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Identification of Polysaccharides from Indian Sugar

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ABSTRACT

In the present study arabinogalactan polysaccharides has been reported from raw and refined cane sugars. These were isolated by the alcoholic precipitation of their aqueous solution and purified by various fractionation methods. Various proportion of neutral monosaccharide's present in the various fractions produced during the isolation of polysaccharides from sugar crystal. The obtained results were further confirmed by nuclear magnetic resonance chromatography techniques.

Keywords: Polysaccharides, sugar crystal, Indian plantation white sugar, chromatography.

INTRODUCTION

Sugarcane being an efficient producer of sucrose is also host to wide variety of polysaccharides. Polysaccharides are polymeric molecules made up of a large number of simple carbohydrate units called monosaccharaides usually more than ten to hundreds or even thousands of such units. The polysaccharide molecule may be in the form of straight chain, branched chain or even coiled chain and may contain only one type of monosaccharide or different type of these units. In plants and animals polysaccharides serve as structures giving them strength and rigidity (e.g. cellulose) and also act as metabolic reserves of energy (e.g. starch and glycogen).

The well-known sugarcane polysaccharides are cellulose, hemicellulose, starch and dextran. The major polysaccharides which gets extracted from sugarcane into cane juice during the milling is starch, cellulose and hemicelluloses are insoluble structural components of cell wall and are not of any importance in cane juice processing. Dextran is a microbial polysaccharide which may occasionally get extracted into cane juice from sugarcane infected with bacteria (*Leuconostoc*). During subsequent boiling starch has the tendency to get incorporated into the raw sugar crystals. The polysaccharides in general causes processing difficulties in sugar manufacture and this effect is not easily diminished as very few of the processing steps remove them. Even during the refining operations these polysaccharides are only partially removed and remain occluded inside the sugar crystal. The production of sugar (sucrose) from sugarcane juice is based on the ability of sucrose to crystallize from thick syrup. It is generally accepted in the literature that polysaccharides have greater tendency to go preferentially into the sugar crystal and thus impact refined sugar quality[1, 2]. These high molecular weight polysaccharides negatively affect sugar processing and

have been implicated in the inclusion of colour in crystals, formation of color on storage processing problems and final product quality issues such as turbidity and acid beverage floc[3].
Another polysaccharides 'dextran' which is formed in sugarcane or cane juice[4] as a result of occasional bacterial contamination is responsible not only for sucrose loss but due to its high positive specific rotation ($+200^0$) shows high misleading polarization values. It also results in viscosity increase. High viscosities decrease the sucrose crystallization rate, the decrease being more pronounced in low grade boiling. Other effects of these high viscosities are decreased heat transfer in the evaporators, crystallizers and pans, increased in- boiling, a decrease in both sucrose yield and quality and increased energy cost due to longer processing time. Filtration is also adversely affected by viscosity increased[5]. There is hardly any study carried out to investigate the presence of any major polysaccharides other than starch or dextran in these granulated plantation white sugars. Such a study will on the one hand give information about the nature of polysaccharides if any present in our factory juices and on the other hand it will reveal the inadequacies of the present sugar manufacturing process and subsequently will guide to the means for its improvement. The success of this investigation may lead the sugar industry in our country to adopt improved clarification/refining techniques for the production of polysaccharide free sugar, which will be most suitable for the needs of various industries.

MATERIALS AND METHODS

Isolation of Alcohol Perceptible Polysaccharide from Plantation White Cane Sugar : To 200 g. sugar dissolved in 200 cm^3 water was added 5cm^3 of acetic acid and 600 cm^3 ethanol. The solution was filtered with suction through a ½ inch thick mat of 'Celite' analytical filter aid on a sintered glass funnel. The filter mat was washed with five 200 cm^3 portions of ethanol to remove sugars, then was washed again with two 200 cm^3 portions of boiling water to dissolve the precipitated polysaccharides and recovered again using ethanol to get the crude polysaccharide.

Purification: Polysaccharide (500 mg) dissolved in 50 ml of 0.02 M phosphate buffer (pH- 7.0) was incubated under a few drops of toluene for 20h at 38^0C with 0.3 ml of dextranase and 1.0 mg of α-amylase. After the incubation, ethanol (150ml) was added and the resulting precipitate was collected by centrifugation. The precipitate was dissolved in water and dialyzed against deionized water for 72 h. The water soluble portion dialyzate obtained as just described was divided into 3 fractions by precipitation with ethanol; insoluble in 50% of aqueous ethanol, insoluble in the ethanol concentration range 50-67.5% and those soluble in 67.5% ethanol. The water soluble fraction 10.5% precipitated in ethanol in the concentration range 50-67.5%. The major fractions, the 0-50% ethanol precipitate representing 67% of the starting material, was 64% protein (from the relationship elemental N×6.25) and 2.2% carbohydrate (by phenol- sulphuric acid colorimetry against a reference of glucose). Chromatography twice of the 50-67% ethanol fraction (0.44g) on sepharose CL – 6B in 7M area on a 90×2.5 cm column led to the recovery of 0.33 g of polysaccharides and a small proportion of material absorbing UV radiation at 278 nm.
Further purification was achieved by ion- exchange chromatography on Biorad macro porous resin AG MP-1 (PO_4). The sample was equilibrated by dialysis to 5 mM phosphate, pH- 7.5 for loading. After elution of unretained material from the column, the polysaccharide was released by a linear gradient to 0.5 m sodium chloride prepared in phosphate buffer. Recovery was 0.19 g of polysaccharide free from U.V. absorbing material.

Structural Analysis : Methylation was effected by as discussed in the literature[6] method the methylated polysaccharides (2-10mg) was dissolved in 2ml of 1.5 m HCl in dry methanol and the mixture in sealed tube was kept for 22h at 85^0C. The mixture was made neutral with silver carbonate, filtered and the filtrate was made deionised with Amberlite IR – 120 (H^+ form) resin and evaporated to dryness under diminished pressure at 40^0C. The resulting methyl glycosides were then hydrolyzed with 2 ml of 0.6 M sulphuric acid for 4 h at 100^0C. The glycosidic linkages involved in the structure have been established, but their distributions are fine structures are not known. From the data, the arabinogalactan is envisaged as having a

framework of (1-3) linked β-D- galactose residues of which two out of every three carry a galactosyl or aralunosyl side chain attached at O-6. The essential structural features of the arabinogalactan establishes it as a type widely distributed in plant world. The negative specific rotation ($[\alpha]^{20}_{589}$-56^0) indicates the β-configuration in D- galactose and α in L -arabinose.

The arabinose occurs only in furanose form. This conclusion is supported by data from partial acid hydrolysis and ^{13}C- n.m.r spectroscopy. Partial acid hydrolysis involved two procedures described by Adams[6-8]. In the first instance the mild hydrolytic step released only arabinose but significant quantities were also present in total acid hyrolyzate. In the second procedure, all arabinose was cleaved in the mild acid stage, but some scission of galactose occurred as well. Oligosaccharides were also present when examined my paper chromatography fail to produce but any pink colour characteristic of pentoses when sprayed with p-anisidine hydrochloride. The ^{13}C-n.m.r. spectrum was obtained with the deuterated polysaccharides in deuterium oxide at a concentration of 50mg ml^{-1} in a 10-mm probe and a JEOLFX – 100 spectrometer at 85^0C. The collection was by overnight acquisition under deuterium lock and the spectrum is referenced to external T.M.S. The spectrum is shown in figure 1 together with the measured chemical shifts ^{13}C-n.m.r. spectrum signals at 110.9 and 109.6 ppm arise from furanoside residues where as that at 105.1 ppm would be from the anomeric carbon atom in β- (1-3) linked galactose[9].

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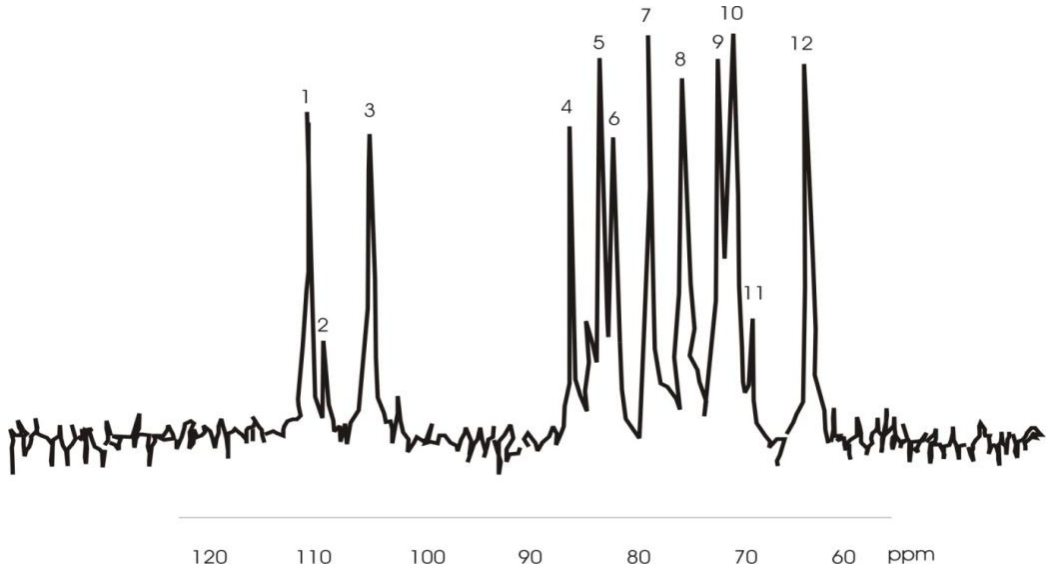

Fig. 1: ^{13}C-n.m.r. spectrum polysaccharide at 85^0. Measured chemical shifts (p.p.m.) are shown for the numbered signals (1) 110.9 (2) 109.6 (3) 105.1 (4) 86.0 (5) 83.2 (6) 82.0 (7) 78.7 (8) 75.4 (9) 71.8 (10) 70.4 (11) 69.0 and (12) 63.4

RESULTS AND DISCUSSION

Glucuronic acid was isolated from the total acid hydrolysate by anion exchange chromatography and was identified by chromatography on paper using 18:3:1:4 ethyl acetate – acetic acid – formic acid – water. D-Glucuronic acid is present as a monosaccharide component in a concentration that enables retention on an ion exchange resin but is insufficient to be detectable in the ^{13}C-n.m.r. spectrum. An arabinogalactan polysaccharide isolated from Indian plantation white cane sugar, consists of L-arabinose and D-galactose units and classified as 3,6 – arabino disutstituted galactan type. From the data, arabinogalactan as having a frame work of (1-3) linked β-D-galactose residues of which two out of every three carry a galactosyl or arabinosyl side chain attached at 0-6. L-arabinofuranosyl residues as single units and small side chain predominate at the branches. Galactosyl residues are attached in small chain linked β(1→6). It is little

richer in arabinose then is normally found, and this sugar exists solely in the furanosidic form. This conclusion is supported by data from partial acid hydrolysis and ^{13}C-n.m.r. spectroscopy. Specific rotation found to the negative $[\alpha]^{20}_{589}$-56^0 which indicates β-configuration in D-galactose and α in L-arabinose. ^{13}C-n.m.r. also supports the presence of anomeric carbon in β-(1→3) linked galactose.

Researchers[**10]** have isolated HMW component in juice polysaccharides fraction and analyzed that as arabinogalactan by Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Ultrafilteration (UF) of sugarcane juice with polysulphone (PS)/Polyether sulphone (PES) membranes is characterized by significant fouling. In earlier work[11] a high molecular weight component (HMW) in the juice polysaccharide fraction was observed to interest with the membrane surface. This study focuses on understanding the polysaccharide fouling using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). Further the HMW, component in the polysaccharide was isolated and characterized by FTIR and nuclear magnetic resonance (NMR) spectroscopy[12]. The HMW fraction appears to contain arabinogalactan protein along with phenolics and some lipids. Analysis by the resistance in series model indicated cake fouling to be the dominant mechanism. Study[13] reported an arbinogalactan polysaccharide to be a common constituent of Australian sugarcane. From methylation data arabinogalactan was envisaged as having a framework of (1→3) linked β-D- galactose residues of which two out of every three carried a galactosyl or arabinosyl side chain attached at O-6. Researchers [14] have precipitated cane polysaccharide with ethnol under conditions which prevented formulation of dextran and inclusion of starch [9]. They were hydrolyzed with H_2SO_4 and the resultant sugars were determined by gas liquid chromatography (GLC)[15]. The main constituent was galactose with smaller amount of arabinose and traces of xylose, mannose and glucose. The polysaccharide thus appear to consist mainly of arabiongalactan which is believed to be as cause of floc formation by refined cane sugar in beverages. These non-sugars including polysaccharide are responsible for sugar crystallization rate, retard sugar crystal growth and reduce sugar quality [16-22].

APPLICATIONS

The results of this investigation are useful to the sugar industry in our country to adopt improved clarification/refining techniques for the production of polysaccharide free sugar, which will be most suitable for the needs of various industries.

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