

Article

Determination of Viscoelastic and Physicochemical Interactions of Dextran Type Exopolysaccharides (EPS) with Different Starch Samples

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Abstract: In this study, the rheological properties of three distinct dextrans with different levels of (1 → 6)-linked α-D-glucose/(1 → 3)-linked α-D-glucose units from three lactic acid bacteria (LAB) strains were determined. Dextran PDER21 was further selected following the rheological measurements and its interactions with maize, wheat and waxy maize starches were determined by characterizing the viscoelastic and pasting properties of the dextran–starch mixtures. Fourier transform infrared (FTIR) spectroscopy analysis was also applied to unveil this interaction. The presence of dextran PDER21 in the standard maize starch increased the elastic behavior, while its increased amounts enhanced the elastic properties of wheat and waxy maize starches. The temperature sweep test showed the solid-like property of starch–dextran mixtures in the studied temperature range (4–70 °C). Dextran PDER21 affected the pasting properties of starches. Especially, high levels of the peak, through and final viscosity values were reached with the blends of standard maize starch–1% dextran, wheat starch–0.5% dextran and waxy maize starch–0.5% dextran PDER21. Finally, the interactions were also confirmed by FTIR analysis as no alterations in the starch FTIR spectra were observed at different levels of dextran in different starch samples.

Keywords: lactic acid bacteria; dextran; starch; rheology; FTIR



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1. Introduction

Starch is one of the main polymers used in the food industry and it is mainly used as a stabilizer and texture modifier in food applications due to its pasting and rheological properties [1]. It contains two types of polysaccharides, including linear and branched molecules called amylose and amylopectin [2]. The ratio of amylose to amylopectin in starch affects the characteristic properties of starch [3]. It is classified according to the amount of amylose content as waxy (less than 15%), normal (20–35%) and high amylose (higher than 40%) starch [4]. The poor mechanical, physical and water solubility properties of natural starch limit its use [5]. Natural starch can be modified by various methods to improve their functional properties. Modification studies are carried out using chemical, physical, enzymatic and genetic methods. Although the commercial applicability of chemical modification is easy, it might not meet the natural expectations of consumers [6]. Modifying starch with hydrocolloids is a safe process preferred in food applications [7]. Food hydrocolloids are polysaccharides and proteins that dissolve or disperse in water to form colloidal systems [8]. Hydrocolloids can change the retrogradation, pasting and rheological properties and water holding capacity of starch [9–11]. The changes in starch with the addition of hydrocolloids are the result of the interaction of the hydroxyl group in

the structure of hydrocolloids and starch. The mixing ratio, concentrations and molecular weight of hydrocolloids and starches also affect the polymer properties [12].

Exopolysaccharides (EPS) are unique polysaccharides produced by different microorganisms including LAB strains, with their physicochemical, technological and functional properties [13]. EPSs can contain distinct sugar monomers linked by glycosidic linkages in their repeating unit structures. Single-type sugar monomers containing EPSs are called homopolysaccharides, while those containing different monosaccharide subgroups are called heteropolysaccharides. Differences in the polymeric structure of EPSs result in the formation of different functional and technological properties [14,15]. EPSs produced by LAB have an important role in fermented food products such as yoghurt, cheese and fermented sausage [16–18]. *Leuconostoc*, *Weissella*, *Streptococcus*, *Lactococcus*, *Lactobacillus*, *Pediococcus* and *Enterococcus* species have been reported as LAB species capable of producing different EPSs [19].

EPSs affect the texture properties and stability of the final product in addition to improving mouth feel and taste [18]. EPSs from LAB strains have potential use in food applications as they have been granted GRAS (generally regarded as safe) status. They are used for several purposes such as texture improvers, emulsifiers, thickeners and stabilizers in the food industry instead of chemical additives [13,14,20]. They also provide prebiotic properties and antioxidant, antiviral and antitumor activities [13]. The physicochemical properties of food products, including starch-containing products, are one of the fundamental characteristics in terms of quality parameters. The variety in structural features increases the attention of EPSs as functional polymers. In addition, the ability to improve the rheological properties of food products by different EPSs is an important function in determining their structural roles. Studies are required to understand the functional and technological roles of EPSs in their interactions with certain food ingredients. Tang et al. [21] tested the interactions of high molecular weight dextran synthesized by *Weissella confusa* QS813 with wheat starch and reported that this dextran improved the freeze–thaw stability and uniformity of starch gel. More information is required to understand the interactions of distinct EPSs with different starch models.

Recently, three different dextran structures containing distinct levels of (1 → 6)-linked α -D-glucose/(1 → 3)-linked α -D-glucose units were identified produced by *Leuconostoc mesenteroides* BI20, *Weissella cibaria* MED17 and *Weissella cibaria* PDER21 strains [22–24]. There are many studies in the literature on starch modifications and EPSs separately; however, research is required in the field of examining their interactions. This study aimed to test the interactions of these three dextrans with common starch types such as standard maize starch, native wheat starch and waxy maize starch by determining the rheological behavior of starch gels prepared with different levels of the selected dextran.

2. Materials and Methods

2.1. Materials

Dextran-type glucans from *Leuconostoc mesenteroides* BI-20, *Weissella cibaria* MED17, and *Weissella cibaria* PDER21 strains were extracted in the laboratory. The standard maize starch, wheat starch and waxy maize starch were obtained from Cargill, Turkiye.

2.2. Preparation of Solutions for Three Distinct Dextrans

In this study, dextran-type glucans containing different levels of (1 → 6)-linked α -D-glucose/(1 → 3)-linked α -D-glucose units from *Leuconostoc mesenteroides* BI-20 [22], *Weissella cibaria* MED17 [23] and *Weissella cibaria* PDER21 strains [24] were used. To extract the dextrans from LAB strains, a previously described methodology was used [23]. Briefly, a modified BHI broth containing 30 gL⁻¹ was used as the cultivation medium for the EPS extraction and the standard methodology of the EtOH precipitation of EPS, followed by removal of the proteins from the EPS solutions by TCA sedimentation, was performed. The same procedures of extraction and purification were applied to all three LAB strains. For the pre-evaluation of the dextrans in terms of rheology measurements, 1%, 3% and 5%

solutions of three dextrans were prepared in distilled water. Dextran samples were stored at 4 °C for further analysis.

2.3. Rheological Analysis

For determining the rheological behaviors of three different dextrans, viscosity analysis, frequency sweep analysis and temperature viscosity analysis were applied. According to the results of this analysis, one dextran type was selected (dextran PDER21) for use in starch gelation analysis. Analyses were carried out using a rheometer (Anton Paar, MCR-302, Austria).

2.3.1. Viscosity Determination

Analyses were carried out between 0.1–100 s⁻¹ shear rate values and shear stress values were obtained with a rheometer using a double gap probe configuration [25].

2.3.2. Amplitude Sweep

Analyses were carried out using a rheometer (Anton Paar, MCR-302, Austria) with a parallel plate (50 mm diameter) between 0.0001–1% strain with a 1 mm gap distance. Storage modulus (G') and loss modulus (G'') values and linear viscoelastic region were obtained [25].

2.3.3. Frequency Sweep

Using the rheometer (Anton Paar, MCR-302, Austria) with a 50 mm diameter parallel plate with a 1 mm gap distance, storage modulus (G') and loss modulus (G'') values were obtained between 0.1–100 rad/s frequency values [26].

2.3.4. Temperature Sweep

Using a temperature-controlled rheometer (Anton Paar, MCR-302, Austria), viscosity values of the samples were determined between 4–70 °C [25].

2.4. Preparation of Dextran-Starch Mixtures

Dextran PDER21 (0.5% and 1%) were selected among the tested dextrans to determine their interactions with starch. Starches were gelled by adding them to water at a ratio of 5% (w/v). EPSs were added to the mixture at a rate of 0.5% and 1% (w/v). The samples were gelled at 95 °C for 8 min. Gels were then cooled for a while at room temperature and were further tested as described above.

Determination of Starch Gelation

A rheometer (Anton Paar, MCR-302, Austria) with a starch cell design was used to determine the pasting properties of starches. Analysis was carried out using the same proportions of starch, EPS and water used in the preparation of dextran–starch mixtures. First, 1 g (5%, w/v) of starch without EPS and with 0.1 and 0.2 g (0.5% and 1%, w/v) EPS were poured into the RVA canister, followed by 20 mL of distilled water. The sample was kept at 50 °C for 1 min before being heated to 95 °C for 7.5 min and then held at that temperature for another 5 min. After that, the sample was cooled to 50 °C for 7.5 min and kept at that temperature for 2 min. In the pre-shear phase, the rotation speed was 960 rpm for 10 s, then 160 rpm for the rest of the analysis [10].

2.5. FTIR Analysis

An ATR accessory (single bounce) was used in all spectral acquisition. Spectral measurement parameters of resolution and accumulation were selected as 4 cm⁻¹ and 16 scans, 0–4500 cm⁻¹ in wave range, respectively. OPUS program Version 7.2 (Bruker GmbH) was employed for instrument control and data acquisition. The ATR crystal was cleaned with distilled water before each spectral acquisition. The background air spectrum was scanned before each acquisition.

2.6. Statistical Analysis

ANOVA was performed to determine if the EPS content significantly affected the dependent pasting parameters. Tukey test was employed to compare the samples with respect to the determined parameter. α was selected as 0.05 and all of the statistical analyses were performed using SPSS software.

3. Results and Discussion

3.1. Rheological Properties of Dextran Solutions

The frequency sweep, viscosity and temperature viscosity analysis of 1%, 3% and 5% solutions of dextrans BI-20, MED17 and PDER21 were carried out and the results for the 3% samples are shown in Figure 1a–c. Viscoelastic parameters: G' and G'' values were determined as a function of angular frequency. Dextran PDER21 solution had gel-like behavior where the G' was higher than G'' for the experimental range of frequency. In addition, for the same dextran sample, the magnitudes of both moduli increased with increasing frequency, and no crossover point was observed. Therefore, dextran PDER21 exhibited solid behaviors with elastic and recoverable deformations [25]. Other dextran solutions showed gel-like to fluid-like transitions and crossover points. Moreover, dextran PDER21 had higher network strength than other EPSs with higher G' and G'' values [26].

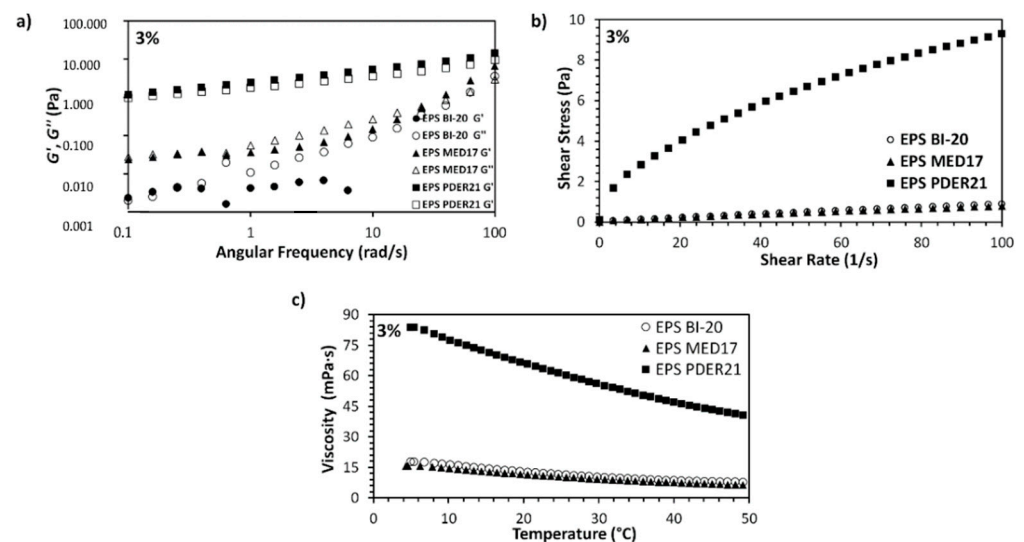


Figure 1. Graphs of frequency sweep. (a) viscosity (b) and temperature viscosity (c) analysis comparing 3% concentrations of three different EPS.

The change in shear stress depending on the shear rate of the dextrans can be seen in Figure 1b. According to the shear stress-shear rate graph, the dextran PDER21 solution demonstrated non-Newtonian shear thinning behavior. The apparent viscosity of dextran PDER21 solutions decreased with increasing shear rate [27]. In addition, it was seen that other dextrans have lower viscosity values (shear stress/shear rate) than PDER21. The apparent viscosity of dextran solutions decreased with increasing temperature from 4 to 50 °C. For all temperature values, dextran BI-20 and MED17 solutions had similar viscosity values, while dextran PDER21 solutions had higher viscosity values in comparison to the aforementioned dextrans. To better understand the effect of dextran on starch, dextran PDER21 was selected for further tests.

3.2. Rheological Properties of the Dextran-Starch Mixture

The amplitude sweep (Figure 2), frequency sweep (Figure 3) and temperature sweep (Figure 4) analysis were carried out for mixtures containing standard maize starch, wheat starch or waxy maize starch alone or with different amounts of dextran PDER21 (0.5 and 1%).

The amplitude sweep analyses were carried out in the strain range between 0.0001–1%, and graphs of strain to G' and G'' values and the linear viscoelastic (LVE) region of mixtures were determined. The linear viscoelastic region is defined as the region in which the test is performed without destroying the structure of the sample. The G' and G'' values were almost constant and independent of strain in this region [28]. After this critical value, G' and G'' were not constant. The samples showed LVE region up to 0.1% strain. The LVE region of soft solid foods was reported in the range of 0.1–2% and colloidal gels had lower LVE values than 0.1, whereas natural biopolymer gels had LVE values of 1 [29,30]. It can be observed from Figure 2a–c that storage modulus G' was greater than loss modulus G'' for all samples in the LVE region.

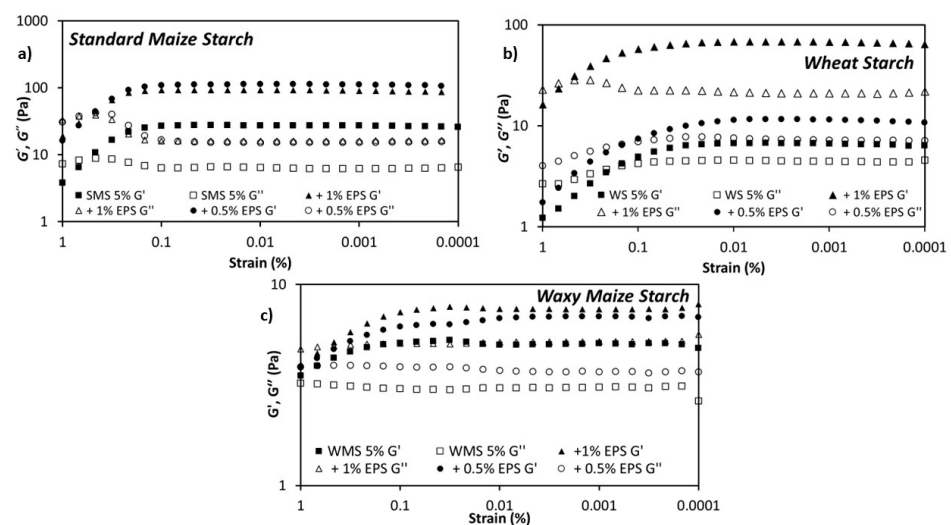


Figure 2. Amplitude sweep analysis graphs of EPS PDER21 gelled with standard maize starch (a), wheat starch (b) and waxy maize starch (c).

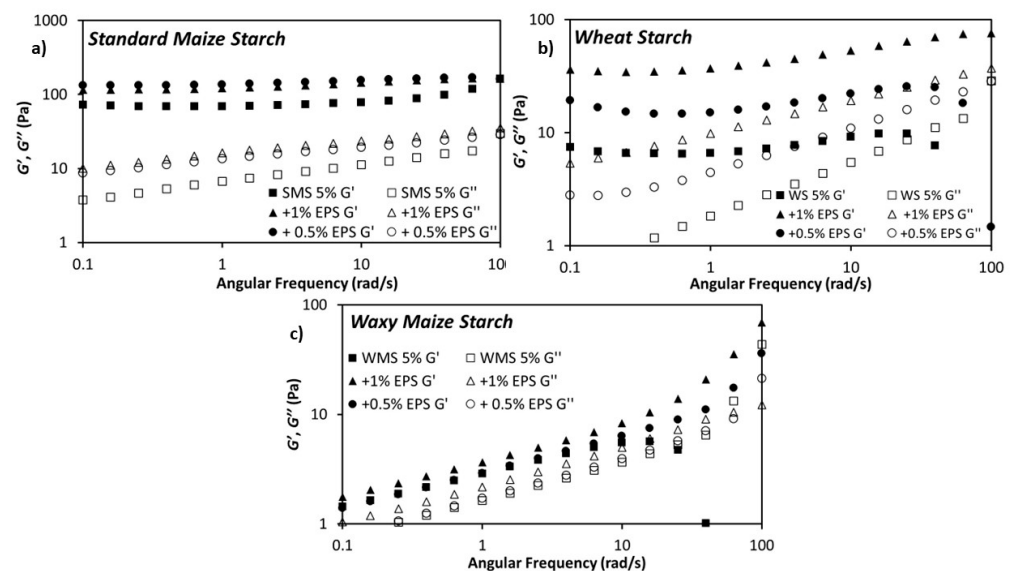


Figure 3. Frequency sweep analysis graphics of EPS PDER21 gelled with standard maize starch (a), wheat starch (b) and waxy maize starch (c).

The frequency sweep analyses were performed to measure the viscoelastic properties of dextran PDER21 and starch mixtures. As can be observed from Figure 3a–c, standard maize starch and standard maize starch–dextran mixtures had a solid-like elastic structure with higher G' than G'' for all angular frequency values performed, and there were no crossover points. Both G' and G'' moduli increased with the addition of dextran to starch, and similar

values were observed with the change in dextran PDER21 amount. In this case, it can be said that the elastic behavior of the starch solution increased according to the presence of dextran PDER21. Wheat starch containing 1% dextran PDER21 showed elastic behavior with higher G' than the G'' values at angular frequencies without a crossover point. The sample solutions containing only wheat starch and wheat starch–0.5% dextran PDER21 had a crossover point representing a transition from an elastic structure to a liquid structure at high angular frequency values measured. Higher G' than G'' results in a stable network and high elasticity [31]. Therefore, it can be said that the increased amount of dextran PDER21 caused a higher elastic modulus and an increase in elastic properties. In the experiments performed with waxy maize starch, it was observed that there was a crossover point and elastic–viscous transformation at higher levels of the angular frequency values that were measured only in the control sample that did not contain dextran PDER21. For both of the dextran PDER21 added samples, G' was higher than G'' over the entire measurement range. In addition, G' and G'' values increased as the dextran PDER21 ratio increased; in other words, dextran PDER21 increased the solid-like structure of waxy maize starch.

The effects of temperature on the viscoelastic behavior of starches and dextran PDER21 mixture at different concentrations are given in Figure 4a–c. In the temperature range studied (4–70 °C), G' was higher than G'' for all starch types and dextran PDER21 concentrations, indicating that all solutions retained their solid-like property. The G' and G'' variation trends of solutions with and without dextran PDER21 for standard maize starch were very similar and did not show a sharp change. There were slight increases in the G' values in the initial temperatures, while decreases occurred in the advancing temperatures. When the wheat starch results were examined, it was seen that there was a slow increase in the G' values for the control sample and a slow decrease in the samples containing dextran PDER21 as the temperature increased. It was thought that the decrease in the G' value with the increase in temperature could result in a decrease in the intermolecular interaction and energy required for the flow and an increase in the fluidity of the solution [25]. Furthermore, in solutions containing waxy maize starch and dextran PDER21, G' values decreased as the temperature increased and increased at the higher temperature levels. The reason for the steep increase in G' and G'' values in the final temperature values can be attributed to starch gelation [32].

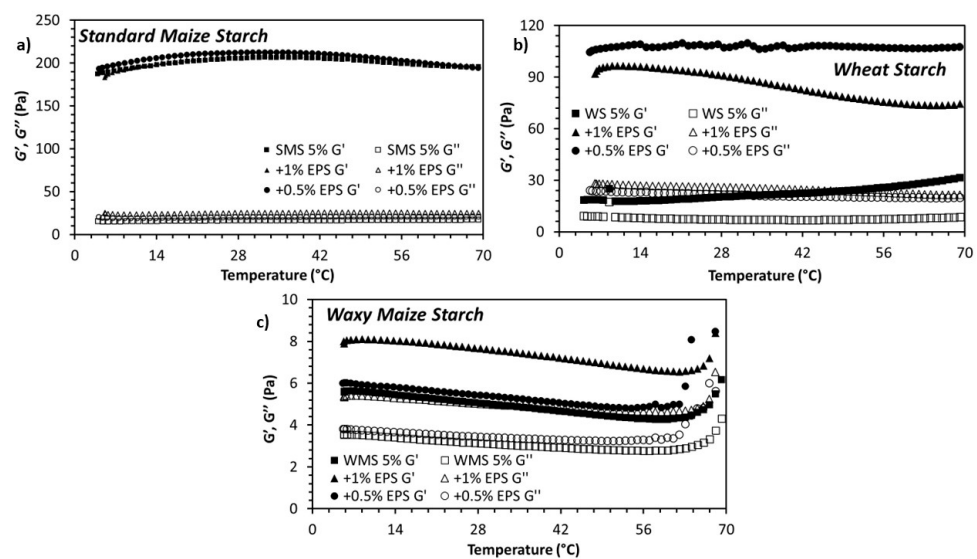


Figure 4. Temperature sweep analysis graphics of EPS PDER21 gelled with standard maize starch (a), wheat starch (b) and waxy maize starch (c).

Pasting properties of standard maize starch, wheat starch and waxy maize starch with dextran PDER21 are presented in Table 1. Pasting properties, namely, peak viscosity (maximum viscosity of hot paste), holding strength viscosity (or trough viscosity: minimum

viscosity of hot paste), breakdown viscosity (the difference between peak and holding strength viscosity), final viscosity (viscosity at the end of test after cooling) and peak time (time to peak viscosity) are given in Table 1 [33]. The peak viscosity of starches is related to water binding capacity and the swelling properties of starches during heating [34]. The peak viscosity of different solutions changed between 2740 to 8832 cP. The standard maize starch–0.5% dextran PDER21 blend had the maximum peak viscosity value. The peak viscosity of starches without dextran PDER21 was lower; in other words, dextran PDER21 addition increased the peak viscosity. Therefore, dextran PDER21 increased the pasting and thickening properties of different starches. The viscosity properties of starches can vary with the addition of different type of hydrocolloids. Liu et al. [35] studied chestnut starch and carboxymethyl chitosan, xanthan gum and sodium alginate. They reported that the decrease in peak viscosity was due to the added hydrocolloid preventing water and starch interaction and swelling, while the increase was due to the synergy between hydrocolloid and starch. The peak time of the sample solutions varied between 0.87 to 12.9 min. Except for the wheat starch–1% dextran PDER21 sample, the peak time values of samples showed that an increased amount of dextran PDER21 increased the time required to reach the peak viscosity and delay the paste formation. The final viscosity indicates the viscous paste consistency after heating and cooling and the ability of the sample to form a viscous gel after cooking [36]. The final viscosity values ranged between 474.8–4707 cP for standard maize starch, 234.4–2166 cP for wheat starch and 1768–3212 cP for waxy maize starch mixtures. Waxy maize starch with a low amylose content had higher final viscosity values resulting in higher stability [3]. When all values are examined, it was seen that standard maize starch containing 1% dextran PDER21, wheat starch containing 0.5% dextran PDER21 and waxy maize starch containing 0.5% dextran PDER21 had higher final viscosity than the others. Breakdown viscosity shows the difference between peak and trough viscosity and the stability of samples [36]. Lower values of breakdown viscosity indicate that starch tends to show higher resistance to shear force during heating [37]. The samples showing the lowest breakdown viscosity value were those of waxy maize starch and the mixtures of waxy maize starch–0.5 and 1% dextran PDER21 and wheat starch–0.5% dextran PDER21. For all samples except wheat starch–0.5% dextran PDER21, dextran addition increased significantly ($p < 0.05$) the breakdown viscosity. This finding was well supported by several previous papers concentrated on the rheology properties of several hydrocolloids and starches. The addition of guar gum and xanthan gum to tapioca starch [38]; guar gum and locust bean gum to yam, taro, sweet potato and yam bean starch [39]; gum *Cordia* to corn starch [40]; and galactomannans to tapioca starch [41] showed similar tendencies. The high breakdown viscosity in starch–hydrocolloid mixtures was explained by the addition of hydrocolloids, which increased the effect of shear force on the swollen granules in gelatinization and increased the breakdown of the granules [38,42,43]. Setback viscosity values give information about the retrogradation of starch after cooling [44]. Setback viscosity values of waxy maize starch samples were found lower because the low amylose content of waxy starch inhibited swelling [45]. The setback values of starch and dextran PDER21 mixtures were higher than that of the plain starch samples, except for wheat starch and 0.5% dextran. The increase in the setback values can be explained by the fact that the added hydrocolloids decrease the mobility of amylose, the amylose molecules become closer to each other, and thus, the retrogradation takes place rapidly [41]. Similarly, an increase in setback viscosity values was observed in the interactions of guar gum, locust bean gum with yam, taro, sweet potato and yam bean starch [39] and guar gum and xanthan gum with high-amylose corn starch and waxy corn starch [42]. However, there are different findings in the literature on the effects of hydrocolloid addition to starch on setback viscosity. Alam et al. [46] found that the addition of xanthan gum and arabic gum (0.2–0.6%) to taro starch caused a decrease in the setback value, while the addition of guar gum and CMC caused an increase. Furthermore, Lutfi et al. [43] reported that gum acacia decreased, while xanthan and guar gum increased the setback viscosity of water chestnut starch.

Table 1. Pasting properties of different starches and EPS PDER 21 blends.

| Starch Type (5%) | EPS Content (%) | Peak Viscosity (cP) | Peak Time (min) | Holding Strength Viscosity (cP) | Holding Strength Time (min) | Breakdown Viscosity (cP) | Final Viscosity (cP) | Setback from Peak (cP) |
|-----------------------|-----------------|---------------------|--------------------|---------------------------------|-----------------------------|--------------------------|----------------------|------------------------|
| Standard maize starch | 0 | 3625 ^B | 3.43 ^C | 456 ^C | 17.3 ^B | 3170 ^C | 575.7 ^B | 3049 ^C |
| | 0.5 | 8832 ^A | 4.83 ^B | 549 ^B | 20.8 ^A | 8283 ^A | 474.8 ^C | 8357 ^A |
| | 1.0 | 8625 ^A | 12.90 ^A | 4305 ^A | 16.7 ^B | 4320 ^B | 4707 ^A | 3918 ^B |
| Wheat starch | 0 | 3477 ^C | 2.13 ^B | 183 ^B | 17.1 ^B | 3294 ^B | 327.1 ^B | 3150 ^B |
| | 0.5 | 3753 ^B | 3.70 ^A | 1596 ^A | 10.0 ^C | 2156 ^C | 2166 ^A | 1587 ^C |
| | 1.0 | 6596 ^A | 0.87 ^C | 186 ^B | 19.1 ^A | 6410 ^A | 234.4 ^C | 6361 ^A |
| Waxy maize starch | 0 | 2740 ^C | 5.70 ^A | 1730 ^C | 17.1 ^B | 1010 ^C | 1768 ^C | 972.6 ^C |
| | 0.5 | 5149 ^B | 5.85 ^A | 3239 ^A | 15.0 ^C | 1910 ^B | 3212 ^A | 1937 ^B |
| | 1.0 | 5994 ^A | 6.23 ^A | 2640 ^B | 21.2 ^A | 3354 ^A | 2506 ^B | 3488 ^A |

Different letters in the same column show significant differences within the same starch ($p < 0.05$).

In summary, the type of starch and the addition of dextran PDER21 significantly affect the pasting properties of starch. The addition of dextran PDER21 changed the peak viscosity, final viscosity and setback values of starches. The mixtures containing standard maize starch–1% dextran PDER21, wheat starch–0.5% dextran PDER21 and both waxy maize starch–dextran PDER21 showed higher peak and final viscosity values.

3.3. FTIR Analysis

The FTIR spectra ($4000\text{--}400\text{ cm}^{-1}$) of standard maize, wheat and waxy maize starch and different proportions of dextran PDER21 mixtures are given in Figure 5a–c. As can be seen from the figures, the incorporation of dextran PDER21 into starches did not change the appearance of the FTIR spectra and, hence, did not change the main polymer structure. Hydrogen bonds were formed between starches and dextran PDER21; no covalent bond was formed. Similar results were reported by studies on different starch and hydrocolloid mixtures [47,48]. The wide peak between $3000\text{--}3600\text{ cm}^{-1}$ seen in all graphs could be attributed to O–H stretching of intramolecular and intermolecular hydrogen bonds [49]. The same peak was also seen in the spectrum of dextran PDER21 [24]. The sharp peak at about 1600 cm^{-1} was attributed to the water content of the samples [50]. For samples containing dextran PDER21, the sharp peak at about 1600 cm^{-1} was found to be related to the stretching vibration of carboxyl and C=O groups [24]. The peaks between $1000\text{--}1100\text{ cm}^{-1}$ are the characteristic peaks of polysaccharide structures resulting from C–O and C–C stretching vibrations [24,39].

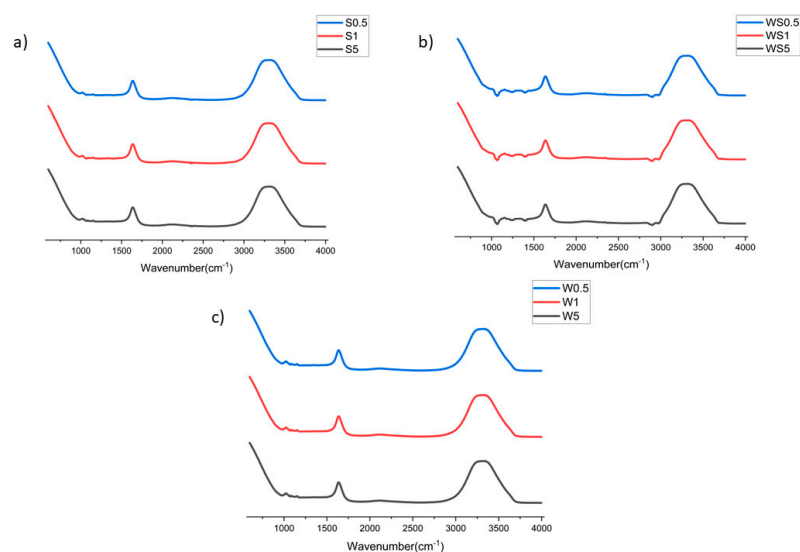


Figure 5. FTIR spectra of EPS PDER21 and standard maize starch (a), wheat starch (b) and waxy maize starch (c).

4. Conclusions

In this study, the rheological properties of dextran BI-20, dextran MED17 and dextran PDER21 solutions prepared in three different ratios were examined and dextran PDER21 was selected to observe the effect of dextrans on starch according to its viscoelastic properties. Amplitude sweep, frequency sweep and temperature sweep analyses and pasting properties of solutions containing standard natural maize starch, natural wheat starch and waxy corn starch and dextran PDER21 at different rates were determined. The results of the rheological measurements showed that dextran-PDER21-added starches exhibited more elastic behavior. As a result of the potential interactions between starch and dextran PDER21, the polymer structure of starch and the FTIR spectrum did not change in the interacted sample in comparison to the control. It can be suggested that adding dextran PDER21 to starches can be a potential alternative to chemical methods used to modify the properties of starches according to the needs of the food industry.

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