World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:4, No:8, 2010

Fermentative Production of Dextran using Food Industry Wastes

Marzieh Moosavi-Nasab, Mohsen Gavahian, Ali R. Yousefi and Hamed Askari

***Abstract*—**Dextran is a D-glucose polymer which is produced by *Leuconostoc mesenteroides* grown in a sucrose-rich media. The organism was obtained from the Persian Type Culture Collection (PTCC) and was transferred in MRS broth medium at 30°C and pH 6.8 for 24 h. After preparation of inoculums, organisms were inoculated into five liquid fermentation media containing either molasses or cheese whey or different combinations of cheese whey and molasses. After certain fermentation period, the produced dextran was separated and dried. Dextran yield was calculated and significant differences in different media were observed. Furthermore, FT-IR analysis was performed and the results showed that there were no significant differences in the produced dextran structures.

***Keywords*—**Dextran, *Leuconostoc mesenteroides*, Molasses, Whey

I. Introduction

DEXTRAN, $(C_6H_{10}O_5)_n$ is a polysaccharide consisting of glucose monomers linked mainly (95%) by α(1–6) bonds [6]. Commercial applications for dextran are generally in pharmaceutical industry, but new applications are being considered in food and textile industries [3]. Dextran for pharmaceutical applications usually is produced by *Leuconostoc mesenteroides* NRRL B512(f) [5]. The bacterium is grown in a sucrose-rich media releasing an enzyme, dextransucrase, which converts excess sucrose to dextran and fructose [5]. Acceptor molecules, such as maltose in culture media can influence dextran molecular weight by allowing the growing chain to be separated from the enzyme active site and transferred to the acceptor [4]. A reduction in dextran molecular weight was observed together with less viscous and more Newtonian solutions, when working at lower temperatures [1].

M. Moosavi-Nasab is with the Department of Food Science and Technology, College of Agriculture, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (corresponding author to provide phone: +98-711-6138227; fax: +98-711-2287029; e-mail: marzieh.moosavi-nasab@mail.mcgill.ca).

M. Gavahian is a graduate student at the Department of Food Science and Technology, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (e-mail: mohsengavahian@yahoo.com).

A. R. Yousefi is a graduate student at the Department of Food Science and Technology, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (e-mail: aliyousefey@gmail.com).

H. Askari was a graduate student at the Department of Food Science and Technology, Shiraz University, Shiraz, Fars 7144165186 I.R.Iran (e-mail: askari.hamed.1983@gmail.com).

Molasses is a cheap source of sucrose. It is the waste of sugar refinery companies which can be an environmental problem.

Another potential area of economical benefit is new uses of milk sugar, which presents a challenge to dairy industry. Indeed, the market for lactose in pharmaceutical industry is over-saturated and all the routes to chemical modification of this sugar in products, such as lactulose, lactitol and detergents involve only small bazaar. The dairy industry is investigating markets for by-products containing high lactose content, such as whey. The worldwide lactose surplus is actually 550,000 T per year [5].

The aim of the present investigation was to study the production of dextran from molasses (by-product of sugar refineries) and cheese whey (by-product of dairy industry) and the influence of the cheese whey concentration on dextran yield and it`s molecular weight and structure.

II. Materials and Methods

A. Stock Culture

The organism exploited for the production of dextran in this work was a strain of *Leuconostoc mesenteroides* NRRL B512 (f) that was obtained from the Persian Type Culture Collection (PTCC) strain number 1591. The microorganism was maintained in test tubes containing MRS broth medium at 30 °C and pH 6.8 for 24 h.

The stock cultures were stored at 5°C to slow down the growth and dextran production. The rate of dextran production in the stock culture test tubes was regularly checked to ensure that mutation did not happen. Visual inspection of colonies under the microscope was an additional check for stability of *L. mesenteroides* strain.

B. Inoculum Preparation

A culture medium composed of MRS broth was used for preparation of inoculum. About 200 ml of this medium in 500 ml Erlenmeyer flasks was autoclaved at 121˚C for 15 minutes prior to inoculation. After cooling to room temperature, the flasks were inoculated with the stock culture and incubated in a Benmarin shaker (240 rpm) set at 30˚C at pH, 6.8. The organisms were collected by centrifugation (2500×g, 20 min) and resuspended in liquid medium and the bacteria were inoculated into liquid fermentation media in Erlenmeyer flasks.

C. Dextran production

After preparation of inoculums, organisms were inoculated into five liquid fermentation media in Erlenmeyer flasks containing molasses (Brix 40) as a carbon source or cheese whey as a nitrogen source. These media (in a final volume of 75 ml) contained cheese whey (7% w/v) or molasses (Brix 40) or combination of them; without any complement. Table I shows the composition of different fermentation media used in this study. For avoiding any suspended solid and undesirable precipitations (specially in the case of whey because of protein denaturation under heating), all fermentation media were first autoclaved at 120˚C for 20 minutes and then filtered by filter paper with mesh size of 53-125 µm and then centrifuged at 2500×g for 20 minutes. The supernatant was used for fermentation purpose. Each fermentation medium was prepared in a 250 ml flask containing 75 ml medium. The inoculant (with O.D. = 0.6) was added to the fermentation media described in Table I. Thus, the final medium going into the fermentor contained 10% of the inoculants (v/v). A blank sample was used with fermentation media (without inoculation).

Fermentation occurred in a Benmarin shaker (150 rpm) set at 30 ˚C and pH, 6.8. Samples were taken from fermentation media after 48 h. For all experiments triplicate flasks were used for each treatment. After growth and centrifugation, samples of supernatants were removed and analyzed for their EPS content. The supernatants from each treatment were pooled prior to the alcohol precipitation step. EPS was dried under vacuum oven at 40ºC. The dried EPS samples were weighed and values were reported as mg of the produced dextran per 75ml of the original medium.

D. Production yield of exopolysaccharide

Exopolysaccharide was precipitated from the fermentation broth using 3 volumes of 96 % ethanol, dried at 40 ˚C and then milled to the mesh size of 53-125 µm. Yield of produced exopolysaccharide was calculated as percentage of polysaccharide present in the broth. All experiments were performed in triplicates and average values are reported.

E. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of the produced exopolysaccharides from different media (Molasses, M-W 2%, M-W 6%, and M-W 10%) were obtained at a resolution of 4 cm^{-1}. The sample was incorporated into KBr (spectroscopic grade) and pressed into a 2 mm pellet. IR spectra were recorded in the transmittance mode from 4000 to 400 cm^{-1}, using a Brucker spectrometer (EQUINOX 55, Germany).

F. Statistical analysis

All experiments were performed in triplicates. Data were analyzed by a one-way ANOVA followed by Duncan test to compare treatment means to the control treatment. Significant size was at level of 0.05.

TABLE I
THE COMPOSITION OF FERMENTATION MEDIA USED FOR DEXTRAN PRODUCTION

Fermentation media	Mode of preparation
Molasses	Sugar refinery by-product was diluted to reach the Brix 40
Cheese whey	Cheese whey powder was dissolved in distilled water at concentration of 6% (w/v)
Molasses-whey 2% (M-W 2%)	Cheese whey powder was mixed with molasses (BX. 40) at concentration of 2% (w/v)
Molasses-whey 6% (M-W 6%)	Cheese whey powder was mixed with molasses (BX. 40) at concentration of 6% (w/v)
Molasses-whey 10% (M-W 10%)	Cheese whey powder was mixed with molasses (BX. 40) at concentration of 10% (w/v)

III. RESULTS AND DISCUSSION

A. Dextran yield in different fermentation media

Fig. 1 shows the result of dextran production in 75 ml fermentation media. Results showed that maximum yield was achieved in M-W 10% and minimum yield was obtained in M-W 2%; in whey no dextran was produced. Combination of nutrients and minerals maybe cause to produce more dextran yield.

B. ANOVA test analysis:

The yield for each sampling time was tested by Duncan test. The obtained results showed four significant groups. The results shown in Table II demonstrate that the yield in different media (except in case of molasses and M-W 6%) were significantly different relative to all other yields at level of 0.05.

C. FTIR results

Fig. 2 shows the FT-IR spectra of the produced dextrans in different media. The bands at 585 cm^{-1} indicate that glycosidic bond exists. The bands at 1047 cm^{-1} are attributed to C-C or C-O stretching vibration. The bands at 1416 cm^{-1} indicate that $-COO^-$ symmetrical stretching of carboxylic groups exist.

The bands at 1600 cm^{-1} indicate that $-COO^-$ asymmetrical stretching of carboxylic groups exist. The bands at 2926 cm^{-1} indicate the presence of -CH stretching of CH_2 and CH_3 groups. The band at 3396 cm^{-1} is attributed to -OH stretching. The results show that there is no significant difference in structures between the produced dextrans in all fermentation media.

TABLE II
ANOVA FOR DEXTRAN YIELD IN DIFFERENT CULTURE MEDIA

Treatment	Yield
Whey	0.1^{a}±0.017
M-W 2%	4.79^{b}±0.291
Molasses	5.98^{c}±0.789
M-W 6%	6.73^{c}±0.721
M-W 10%	9.51^{d}±0.369

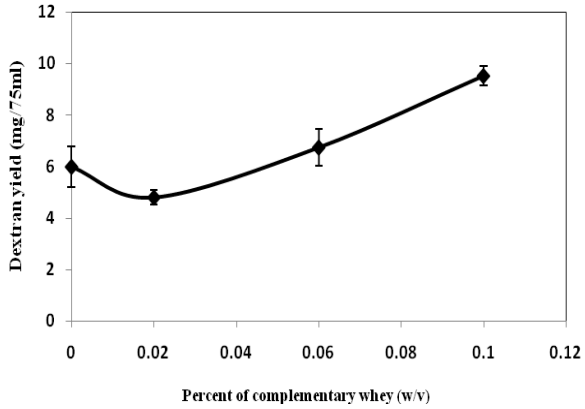

Fig. 1 Effect of whey and molasses combination ratio on dextran yield.

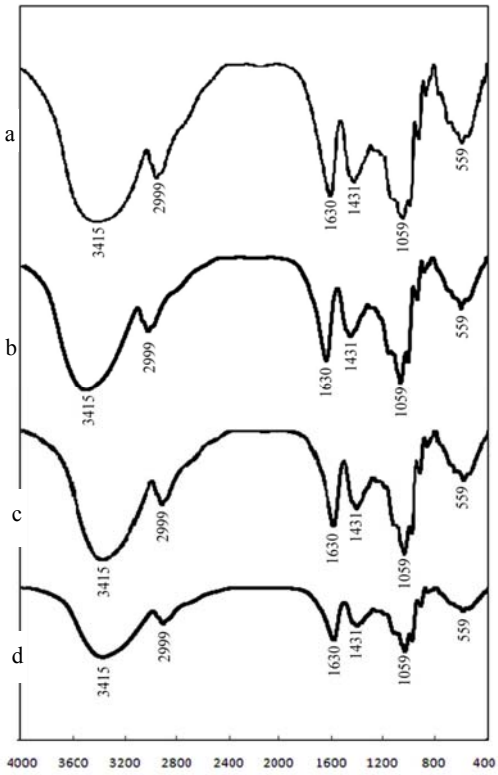

Fig. 2 FT-IR spectra of the produced dextran in (a) molasses (b) M-W 2% (c) M-W 6% and (d) M-W 10%.

REFERENCES

[1] A. Tecante, A. Lopez-Munguia, and A. Garcia-Rejon, "Rheological characterization of dextran-enzymatic synthesis media," J. Appl. Polym.Sci., vol. 31, pp. 2337–2350, 1986.

[2] D. Kim, J. F. Robyt, S. Y. Lee, J. H. Lee, and Y. M. Kim, "Dextran molecular size and degree of branching as a function of sucrose concentration, pH, and temperature of reaction of *Leuconostoc mesenteroides* B-512FMCM dextransucrase, " . Carbohydrate Research, vol. 338, pp. 1183–1189, 2003.

[3] H. J. Koepsell, and H. M. Tsuchiya, "Enzymatic synthesis of dextran," J. Bacteriol., vol. 63, pp. 293–295, 1952.

[4] M. Dols, M. Remaud-Simeon, R. Willemot, M. Vignon, and P. Monsan, "Structural characterisation of the maltose acceptor-products synthesized by *Leuconostoc mesenteroides* NRRL B1299 dextransucrase," Carbohydr. Res., vol. 305, pp. 549–559, 1998.

[5] M. Santos, A. Rodriguesb, and J. A. Teixeira, "Production of dextran and fructose from carob pod extract and cheese whey by *Leuconostoc mesenteroides* NRRL B512 (f)," Biochemical Engineering J., vol. 25, pp. 1–6, 2005.

[6] M. Santos, J. Teixeira, and A. Rodrigues, "Production of dextransucrase, dextran and fructose from sucrose using *Leuconostoc mesenteroides* NRRL B512 (f)," Biochemical Engineering J., vol. 4, pp. 177–188, 2000.

[7] S. M. Holt, H. Al-Sheikh, and K. J. Shin, "Characterization of dextran-producing *Leuconostoc* strains," Letters in Applied Microbiology, vol. 32, pp. 185-189, 2001.

[8] V. B. Veljkovic, M. L. Lazic, D. J. Rutic, S.M. Jovanovic, and D.U. Skala, "Effects of aeration on extracellular dextransucrase production by *Leuconostoc mesenteroides*," Enzyme Microb. Technol., vol. 14, pp. 665–668, 1992.