Iran Agricultural Research, Vol. 27, No. 1-2, 2008 and Vol. 28 No. 1, 2009
Printed in the Islamic Republic of Iran
Shiraz University

Characterization of the Dextran Produced by *Leuconostoc mesenteroides* from Date Fruit Extract

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ABSTRACT- In the present study, the production of dextran from date extract and sucrose as carbon sources by the bacterium *Leuconostoc mesenteroides* NRRL B512 (f) was investigated. In comparison to blue dextran (Mw≈2000 Da), dextran molecular weight was reduced (Mw<2000 KDa) when date extract was used as a substrate, while sucrose produced dextran with high molecular weight (Mw>2000 KDa). Flow behavior indices of dextran solutions from date extracts were investigated and rheological parameters were evaluated at concentrations of 1.5, 2.5, 5, together with 10% total solids at 25˚C using a Brookfield rotational viscometer as well as a cone and plate geometry. The experimental results followed the power law model for the best fit and the values of flow behavior index (n) were less than unity (0.49 .93) at all concentrations, revealing the shear thinning nature of the produced dextran. Furthermore, dependence of apparent viscosity on concentration was confirmed using the power law model.

Keywords: Dextran, Date extract, *Leuconostoc mesenteroides*, Rheological behavior

INTRODUCTION

Microbial exopolysaccharides (EPS) are a class of biothickeners that are found in two forms of capsules and slime. Dextran, xanthan, gellan, pullulan, yeast glucan and bacterial alginate are the examples of industrially important microbial exopolysaccharides (3, 19). Many food grade microorganisms produce EPS, in particular lactic acid bacteria (2). These EPSs are used extensively in many applications such as biofilteration of drinking water, hydraulic fracturing fluids in enhanced oil/gas production, immobilized biofilm reactors, biomedical materials and food additives. All these applications are based on the ability of exopolymers to modulate rheological properties of materials (9). Dextran $(C_6H_{10}O_5)n$ belongs to the group of homopolysaccharides consisting of glucose monomers linked mainly (95%) by α-1,6 glycosidic bonds together with a few α-1,2 and α-1,3 branched glycosidic linkages depending on the specificity of the particular dextran producing enzyme, namely dextransucrase (14). Dextran has various industrial applications in food, pharmaceutical and chemical industries as emulsifier, carrier, stabilizer, etc. Cross-linked dextran known

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as sephadex is widely used for separation and purification of various products such as proteins in research and industry. In food industry, it is used as a thickener for jam and ice cream. It prevents crystallization of sugar, improves moisture retention, and maintains flavor and appearance of various foodstuffs (6, 18). Dextran is produced by species of *Leuconostoc, Streptococcus* and *Acetobacter* (10). However, dextran for human application is usually produced by *Leuconostoc mesenteroides* NRRL B512 (f) (1). When this strain is grown on sucrose rich media, the production of an extracellular ezyme, dextransucrase (EC 2.4.1.5), is induced.

Dextransucrase produces dextran using two pathways, first, hydrolyzing the sucrose and binding the glycosyl moiety, thereafter, building up the dextran chain by an insertion mechanism (21):

$$C_{12}H_{12}O_{11} \longrightarrow (C_6H_{10}O_5)\ n + n\ C_6H_{12}O_6$$

It has been reported that the dextransucrase of *L. mesenteroides* is also expressed in the presence of carbon sources other than sucrose, but at very low levels (11). Various factors affect the molecular weight of dextran including working at low temperatures, presence of acceptors such as maltose, fructose and glucose, resulting in the production of dextrans with low molecular weight (20).

Date fruit is one of the main agricultural products whose sugar content is ~ 60% of its dry weight. About 30% of the produced dates are not sold at markets due to undesirability. Thus, they must be used in converting industries (10). Since date fruit contains high sugar content, it was used as a carbon source in this study.

The present study aimed at producing dextran by *L. mesenteroides* NRRL B512 (f) from date extract as a substrate and to assess the molecular weight and rheological behavior of the resulting dextran.

MATERIALS AND METHODS

Preparation of date extract

Date extract was collected from authenticated date palms in Bushehr city, Iran. Fruit flesh (500 g) were mixed with distilled water (1500 mL) using a blender. It was then centrifuged at 4°C for 20 minutes at 4000 x g, and the supernatant was collected, lyophilized and stored at -20°C until use (22).

Strain, inoculums and culture media

The strain used in this study was *L. mesenteroides* NRRL B512 (f). This strain was stored at 4˚C in MRS agar slants until needed for the preparation of fermentation inocula. The fermentation inocula were prepared such that the final medium going into the fermentor was 5% (v/v) of the initial fermentation medium. The organism was transferred from the stock culture to MRS broth. The inoculum was grown for 16 to 17 h at 30˚C in agitated flasks. To assess the influence of the date fruit extract on dextran properties, two runs were performed. The first fermentation run was a typical fermentation consisting sucrose (20 g/L), yeast extract (20 g/L) and K_2HPO_4 (8 g/L) (17). The other run was similar to the first one; however, considering sucrose concentration of %2.8 in the date fruit extract, the similar sucrose concentration of the previous medium was used. The pH of the media was adjusted to 7.0 prior to autoclaving at 121˚C for 15 min.

Determination of bacterial growth

Bacterial growth was determined and monitored using a Jen-Wey UV spectrophotometer model 6405. A calibration graph with dry weight versus optical density at 660 nm gave the biomass concentration in g cells/L (17).

Sugar assay

The unreacted sucrose was monitored by TLC using ethyl acetate, 1-propanol, and water at the ratio of 8:7:2 as a mobile phase. Spray reagent used for visualization contained 4% α-naphtol in 20:80, ethanol: 2N sulfuric acid. As such, sucrose and fructose could be detected in a concentration as low as 0.05% (7, 8).

Precipitation of dexran

The dextran in the culture medium was precipitated after 18 h of fermentation, using chilled ethanol. In the first step, an equal amount of ethanol was added, stirred well and centrifuged at 10,000 rpm for 15 minutes, and the supernatant was decanted. In the second step, chilled ethanol was added with constant stirring until precipitates of dextran appeared. It was then left to stand for 5–10 minutes, when a supernatant was again decanted. After standing for 10 minutes, chilled ethanol was added again and dextran was precipitated in a very fine form. The precipitated dextran was dried under a vacuum over calcium chloride at 30°C (10).

Purification of dextran

To 10 g of dextran, 200 mL cold water was added. Next, 100 ml water was added step wise to make a paste of dextran in water. Dextran was then precipitated with chilled ethanol. This cycle of redissolving, precipitation and washing was repeated three times. The dextran was dried under vacuum over calcium chloride at 30°C (10).

Determination of molecular weight

The average molecular weight of the dextran produced by *L. mesenteroides* was determined by gel permeation chromatography on LKB gel filtration system using blue dextran 2000 (average Mw 200 KD) as a standard. The sample was applied through an automatic sample applicator on XK16/70 glass column packed with sephacryl-S-200HR. It was eluted with 0.05 M phosphate buffer (pH 7.0) at a constant flow rate of 20 mL/h. The fractions (40 drops/Fr.) were collected through the automatic fraction collector Ultro Rac II (Model LKB 2070).

Rheological measurements

Rheological behaviors of dextran solutions at different concentrations (1.5-10% w/v) were investigated using a rotational viscometer (Brookfield, DVII+Pro, USA) with a cone and plate geometry in the shear rate range of 1.9-226.5 s^{-1}. Flow behavior characteristics were analyzed from the apparent viscosity and shear rate relationship data. Apparent viscosity measurements were taken at different shear rate ranges (1.9 to 226.5 s^{-1}).Variation in apparent viscosity as a function of dextran concentration was also investigated. A power law model that relates the apparent viscosity to shear rate was used to characterize the flow behavior index (n) of the dextran (4, 8).

$$\eta = k\gamma^{n-1} \qquad \text{Eq. (1)}$$

The effects of concentration on flow parameters were examined at the shear rates used in this study. The following power law equation, suggested by Rao, et al. (1984) was used (12).

$$\eta_a = MC^a \qquad \text{Eq. (2)}$$

Where η_a is apparent viscosity, C is the concentration of dextran (%), and "M" and "a" are the constants to be determined from the log-log plot of apparent viscosity versus dextran concentration.

RESULTS AND DISCUSSION

Bacterial growth and consumption of sucrose

Tsuchiya et al. (1952) investigated the effect of sucrose concentration in culture media on the production of dextransucrase. They showed that an increase in sucrose concentration of the culture media induced better enzyme production; however, an increment of dextran resulted in difficult removal of cells from culture media. Thus, they concluded that 2% (w/v) sucrose was the optimum level for the production of dextransucrase (21).

Using TLC, the sucrose concentration of date fruit extract was estimated to be 3.5%, and based on that the amount of date extract to contain 2% sucrose was calculated. During fermentation, samples were collected every 2 h and remaining sugar content and cellular growth were measured. As shown in Fig 1, the biomass in the media containing date extract was less than pure sucrose, while the concentration of used sucrose by *Leuconostoc mesenteroides* was higher than media containing sucrose, indicating that less extra cellular enzyme was produced. However, this enzyme concentration was sufficient for dextran production (16).

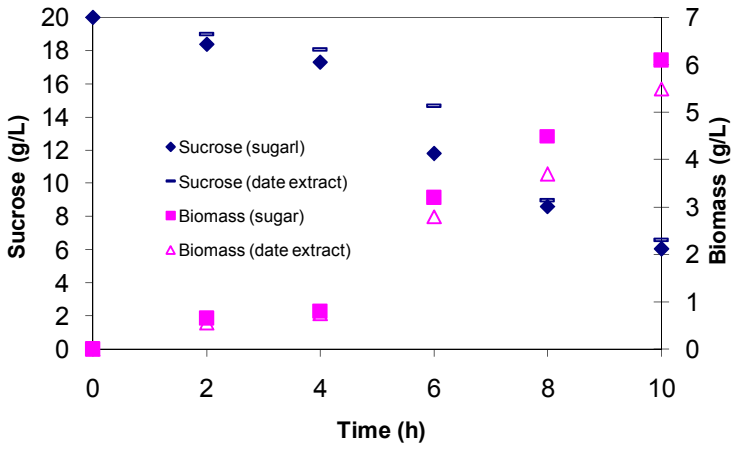

Fig. 1. Different concentrations of sucrose & biomass in media containing sucrose and date extract during fermentation

Rheological behavior and the effect of concentration

As shown in Fig. 2, the viscosity versus shear rate curves were characterized by more increments in the shear rate as a decrease in viscosity occurred. The highest viscosity values were obtained at the lowest shear rate (1.9 s^{-1}) and vice versa (226.5 s^{-1}).

Thus, the flow behavior characteristics were influenced by shear rate and dextran concentration.

Flow behavior (n) and consistency indices (k) were calculated by the power law equation using the data shown in Fig.2 and the results are shown in Table 1. The flow behavior index (n) which represents the degree of pseudoplastic behavior at all concentrations was shown to be less than 1.0 (0.48-0.93) which confirmed the pseudoplastic behavior of the dextran solutions.

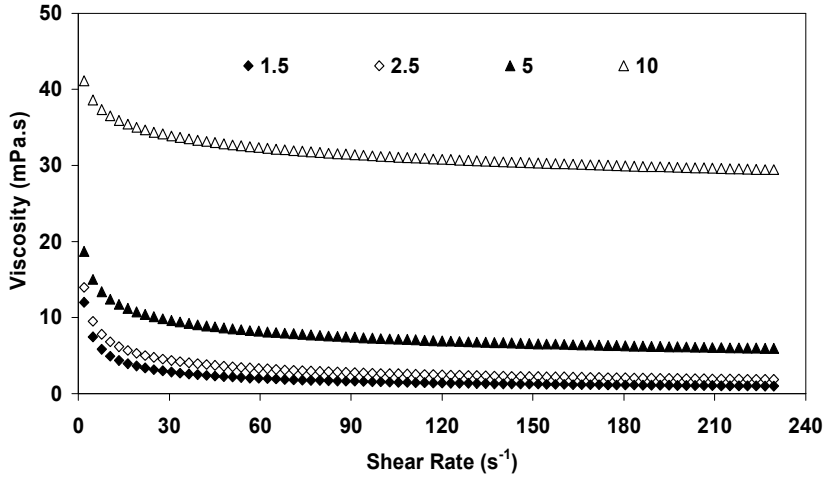

Fig. 2. Apparent viscosity vs shear rate of dextran dispersions in water at concentrations of 1.5-10% w/v

Using dynamic oscillatory shear testing, Padmanabhan et al. (2005) showed that the viscosity of dextran at 250 mg/mL decreased with an increase in shear rate, which is a typical characteristic of a pseudoplastic fluid (9). Moreover, the consistency coefficient (k) which is a measure of viscosity increased with the increment of dextran concentration (Table 1). The relationship between apparent viscosity and concentration was estimated from Eq. 2, described earlier. This is depicted at some fixed shear rate for dextran solutions in Fig. 2. The values of "M" and "a" were determined as the intercept and slope of the lines obtained from the plot.

Table 1. Effect of various concentrations of dextran on the rheological parameters[a]

Concentration (%w/v)	K (mpa/s)	N
1.5	$16.82^{b}\pm4.86$	$0.48^{c}\pm0.10$
2.5	$18.37^{b}\pm1.22$	$0.58^{bc}\pm0.04$
5	$21.88^{b}\pm4.13$	$0.76^{ab}\pm0.10$
10	$43.07^{a}\pm5.46$	$0.93^{a}\pm0.13$

[a] In each column different letters indicate significant difference (P < 0.05)

As shown in Fig. 3 viscosity of dextran solutions increased with an increase in dextran concentration. At higher shear rates, the slope of log-log plot curves of ap-

parent viscosity vs. concentration increased, while "M" showed a decreasing trend demonstrating a more obvious effect of concentration on viscosity at high shear rates.

Mothé and Rao (1999) and Ross-Murphy (1994) stated that at low concentrations, the polymer chains are not in contact with each other; the polymer coils have infinite dilution radii and viscosities show slight shear rate dependence. With increasing polymer concentration, the coils begin to overlap with each other (8, 15). Ioan et al. (2001) studied the semi-dilute solutions of dextran using static light scattering, photon correlation spectroscopy (PCS), and viscometry. They showed that dextrans formed entanglements as the solution concentration increased. This limited water penetration and restricted the fluidity of dextran. Consequently, higher viscosity was observed at higher concentrations (5).

Table 2. Results derived from power equation $\eta_a = MC^a$ for dextran solution

Shear rate (s^{-1})	M	a	R^2
1.92	**4.100**	**0.934**	**0.961**
7.68	**4.200**	**0.882**	**0.981**
19.2	**2.100**	**1.106**	**0.909**
65.28	**0.700**	**1.571**	**0.966**
151.68	**0.700**	**1.541**	**0.980**
226.56	**0.700**	**1.512**	**0.985**

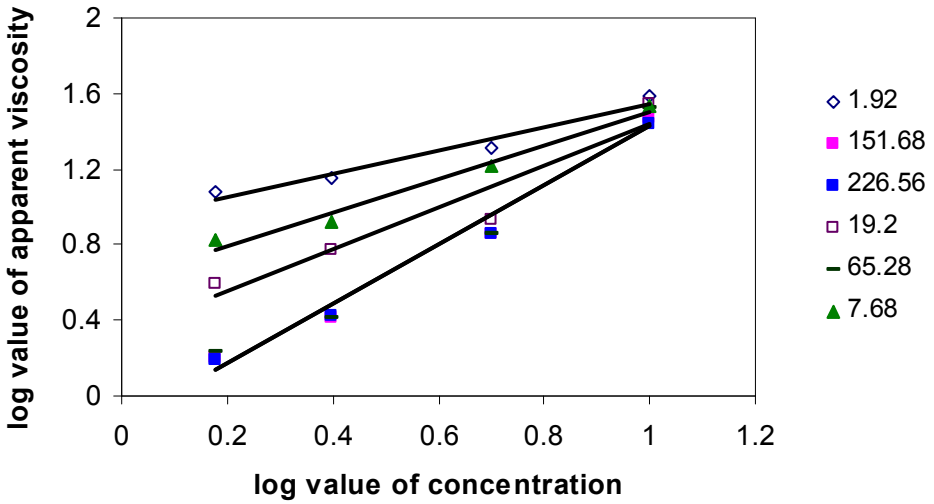

Fig. 3. Effect of dextran concentration on apparent viscosity at different shear rates

Molecular weight distribution of dextran

As shown in Fig. 4, the results suggested that dextran produced from sucrose had higher molecular weight (M_W > 2000 KDa) based on the molecular mass distribution of blue dextran (approximately 2000 KDa). The presence of other sugars such as fructose and glucose in culture media containing date extract had a significant effect on molecular weight of the produced dextran. Santos et al. proved that dextran molecular weight fell from the range of 1890-10000 KDa using only sucrose as a substrate, to values between 240-400 KDa when other sugars were used along with sucrose (16). Therefore, when other sugars are added to the reaction mixture, dextran-

sucrase can also transfer glycosyl residues from sucrose to the non-reducing end of these molecules forming the so called acceptor products (13).

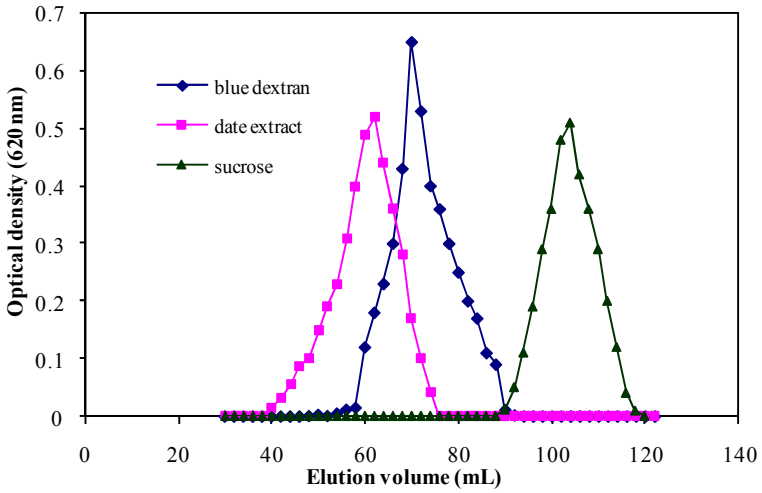

Fig. 4. Molecular mass distribution of dextran from sucrose and date extract

Conclusions

This study showed that *Leuconostoc mesenteroides* NRRL B512 could use existent carbon in date fruit extract for dextran production. Rheological behavior of the resulting dextran was markedly influenced by shear rate and dextran concentration. Moreover, the low molecular weight of produced dextran indicated that by manipulating the fermentation media composition, the molecular weight of dextran can be controlled.

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ویژگیهای صمغ دکستران تولید شده از شیره خرما با استفاده از باکتری لوکونوستاک مزنتروئیدس

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چکیده- در این تحقیق تولید دکستران با استفاده از باکتری لوکونوستاک مزنتروئیدس NRRL B512 (f) از دو منبع کربنی سوکروز و شیره خرما مورد بررسی قرار گرفت. بر اساس نتایج به دست آمده، وزن مولکولی دکستران حاصل از شیره خرما در مقایسه با بلو دکستران (Mw≈2000Da) کاهش یافت (Mw<2000 KDa)، این درحالیست که سوکروز دکسترانی با وزن مولکولی بالاتر از بلو دکستران را تولید نمود .(Mw>2000 KDa) مؤلفه های جریانی محلول های دکستران حاصل از شیره خرما در غلظت های ۱/۵، ۲/۵، ۵ و ۱۰ درصد و دمای 25°C با استفاده از ویسکومتر چرخشی بروکفیلد با ژئومتری مخروط صفحه مورد ارزیابی قرار گرفت. نتایج بدست آمده بعد از قرار گرفتن در مدل قانون توان حاکی از آن بود که شاخص رفتاری جریان تمامی محلول های فوق مقادیر کمتر از واحد را نشان می دهند (۰/۹۳ – ۰/۴۹) و در تمامی غلظت های ذکر شده، محلولهای دکستران رفتاری رقیق شونده با برش سودوپلاستیک) را بروز دادند. همچنین وابستگی ویسکوزیته ظاهری به غلظت نیز با استفاده از قانون توان به اثبات رسید.

واژه های کلیدی: دکستران، رفتار رئولوژیکی، شیره خرما، لوکونوستاک مزنتروئیدس

*به ترتیب استادیار و دانشجویان پیشین کارشناسی ارشد

** مکاتبه کننده