

Article

The Rheological Behavior of Polysaccharides from Mulberry Leaves (*Morus alba* L.)

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Received: 22 July 2020; Accepted: 25 August 2020; Published: 27 August 2020



Abstract: In this study, mulberry leaves polysaccharides (MLPs) namely HBSS (extracted with hot buffer soluble solids), CHSS (extracted with chelating agent soluble solids), DASS (extracted with diluted alkali soluble solids), and CASS (extracted with concentrated alkali soluble solids) were obtained using four different solvents and examined for their rheological potential. Different MLPs solutions harbored obvious disparity for viscosity and displayed a shear-thinning behavior at the tested range. Among all the fractions, DASS possessed the highest apparent viscosity at 0.5–2.5%. The apparent viscosity of MLPs solutions declined at acidic pH, alkaline pH, and higher temperature (90 °C). The HBSS fraction showed the best heat stability of all the fractions. All the fractions displayed noticeable differences in apparent viscosity in response to Na⁺ and Ca²⁺ at 20 °C. Both the modules such as G' (storage modulus) and G'' (loss modulus) showed augmentation with oscillation frequency. Initially, the value of G'' was higher than G' of MLPs at lower frequency and lower concentration, and the MLPs displayed stronger viscous nature; whereas, G' was consistently higher at higher frequency and higher concentration, and the MLPs displayed stronger elastic characteristic. From our data, it was indicated that these MLPs can be used as promising natural materials (thickeners, gelling agents, binding agents, stabilizers) for their direct application to the food industry.

Keywords: mulberry leaves; polysaccharides; sequential extract; rheological properties

1. Introduction

Over the years, mulberry has been recognized as a Chinese conventional plant beneficial for the human health and its leaves are used to feed silkworms during the silk production process [1,2]. Mulberry leaves are reported to possess various biological activities and can be consumed as medicine to improve eyesight, reduce fever, lower blood pressure, and protect the liver [3]. Plant polysaccharides are gaining a huge attention due to their multifaceted attributes such as anticoagulant [4], antioxidant [5–8], anti-cancerous [9,10], antibacterial [11], immunomodulatory [12], anti-inflammatory [13], and antihyperglycemic properties [14]. Previous studies have reported some methods of extraction of polysaccharides from mulberry, including hot water extraction [15], ethanol extraction [16], microwave-assisted extraction [17], enzyme-assisted extraction [18], ultrasound-assisted extraction [19],

and electric-field-assisted extraction [15], etc. Mulberry polysaccharides (MLPs) displayed antioxidant activity [20,21], anti-inflammatory [22], hypoglycemic activity [23,24], antihyperglycemic, and anti-hyperlipidemia activities [17].

Polysaccharide is a long-chain macromolecule polymer, and often used as a thickener and gelling agent in the food industry due to their high ability to retain water and form hydrogels. The rheological characteristic of polysaccharides is related to the thickeners, gelling agents, stabilizers, and binding agents of samples [25–29], which is responsible for their use in food, pharmaceutical, and cosmetic industries as natural material. Polysaccharides are viscoelastic materials which always display liquid and solid properties simultaneously. The rheological behavior of polysaccharides is affected by some factors, e.g., concentration, temperature, sugars, pH, and salts [28,29].

To the best of our literature search, the rheological properties of MLPs are rarely studied [30,31] and there are no reports about the sequential extraction of MLPs except our previous study [32]. In the recent years, our laboratory has focused more on plant derived polysaccharides and their physicochemical properties [9,24–28]. In our previous research, four different polysaccharides obtained by hot buffer soluble solids (HBSS), chelating agent soluble solids (CHSS), dilute alkaline soluble solids (DASS), and concentrated alkaline soluble solids (CASS) were prepared. The FTIR spectrum demonstrated that four members of MLPs displayed the typical characters of polysaccharides [32]. The monosaccharide components of four members displayed significant differences, galactose and arabinose were the major components in HBSS, glucose was the major sugar in DASS and CASS, whereas the major sugars in CHSS were arabinose. The molecular weights of HBSS, CHSS, DASS, and CASS were 7.812×10^3 , 3.279×10^3 , 6.912×10^3 , and 1.408×10^3 kDa, respectively. HBSS and DASS possessed the highest DPPH radical scavenging activity. The reducing power of HBSS was higher than that of the other three. DASS exhibited the strongest ABTS radical scavenging activity. CHSS displayed the best Fe^{2+} -chelating ability [32].

To extend the utilization of MLPs in the food industry, the current study was crucial to affirm the rheological behavior of the extracted four polysaccharides, such as differences in apparent viscosity and influences of different treatments. Then after, the effects of different concentrations of MLPs, pH, temperature, salt ions, and oscillatory shear measurements on apparent viscosity were examined.

2. Results and Discussion

2.1. Apparent Viscosity of Four MLPs Fractions

Four kinds of MLPs at 1% concentration showed different rheological behaviors at 20 °C as shown in Figure 1. The apparent viscosity of samples was observed in the following order: DASS > CHSS > HBSS > CASS. The obtained curves declined over the range of 0.01–1000 s^{-1} and showed different behaviors. MLPs solutions had almost similar apparent viscosity at a low shear rate; whereas, apparent viscosity of HBSS and CASS displayed a slight increase at 100–1000 s^{-1} . Our results confirm that these two MLPs fractions can be used as novel commercial gum materials due to their high viscosity. In addition, the apparent viscosity of DASS was higher than other members, which demonstrated that DASS was better used as thickening agents in the food industry.

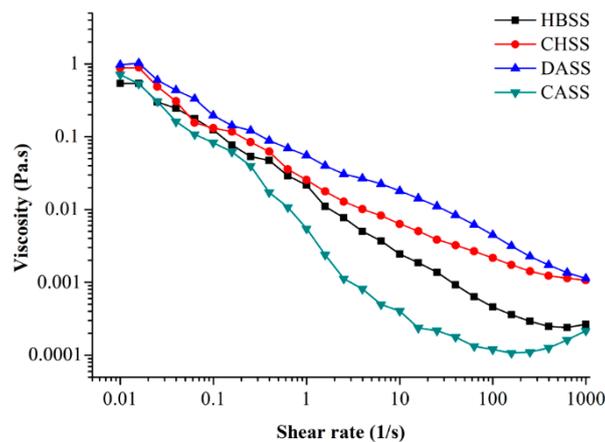


Figure 1. Effect of 1% mulberry polysaccharides (MLPs) on apparent viscosity with rising shear rate at 20 °C.

2.2. Effect of Different MLPs Concentrations on Apparent Viscosity

The different tested concentrations led to significant change in apparent viscosity measurements at 20 °C as shown in Figure 2. The concentrations of samples solutions were 0.5%, 0.8%, 1.0%, 1.5%, 2.0%, and 2.5%. Under the selected range, the apparent viscosity of MLPs fractions was decreased with the increasing shear rate. These results demonstrated the typical pseudoplastic fluid with shear-thinning and non-Newtonian fluidic characteristics which was consistent with the previous report [33]. In general, polymer solutions had the same behavior which may be related to the molecular weight [34,35]. Moreover, the apparent viscosity increased with rising MLPs concentrations which might be due to the hydrodynamic and thermodynamic interactions of macromolecules and strengthening of aggregates [36]. At lower concentrations, previous studies have reported some entanglements in the polymer molecular chains; on the other hand, the easy formation of gel network at high concentrations was reported [37]. Hydrocolloids (pectin) had highly apparent viscosity at lower concentration [38]. Whereas, DASS showed the highest apparent viscosity and CASS showed the lowest viscosity value. These data indicated that the DASS fraction can be used as thickener, gelling agent, binding agent in the food, pharmaceutical, and cosmetic industries.

The Ostwald-DeWaele equation $\eta = m \times \gamma \times n^{-1}$ was fitted to the shear thinning region of the samples at various concentrations, where m was the consistency index and n was the flow behavior index. The value of n reflects the degree of deviation between solution fluid and Newtonian fluid ($n = 1$ is a significant characteristic of Newtonian fluid). The consistency index m of HBSS, CHSS, DASS, and CASS increased significantly (Table 1). However, the flow index n decreased significantly, which reflected the dependence of apparent viscosity on concentration (Table 1). In the present research, the n value of HBSS was less than the other three components, which may be due to the different extraction solvents and structures of the four polysaccharides.

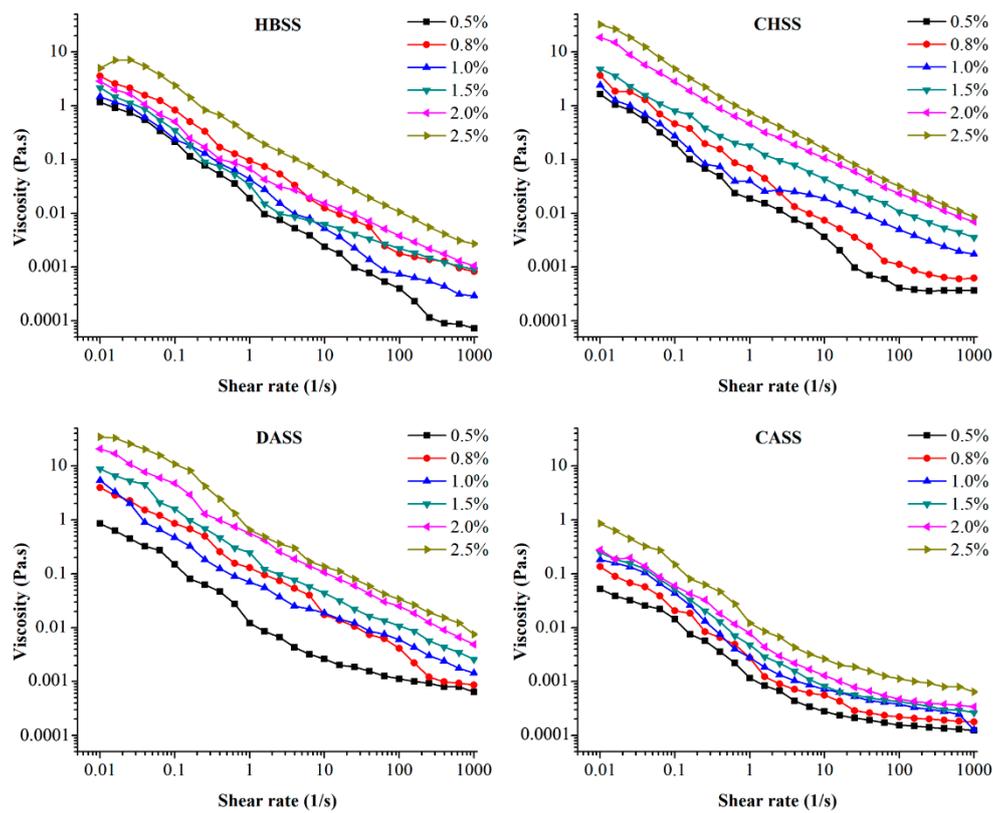


Figure 2. Variation of apparent viscosity of MLPs at different concentrations with rising shear rate at 20 °C.

Table 1. Exponent n and coefficient m of $\eta = m \times \gamma \times n^{-1}$ as a function of concentration for MLPs in water at 20 °C.

Concentration (mg/mL)	HBSS			CHSS			DASS			CASS		
	m	n	R^2	m	n	R^2	m	n	R^2	m	n	R^2
1.0	0.0277	0.051	0.98	0.195	0.629	0.94	0.092	0.578	0.93	0.002	0.674	0.92
5	0.013	0.46	0.94	0.328	0.638	0.92	0.159	0.54	0.95	0.002	0.651	0.94
10	0.017	0.418	0.96	0.682	0.535	0.95	0.285	0.511	0.93	0.004	0.569	0.95
15	0.023	0.380	0.95	1.399	0.541	0.94	0.497	0.476	0.96	0.007	0.484	0.96
20	0.066	0.385	0.98	2.852	0.496	0.96	0.817	0.464	0.95	0.009	0.379	0.95
25	0.3265	0.259	0.99	3.012	0.428	0.96	0.922	0.421	0.96	0.011	0.325	0.95

R^2 represents correlation coefficient.

2.3. Effect of pH on Apparent Viscosity

As known, pH could change the apparent viscosity of samples solutions which was revealed through the change of apparent viscosity of 1.0% samples solutions at different pH values as shown in Figure 3. Our results showed the decreased viscosity for all the four fractions with the rising shear rate at 0.01–1000 s⁻¹ for all conditions. Particularly, the viscosity values of MLPs at pH 4 or pH10 were less than those at pH 7 which might be due to ionic interactions or composition modification (acid or alkali) which can lead to conformational changes [39,40]. Therefore, the change of pH value could decrease apparent viscosity due to the influence on breaking the bond which can further lower the molecular weight [41].

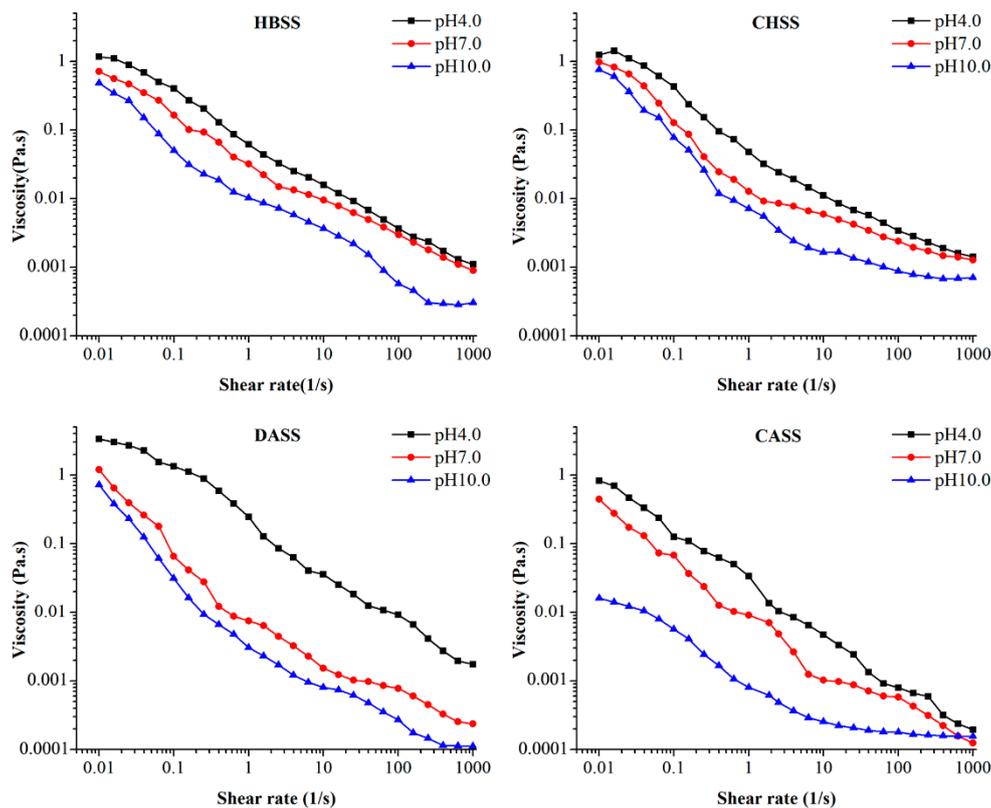


Figure 3. Variation of apparent viscosity of 1% MLPs at different pH values at 20 °C.

2.4. Effect of Temperature Range on Apparent Viscosity

For this, apparent viscosity of MLPs fractions was determined at the same range (1.0%) at a stable shear rate (100 s^{-1}) as shown in Figure 4. The viscosity values of four fractions were lowered with the rising temperature (20 to 90 °C). Four samples solutions showed the change in the apparent viscosity, such as 53.42%, 68.73%, 59.25%, 83.87% for HBSS, CHSS, DASS, and CASS, respectively. The major alteration in the apparent viscosity was observed for CASS; whereas, HBSS displayed the least change of all samples. Moreover, HBSS possessed the stronger viscosity over the temperature range of 20–90 °C. From the above data, we can conclude that the larger change in the viscosity of samples led to the weaker heat stability. These results indicate that the HBSS fraction can preferably be applied in baking processing.

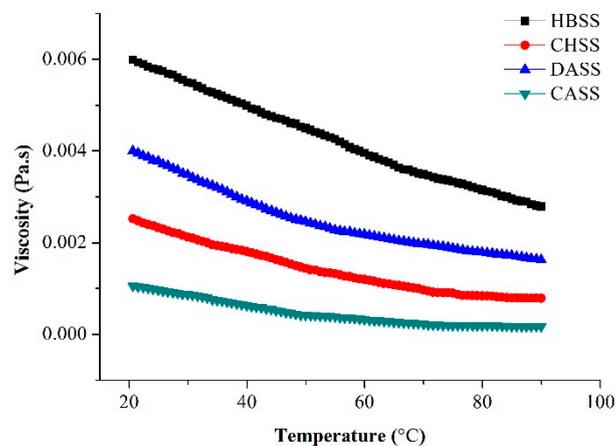


Figure 4. Effect of apparent viscosity of 1% MLPs with rising temperature.

2.5. Effect of Various Temperature Treatments on Apparent Viscosity

As shown in Figure 5, various temperature treatments caused significant change in the apparent viscosity measurements. The apparent viscosity was noticeably changed after heating or freezing treatment and the change in the viscosity values of CHSS and CASS was higher than the remaining fractions. The apparent viscosity of samples solutions was increased after the freezing process ($-20\text{ }^{\circ}\text{C}$) as compared to heating ($100\text{ }^{\circ}\text{C}$). The apparent viscosity value of DASS was the highest among all samples after the freezing treatment, which indicated that DASS can be more suitable as a stabilizer in freeze processing. As previously reported, high temperature could increase the thermal motion of molecules and intermolecular distance, which could further weaken the interactions and recede the apparent viscosity [42]. The change in the apparent viscosity might be due to transitions in the consequent phase of water and the change in composition as described in the previous studies [43].

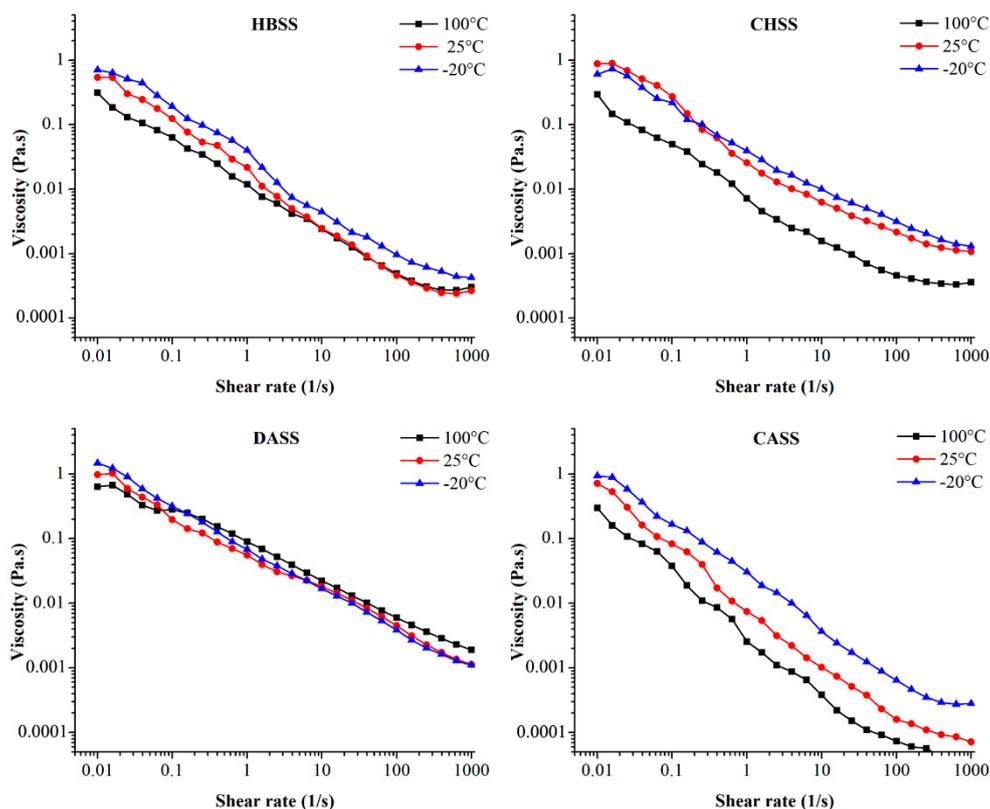


Figure 5. Variation of apparent viscosity of 1% MLPs with different temperature treatments.

2.6. Effect of Various Salts on Apparent Viscosity

The effect of Na^+ on apparent viscosity was displayed in Figure 6. The apparent viscosity of MLPs was reduced with the growing shear rate at $0.01\text{--}1000\text{ s}^{-1}$ with the addition of different concentrations of Na^+ . The results indicated that four kinds of samples solutions showed completely different behaviors from each other. Initially, the viscosity value for HBSS was declined followed by an increase at higher concentration of Na^+ . On the contrary, CHSS showed the opposite trend with the increased viscosity which was followed by a decrease with the increase in concentration of Na^+ . Furthermore, the apparent viscosity of DASS was declined after the addition of Na^+ and the values were gradually decreased with the increasing Na^+ concentration. The anionic or cationic polysaccharides had higher apparent viscosity due to the negative contacts in the branches [44]. The observed decrease in the apparent viscosity might be due to the charge shielding effect which could increase the concentrations of counter-ions and lead to molecules contractions [45].

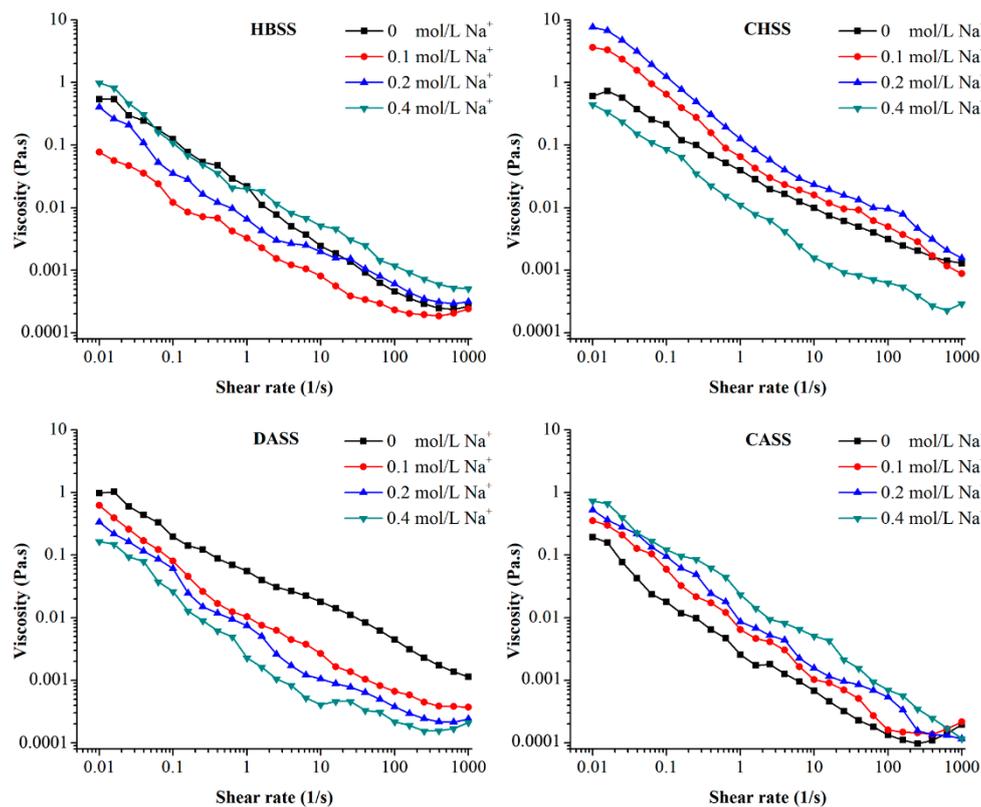


Figure 6. Variation of apparent viscosity of 1% MLPs with addition of different concentrations of NaCl at 20 °C.

The effect of Ca^{2+} on apparent viscosity of four fractions' solutions was shown in Figure 7. The prominent differences in apparent viscosity were observed due to the presence of Ca^{2+} for all the samples. In the case of HBSS and CASS, the viscosity was improved with the addition of Ca^{2+} and it was constantly increased with the rising concentration of Ca^{2+} . On the other hand, CHSS and DASS showed decreased viscosity values in the presence of high concentration of Ca^{2+} which was followed by a slight increase in the viscosity. The apparent viscosity might increase due to the increased intermolecular association in the case of HBSS and CASS fractions [40].

2.7. The Linear Viscoelastic Region Measurements of MLPs

MLPs with the concentration of 0.5%, 1.0%, and 2.0% were measured with the stress ranging from 0.1% to 1000% in order to make sure that the structure of samples was protected [46]. The obtained results were depicted in Figure 8 which indicated that the 1% shape change could be used as dynamic oscillatory measurements.

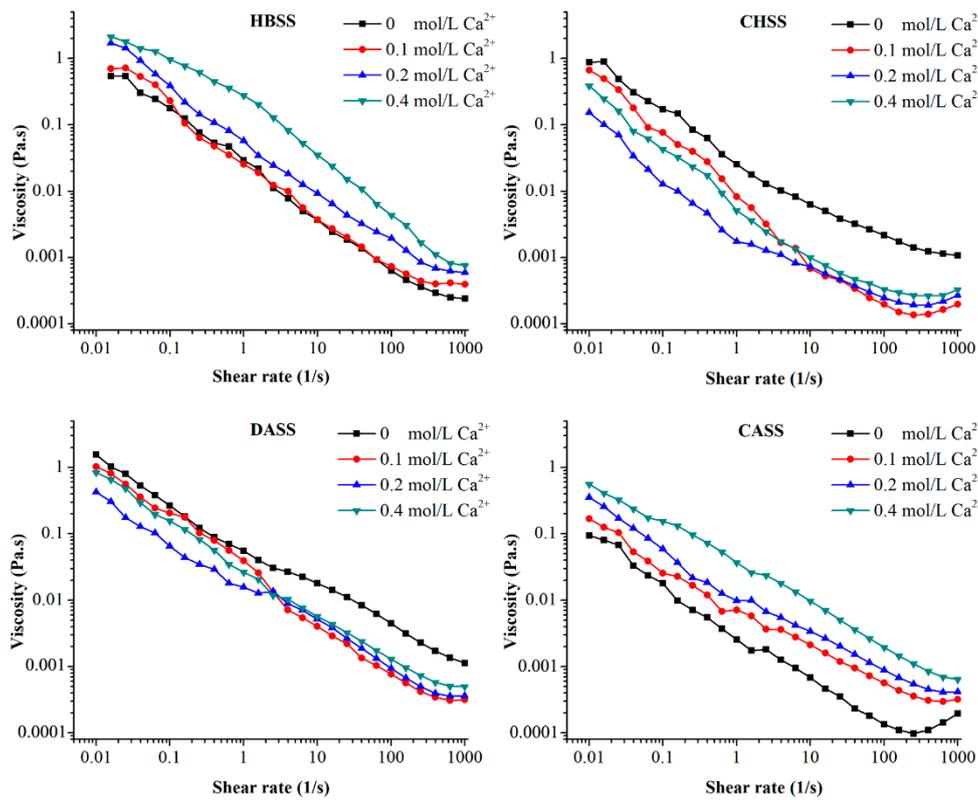


Figure 7. Variation of apparent viscosity of 1% MLPs with addition of different concentrations of CaCl₂ at 20 °C.

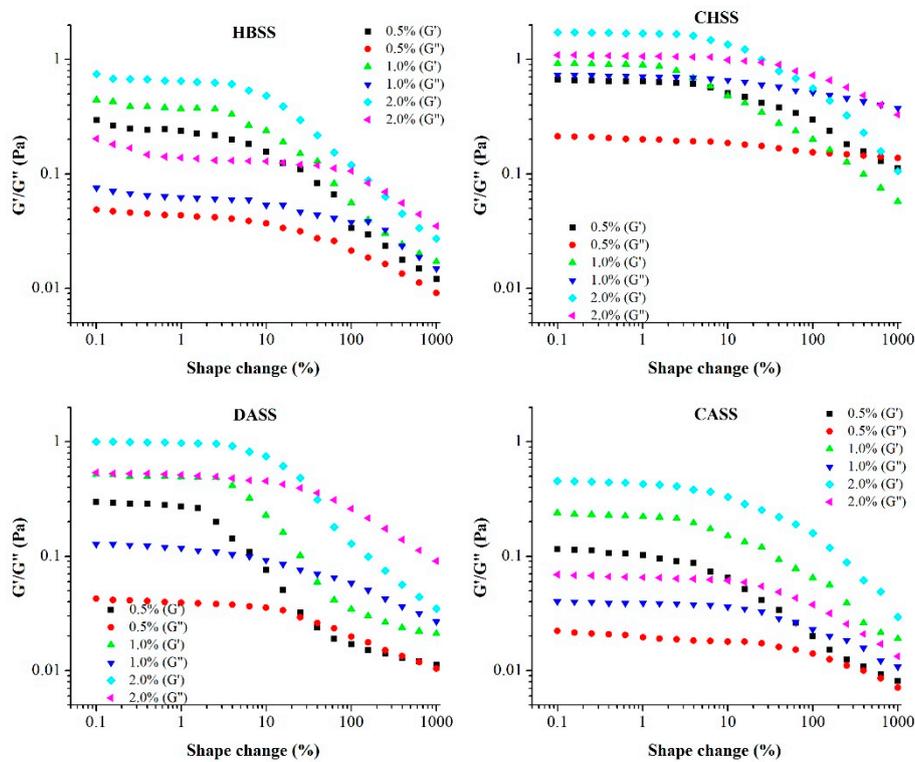


Figure 8. Strain sweeps measurements at 1.0 Hz of MLPs at different concentrations to determine the G' (storage modulus) and G'' (loss modulus).

2.8. Oscillatory M

Viscoelastic properties were determined to evaluate the viscous and elastic characteristics of samples [47]. The change in storage modulus (G') and loss modulus (G'') of MLPs (5, 10, and 20 mg/mL) was determined based on the above mentioned linear viscoelastic region measurements at 1% oscillation intensity in Figure 9. The curves of G' and G'' ascended with the growing shear oscillation frequency (0.01–100 Hz). Initially, the value of G'' was higher than G' at lower frequency and lower concentration; whereas, G' was consistently higher at higher frequency and higher concentration, which showed the characteristic of samples solutions. The phenomenon of G' increased on the account of increasing the number and average size of junction points [48]. The crossover frequency displayed the viscoelastic behavior of samples. As reported previously, the lower crossover value often leads to the larger elastic nature [49]. The fractions displayed stronger viscous nature when G'' was higher than G' ; whereas, a stronger elastic characteristic was observed at higher G' as compared to G'' . Our data affirmed the gel-like characteristics of the tested samples in the case of $G' > G''$. Moreover, a liquid-like behavior was noticed in the case of $G' < G''$. The weak gel properties were observed when G' was just above or on the G'' . All these findings were in agreement with the previously reported data [50]. Our results suggest that MLPs with large viscosity and elasticity could contribute to the food processing.

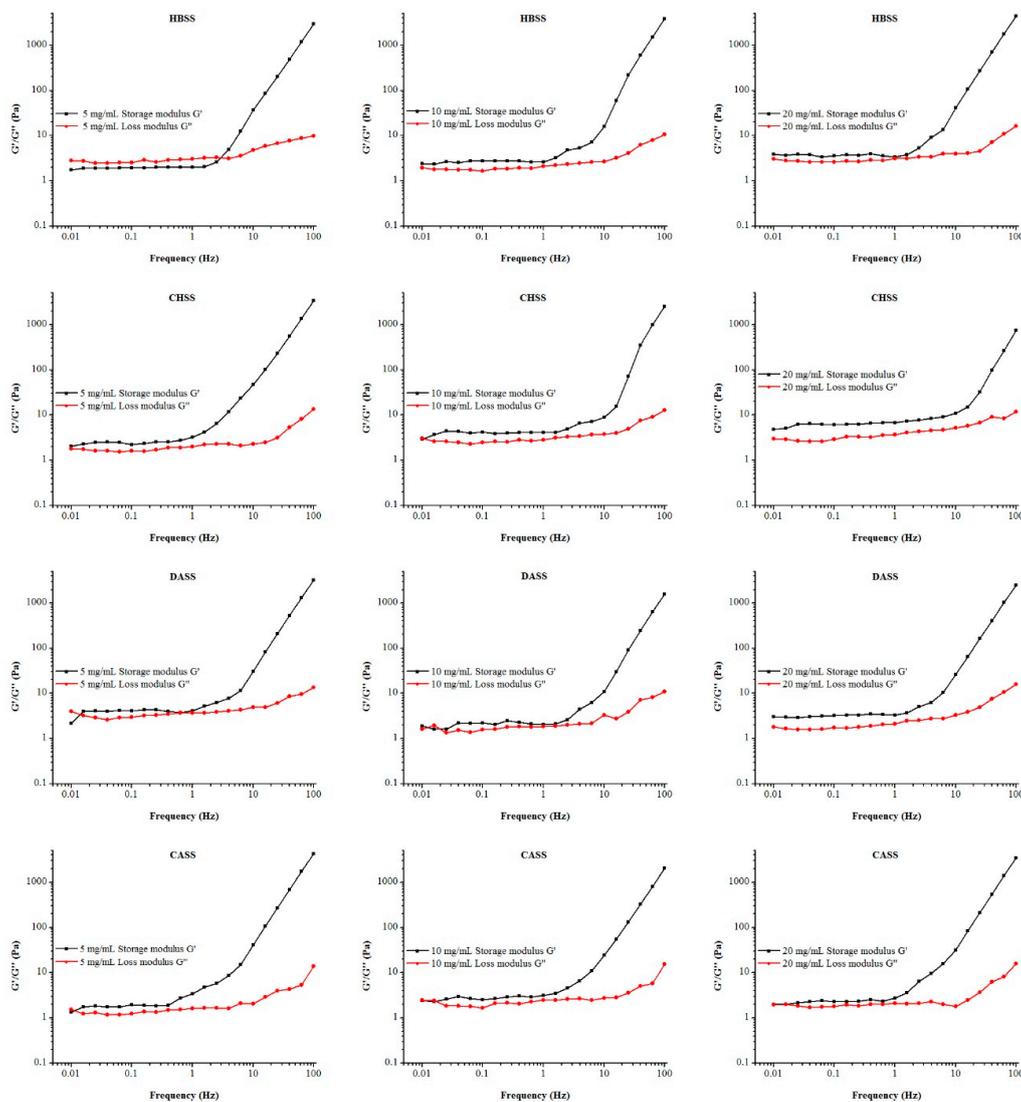


Figure 9. Frequency sweeps at 1% strain to determine the G' (storage modulus) and G'' (loss modulus) of MLPs at different concentrations.

3. Materials and Methods

3.1. Materials and Chemicals

Initially, dried mulberry leaves (*Morus alba* L.) were procured as mentioned in our previous study [32] and grounded into a powder (60 mesh) before the extraction of polysaccharides.

3.2. Sequential Extraction and Purification of MLPs

Four MLPs (HBSS, CHSS, DASS, and CASS) were sequentially extracted by our previous method [24]. The purified samples were obtained by using a DEAE-Cellulose column (DEAE Cellulose-52 (Sigma-Aldrich, St. Louis, MO, USA) using which eluent was 0.1 mol/L of NaCl solutions at a flow rate of 1 mL/min. The obtained fractions were dialyzed (4 °C), freeze-dried, and studied for their rheological behavior.

3.3. Rheological Measurements

The rheological potential of MLPs was determined using the previous method [25,26,28,51,52]. For this, MLPs with different concentrations (0.5%, 0.8%, 1.0%, 1.5%, 2.0%, and 2.5%) were completely dissolved in distilled water and allowed to stand for 30 min at normal conditions. The samples were investigated for their apparent viscosity using a Discovery Hybrid Rheometer-3 (DHR-3) (TA instruments, New Castle, DE, USA) outfitted with a cone-and-plate geometry (diameter 40 mm, cone angle 2°). The steady-shear rate was 0.01–1000 s⁻¹ and the temperature selected was 20 °C.

3.4. Effect of Different MLPs Concentrations on Apparent Viscosity

The MLPs solutions at different concentrations (0.5%, 0.8%, 1.0%, 1.5%, 2.0%, and 2.5%) were studied under the selected range of 0.01–1000 s⁻¹ at 20 °C [33,52].

3.5. Effect of Acidic and Alkaline pH on Apparent Viscosity of MLPs

Briefly, 1.0% of MLPs solutions were added to NaOH or HCl to adjust the pH values from 4.0 to 10.0, respectively to measure the apparent viscosity under the selected range of 0.01–1000 s⁻¹ at 20 °C [33,52].

3.6. Effect of Temperature on Apparent Viscosity of MLPs

One percent of MLPs solutions was selected to evaluate the effects of different temperature ranges (20–90 °C) followed by the measurement of apparent viscosity at a shear rate of 100 s⁻¹ [33].

3.7. Effect of Various Temperature Treatments on Apparent Viscosity of MLPs

The MLPs samples were exposed to different temperature conditions such as room temperature (25 °C for 2 h), very high temperature (100 °C for 2 h), and freezing temperature (−20 °C for 2 h), respectively. Subsequently, the MLPs solutions were placed at room temperature before the experiment. The apparent viscosity of 1.0% samples solutions after different temperature treatments was measured under the selected range of 0.01–1000 s⁻¹ at 20 °C [33,52].

3.8. Effect of Various Salts on Apparent Viscosity of MLPs

MLPs were dissolved in NaCl, CaCl₂ at the concentrations of 0, 0.1, 0.2, and 0.4 mol/L. The apparent viscosity of 1.0% samples solutions was determined under the selected range of 0.01–1000 s⁻¹ at 20 °C [33,52].

3.9. Oscillatory Shear Measurements of MLPs

To measure the storage modulus (G') and loss modulus (G'') of MLPs fractions at different concentrations of 5, 10, and 20 mg/mL, oscillatory shear determinations were used as per the previous

reports [26,27]. The G' and G'' were determined to study the frequency dependence at 0.01–100 Hz and 1% oscillation strain.

3.10. Statistical Analysis

For the data analysis, one-way analysis of variance (ANOVA) was performed by using Origin Lab (Origin Pro 8.0) software (Northampton, Massachusetts, USA).

4. Conclusions

Due to the lack of data on rheological properties of MLPs, the present study was executed to affirm the rheological attributes of four kinds of MLPs fractions which were extracted from mulberry leaves using four different solvents. According to the obtained results, MLPs exhibited shear-thinning characteristic and non-Newtonian fluid behavior. The viscosity of the MLPs fractions was significantly affected under acid, alkaline conditions, different ions concentrations, and high temperature. DASS possessed the highest apparent viscosity, while HBSS was found the most heat stable fraction of all the samples. Moreover, the increase in the G' and G'' ultimately led to the different rheological behavior. The above results confirmed that MLPs with a particular emphasis on DASS seem to be the promising materials for their diversified utilization in the food industry.

Author Contributions: Conceptualization, Z.-J.W.; methodology, B.-Y.L. and L.L.; software, K.T., C.T. and D.-Y.Z.; validation, J.-G.Z.; investigation, B.-Y.L. and L.L.; writing—original draft preparation, B.-Y.L.; writing—review and editing, K.T., C.T. and Z.-J.W.; funding acquisition, K.T., J.-G.Z., and Z.-J.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (31850410476), the Major Projects of Science and Technology in Anhui Province (201903a06020021, 18030701158, 1804b06020347, 18030701144, 18030701161, 17030701058), and Master Workstation of Traditional Manual Skills in Anhui Finance and Trade Vocational College.

Conflicts of Interest: The authors declare no conflict of interest.

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