{T_0} - \frac{1}{T}\right) \tag{4}$$

K_0 and E_a values are calculated by plotting $\left(\frac{1}{T_0} - \frac{1}{T}\right)$ against ln K.

Conversely, a correlation coefficient (R^2) was computed to assess the strength of the relationship between the consistency index and activation energy. Correlation coefficient values exceeding 0.8 are typically considered strong. R^2 is valuable as it indicates the proportion of variance in one variable that can be predicted from another variable [22].

SEM Analysis

The surface structure of the prepared beads was examined using scanning electron microscopy (SEM) (JEOL, JSM-5800, Japan). The beads were affixed to a brass stud with double-sided adhesive tape and gold-coated under vacuum using ion sputtering to produce a thin layer of gold (3-5 nm) for 75 seconds. SEM images were captured at an accelerating voltage of 15 kV and a chamber pressure of 1.0 mm Hg to study the morphology. The magnification levels for the samples were set at ×10000 and ×25000.

Statistical Analysis

The fit of the rheological model used to determine the flow behavior with the experimental data was determined using Microsoft Excel (Microsoft Office 365 ProPlus, version 1810). The coefficient of determination (R^2) close to 1 was taken into consideration as an indicator of goodness of fit, while the root mean square error of the mean square (RMSE) and chi-square (χ^2) values were considered to increase the fit. For RMSE, values less than 0.1 represent perfect fit [23]. RMSE and χ^2 values were calculated using Equation 5. and 6. It is possible to detect differences between empirical data and model predictions using the following statistical tools.

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N} \left(M_{predicted,i} - M_{actual,i}\right)^2\right]^{1/2} \quad (5)$$

$$\chi^2 = \frac{\sum_{i=1}^{N} \left(M_{actual,i} - M_{predicted,i}\right)^2}{N-n} \quad (6)$$

Here, $M_{actual,I}$ is the measured value in the i. experimental analysis, $M_{predicted,i}$ is the predicted value in the i. analysis in the model, N is the number of experimental data and n is the amount of coefficients in the model used [2].

RESULTS and DISCUSSION

Rheological Measurements

Rheology provides valuable insights into the type of fluid, solution state, and phase transformation of polysaccharide solutions. Studying the rheological properties of polysaccharides is significant for enhancing their development and utilization in food-related industries [24]. This study examined how solutions of polysaccharides at various concentrations (1, 2, 3 and 4%, w/v) [25] extracted using different ratios of okra to distilled water (1:10 and 1:30), behave under different temperatures (10, 20, 40, 60 and 80°C).

After conducting the extraction process with a solid-to-solvent ratio of 1:10, the apparent viscosity of the samples increased with higher concentrations (1, 2, 3 and 4%) (Figure 1). Higher polysaccharide concentrations can often result in higher viscosity and more pronounced flow properties. This means that the polysaccharide solution shows more resistance when more force is applied. Furthermore, the amount of polysaccharide can also influence other rheological properties such as gel formation. Higher polysaccharide concentrations can generally lead to the formation of a firmer and less fluid gel, while lower concentrations can result in the formation of a looser and more fluid gel. Therefore, the percentage of polysaccharide solution can have a significant influence on the properties studied in rheology studies. However, it was noted that the apparent viscosity decreased as the shear rate increased within each concentration, showing shear-thinning behavior characteristic of a pseudoplastic fluid. All samples exhibited pseudoplastic behavior, maintaining constant viscosity values at high shear rates. The shear behavior of okra mucilage arises due to the intricate composition of its complex mixture, comprising polysaccharides, protein, and mineral components. Additionally, when subjected to shear, each sample of okra mucilage exhibits a plateau region at elevated shear rates. These constraints emerge because escalating shear rates compel the structural constituents to align with the direction of fluid flow[9]. When the shear rate increases, the extended polymer molecules within the substance gradually align themselves in the direction of the flow, leading to a reduction in interactions between neighboring polymer chains. This alignment phenomenon ultimately causes the observed shear-thinning properties as the shear rate rises [26].

In contrast, when the extraction process was conducted with a solid-to-solvent ratio of 1:30, the apparent viscosity declined as the shear rate increased (Figure 1). However, unlike the 1:10 ratio, no significant viscosity differences were observed among the concentrations. The viscosity values were quite similar to each other at the 1:30 ratio. The decrease in viscosity as the shear rate increases can be associated with hydrodynamic forces and the structural breakdown of less stable molecules resulting from an increase in component alignment.

Comprehending the alterations in apparent viscosity with temperature holds significant importance within the food industry, given the diverse range of temperatures involved in numerous heating processes, and the way viscosity responds to temperature can differ depending on the hydrocolloid source. It can be seen from the figures that at all concentrations of 1:10, at constant shear rate, as the temperature increases, the viscosity and therefore the resistance to flow decreases. At increased temperatures, molecular movement intensifies, leading to an increase in intermolecular distance and a weakening of interactions, consequently resulting in a decrease in the apparent viscosity of water-soluble systems. Additionally, various bonding forces like hydrogen, electrostatic, and hydrophobic interactions between molecules may weaken under high temperatures, leading to a similar adverse effect on apparent viscosity [26]. In the graph depicting a 1:10 dilution with a 3% concentration, it's noted that at the outset of the measurement, particularly at low shear stress levels, the viscosity value at 20°C is observed to be lower than that at 10°C. This discrepancy may stem from the fact that the initial data was collected prior to the rheometer system achieving the necessary stabilization time to reach a consistent operational state. Alternatively, variations in environmental conditions could have influenced the precision of the data.

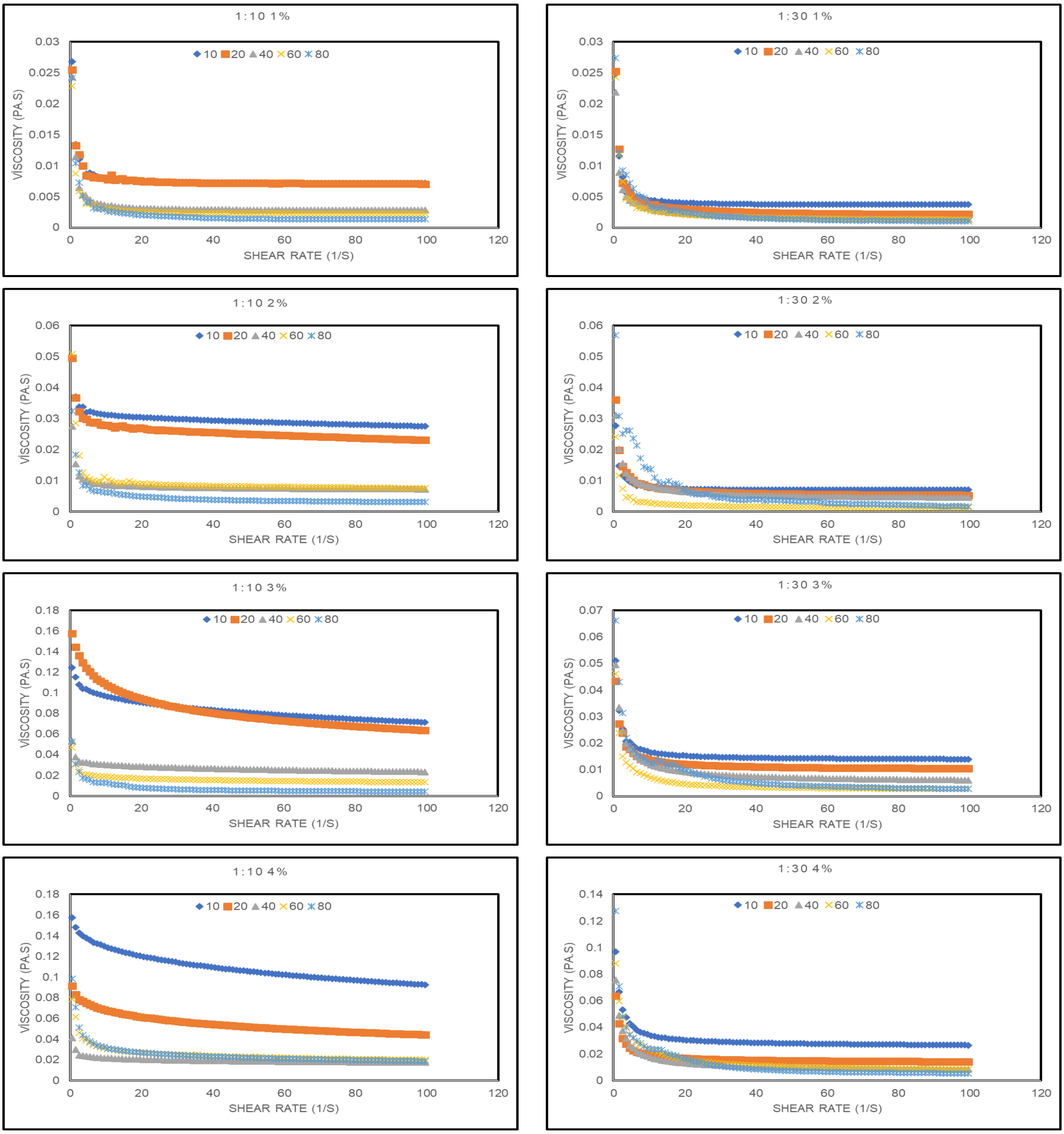

Figure 1. Viscosity - Shear Rate graphs of 1, 2, 3, 4% concentrations of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio measured at different temperatures.

As a result of the 1:10 solid/solvent ratio extraction process, when the apparent viscosity versus shear rate data of the samples at different temperatures are transferred to the graphs, it is seen that the viscosity increases with increasing concentration at all temperatures at constant shear rate (Figure 2). Our study yielded results similar to those of previous studies on the rheological properties of okra polysaccharides [11, 27-29].

At all concentrations of 1:30, at constant shear rate, viscosity decreases with increasing temperature, but the difference in viscosity between temperatures is very small (Figure 2). Consequently, the viscosity of all samples decreased as temperature rose, indicating their temperature-sensitive viscosity property. This occurrence can be attributed to the heightened thermal motion of molecules at elevated temperatures, which weakens intermolecular interaction forces, resulting in decreased viscosity [24].

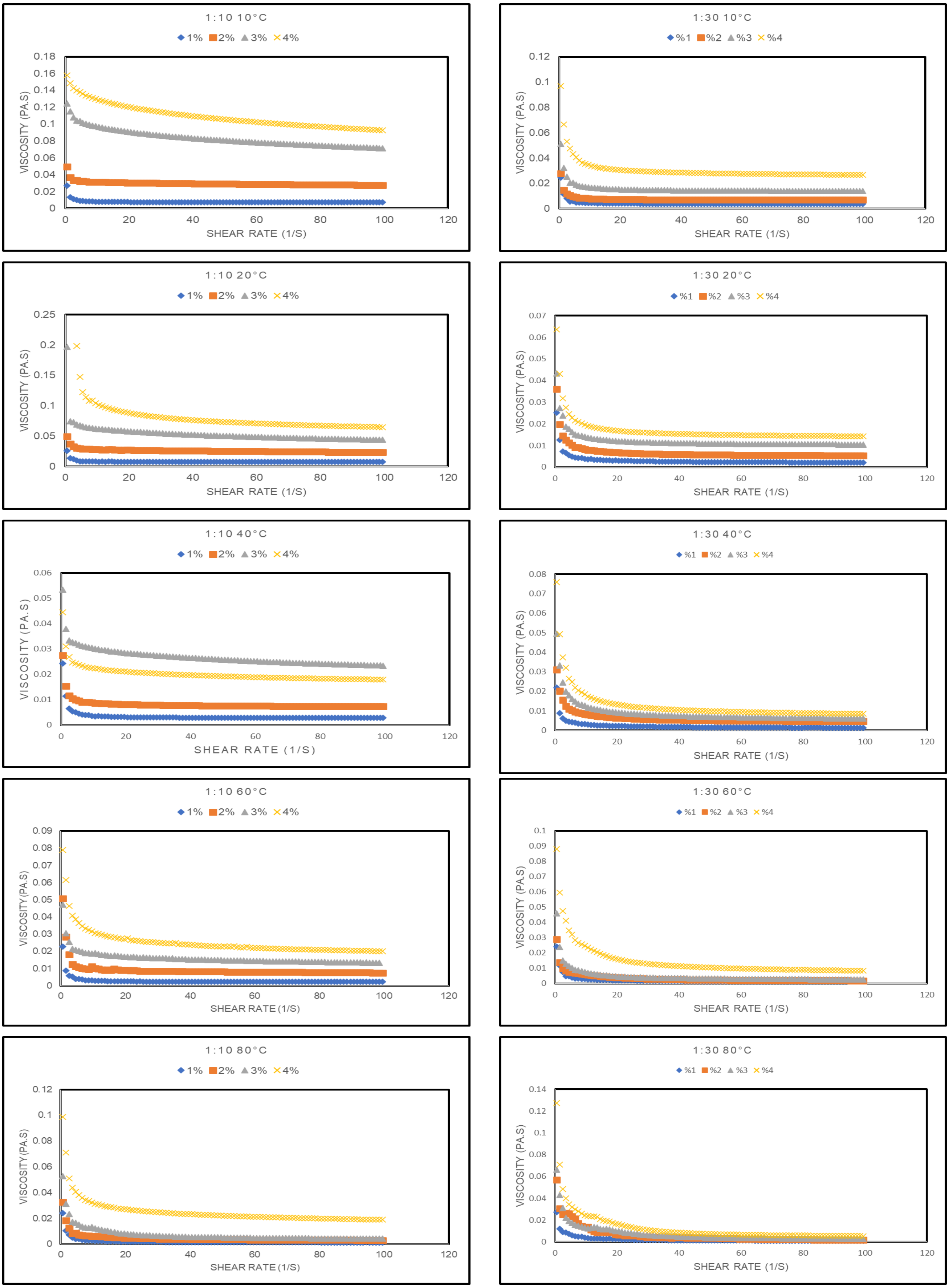

Figure 2. Viscosity - Shear Rate graphs of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio at different concentrations at 10, 20, 40, 60 and 80°C.

Viscosity - shear rate graphs of polysaccharides obtained from ultrasound assisted extractions using 1:10 and 1:30 okra/distilled water ratios were very different from each other. Polysaccharides play a pivotal role in rheology owing to the substantial volume they occupy when hydrated, compared to the non-hydrated chain's total volume. Intrinsic viscosity assessments hold significant value in biopolymer analysis, providing insights into the volume enclosed by individual polymer molecules [11].

As a result of the 1:30 solid/solvent ratio extraction process, when the apparent viscosity versus shear rate data of the samples at different temperatures were analyzed, the viscosity increased as the concentration increased at all temperatures of 1:30 solid/solvent ratio at constant shear rate (Figure 2). In general, it can be concluded that the viscosity values of all polysaccharide solutions increased as the concentration increased.

Zaharuddin et al. [29] showed in their study that the viscosity of a 1% solution of okra mucilage was greater than that of a lower concentration (0.5% solution) and this proved that viscosity increases with concentration. He said that higher viscosity means more viscosity and denser material with more important cross-linked molecules. This has been shown to facilitate more efficient retention of ingredients within tablets.

The flow behavior of the samples was explained by applying the power law (exponential) model to the shear stress and shear rate experimental data. The experimental data exhibited a strong fit ($R^2 > 0.99$) with the Power Law model. Qasem et al. [30] suggested that the pseudoplasticity observed in solutions containing macromolecules arises from the disentanglement of long-chain molecules, leading to decreased intermolecular resistance to flow during shear conditions. Graphs depicting shear stress versus shear rate curves for ratios of 1:10 and 1:30, across various concentrations and temperatures, are provided in Figure 3 and Figure 4.

As can be seen from the shear stress - shear rate graphs above, the shear stress increased with increasing shear rate and this increase is non-linear. The natural logarithms of the curves were utilized alongside nonlinear regression to ascertain the K and n values of the equation. The outcomes of the nonlinear regression for the conducted measurements are detailed as K and n values and are displayed in Table 1.

Shear stress values show lower values at high temperatures and low concentrations, which is a typical behavior of pseudoplastic (non-Newtonian) fluids [31]. The rheological properties of okra polysaccharide can be described by the coefficient of consistency and the flow behavior index. The consistency coefficient gauges the resistance of the sample to flow, while the flow behavior index delineates the rheological characteristics of a substance. Various materials exhibit distinct flow behavior indices [32]. The samples' flow behavior was analyzed concerning both concentration and temperature.

The flow behavior index, represented by "n," reflects how much the sample's flow deviates from the Newtonian flow, where $n = 1$. As shown in Table 1, all samples exhibit n values below 1, indicating pseudoplastic behavior. This aligns with previous research findings, suggesting that apparent viscosity decreases with increasing shear rates. Smaller n values reflect greater pseudoplasticity. As evident from the table, the n value decreases with rising temperature, indicating an increase in the product's pseudoplastic properties as temperature increases.

The viscosity coefficient (K) describes the viscosity of the sample. In other words, high K values define high viscosity [26]. It is seen that the K values decrease as the temperature increases at the same concentration and increase as the concentration increases at the same temperatures. At higher temperatures, a reduction in the consistency coefficient (K) was noted, indicating a decrease in viscosity with increasing temperature.

The fit of the power law model to the empirical data was evaluated using three statistical parameters: coefficient of determination (R^2), root mean squared error (RMSE), and chi-square (χ^2). Using these statistical tools, it is possible to detect differences between empirical data and model predictions. In addition to considering the coefficient of determination (R^2) close to 1 as an indicator of fit, it is accepted that the fit increases with lower root mean square error (RMSE) and chi-square (χ^2) values [33]. If the root mean square error is less than 0.1, it represents perfect fit [23]. The closer the chi-square (χ^2) values are to zero, the better the models fit the data. The results obtained are shown in Table 1 and according to these results, the power law model fits the flow curves of okra polysaccharides.

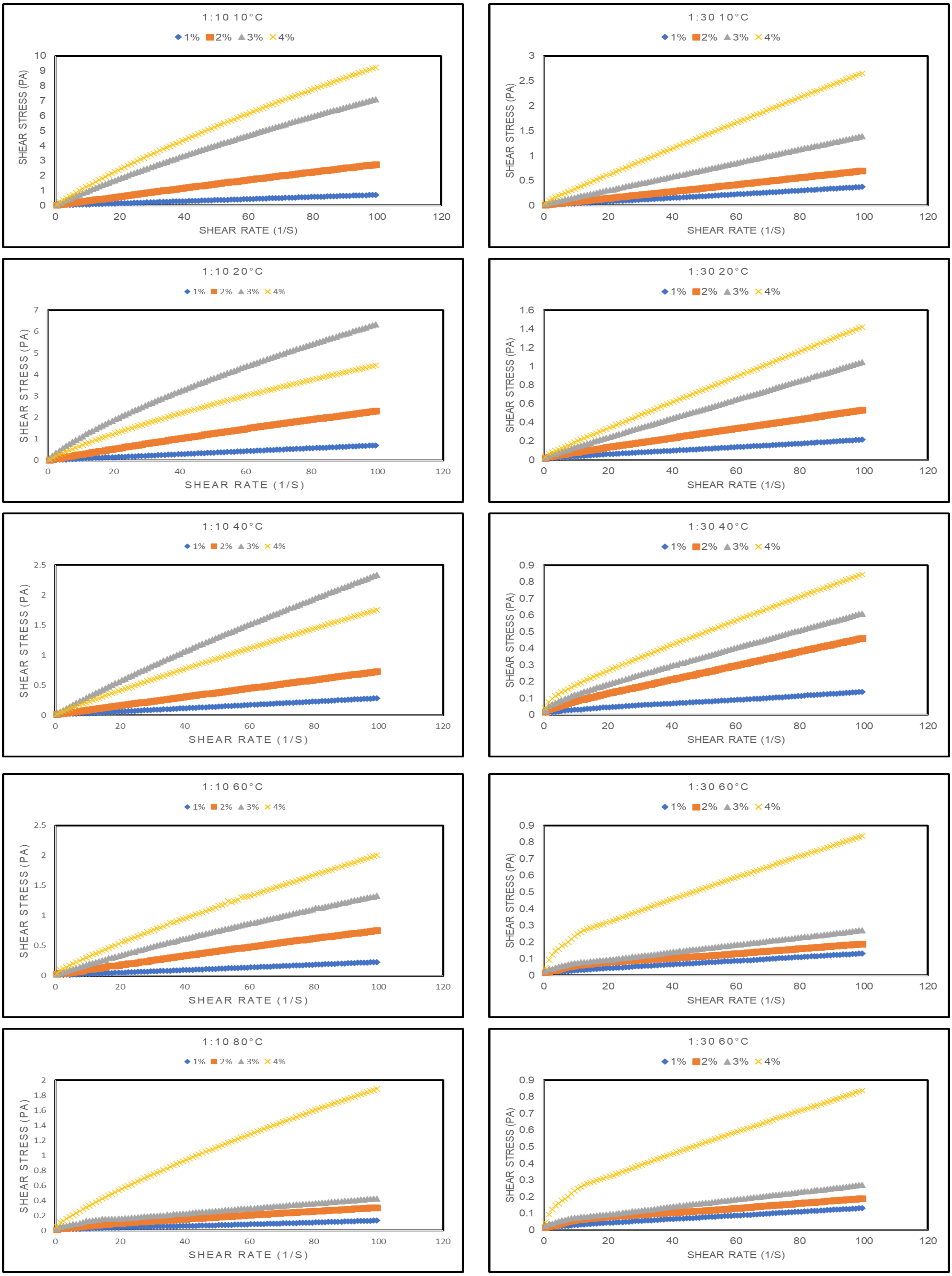

Figure 3. S Shear Stress - Shear Rate graphs of 1, 2, 3 and 4% concentrations of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio measured at different temperatures.

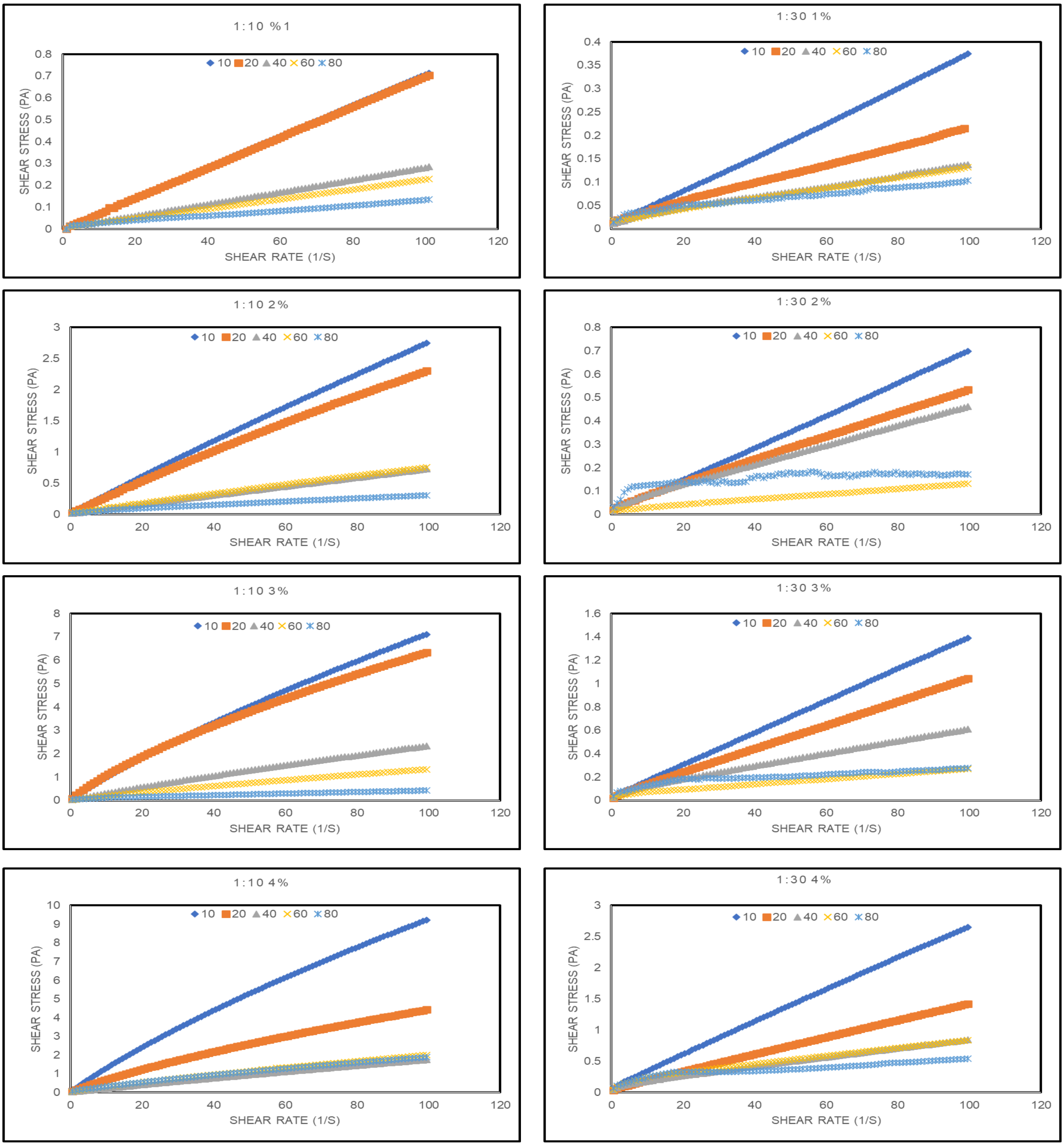

Figure 4. Shear Stress- Shear Rate graphs of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio at different concentrations 10, 20, 40, 60 and 80°C.

Temperature Dependency (Arrhenius Equation)

The Arrhenius model was employed to characterize how temperature influences the rheological properties. Assessing the temperature effect was vital due to the potential impact of different heating temperatures on food items incorporating gums and starches throughout processing. The impact of temperature on the apparent viscosity of okra polysaccharide samples was examined across a temperature range of 10 – 80°C. Following the measurements, the consistency coefficient was graphed, and the influence of temperature on the consistency index was analyzed using the Arrhenius equation. Activation energies, consistency factors and correlation coefficients are shown in Table 2.

Table 1. Rheological parameters of okra polysaccharides with different solid/solvent ratios at different temperatures and concentrations

Concentration	Temperature (°C)	Solid/Solvent Ratio (g/mL) 1/10					1/30				
		K	n	R²	χ2	RMSE	K	n	R²	χ^2	RMSE
1%	10	0.009103	0.9418	0.99905	0.0000416	0.0063790	0.00745	0.87655	0.99685	0.0000301	0.005427
	20	0.008565	0.92075	0.99735	0.0000596	0.0075488	0.006951	0.73665	0.9948	0.0000225	0.004671
	40	0.005908	0.8932	0.9964	0.0000228	0.0047241	0.006361	0.63855	0.98935	0.0000180	0.004197
	60	0.005067	0.86685	0.99505	0.0000218	0.004619	0.005572	0.6613	0.9953	0.0000071	0.002617
	80	0.004704	0.67025	0.98515	0.0000251	0.004937	0.004373	0.36455	0.95845	0.0000279	0.005123
2%	10	0.03506	0.94725	0.9999	0.0000795	0.0088209	0.019602	0.92585	0.9982	0.0000967	0.009649
	20	0.030036	0.9277	0.9998	0.0000986	0.0117502	0.013993	0.8173	0.997	0.0000708	0.008323
	40	0.016457	0.9137	0.9995	0.0000200	0.0044217	0.012572	0.73735	0.99455	0.0001056	0.01016
	60	0.015249	0.8645	0.99545	0.0001374	0.010676	0.008936	0.5082	0.982	0.0000351	0.005861
	80	0.012205	0.7258	0.9972	0.0000532	0.006609	0.006512	0.23405	0.88355	0.0001084	0.009986
3%	10	0.1275	0.8815	0.9993	0.0078253	0.0872258	0.032954	0.90805	0.99835	0.0002015	0.013954
	20	0.134985	0.8143	0.9994	0.0018174	0.0421870	0.020143	0.8753	0.99805	0.0002021	0.014056
	40	0.051053	0.8892	0.99965	0.0001209	0.0107004	0.01816	0.6901	0.9938	0.0001664	0.01275
	60	0.036105	0.856	0.9999	0.0018506	0.003717	0.01205	0.54325	0.9758	0.0001005	0.009906
	80	0.03342	0.5363	0.9902	0.0004011	0.019058	0.01044	0.3388	0.9661	0.0002515	0.014775
4%	10	0.2445	0.8523	0.9994	0.0069765	0.0825000	0.095827	0.88285	0.9985	0.0007445	0.026983
	20	0.193125	0.8095	0.9995	0.0013318	0.0354762	0.068926	0.86215	0.9981	0.0003087	0.017384
	40	0.09755	0.9054	1	0.0000040	0.0018884	0.045348	0.66565	0.9938	0.0002706	0.01627
	60	0.064785	0.7387	0.99585	0.0073437	0.008656	0.037323	0.52495	0.99135	0.0006028	0.024275
	80	0.053403	0.7363	0.99855	0.0002170	0.00576	0.026303	0.3679	0.96545	0.0005094	0.022295

The rise in temperature induces alterations in the consistency index, with decreasing values indicating increased resistance to flow. The high R^2 values suggest that the consistency factors of the samples exhibit a good fit to the Arrhenius-type model equation concerning temperature. R^2 values ranging from 0.90 to 1.00 across all samples signify a robust correlation of the Arrhenius model with hydrocolloids in describing the temperature dependencies. A higher activation energy implies a greater impact of temperature on viscosity [22].

An elevation in the activation energy (E_a) of a biological system suggests its reliance on temperature. For both samples with different solid/solute ratios, the activation energy increased with increasing concentration (Table 2). The maximum activation energy value was achieved for 4% concentration (275.8434 kj/mol). A higher E_a value implies the potential for more pronounced changes in viscosity. Therefore, viscosity is more difficult to control at high concentration.

Table 2. The activation energy (E_a) and consistency factor (K_o) values of samples with different solid/solute ratios and concentrations

Solid/Solvent Ratio (g/mL)	Concentration	E_a (kJ/mol)	K_o (Pa.s)	R^2
1/10	%1	123.65	0.0080	0.96
	%2	185.84	0.0280	0.94
	%3	270.30	0.1040	0.91
	%4	275.84	0.1807	0.98
1/30	%1	85.36	0.0070	0.94
	%2	174.18	0.0157	0.96
	%3	183.67	0.0240	0.93
	%4	211.14	0.0723	0.98

Bai et al. [24] demonstrated that the activation energy values for okra polysaccharides ranged from 6.67 to 13.26 kJ/mol. It is thought that the reason for this difference may be the extraction method they applied and the pH values they tested.

Marcotte et al. [34] found that food systems containing xanthan gum exhibited lower activation energy, while systems containing starch and pectin had intermediate values. The increase in activation energy noted with the inclusion of okra extract may be associated with its notable water solubility, as indicated by the extraction procedure.

Scanning Electron Microscope

The macro and microstructures of the samples extracted for 5 minutes with 1:10 and 1:30 okra/distilled water ratios were visualized by scanning electron microscopy at two different magnifications and the irregular, wavy, rough-textured surface and amorphous appearance of okra polymers were shown (Figure 5). SEM images of polysaccharides obtained through ultrasound-assisted extraction at identical durations but varying solid-to-solvent ratios demonstrate noticeable morphological differences. Lower solid-to-solvent ratios result in denser and more compact polysaccharide structures, with fewer visible void spaces or pores. Conversely, higher solid-to-solvent ratios lead to more porous and interconnected polysaccharide structures, accompanied by an increased presence of void spaces or pores evident in the SEM images. In general, both samples consist of flake bundles, but the flakes in the sample with 1:30 okra/distilled water ratio were found to contain larger holes than the other [35].

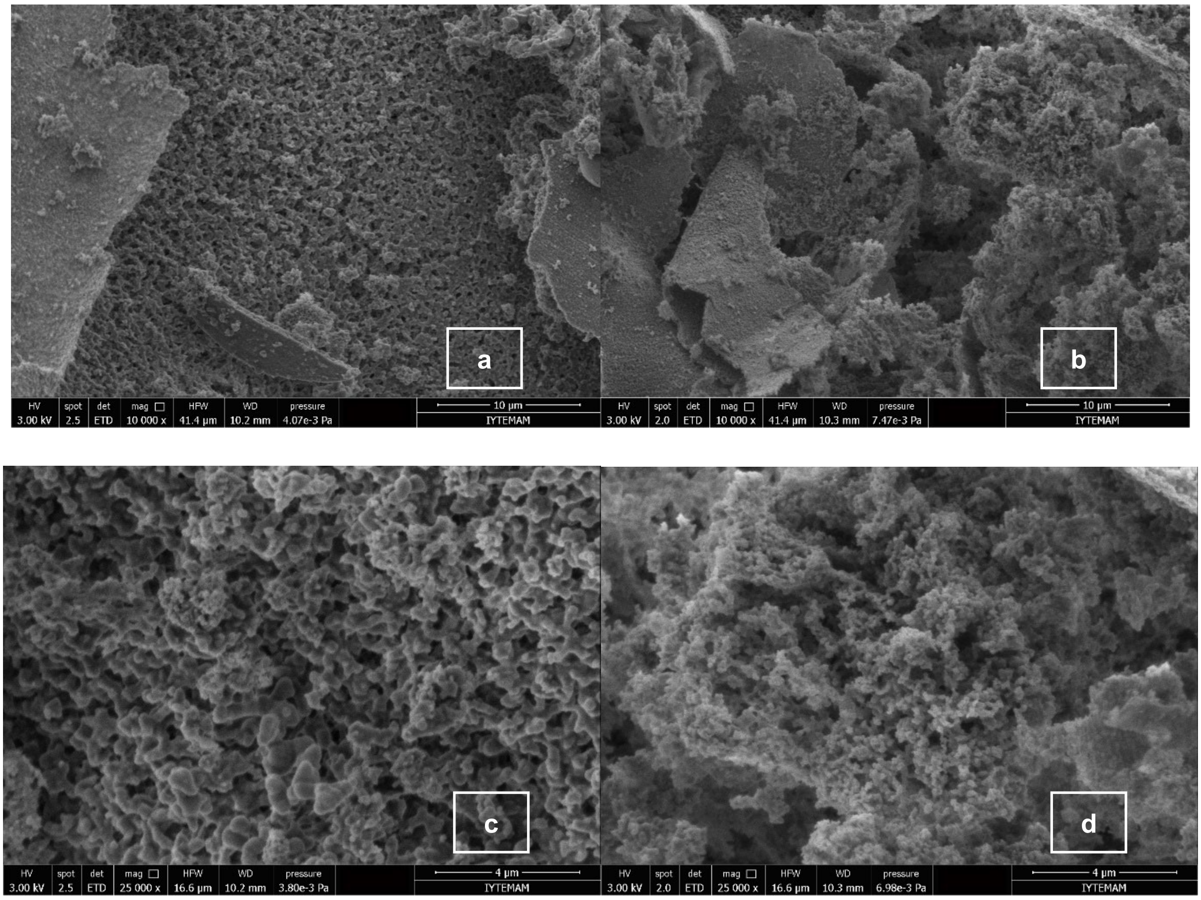

Figure 5. SEM images of polysaccharides obtained as a result of ultrasound treatment at different solid/solvent ratios. a) 1:10 / 10000x magnification, b) 1:30 / 10000x magnification, c) 1:10 / 25000x magnification, d) 1:30 / 25000x magnification

Nagpal et al. [36], characterized the structure of powdered polysaccharides in their study, focusing on the extraction of gum from Abelmoschus esculentus using an ultrasonically assisted method. Their research aimed to explore the physicochemical, functional, and antioxidant properties of the gum for potential applications in food and pharmaceuticals.

The correlation between SEM images and rheology lies in their collective capacity to offer understanding into the structure and characteristics of materials, especially concerning intricate substances such as polymers, gels, and colloidal systems. A substance showcasing a network structure that is more interconnected, as visualized in SEM images, could potentially display elevated viscosity or elasticity owing to heightened resistance against flow or deformation. Conversely, substances characterized by dispersed or irregular structures might flow more readily and showcase reduced viscosity. Large and irregular particles can lead to increased resistance in the flow and increased viscosity, while smaller and regular particles can lead to a lower viscosity flow. Furthermore, structural properties such as porosity also affect the rheological behavior of the material. Higher porosity can increase the fluidity of the material, while lower porosity can result in a higher viscosity flow. The SEM images reveal that the sample containing a 1:10 ratio of okra to distilled water exhibits a larger and irregular network structure, which is more interconnected. This structural characteristic could lead to elevated viscosity as a result of heightened resistance to flow or deformation.

As Zaharuddin et al. [29], also described, the higher the viscosity of a sample, the stickier it is. Higher viscosity leads to denser material with heavier cross-linking of molecules and the structure of okra polysaccharides appears more compact as can be seen in SEM images.

In summary, the outcomes indicate that the proportion of solid to solvent in the ultrasound-assisted extraction technique for isolating polysaccharides impacts the structure of the resulting powdered products, thereby potentially influencing their functional properties.

CONCLUSION

In conclusion, our study delved into the extraction and characterization of mucilaginous polysaccharides from okra utilizing both chemical and physicochemical techniques, with a focus on their rheological behavior. Through a systematic investigation encompassing various ratios of okra to distilled water and polysaccharide concentrations, we elucidated the non-Newtonian pseudoplastic flow characteristics of okra polysaccharides across a temperature range. The derived parameters, including flow behavior index and consistency coefficient, provided insights into the viscosity behavior of the extracted polysaccharides under different conditions.

Furthermore, our analysis revealed the significant influence of temperature and concentration on the rheological properties of okra polysaccharides. The observed trends in flow behavior index and consistency coefficient with temperature and concentration variations were systematically analyzed. Additionally, the fit of the power law model to the experimental data was rigorously evaluated using statistical parameters, further validating our findings.

Moreover, our exploration of temperature's impact on the apparent viscosities of okra polysaccharide samples, along with the modeling of temperature influence using the Arrhenius equation, provided valuable insights into the thermal behavior of these polysaccharides.

SEM imaging allowed us to visualize the structural characteristics of okra polysaccharides, highlighting differences in morphology based on solid-to-solvent ratios. These observations offer valuable guidance for optimizing extraction protocols and enhancing the industrial applications of okra mucilages based on their rheological properties.

In essence, our study contributes to the understanding of okra polysaccharides' rheological behavior and lays the groundwork for further research aimed at optimizing extraction processes and leveraging these polysaccharides in various industrial applications.

As a result, these polysaccharides obtained as a result of the analyses can be widely used in the food industry as a thickener thanks to their sticky and gamy structure, in wastewater treatment systems due to the high adsorption capacity of their wastes, and in pharmaceuticals as emulsifying and suspending agents.

REFERENCES

[1] Wang, K., Li, M., Wen, X., Chen, X., He, Z., Ni, Y. (2018). Optimization of ultrasound-assisted extraction of okra (*Abelmoschus esculentus* (L.) moench) polysaccharides based on response surface methodology and antioxidant activity. *International Journal of Biological Macromolecules*, 15(114), 1056-1063.

[2] Demiray, E., Tülek, Y. (2016). Güneşte kurutulmuş bamyaların rehidrasyon kinetiği. *Akademik Gıda*, 14(4), 368-374.

[3] Rajalakshmi, M., Sangeetha, S. (2023). Okra mucilage - method of extraction and a novel strategy for pharmaceutical drug delivery system. *Journal of Pharmaceutical Negative Results*, 14, 2473-2481.

[4] Öncü Glaue, Ş., Akcan, T., Tavman, Ş. (2023). Thermal properties of ultrasound-extracted okra mucilage. *Applied Sciences*, 13(11).

[5] Ormanlı, E., Bayraktar, O., Şahar, U., Tavman, S., Kumcuoglu, S. (2022). Development and characterization of films based on okra polysaccharides and whey protein isolate. *Journal of Food Measurement and Characterization*, 17(1), 264-277.

[6] Lengsfeld, C., Titgemeyer, F., Faller, G., Hensel, A. (2004). Glycosylated compounds from okra inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. *Journal of Agricultural and Food Chemistry*, 52(6), 1495-1503.

[7] Deters, A.M., Lengsfeld, C., Hensel, A. (2005). Oligo- and polysaccharides exhibit a structure-dependent bioactivity on human keratinocytes in vitro. *Journal of Ethnopharmacology*, 102(3), 391-399.

[8] Kumcuoglu, S., Yılmaz, T., Tavman, S. (2014). Ultrasound assisted extraction of lycopene from tomato processing wastes. *Journal of Food Science and Technology*, 51(12), 4102-4107.

[9] Zhu, W., Obara, H. (2022). The pre-shearing effect on the rheological properties of okra mucilage. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 648, 129257-129266.

[10] Georgiadis, N., Ritzoulis, C., Sioura, G., Kornezou, P., Vasiliadou, C., Tsioptsias, C. (2011). Contribution of okra extracts to the stability and rheology of oil-in-water emulsions. *Food Hydrocolloids*, 25(5), 991-999.

[11] Kontogiorgos, V., Margelou, I., Georgiadis, N., Ritzoulis, C. (2012). Rheological characterization of okra pectins. *Food Hydrocolloids*, 29(2), 356-362.

[12] Goksen, G., Demir, D., Dhama, K., Kumar, M., Shao, P., Xie, F., Echegaray, N., Lorenzo, J.M. (2023). Mucilage polysaccharide as a plant secretion: Potential trends in food and biomedical applications. *International Journal of Biological Macromolecules*, 230, 123146-123163.

[13] Safwa, S.M., Rana, M.R., Ahmed, T., Rahman, S., Kabir, M.A.B. (2023). Maximization and characterization of ultrasonic-assisted extraction of taro corms mucilage using response surface optimization and comparison with conventional methods. *Food Analytical Methods*, 16(11), 1724-1737.

[14] Chalapud, M.C., Carrín, M.E. (2023). Ultrasound-assisted extraction of oilseeds-sustainability processes to obtain traditional and non-traditional food ingredients: A review. *Comprehensive Reviews in Food Science and Food Safety*, 22(3),

2161-2196.
[15] Sengkhamparn, N., Sagis, L.M.C., de Vries, R., Schols, H.A., Sajjaanantakul, T., Voragen, A.G.J. (2010). Physicochemical properties of pectins from okra (A*belmoschus esculentus* (L.) *moench*). *Food Hydrocolloids*, 24(1), 35-41.
[16] Ndjouenkeu, R., Goycoolea, F.M., Morrisav, E.R. (1996). Rheology of okra and dika nut polysaccharides. *Carbohydrate Polymers*, 29(1), 263-269.
[17] Savouré, T., Dornier, M., Vachoud, L., Collignan, A. (2020). Clustering of instrumental methods to characterize the texture and the rheology of slimy okra (*Abelmoschus esculentus*) suspensions. *Journal of texture studies*, 51(3), 426-443.
[18] Ding, M., Liu, Y., Ye, Y.F., Zhang, J.C., Wang, J.H. (2021). Polysaccharides from the lignified okra: Physicochemical properties and rheological properties. *Bioactive Carbohydrates and Dietary Fibre*, *26*, 100274.
[19] Turabi, E., Sumnu, G., Sahin, S. (2008). Rheological properties and quality of rice cakes formulated with different gums and an emulsifier blend. *Food Hydrocolloids*, 22(2), 305-312.
[20] Cevoli, C., Balestra, F., Ragni, L., Fabbri, A. (2013). Rheological characterisation of selected food hydrocolloids by traditional and simplified techniques. *Food Hydrocolloids*, 33(1), 142-150.
[21] Rao, M., Rizvi, S. (1995). Engineering properties of foods, marcel decker. *Inc., New York, USA*.
[22] Yılmaz, V.M.B., Süfer, Ö., Kumcuoğlu, S. (2017). Effects of temperature and hydrocolloids on the rheological characteristics of coating batters. *Journal of Food Measurement and Characterization*, 11(3), 1159-1166.
[23] Mohammadi Moghaddam, T., Razavi, S.M.A., Malekzadegan, F., Shaker Ardekani, A. (2009). Chemical composition and rheological characterization of pistachio green hull's marmalade. *Journal of Texture Studies*, 40(4), 390-405.
[24] Bai, L., Zhu, P., Wang, W., Wang, M. (2020). The influence of extraction ph on the chemical compositions, macromolecular characteristics, and rheological properties of polysaccharide: The case of okra polysaccharide. *Food Hydrocolloids*, 102, 105586-105596.
[25] Xu, K., Guo, M., Du, J. (2017). Molecular characteristics and rheological properties of water-extractable polysaccharides derived from okra (*Abelmoschus esculentus* L.). *International journal of food properties*, 20(1), 899-909.
[26] Li, Y., Wang, X., Lv, X., Wang, X., Wang, X., Cui, J., Yan, M. (2020). Extractions and rheological properties of polysaccharide from okra pulp under mild conditions. *International Journal of Biological Macromolecules*, 148, 510-517.
[27] Alamri, M.S., Mohamed, A.A., Hussain, S. (2013). Effects of alkaline-soluble okra gum on rheological and thermal properties of systems with wheat or corn starch. *Food Hydrocolloids*, 30(2), 541-551.
[28] Woolfe, M.L., Chaplin, M.F., Otchere, G. (1977). Studies on the mucilages extracted from okra fruits (*Hibiscus esculentus* L.) and baobab leaves (*Adansonia digitata* L.). *Journal of the Science of Food and Agriculture*, 28(6), 519-529.
[29] Zaharuddin, N.D., Noordin, M.I., Kadivar, A. (2014). The use of *Hibiscus esculentus* (okra) gum in sustaining the release of propranolol hydrochloride in a solid oral dosage form. *BioMed Research International*, 2014.
[30] Qasem, A.A.A., Alamri, M.S., Mohamed, A.A., Hussain, S., Mahmood, K., Ibraheem, M.A. (2017). Effect of okra gum on pasting and rheological properties of cake-batter. *Journal of Food Measurement and Characterization*, 11(2), 827-834.
[31] Santos, P.H., da Silva, L.H.M., Rodrigues, A.M.C., Souza, J.A.R. (2016). Influence of temperature, concentration and shear rate on the rheological behavior of malay apple (*Syzygium malaccense*) juice. *Brazilian Journal of Food Technology*, 19.
[32] Zhou, M., Parkhurst, A., Voss, H., Weller, C. (2004). Estimating rheological properties of yogurt using different versions of the Freundlich model and design matrices. *Conference on Applied Statistics in Agriculture.*
[33] Beigi, S., Sobati, M.A., Charkhi, A. (2015). An experimental investigation on drying kinetics of calcium carbonate. *Journal of Particle Science and Technology*, 1(3), 153-162.
[34] Marcotte, M., Taherian Hoshahili, A.R., Ramaswamy, H.S. (2001). Rheological properties of selected hydrocolloids as a function of concentration and temperature. *Food Research International*, 34(8), 695-703.
[35] Wang, C., Li, J., Cao, Y., Huang, J., Lin, H., Zhao, T., Liu, L., Shen, P., Julian McClements, D., Chen, J., Liu, C., Liu, J., Li, Q. (2023). Extraction and characterization of pectic polysaccharides from choerospondias axillaris peels: Comparison of hot water and ultrasound-assisted extraction methods. *Food Chemistry*, 401, 134156-134165.
[36] Nagpal, M., Aggarwal, G., Jain, K., Madan, J. (2017). Extraction of gum from *Abelmoschus esculentus*: Physicochemical peculiarity and antioxidant prepatent. *Asian Journal of Pharmaceutical and Clinical Research,* 10(9), 174-179.