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STUDIES ON THE ABILITY OF DEXTRAN PRODUCED BY *LEUCONOSTOC MESENTEROIDES* TO STABILIZE SUGAR SYRUPS

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Preparation of a high-viscosity syrup that contained 80 and 85% sucrose was studied. Dextran produced by a locally isolated strain of *Leuconostoc mesenteroides* and its spent culture medium were used as stabilizers. The spent culture medium with a solid content of 11.5% contained 44% dextran, 40% reducing sugars and 12% non-reducing sugars. The stabilizing properties of dextran and the spent culture medium were compared with those of commercial stabilizers such as liquid glucose, sodium alginate and carboxymethyl cellulose.

Key words: Gum dextran, Stabilizer, Sugar syrup.

Introduction

In various pharmaceutical tinctures or syrups and in certain foodstuffs, such as 'Sherbets' and beverages, sugar is added for sweetening, to enhance storage ability and to give consistency and body. Concentrated syrups containing 80% sucrose or more are used for this purpose. To control crystallization, formation of films and to inhibit synerism namely, the release of water from the concentrated sucrose syrup, small amounts of stabilizers are added. The stabilizers are long-chain polymers that dissolve or disperse in water and have a thickening or viscosity-building effect, which stabilizes the syrups. These compounds are usually called hydrocolloids or, more commonly, gums.

In Pakistan, 'Sherbets' are among the most popular drinks. Generally, they are made of flavoured concentrated sucrose syrups of high viscosity. Liquid glucose is the only stabilizer that is manufactured in Pakistan while other stabilizers, such as sodium alginate and carboxymethyl cellulose (CMC) are imported. Dextran is used as a stabilizer in ice creams and in concentrated sucrose syrups of exceptionally high viscosity. As reported previously [1,2], the preparation and characterization of dextran from sucrose and molasses generated by a locally isolated strain of *L. mesenteroides*. In this communication we describe the preparation of concentrated sucrose syrups of exceptionally high viscosity for use in 'Sherbets', 'murrabbahs' (fruits preserved in concentrated sugar syrup), ice creams, sweetmeats and other foodstuffs. The syrups remain stable and do not crystallize upon storage for a long period of time.

Materials and Methods

Commercial 'Sherbets', liquid glucose, sodium chloride, wheat bran and rice polishings were purchased from the local market, CMC was of food grade from Japan. Yeast extract and tryptone were of Bacto grade (Difco) sodium alginate was of G.R. grade (BDH). Commercial grade alcohol and acetone were used after distillation, all other reagents were of analytical grade.

Moisture content. The moisture content of liquid glucose, commercial 'Sherbets' and sucrose syrups prepared in the laboratory, was determined at 110° and that of dextran at room temperature, by use of calcium chloride under reduced pressure.

Dextran and sucrose content. Dextran in the broth was quantified colorimetrically by the Anthrone method [3]. A 0.001% aqueous solution of dextran (Mr, 60,000 - 90,000) was used as the standard. The yield of dextran was also determined by precipitation with alcohol/acetone. Sucrose in 'sherbets' was quantified by the Anthrone method with a 0.001% aqueous solution of sucrose as the standard.

Reducing sugars. Reducing sugars were quantified colorometrically by the Nelson Somogyi method [4,5] with a 0.001% solution of glucose as the standard.

Viscosity. Viscosity of sucrose syrups, 'Sherbets', stabilizers and culture medium was determined with a Redwood viscometer, No. 2 (Gallenkamp). Its container holds approximately 150 ml of solution. The efflux time of 100 ml of solution was noted in seconds.

Organism. The strain *L. mesenteroides* was isolated from a mixture of fermented vegetables, such as cauliflower, cabbage, pumpkin and tomato [6]. Small pieces of vegetables were inoculated in 5-10% sucrose broth medium and incubated at 25° for 3-4 days for non-selective enrichment. The tubes showing high viscosity in broth were used for selective isolation of *L. mesenteroides* on sucrose agar medium containing 0.005% sodium azide. The slime producing colonies of Gram-positive diplococci, obtained after 48 hrs incubation, were isolated and identified by the usual methods [7].

Media. Two types of medium were used for the growth of *L. mesenteroides* and for production of dextran. The composition of the first medium was (g/100 ml): sucrose 10.0, tryptone 1.0, yeast extract 0.5, sodium chloride 0.10, and di-potassium hydrogen orthophosphate 0.1. The second medium contained (g/100 ml): sucrose 10.0, yeast extract 0.5, peptone 0.5, di-potassium hydrogen orthophosphate 1.5 manganous chloride 0.001, magnesium sulphate 0.001 and calcium chloride 0.005. The pH was adjusted to 8.0 with sodium hydroxide and sterilization was carried out at 15 lb/psi for 20 mins. Extract of rice polishing and wheat bran were used as substitutes for imported tryptone/peptone and yeast extract. Preparation of extracts of rice polishing and wheat bran and of dextran were carried out as reported earlier [1].

Preparation of spent culture medium. The spent culture medium was prepared by inoculating 10 ml sterile broth with a loopful of rapidly growing culture of *L. mesenteroides*. It was incubated at 25° for 18 hrs. The 10 ml of 18 hrs broth culture was transferred to 90 ml sterile medium and incubated at 25° for further 18 hrs. The 100 ml inocula obtained was used to inoculate 900 ml medium and incubated at 25° for 18 hrs. The highly viscous spent culture medium was sterilized by autoclaving at 15 lb/psi for 20 mins. Dextran was precipitated with cooled ethanol from the spent culture medium. The spent culture medium contained 44% dextran, 40% reducing sugar and 12% non-reducing sugar.

Enzyme activity. The spent culture of *L. mesenteroides* was prepared in broth medium with 2% sucrose. The cells were removed by centrifugation at 15000 rpm for 20 mins at 0°. The supernatant was used for the determination of dextran-sucrose activity by the standard assay method of Kobayashi *et al.* [8].

Preparation of sucrose solutions. A known weight of succrose was dissolved by mechanical stirring in 140 ml of water kept in a boiling water bath. At the same time, methyl parahydroxybenzoate at 0.1% and propyl parahydroxybenzoate at 0.05% were added as preservatives. After complete dissolution of sugar, the solution was made up to 200 ml. Solutions of sucrose with stabilizers and with sterile spent medium from a culture of *L. mesenteroides* were prepared by dissolving the required quantity of stabilizers in concentrated sugar syrups with preservatives. After complete dissolution, the volume was made up to 200 ml. The viscosity and density of each preparation was determined at 30°.

Results and Discussion

Table 1 shows the efflux times and densities of sugar solutions of various concentrations without stabilizers. As indicated in the table, the efflux time of water at 30° was 7 secs, while the efflux times of 80% and 85% sucrose solutions were 45 and 66 sec., respectively. The efflux times and densities of different imported and locally manufactured commercial stabilizers, at various concentrations, are shown in Table 2. Liquid glucose, the only commercial stabilizer produced in Pakistan, when used at concentrations of 90% and 95% had efflux times of 47 and 80 secs. respectively. Sodium alginate and CMC, which are imported and expensive in Pakistan, were used in small amounts to give the same efflux times.

In Table 3 the efflux times of the sterile spent medium after culture of *L. mesenteroides* in two different culture media, as well as of the dextran obtained by precipitation with alcohol/acetone from the spent culture media, are provided. An aqueous solution of 10% dextran had an efflux time of 60 secs. The sterile culture medium after growth of *L. mesenteroides* in medium No. 1 was very viscous and had an efflux time of 720 secs, while the viscosity of spent medium No. 2 was relatively low and the efflux time was only 45 secs. Although the viscosity of the spent culture medium No. 2 was lower than

TABLE 1. EFFLUX TIMES AND DENSITIES OF SOLUTIONS OF SUCROSE PREPARED AT VARIOUS CONCENTRATIONS AT 30°.

Sucrose solution percent (w/v)	Efflux time (sec)	Density
0 (water)	7.0	1.00
40	9.0	1.16
50	11.0	1.20
60	12.5	1.23
70	19.0	1.27
80	45.0	1.31
85	66.0	1.34

TABLE 2. EFFLUX TIMES AND DENSITIES OF VARIOUS COMMERCIAL SUGAR-SYRUP STABILIZERS AT 30°.

Product	Percent solution	Efflux time (sec)	Density
1. Liquid glucose	85	28.0	1.27
(v/v)	90	47.0	1.30
	95	80.0	1.31
2. Sodium alginate	2	12.0	1.01
(w/v)	4	18.0	1.02
	6	38.0	1.02
	8	77.5	1.04
3. Carboxymethyl	0.2	16.0	1.00
cellulose (w/v)	0.4	45.0	1.00
	0.6	54.0	1.00
	0.8	234.0	1.01

that of the spent culture medium No. 1, the amount of dextran obtained by precipitation from the two media was nearly the same. This result is due to the production of low-molecular-weight dextran in medium No. 2, as confirmed by the relative molecular-weight distribution of the native dextran produced in the two media after gel-permeation chromatography with blue dextran as the reference standard. Moreover, in medium No. 2, the activity of dextran sucrose was found to be 2 times higher than that in medium No. 1. This difference may be due to the presence of essential elements, such as Mn^{2+}, Mg^{2+} and Ca^{2+} ions that may accelerate the production of enzymes which in turn generate dextrans of low molecular weights. The efflux time of unfermented, sterile sucrose broth was found to be equal to that of water.

TABLE 3. EFFLUX TIMES OF STERILE SUCROSE-BROTH, SPENT CULTURE MEDIUM AND DEXTRAN PRODUCED BY *L. MESENTEROIDES*.

Product	Percent solution (scc)	Efflux time (w/v)
1. Sterile sucrose broth medium		
Medium No. 1	–	7
Medium No. 2	–	7
2. Sterile spent medium from culture of *L. mesenteroides*		
Medium No. 1	–	720
Medium No. 2	–	45
3. Dextran	2	7
	4	13
	6	20
	8	37
	10	60

TABLE 4. EFFLUX TIMES, DENSITIES AND PERCENTAGE OF SUGAR OF TWO COMMERCIAL SHERBETS.

Commercial 'Sherbets'	Percent sugar	Efflux time (sec)	Density
No.1	76	393	1.38
No.2	82	405	1.39

For, comparative purpose, two well known commercial 'sherbets' were purchased from the market and their sugar contents, efflux times and densities were determined as shown in Table 4. Since the commercial 'Sherbets' had sugar contents of 76 to 82%, we prepared our sucrose syrups at 80 and 85% with various stabilizers to achieve the same efflux times as those of the commercial 'Sherbets'. The efflux times and densities of our syrups with stabilizers are shown in Table 5. As indicated in the table, 23 g of liquid glucose, when added to a 80% sucrose solution, gave an efflux time of 385 secs. Almost the same viscosity was attained with 40 g of sterile spent culture medium No. 1, which is equivalent to 4 g of sucrose or with less than 5 g of dextran obtained from the spent culture medium by precipitation. Similarly, 3.5 g dextran or 40 g of spent culture medium, when added to 85% sucrose syrup, gave approximately the same efflux times as those obtained with 1.7 g of sodium alginate or with about 16 g of liquid glucose. The appearance, consistency and stability of the syrups prepared with different stabilizers was the same.

TABLE 5. EFFLUX TIMES AND DENSITIES OF SUCROSE SYRUPS PREPARED WITH DIFFERENT STABILIZERS.

Stabilizers	Sucrose solution (80%, w/v)			Sucrose solution (85%, w/v)		
	Stabilizer added (g/100 ml)	Efflux time (sec)	Density	Stabilizer added (g/100 ml)	Efflux time (sec)	Density
Liquid glucose	22.50	360	1.37	15.50	402	1.36
	23.00	385	1.37	16.00	525	1.36
Dextran	4.50	340	1.29	3.50	465	1.33
	5.00	487	1.33	-	-	-
Spent Culture	40.00	390	1.31	40.00	460	1.32
medium	-	-	-	38.50	365	1.32
Sodium alginate	2.50	380	1.31	2.00	502	1.30
	-	-	-	1.70	468	1.30
CMC	0.25	305	1.30	0.16	490	1.33
	0.30	395	1.30	0.15	390	1.33

From these results it can be concluded that the sterile spent medium from culture of *L.mesenteroides*, which contains products of the fermentation of sucrose or the dextran obtained by precipitation from the sterile spent medium can be used as a syrup stabilizer. The use of the spent medium should be more economical than the use of liquid glucose. A total of 23 g of liquid glucose is needed to raise the viscosity of an aliquot sugar syrup to the desired level while spent medium from a culture of *L. mesenteroides* that contains 4 g of sucrose is sufficient for this purpose. The price of liquid glucose is higher than that of sucrose. The preparation of the spent culture medium is very simple. The fermentation process does not require aeration or agitation and practically all the ingredients used in the medium are indigenous. Yeast extract and peptone can be replaced by extract of rice polishing or wheat bran, which are locally available in abundance at a very low price. Thus, the use of spent culture medium should be very economi cal. Moreover, vitamins and essential minerals are present in the spent culture medium providing additional advantages.

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