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Research Article

Characterization and Rheological Behavior of Dextran from *Weissella confusa* R003

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Dextran from *Weissella confusa* R003 isolated from sugar cane juice was purified and characterized. Dextran synthesis was performed by fermenting *W. confusa* R003 in MRS medium containing 10% (w/v) sucrose with continuous shaking at 125 rpm and at 30°C. For 24 hours, the 50% efficiency yield was obtained. Dextran in the culture medium was purified by ethanol precipitation. Structural analysis of dextran using ^{1}H NMR, ^{13}C NMR, and 2D NMR techniques showed the existence of glucoses with 97.4% α (1→6) linkage in the main chains and 2.6% α (1→3) in branches. The estimation of molecular weight by dynamic light scattering exhibited average molecular weight of 1.0×10^4 kDa. At low concentration (2.5% w/v), dextran behaved like liquid structure, while, increasing the concentration (5.0 and 10.0% w/v), it was revealed as viscoelastic behavior. The highest gelling phenomenon was found in the concentration of 10% w/v and at 37°C. Due to its production and properties, it may be suitable for commercial production and application in the field of foods as well as hydrogel.

1. Introduction

Dextran is a homopolysaccharide (HoPS) consisting of D-glucose units linked by α (1→6) glycosidic bonds in more than 50% of the main chain and generally branched with α (1→2), α (1→3), or α (1→4) glycosidic linkages [1, 2]. Synthesizing dextran via biosynthesis can be done by using lactic acid bacteria (LAB) such as *Leuconostoc, Lactobacillus, Streptococcus,* and *Weissella* [1, 3, 4]. These bacteria are able to secrete dextransucrase, which synthesizes dextran by transferring glucose residues from sucrose to the reducing tail of growing chains [5]. Thus, dextrans are varied in their chemical structure, molecular weight, as well as branch linkage pattern depending on the bacterial strain [4, 6, 7]. This results in variable rheological behavior of dextran.

Today, several biopolymers including dextran are used commercially. Not only native dextran but also dextran derivatives have received attention for applications in food, pharmaceuticals, chemicals, cosmetics, and frozen dairy products [1, 8–10]. Additionally, several biopolymers including dextran are utilized as hydrogels, especially in the field of tissue engineering where they are used as matrices to repair and regenerate deteriorated tissues and organs [11].

Due to their unique properties that can regulate the rheological behavior of the final product, dextrans have been used in manufacturing [12]. Dextran, especially for food applications, was originally overlooked due to its high solubility or lack of viscosity, so other biopolymers have been widely used for of their thickening and gelling properties [13]. Most aqueous dextrans are non-Newtonian pseudoplastic materials in which the viscosity always decreases while the shear rate increases. In previous studies, 5.0 mg/mL aqueous dextran from *Pediococcus pentosaceus* and *L. mesenteroides* NRRL B-640 have shown non-Newtonian pseudoplastic behavior when shear rates of 0.05–500 s^{-1} and 0.1–1000 s^{-1} were applied, respectively [14, 15]. Moreover, the aqueous dextran produced by *L. mesenteroides* with a molecular weight between 1.74×10^8 and 4.41×10^8 Da exhibits pseudoplastic

behavior in the shear field [16]. At high concentrations, aqueous dextran is a viscoelastic material. Padmanabhan has reported that, using dynamic frequency sweep, 250 mg/mL dextran from *L. mesenteroides* NRRL B-523 is an entangled biopolymer in which solid-like behavior dominates in the high frequency range while liquid-like behavior dominates in the low frequency range [12].

In various applications, such as blood plasma substitution, low molecular weight 40–100 kDa dextran is suitable [17]. Foods and hydrogels, however, need high viscosity dextran, which means adding low molecular weight dextran to increase the viscosity of the final product. Consequently, dextran that exhibits more gel-like behavior or high viscosity is preferable for commercial applications since the amount used is minimal; thus, the cost is reduced. The common factors that affect dextran viscosity are molecular weight and branch linkages.

Generally, dextrans from the genus *Weissella* are highly linear with few branches. Based on a previous report, *W. confusa* E392 produces dextran, composed of 97.3% α (1→6) linear linkages and 2.7% α (1→3) branch linkages [4]. *W. confusa* Cab3 dextransucrase synthesizes dextran with 97% α (1→6) and 3% α (1→3) linkages [18], whereas *W. cibaria* CMGDEX3 produces a linear dextran with 3.4% α (1→3) branch linkages [1]. Dextrans from the genus *Weissella* sp. have been studied, but the rheological properties are rarely mentioned. Aqueous dextran may exhibit different rheological characteristics depending on the molecular weight, branch linkages, concentrations, and testing temperature. Therefore, further understanding about polymer rheology must be conducted to allow for precise applications.

In this work, we present the properties of dextran from a new strain, *W. confusa* R003, which was isolated from sugar cane juice. The dextran from this strain was selected due to its high viscosity, which causes problems in sugar production. The dextran was produced and purified, and the structure was analyzed using ^{1}H-NMR, ^{13}C-NMR, and 2D NMR spectroscopy. The molecular weight and surface morphology were evaluated. Importantly, the rheological properties and factors affecting its rheological behavior were also determined.

2. Materials and Methods

2.1. Materials. Dextran T2000 and standard dextran 1–670 kDa were purchased from Sigma. All other chemicals in this research were of analytical grade.

2.2. Bacterial Strain and Identification. The bacterial strain was isolated from sugar cane juice from the sugar industry in Thailand by Milintawisamai et al. (2009) [19]. The strain was stored at −70°C until use. The bacterial strain was identified by 16S rDNA gene sequencing analysis [20]. The two sets of primers were 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′)/800R (5′-TAC CAG GGT ATC TAA TCC-3′) and 518F (5′-CCA GCA GCC GCG GTA ATA-3′)/1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) to give two partial sequences; overlapping provided the full-length sequence. The full sequence of 16S rDNA gene was aligned using the NCBI database. The gene sequence of *Weissella confusa* R003 was submitted to the GenBank database.

2.3. Dextran Production. *W. confusa* R003 was cultured in modified De Man, Rogosa, and Sharpe (MRS) medium [21] containing (g/L) sucrose, 100.0; Bacto-peptone, 10.0; yeast extract, 4.0; K_2HPO_4, 2.0; meat extract, 8.0; $MgSO_4 \cdot 7H_2O$, 0.40; $MnSO_4 \cdot H_2O$, 0.05; $CH_3COONa \cdot 3H_2O$, 8.29; $(NH_4)_2C_6H_6O_7$, 2.0; Tween 80, 1.0. The pH of the medium was adjusted to 7.5 before sterilization at 121°C for 15 min. *W. confusa* R003 was incubated in MRS at 30°C with a shaking rate of 125 rpm for 24 h. Time course of dextran production was monitored from culture medium for 0 to 48 hours. Dextran was monitored by collecting culture medium by centrifugation at 6000g for 20 min in order to remove the bacterial cell in the first step and then precipitating by chilled ethanol according to the method of Sarwat et al. (2008) in the second step [8]. The precipitated dextran was dried and weighed. The cell growth was monitored by a UV-Vis spectrometer at 600 nm. Sucrose content was monitored by high-performance liquid chromatography (HPLC). The samples were prepared and subjected to LC-20AB and SIL-20A autosampler system (Shimadzu, Japan). The sugar was separated by Shodex Asahipak NH2P-50 4E at 40°C using water and acetonitrile (35 : 65) as the mobile phase. The flow rate was fixed at 1 ml/min and the elution was monitored by a RID-10A refractive index detector (Shimadzu, Japan).

2.4. Purification of Dextran. Dextran was harvested from the culture of *W. confusa* R003 at 24 hours and then precipitated the culture medium by chilled ethanol according to the method described by Sarwat et al. (2008) [8]. The ratio of supernatant per ethanol was modified from the method described by Sarwat et al. (2008) from the ratio of 1 : 1 to 3 : 2. After adding chilled ethanol, the solution was vigorously stirred with a magnetic stirrer until the precipitate appeared. The precipitated dextran was redissolved in distilled water and then precipitated again as mentioned above. This step was repeated twice in order to remove the impurities. The purified dextran was subjected to drying by freeze drying processes which were performed by a ScanvacCoolSafe Freeze Dryer (LaboGene, Denmark).

2.5. Scanning Electron Microscopy (SEM). The freeze dried dextran was subjected to SEM. The 100 mg of the purified dextran was scattered on stubs and fixed by double sided tape, respectively. The sample was coated with gold particles using the current of 20 mA for 3 min. The surface morphologies were investigated using a SNE-4500M desktop scanning electron microscope (SEC) by applying the voltage at 15 kV.

2.6. Estimation of Molecular Weight. The molecular weight of the purified dextran was determined by high-performance liquid chromatography (HPLC) and dynamic light scattering (DLS). HPLC was carried out using the method as described by Wu et al. (2011) [22] using a UFLC-HPLC system (Shimadzu, Japan). The dextran was dissolved in water and separated by TSKgel G5000PW (7.5 mm × 30 cm; Tosoh, Shanghai, China) at 60°C using distilled water as an eluent

with a flow rate of 0.6 mL/min. The polymer was monitored by an RID-10A refractive index detector (Shimadzu, Japan). DLS was performed using a Zetasizer Nanoseries model S4700 (Malvern Instruments, UK). The dextran molecular weight was estimated by DLS [23]. Water was used as a dispersant where the viscosity and reflective index (RI) were 0.8872 cP and 1.33, respectively, at 25°C. The purified dextran was dispersed in water to the concentration of 1×10^{-6}–6×10^{-6} g/mL. The hydrodynamic diameter of the dextran was directly read from the instrument. The molecular weight was estimated via empirical mass versus size calibration curves by using the definite molecular weight of globular protein (4.71×10^5 kDa), linear polymer (1.98×10^4 kDa), brush polymer (1.82×10^5 kDa), and starburst polymer (1.62×10^7 kDa).

2.7. Structural Analysis. Structural analysis of dextran was performed by NMR spectroscopy using an Avance III NMR spectrometer (Bruker). For ^{1}H NMR, 5 mg purified dextran was dissolved in 0.5 mL of D_2O and the process was operated at 500 MHz at 40°C. ^{13}C NMR and 2D NMR, heteronuclear single-quantum coherence (HSQC), and heteronuclear two-bond correlation (H2BC) were conducted at 125 MHz and 40°C.

2.8. Rheological Analysis. To study rheological properties, dextran gels were prepared. The freeze dried dextran was dissolved in distilled water to final concentrations of 2.5, 5.0, and 10.0% (w/v) to prepare dextran gels. All samples were vigorously mixed at room temperature for 12 h to ensure the gels had completely swelled. The samples were tested on a plate-plate combination rheometer (Haake Mars Rheometer, 379-0200, Thermo Electron GmBH, Karlsruhe, Germany; rotor: C35/1°, D = 60 mm). Two functions of measurement, that is, oscillatory stress sweep and frequency sweep, were used. Oscillatory stress sweep was conducted by fixing the frequency at 1 Hz while oscillatory frequency sweep experiments were conducted at a fixed shear stress of 1 Pa. G' (solid-like structure), G'' (liquid-like structure), and viscosity of the samples were measured at 25, 37, and 50°C.

3. Results and Discussion

3.1. Bacterial Strain and Identification. *W. confusa* R003 isolated from sugar cane juice from a sugar factory in Thailand showed rapid growth and slimy mucoid colonies on modified MRS agar after incubation at 30°C for 24 h. The 16S rDNA sequence showed 99% sequence similarity to *W. confusa*. The 16S rDNA sequence was submitted to the GenBank database with accession number KF312398.

3.2. Dextran Production and Purification. The time course of dextran production by *W. confusa* R003 in modified MRS medium is shown in Figure 1. Dextran production dramatically increased according to cell growth within 8 h of incubation. The highest dextran amount was detected at 24 h (25 g/L), while the highest OD600 was detected at 12 h. After that incubation time, there was a possibility that pH and nutrients in the culture medium decreased, leading to a decline phase of the bacteria. However, dextran could still

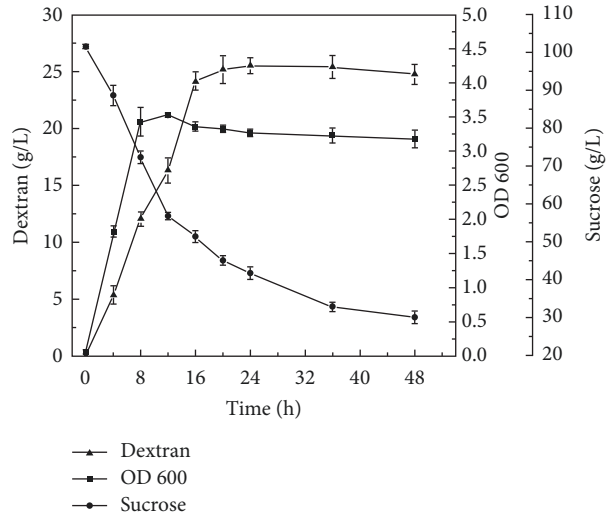
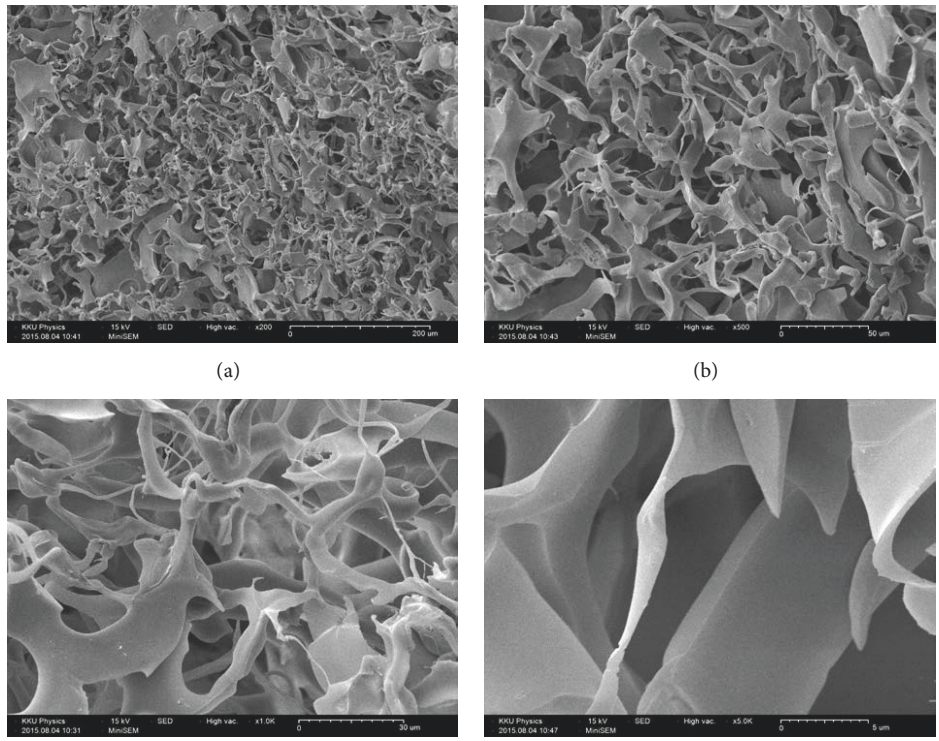


Figure 1: Time course of dextran production, cell growth, and sucrose consumption of *W. confusa* R003 in MRS medium pH 7.5, containing 10% w/v sucrose at 30°C with a shaking rate of 125 rpm.

be synthesized via dextransucrase until sucrose diminished. The sucrose contents in culture broth dramatically decreased within 0–24 h and then it has slowly decreased in 24–48 h. At 0, 24, and 48 h, the amount of sucrose was approximately 100, 41, and 30 g/L, respectively. The high consumption period of sucrose was found in the same range of high dextran production and cell growth. This result suggested that the sucrose was changed to dextran and suitable time for dextran production was 24 h. From previous reports, the initial concentration of sucrose affected the production yield of dextran. In this research, the concentration of sucrose was started at 100 g/L. According to our preliminary studies, using an initial concentration of sucrose of more than 100 g/L contributed to the high viscosity of the culture medium, resulting in difficulty separating bacterial cells from the culture medium, while using an initial concentration of sucrose less than 100 g/L resulted in low dextran production yield. In the mechanism of dextran synthesis, glucosyl moieties from sucrose molecules are transferred to the reducing end of the dextran chain [24]. Therefore, the efficiency yield of dextran production could be predicated from the weight ratio of dextran and the initial glucose moiety. *W. confusa* R003 was able to produce dextran with an efficiency yield 50%. The production yield of the strain was less than the 92.4% from *W. confusa* Cab3 [25] used to optimize the medium containing 6.06% (w/v) sucrose. However, it was higher than the 32% observed from *W. cibaria* CMGDEX3, which was carried out on MRS medium containing 15% (w/v) sucrose [1]. Therefore, the results show the potential for dextran production by this strain, which may be further improved by medium optimization and applied on an industrial scale.

3.3. Scanning Electron Microscopy. SEM revealed the surface morphologies of the freeze dried dextrans. At the low magnification of 200x and 500x (Figures 2(a) and 2(b)), it appeared as fibrous groups that folded with each other while, at the higher magnification of 1000x and 5000x (Figures



(a) (b)

(c) (d)

Figure 2: Surface morphology of dextran from *W. confusa* R003 by scanning electron microscopy (SEM): (a) 200x; (b) 500x; (c) 1000x; (d) 5000x.

2(c) and 2(d)), a network of sheets permeated with pores was observed. This result implied interactions in an aqueous system between the dextran molecules such that their chains were weaved in with nearby molecules due to hydrophobic interactions, while hydrophilic regions formed interactions with water. The surface morphologies may be affected by the method of sample preparation, which would subsequently affect their rheological behaviors. Moreover, this result was different from a previous report on the dextran produced by dextransucrase from *Leuconostoc mesenteroides* NRRL B-640, which exhibited a cubical or web-like structure [15]. The distinctions in surface morphology of dextrans revealed from SEM might be a result of the differences in their chemical bonding. The *W. confusa* R003 dextran was composed of 2.6% α (1→3) branch linkages while *L. mesenteroides* NRRL B-640 was a linear chain of α (1→6) linkages. The branched dextran was less soluble in water comparing to a linear dextran. It was possible that the *W. confusa* R003 dextran has diverged in dispersion character in the solution. Therefore, it exhibited sheet-like structure and was highly porous. Moreover, Zhou et al. (2018) have described that difference in microstructures and surface morphologies result from microorganism strains and monosaccharide composition and structure [26].

3.4. Dextran Molecular Weight. The HPLC results showed that the molecular weight of dextran was greater than 2.0×10^3 kDa since it was eluted in the void volume of the column. However, DLS determination revealed two average molecular weights of 1.0×10^4 kDa. The molecular weight of 1.0×10^4 kDa correlates with previous reports of some dextrans produced from LAB such as *L. mesenteroides* KIBGE-IB22 and its mutant strain *L. mesenteroides* KIBGE-IB22 M20, which produced large dextrans with high molecular weights of $1.5–2.0 \times 10^4$ and $2.5–4.0 \times 10^4$ kDa, respectively [27]. *L. mesenteroides* sp. produced $1.0–4.0 \times 10^4$ kDa dextran [28]. *W. confusa* Cab3 produced 1.8×10^4 kDa dextran [18]. However, the aggregation of dextran might have appeared due to hydrophobic and hydrophilic regions within the dextran molecules. Dextran composed of both hydrophobic and hydrophilic regions contributes to interactions in aqueous solution [12].

3.5. Structural Analysis. The ^{1}H NMR spectrum of the dextran is shown in Figure 3(a). The peaks were distributed into two regions, upfield of 3.5–4.0 ppm and downfield of 4.9–5.3 ppm. This was in agreement with the ^{1}H NMR spectrum of dextran where the proton signals at C-2, C-3, C-4, C-5, and C-6 were found in the range of 3-4 ppm while C-1 was found in the 4–6 ppm region [29]. From the spectrum, two peaks of anomeric protons were observed. The high intensity peak at 4.98 ppm was referred to the anomeric proton of the α (1→6) linkages in the main chain [1, 4, 6, 15, 30, 31], whereas the low intensity peak at 5.32 ppm represented the anomeric proton of the α (1→3) branch linkages [1, 4, 31]. A ratio of 97 : 3 was obtained from the integration of the relative intensity of the signal at 4.98 and 5.32 ppm. This result suggests that the dextran was composed of 97.4% α

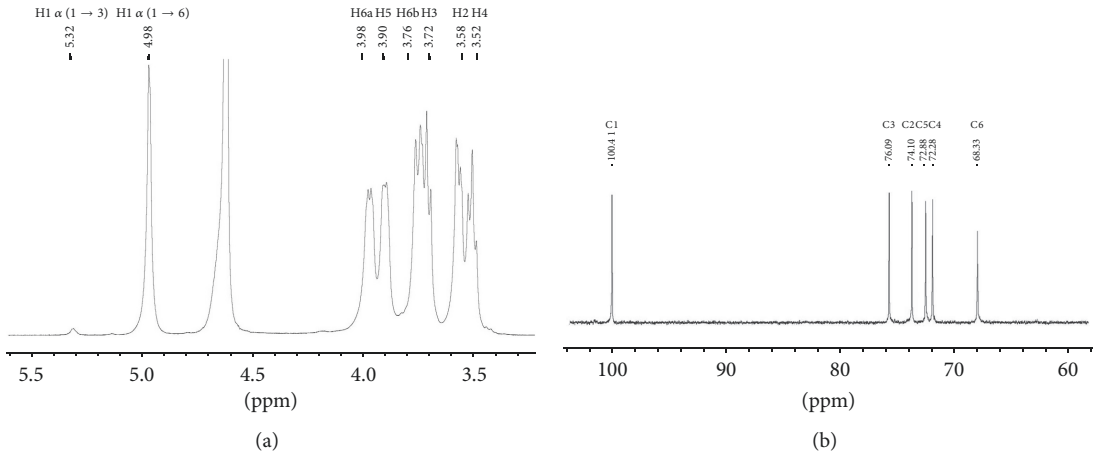


Figure 3: 1D NMR spectrum of purified dextran from *W. confusa* R003 recorded at 40°C in D_2O: (a) ^1H NMR (500 MHz); (b) ^{13}C NMR (125 MHz).

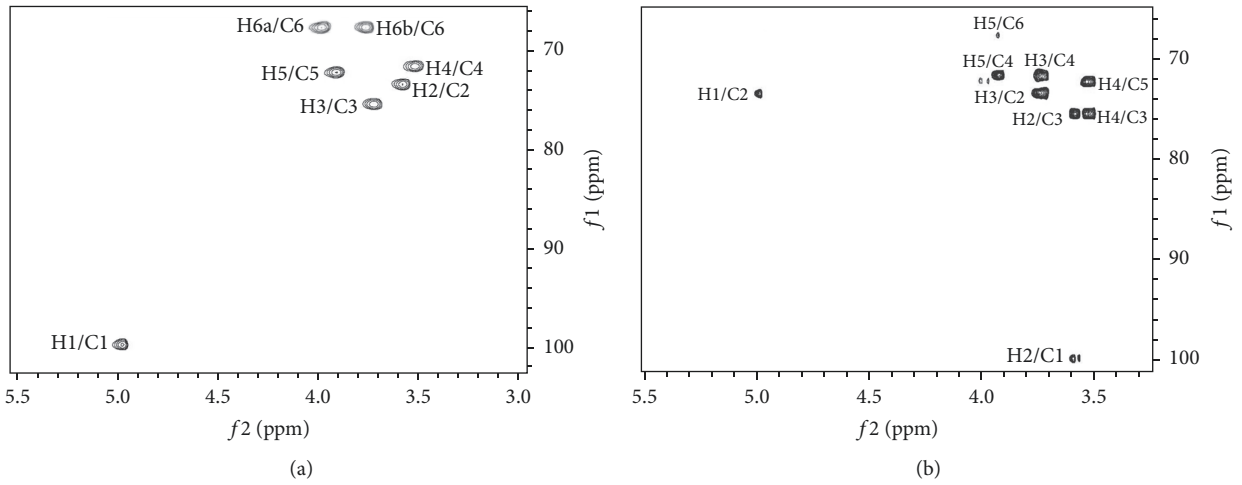


Figure 4: 2D NMR spectrum of purified dextran from *W. confusa* R003 recorded at 125 MHz in D_2O at 40°C: (a) HSQC spectrum; (b) H2BC spectrum.

(1→6) linkages in the main chain and 2.6% α (1→3) branch linkages.

The ^{13}C NMR spectrum revealed six peaks appearing around 68–100 ppm (Figure 3(b)). In previous studies, anomeric carbon signals were generally found downfield, in the region of 95–105 ppm [15]. Carbon signals at C-2, C-3, C-4, and C-5 were found in the range of 70–75 ppm, while C-6 was found in the upfield region at about 60 ppm [1, 4]. Moreover, dextran branch linkage signals were found around 75–85 ppm [15]. In this study, the signal at 100.41 ppm was clearly due to the anomeric carbon C-1, while the signal at 68.33 ppm was due to C-6. This peak was also observed at a lower intensity compared to the other peaks since some parts of the α (1→6) linkages had been replaced by α (1→3) branches. However, the signal referring to branch linkages around 75–85 ppm was absent due to the small amount of α (1→3) linkages. To assign the signals at 72.28, 72.88, 74.10, and 76.09 ppm, the data from 2D NMR were also analyzed.

HSQC and H2BC, which provided the correlation between ^1H and ^{13}C direct bond and two bonds, respectively, were used to confirm the structure of the polysaccharide (Figures 4(a) and 4(b)). From the HSQC spectrum, seven protons in the glucose residue were found to correlate. The correlation of ^1H at 4.98 ppm and ^{13}C at 100.41 ppm confirmed the presence of α (1→6) glycosidic bond. Other assignments from the HSQC and H2BC analysis of each position are presented in Table 1. The NMR results confirmed that the dextran was composed of glucose units linked with 97.4% α (1→6) glycosidic bonds and 2.6% α (1→3) branches. This finding emphasizes the unique structure of dextrans from the genus *Weissella*, which have high linearity and low branching.

3.6. Rheological Analysis. The flow behavior of the dextran solutions in water was studied by oscillatory stress sweep at different concentrations, temperatures, and shear stress levels in order to evaluate these effects. As the rheological characteristics of biopolymer solutions are complex and are affected by the experiment conditions, in this study, the frequency of stress was fixed at 1 Hz while the storage

Table 1: Assignment of $^{1}H/^{13}C$ chemical shift of dextran from *W. confusa* R003 correlated with dextran from *W. confusa* E392.

Atom position		1	2	3	4	5	6
W. confusa R003	^{1}H	4.98	3.58	3.72	3.52	3.90	3.76/3.98
	^{13}C	100.28	74.10	76.09	72.28	72.88	68.33
W. confusa E392[a]	^{1}H	4.98	3.58	3.73	3.52	3.91	3.77/3.98
	^{13}C	99.3	72.9	74.8	71.2	71.8	67.3

[a]Maina et al. [4].

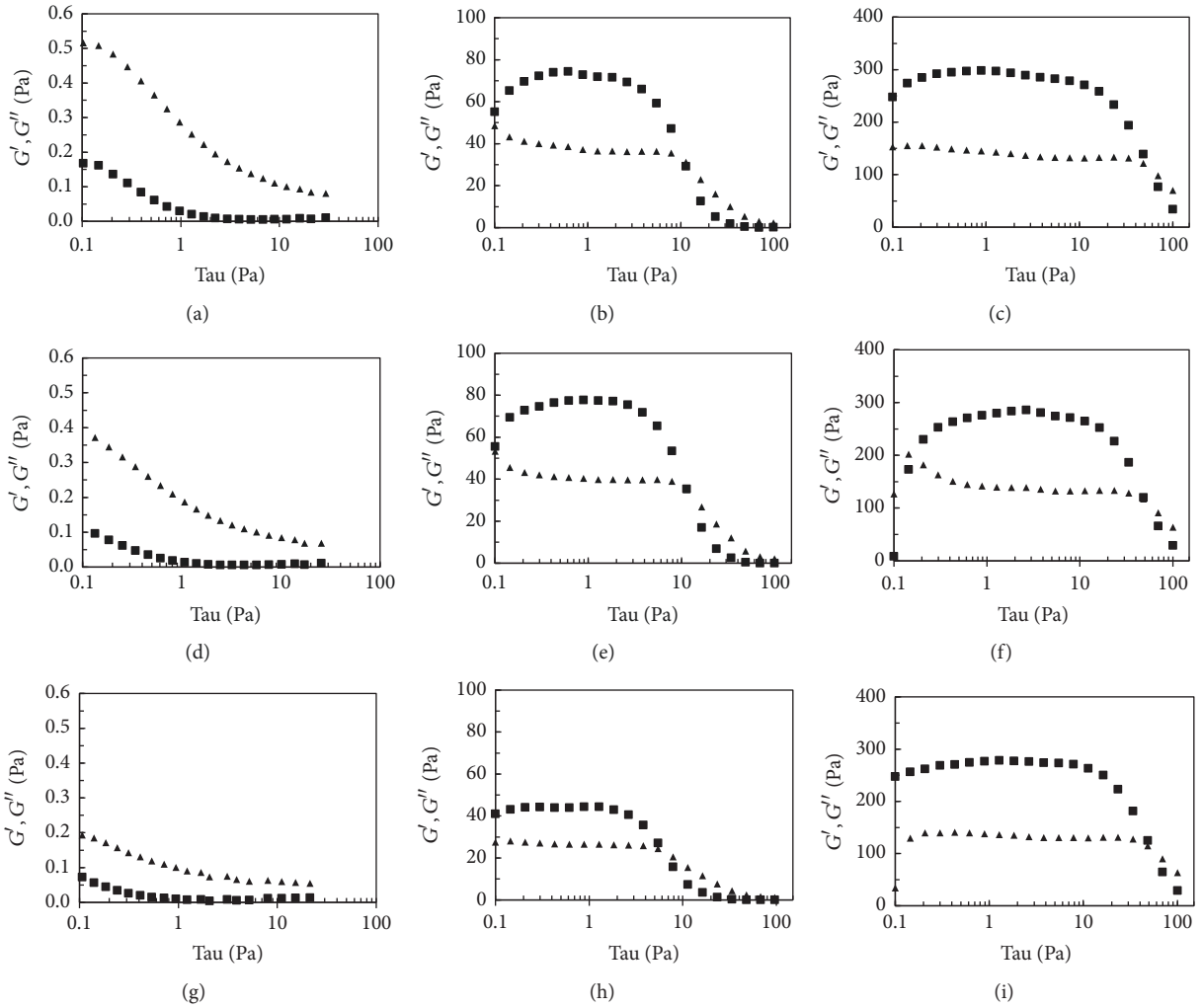


Figure 5: Elastic moduli, G' (■), and viscous moduli, G'' (▲), as a function of shear of the purified dextran from *W. confusa* R003: (a) 2.5% w/v, 25°C; (b) 5.0% w/v, 25°C; (c) 10.0% w/v, 25°C; (d) 2.5% w/v, 37°C; (e) 5.0% w/v, 37°C; (f) 10.0% w/v, 37°C; (g) 2.5% w/v, 50°C; (h) 5.0% w/v, 50°C; (i) 10.0% w/v, 50°C.

modulus (G') and loss modulus (G'') were monitored in the ranges of shear (Tua) 0.1–100.0 Pa. The results are shown in Figures 5(a)–5(i).

The aqueous dextran exhibited liquid-like behavior at 2.5% w/v at the temperatures of 25, 37, and 50°C (Figures 5(a), 5(d), and 5(g)) because of the distinctive G'' over G' observed in the entire range of the applied shear stress. At 25°C, both G' and G'' tended to decrease successively as the shear rate increased; G' started to approach zero around a shear stress of 2.2 Pa. This pattern of the G' and G'' profile was observed when the temperature was increased to 37°C and 50°C at this concentration. This confirmed that the dextran absolutely behaved like a liquid at this concentration. An effect of temperature was found as a reduction in the modulus (G' and G'') when the temperature increased. Comparing the modulus at the initial shear stress showed that G'' was 0.52, 0.37, and 0.12 Pa at 25°, 37°C, and 50°C, respectively, while G' was 0.17, 0.09, and 0.07 Pa at 25°C, 37°C, and 50°C, respectively.

Increasing the dextran concentration led to a transformation from liquid-like to solid-like behavior. At 5.0% w/v dextran, the G' and G'' profile was markedly different from

that of 2.5% w/v dextran (Figures 5(b), 5(e), and 5(h)). At the beginning, with low stress, a linear viscoelastic region (LVR) and then a crossover between G' and G'' were observed. In the LVR range, G' overcame G'' after starting to apply stress and then successively scrolled down to the crossover point. After this point, G'' increased over G'. This indicates that the aqueous dextran changed from solid-like to liquid-like behavior.

Therefore, at 5.0% w/v, dextran acted as a viscoelastic material depending on the stress. At a low level of stress, around 0.1–10.0 Pa, solid-like behavior was prominent, while liquid-like behavior was apparent with a high stress, around 10–100 Pa. The existence of crossover denoted that dextran has a dense structure, which resulted from network interactions between dextran-dextran molecules and dextran-water molecules. When a small amount of shear stress was applied, the samples were able to absorb most of energy while maintaining their structure without deformation. When the applied shear stress reached a yield point, the structure of the samples started to break down and eventually flowed. The prior reductions in modulus (G' and G'') indicated that the structure of the dextran began to break down. However, it may be said that the crossover points were the yield point of the aqueous sample [32]. At 50°C, it rapidly deformed, comparing to the behavior at 25 and 37°C. This may have occurred since, at 50°C, the dextran molecules carried a lot of thermal energy and flow easily occurred although a small amount of stress was applied. The apparent shear stress, strain, and moduli (G' and G'') at the yield point (crossover point) were 10.69 Pa (γ = 24.11%; $G' = G''$ = 32.22 Pa), 11.44 Pa (γ = 23.32%; $G' = G''$ = 34.99 Pa), and 6.16 Pa (γ = 19.38%; $G' = G''$ = 23.26 Pa) at 25, 37, and 50°C, respectively. Moreover, the moduli in the LVR from 25°C was similar to 37°C but markedly higher than the moduli in the LVR at 50°C. It was interesting that the yield point did not decrease when the temperature was increased. The yield stress at 37°C was higher than that at 25°C, even though the dextran molecules had greater thermal energy. These results suggest that the dextran solution at 37°C was able to maintain structure more than the aqueous dextran at 25°C. This phenomenon can be explained in terms of the solubility of the polymer. This dextran has a high molecular weight and branches, so increasing the temperature promoted the solubility of the sample. Normally, dextran in aqueous solution forms a gel via an entanglement network [12]. In an entanglement network, most of the interactions occur via interlacing polymeric chains. Moreover, ionic/hydrophobic interactions and H-bonds are occasionally found in the network. Hence, factors such as the degree of polymerization and the length of the polymeric chain could affect the solubility and rheological behavior of the polymer in an aqueous system. It is possible that dextran solubility at 25°C was less than that at 37°C, resulting in weak entanglement networks and thus a lower crossover point at 25°C compared to 37°C.

High dextran concentrations led to a more solid-like or stronger gel character, that is, 10% w/v dextran (Figures 5(c), 5(f), and 5(i)). The LVR of 10% w/v dextran was longer than that of 5% w/v dextran; likewise, the yield stress of 10% w/v dextran was higher than that of 5% w/v dextran. The apparent

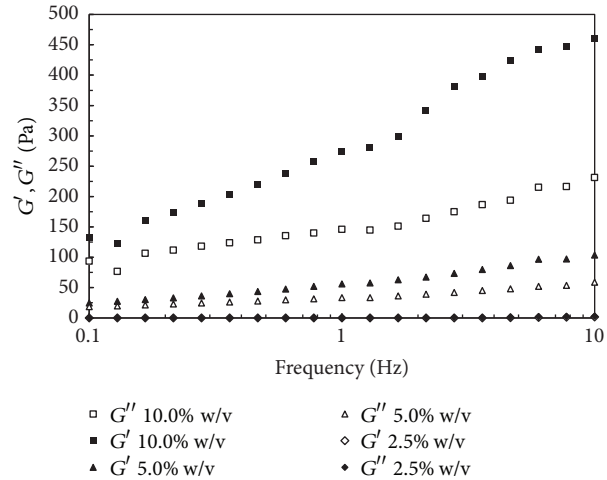


Figure 6: Elastic moduli, G', and viscous moduli, G'', as a function of frequency at 37°C of the purified dextran from *W. confusa* R003 at the concentration of 2.5, 5.0, and 10.0% w/v.

shear stress, strain, and moduli (G' and G'') at the yield point (crossover point) were 55.89 Pa (γ = 36.78%; $G' = G''$ = 114.9 Pa), 11.44 Pa (γ = 23.32%; $G' = G''$ = 34.99 Pa), and 52.76 Pa (γ = 35.58%; $G' = G''$ = 111.2 Pa) at 25, 37, and 50°C, respectively.

From these results, it was concluded that there are three parameters, that is, concentration, temperature, and stress, which affect the rheological behavior of the dextran from *W. confusa* R003. Low concentration dextran at 2.5% w/v behaved like a liquid, while, at 5 and 10% w/v, it behaved like a viscoelastic material. A higher dextran concentration caused more gelation. The optimum temperature for maintaining the structure was 37°C. The rheological behavior of high concentration of 5 and 10% w/v dextran was affected by increasing shear stress, which led to the transformation to liquid-like behavior.

The fluid structure of the purified dextran was also analyzed by frequency sweep in the frequency range of 0.1–10.0 Hz in the temperature of 25, 37, and 50°C. The data obtained at the temperature of 37°C was selected as representative results (Figure 6). Based on the results, the liquid-like behavior of dextran at 2.5% w/v was confirmed. The elastic modulus, G', and viscous modulus, G'', approached zero in the entire frequency range. An increase in the polymer concentration promoted its gelation. In the aqueous solutions of 5.0 and 10.0% w/v dextran, the dextran expressed typical solid-like behavior in which G' dominated over G'' in the entire frequency range at both concentrations. G' was more frequency dependent particularly in the high frequency region. Especially for 10% w/v dextran, the slope of the line in the frequency range of 2.1–10.0 Hz was higher than the slope of the line in the range of 0.1–1.0 Hz. This result indicates that the polymer tended to shift from solid to liquid in the high frequency field. The strong frequency dependence of G' also indicated that its fluid structure was formed via an entanglement network [12]. This result provides beneficial data that can be applied to processing this dextran for use in commercial applications.

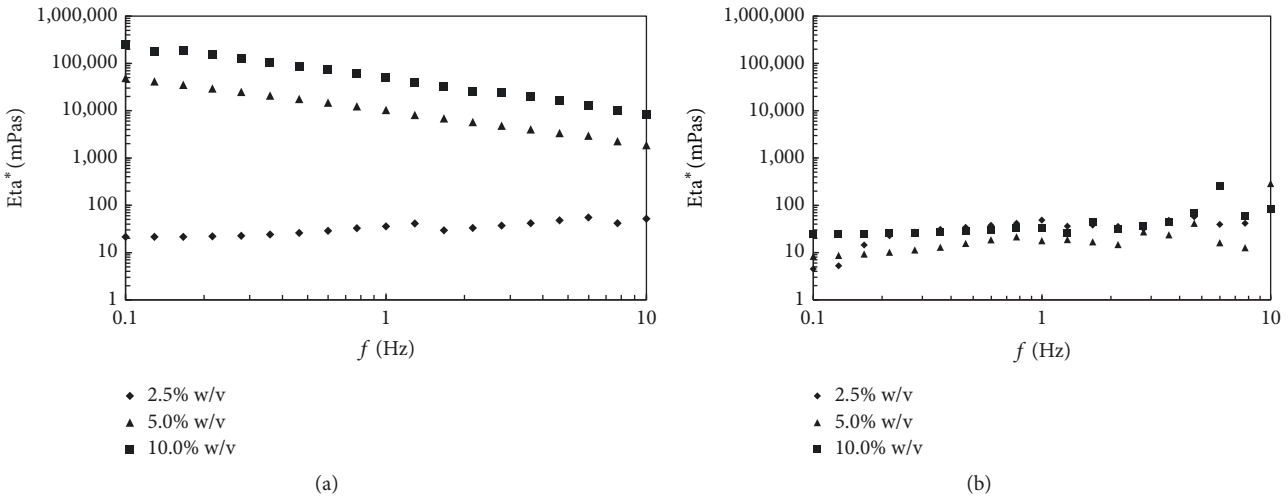


Figure 7: Complex viscosity, Eta*, as a function of frequency: (a) the purified dextran from *W. confusa* R003; (b) commercial dextran T2000.

The complex viscosity (Eta*) of the dextran is depicted in Figure 7(a). The result very clearly shows that the viscosity of the aqueous polymer decreased with a decrease in the polymer concentration. However, the progressive increase in viscosity was not proportional with the increase in concentration. An increase in the dextran concentration from 2.5% w/v to 5.0% w/v led to a large increase in viscosity, while, from 5.0% w/v to 10.0% w/v dextran, the viscosity increased to a lesser extent. Considering a middle point frequency at 1.0 Hz, the observed viscosity of 10% w/v dextran was around 4.7 and 1,300 times higher than that of 5.0 and 2.5% w/v dextran, respectively. Moreover, the viscosity trend from 2.5% w/v was also different than the others since it was slightly stable in the entire frequency range. The observed viscosity of 5.0% w/v and 10.0% w/v dextran was successively decreased as the frequency increased. This result also indicates that a concentration between 2.5 and 5.0% w/v might be in the critical range in which dextran is able to shift from liquid-like to solid-like behavior. Comparing the viscosity of the commercial dextran T2000 (Figure 7(b)), all concentrations (2.5–10% w/v) of the polymer exhibited low viscosity and the same pattern of the viscosity profile. These results are similar to the viscosity profile of 2.5% w/v dextran from *W. confusa* R003. This result indicates that the gelation of dextran T2000 is not promoted by increasing the concentration in the range of 2.5–10.0% w/v. Dextran T2000 has a molecular mass around 2000 kDa and is produced by *Leuconostoc mesenteroides*. Usually, dextrans produced from *L. mesenteroides* are linear with few branches. A previous study reported that the dextrans from *L. mesenteroides* NRRL B-640 and *L. mesenteroides* NRRL AA1 are linear without branches [15, 28]. Meanwhile the dextransucrase from *L. mesenteroides* FT045B synthesizes dextran comprised of only 2.1% α (1→3) branch linkages and 97.9% α (1→6) linkages in the main chain [6]. The highly linear dextran from *L. mesenteroides* resulted in high solubility in aqueous solution and low viscosity. This characteristic is different from the observations on *W. confusa* R003 dextran, in which the viscosity was very sensitive to the concentration. This might be due to differences in the molecular weight as well as branch patterns. *W. confusa* R003 dextran has a higher molecular weight, with more than 2000 kDa and is composed of 2.6% α (1→3) branch linkages and 97.4% α (1→6) linkages. This led to differences from the rheological properties of the dextran from *W. confusa* R003. Due to its high viscosity and ability to behave as a viscoelastic material, it can be used as a gelling agent in commercial applications.

4. Conclusion

Dextran tended to have high attention for commercial applications because of the diverse properties depending on their structures and it can be employed in several fields. In this work, *W. confusa* R003 has expressed the potentiality for dextran production with the 50% efficiency yield and this was expected to improve in the further study. The results from structural analysis have pointed that the R003 dextran was the high molecular mass and low branched biopolymer. With the apparent properties with 10^4 kDa in size and 2.6% α (1→3) branches, this dextran carried the distinct morphology and the rheological feature from other dextrans. The sheet-like and high porous structure resulted in the ability to hold water and formation of the gel. The flow behavior of dextran solution was influenced by the concentration, temperature, and shear level. The dextran exhibited absolutely liquid at 2.5% (w/v) while at 5.0% and 10% (w/v) exhibited both gel- and liquid-like behavior. It also showed noticeably higher viscosity than the commercial dextran T2000. According to the properties of the *W. confusa* R003 dextran, it may be suitable for use as a food additive or in hydrogel preparations for utilization in the medical field. Moreover, the oligosaccharides generating from enzymatic hydrolysis of this dextran may be used as being prebiotic.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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