Pakistan J. Sci. Ind. Res., Vol. 20, No. 6, December 1977

PREPARATION AND CHARACTERIZATION OF DEXTRAN FROM MOLASSES BY THE ACTION OF A LOCALLY ISOLATED STRAIN OF LEUCONOSTOC MESENTEROIDES

S.A.H. ZAIDI, (MRS.) J. JAFRI and (MISS) R. ZUBERI

PCSIR Laboratories, Karachi

(Received July 5, 1977; revised April 3, 1978)

Abstract. The preparation and characterization of a water soluble dextran from molasses by the action of a locally isolated strain of *Leuconostoc mesenteroides* has been studied. Pretreatment of molasses for clarification is not required for the production of dextran, in the culture medium, which reached a maximum of 30% of available sucrose, from molasses in 18 hr. After purification through a simple procedure dextran's ash content of 15.6% reduced to 1.5% and its colour was also improved.

In a previous communication[1] the preparation and characterization of a water soluble dextran from sucrose by the action of a locally isolated strain of *Leuconostoc mesenteroides* has been reported. Non-food sucrose, in enormous quantities, is available from molasses and it would be a big step forward if it could be utilized in the production of dextran, which finds many uses in the pharmaceutical and food industries. The investigations on the production of dextran from cane molasses of local sugar industries are reported in the present paper. Dextran produced from molasses contained an average of 16.0% ash content, which is equivalent to that of the molasses itself, from which it is elaborated. Its colour is also disagreeable and thus makes it useless for industrial use. A simple method for its purification has also been described.

Materials and Methods

Molasses were kindly supplied by Mehran Sugar Mills and Faugi Sugar Mills of Sind with the following average percent analysis: moisture 20.9; sucrose 40.43; reducing sugar 9.7; ash 15.3. Pure Hydrochloric acid was supplied by the Fine Chemical Division of these Laboratories. Commercial grade alcohol after distillation was used, rest of the chemicals were of A.R. Grade. Paper chromatographic technique and methods for the determination of moisture[2] dextran[3] reducing sugars, fructose[4]; viscosity, pH and specific rotation were same as described previously.[1]

Experimental

In eight flasks 900 ml of the sterile medium were taken; and after inoculation with an active 24 hr. culture of the local strain, were incubated at 25–27°. Fermentation process was followed by determining the pH, density and viscosity of the culture medium. The yield of dextran was also noted after 12 hr. incubation. On completion of the process the culture medium, which appeared to be homogeneously dispersed, was diluted with 20% aqueous alcohol (200 ml) and centrifuged twice. The supernatant decanted off, cooled to 15° and 95% alcohol was added slowly with mechanical stirring till its concentration became 50%. The flocculent precipitate thus obtained was freed from moisture through kneading with 99% alcohol three times and then filtered under vacuum. Dextran was further dried under vacuum, on anhydrous $CaCl_2$. It weighed 78 g, being equivalent to 31% of the initial wt of molasses.

Purification of Dextran

Hydrochloric acid 1.4N (125 ml) and alcohol 95% (125 ml) were mixed and cooled to 15°. Dried dextran (10 g) was dispersed mechanically in this solvent mixture for 15 min and filtered under vacuum. Dried alcohol (40 ml) was added in portions during filtration. Dextran 5.3 g was obtained.

In similar way the purified dextran (5 g) was again dispersed in the mixture (125 ml) for another 15 min and filtered. During filtration dried alcohol (20 ml) was added in portions. Dextran 3.56 g was obtained. Details of the results are given in Table 2.

The washings obtained from the two purifications were combined and distilled. The normality of the distilled solvent mixture was almost unchanged and was used again for purification. Results are given in Table 3 .

Results and Discussion

From 250 g molasses containing an average of 100 g sucrose the locally isolated strain of *Leuconostoc mesenteroides* elaborated 78 g of dextran by weight; or 29 g when estimated colorimetrically, (Table 1), in a culture medium containing (g/1) molasses 250 g, acidic aqueous extract at p H2 of 10 g rice polishings or

TABLE 1. CHANGE OF pH AND VISCOSITY OF THE CULTURE MEDIUM (250 g. MOLASSES) AND THE YIELD OF DEXTRAN DURING INCUBATION AT 25°.

Incubation time hr.	pH	Density	Absolute viscosity in c.p. (25°).	Yield of dextran	
				By wt.	by colorimetric estimation
0	8.0	1.099	2.291	–	–
2	6.2	1.21	2.538	–	–
4	6.2	1.21	2.540	–	–
6	6.2	1.210	2.545	–	–
12	5.7	1.120	2.848	40	16.95
24	5.1	1.119	4.652	47	20.00
48	4.6	1.119	10.34	78	32.61
92	4.6	1.118	15.784	78	32.61

TABLE 2. PURIFICATION OF DEXTRAN: 250 ml ACID: ALCOHOL MIXTURE WAS USED FOR 10 g DEXTRAN IN EACH PURIFICATION.

Origin of molasses from which dextran was produced	Nature of Dextran	Moisture	Dextran (colorimetric)	Reducing sugar	Ash
Mehran Sugar Mills.	Crude	9.2%	42.37%	10.2%	15.5%
Mehran Sugar Mills.	Form 1st Purification.	8.9%	61.02%	10.2%	5.2%
Mehran Sugar Mills.	From 2nd Purification.	10.5%	81.30%	9.8%	1.5%
Faugi Sugar Mills.	Crude	9.5%	42.6%	9.9%	14.9%
Faugi Sugar Mills. Tank No. 1	From 1st Purification.	9.1%	62.15%	10.2%	4.9%
Faugi Sugar Mills. Tank No. 1	From 2nd Purification.	10.0%	81.5%	10.1%	1.2%
Faugi Sugar Mills.	Crude	9.0%	42.76%	10.1%	15.10%
Faugi Sugar Mills. Tank No. 2	From 1st Purification.	9.5%	62.10%	9.9%	5.10%
Faugi Sugar Mills. Tank No. 2	From 2nd Purification.	9.5%	80.9%	9.9%	1.19%

TABLE 3. YIELD OF THE PURIFIED DEXTRAN: 10 g CRUDE DEXTRAN WAS USED IN 1ST PURIFICATION WHILE 10 g OF THE PURIFIED DEXTRAN WAS USED IN 2 ND PURIFICATION.

Molasses from which dextran was produced	Wt of dextran after purification (g)	Wt of solid in the filtrate in(g)	Wt of dextran after second purification in(g)	Wt of the solid in the filtrate in(g)
Mehran Sugar Mills.	5.325	3.50	7.12	1.2
Faugi Sugar Mills. Tank No. 1	5.600	3.20	7.21	1.19
Faugi Sugar Mills. Tank No. 2	5.502	3.22	7.19	1.19

TABLE 4. CONSUMPTION OF HYDROCHLORIC ACID: ALCOHOL (1:1) MIXTURE FOR PURIFICATION OF DEXTRAN (250 ml /10 g).

	Vol of acid: alcohol mix.	Vol after use	Normality	Vol after distillation	Normality
1st	Purification				
	250	225	0.679	215	0.603
2nd	Purification				
	250	235	0.675	225	0.603

5.0 g wheat bran, sodium chloride 1.0, and dipotassium hydrogen phosphate 1.0 in 48 hr at 25–27°. The nutrition value of rice polishing and wheat bran deteriorate on keeping and therefore should invariably be used in fresh form. Pretreatment of molasses for the production of dextran is found to be unnecessary. However, it was filtered to remove dirt and other foreign particles before use in the culture medium.

Filtration of 25% solution of molasses (100 ml) through charcoal (10 g) resulted in the reduction of 20% of its total ash and was also accompanied by much improvement in its colour. Removal of calcium through sulphuric acid (1 ml) treatment of 25 g molasses also resulted in a decrease of 20% of the ash, but inversion of approximately 20% of sucrose available in the molasses also took place at 20°. Moreover extra quantity of sodium hydroxide is required to bring back the pH of the culture medium to 8. Pretreatment of molasses, therefore, did not prove useful. Hence solution of molasses was used as such after filtration through cotton.

Purification of dextran has been accomplished in two steps; and is based on the observation that precipitation of total dextran in the culture medium takes place when the concentration of alcohol reaches 50%. Thus the dextran, dispersed in a mixture, in which the concentration of alcohol is already 50%, does not go into solution; while the impurities are dissolved and removed by filtration. In the first step 70% of the total and in the second 52% of the remaining ash is removed (Table 2), with the result that the finally purified dextran, contains only 1.2-1.5% ash. The yield of the purified dextran is quantitative (Table 3); and loss of the solvent mixture is negligible (Table 4). The purified dextran, thus obtained is suitable for industrial use..

Proportion of purified dextran; Moisture 2.8%; Dextran 81.5%; Reducing sugars as glucose 9.0%; Nitrogen 0.43; Ketoses (fructose) Nil.

Composition On complete acid hydrolysis yielded glucose.

Solubility. Readily soluble in water.

Specific rotation. 153 (C = 0.4 in 1.8 N KOH).

Relative Viscosity. 1.206 (2% at 32°).

Acknowledgement. The authors are thankful to Mr. S. Ashfaq Hussain, for useful help in conducting the experiments.

References

1. J. Jafri, R.Zuberi and S.A.H. Zaidi, Pakistan. J.Sci. Ind. Res., **17**, 6, 219 (1974).
2. D.Glick, *Methods of Biochem. Analysis* (Interscience, New York, 1955), vol. II, p. 325.
3. P. B. Hawk, B.L. Oser, W.H. Summerson, *Practical Physiological Chemistry* (McGraw Hill, New York, 1954),p. 568.
4. A. Jeanes and C. A. Wilham and J.C. Miers, J.Biol. Chem., **176**, 603 (1948).