NON-STARCH, SOLUBLE POLYSACCHARIDES OF SUGAR CANE

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Abstract

Non-starch polysaccharides of sugarcane include the cellulose and hemicellulose fractions, and several water-soluble compounds: cell wall polysaccharides, indigenous sugarcane polysaccharide (I.S.P.), sarkaran and sarkaran-like polysaccharides, and other glucans. Emphasis in this paper is on the soluble species. Structures of the compounds, including the recently identified low molecular weight glucan, are reviewed. Roles of the soluble polysaccharides in plant growth and metabolism are outlined. The effects of the compounds on manufacture, processing and utilization of sugar are described. Some analytical procedures for these polysaccharides are discussed, and specific analyses for some of the compounds are considered.

Introduction

The sugarcane plant contains many types of polysaccharides. Cellulose and hemicellulose make up the cell wall material of the plant, in stems and leaves. Starch and other glucans are part of the chain of food storage and synthesis in the plant. Other polysaccharides often found in the sugarcane are products of microbial infections, for example, dextrans, products of *Leuconostoc mesenteroides* infection, and are not part of the plant system.

In 1972, Imrie and Tilbury published an excellent review of polysaccharides in, and associated with, sugar cane.[1] In the years since 1972, additional information has been reported on the polysaccharides then known, and several new polysaccharides, native to the sugarcane plant, have been identified. Some of these new compounds, for example I.S.P., indigenous sugarcane polysaccharide, which can be classified as a type of hemicellulose, are native to all cane plants;[2-5] others, for example the water soluble polysaccharide identified by Blake and Littlemore,[6,7] are found only in stale cane, or cane that has been allowed to stand over from one crop to the next.

The reasons for interest in and research on these polysaccharides are many: their effect on raw sugar production and quality, including quality parameters such as pol, is the most familiar to processors. Their effect on refining efficiency, and product quality of refined sugars in end uses such as soft drinks and alcoholic beverages is also well known. Knowledge of the structure of these compounds, their role in plant metabolism and their biosynthesis is important in the development of varietal characteristics and in breeding for high sucrose, lower starch and soluble polysaccharides, quantity of fibre, and maturity, among other factors.

Polysaccharides found in raw sugar come from either the cane plant, for example starch, or from products of invading microorganisms, for example dextrans. The total polysaccharides in raw sugar[8] are frequently at levels far above the sum of starches and dextrans. Historically, total soluble polysaccharides have been called "gums". A recommended test for total polysaccharides is outlined in Appendix A.

Table 1

Polysaccharides in raw sugars

Sugar	Total polysacch., ppm	Dextran, ppm	Starch, ppm	Other soluble polysacc., %
A	1670	372	390	54,4
B	2030	775	141	54,8
C	2790	1584	382	29,5
D	1390	502	155	52,7
E	3120	1883	424	26,1

Table 1 shows analyses on several raw sugars, of various origins, and the proportions of unidentified soluble polysaccharide material in these sugars. The proportion of this latter is significant, ranging from 25 to 55%. It is of interest to processors, refiners and cane growers to know what this material is, how it is produced by the sugarcane, and what the effects on sugarcane processing and refining can be.

This paper will present a brief summary review on the soluble non-starch polysaccharides of sugarcane. Starches and dextrans will not be included, nor will the insoluble cellulose and hemicellulose materials, which make up the insoluble portion of bagasse.

Properties and structure of each type of polysaccharide are presented. The effects, or potential effects, of each type on processing are discussed, and their possible roles in sugarcane plant metabolism are considered.

Polysaccharides included in this review are: indigenous sugarcane polysaccharide,[2-5] sarkaran and stand-over cane polysaccharide,[6,7,9-11,23-25] Roberts' glucan,[12] beverage floc polysaccharides reported by Japanese workers,[13,14,26] and some polysaccharides reported by Cuban workers, and not identified as any of the above groups.[15,16,17] As some of these compounds are not yet isolated or named, a temporary system of reference is used for them in this paper, e.g. Roberts' glucan; CP, galactomannan from Japan.

Soluble Polysaccharides

Indigenous Sugarcane Polysaccharide (I.S.P.)

In 1964, Roberts reported the presence of an arabinogalactan-type polysaccharide in fresh cane juice: this arabinogalactan travels through processing and into final products. Sutherland,[18] in 1960, had found in cane syrups a hemicellulose-type of polysaccharide, which appears to be an incompletely isolated form of the arabinogalactan.

Roberts subsequently further elucidated the composition of the arabinogalactan[3] and showed that it contained glucuronic acid units,[19] which accounted for its negative charge at low pH's, as in acidified beverages. This polysaccharide

became known as I.S.P. (indigenous sugarcane polysaccharide). It has a negative rotation (−46° to −50°), a molecular weight ranging from 100,000 to 300,000 daltons, has a glucuronic acid content of 7 to 8 % in the freshly extracted state, decreasing as the I.S.P. goes through processing, and is extremely soluble. I.S.P. is one of the factors in a common type of acid beverage floc.[20]

The structure was further elucidated by Blake,[4, 5] who compared I.S.P. from Louisiana cane to a similar polysaccharide from Queensland cane and clarified the structure of I.S.P. as a 3,6-arabinogalactan, with a backbone of beta −1,3 linked galactose residues, with arabinose and some galactose and other residues as side chains at the 6 position. The Australian arabinogalactan appeared to have a lower level of glucuronic acid (also substituted at the 6 position of galactose residues) than did the I.S.P.

All grasses contain hemicelluloses, as part of the cell wall structure of their non-endospermic parts.[21] Sugarcane, a giant grass, is like other grasses in that most of its hemicellulose is made up by polymers of xylans with some other residues, or heteroxylans, which are insoluble and which, with cellulose and lignins, form bagasse. The I.S.P., or arabinogalactan, group may be regarded as soluble hemicellulose, which may, in the plant, serve to link phenolic and flavonoid residues to the insoluble cell wall glycans.

Another component of cell wall material in many plants is called "pectic substance" and contains galacturonic acid residues in its polymeric structure. There are references in the old literature to "pectic substances" in sugarcane. Until recently,[19] it was difficult to distinguish glucuronic from galacturonic acid when both were part of polysaccharide structures. The most common procedure was to titrate hydrolyzed polysaccharide for acid residues, with no identification of the type of acid. Recent studies at S.P.R.I.,[22] have shown no galacturonic acid in sugarcane polysaccharides, but only glucuronic acid. The old references to "pectic substance" probably result from confusion of this glucuronic with the more common galacturonic acid. Galacturonic acid, or "pectic substance" is certainly found in sugarbeets.

I.S.P. is the non-sugar of greatest concentration in cane juice, after organic and amino acids.[12] I.S.P. and similar compounds can have a negative effect on polarization if they are not removed by lead acetate clarification. Initial studies at S.P.R.I. indicates that there is partial removal of these polysaccharides by lead acetate.

Sarkaran

In 1966, Bruijn, in an examination of the polysaccharides in cane allowed to become stale after harvesting, observed another glucose polymer, in addition to dextran. His glucan, which he later named sarkaran, increased in concentration with the cut-to-crush time. Bruijn showed that sarkaran, which is very soluble, with a rotation of +160°, is a straight chain glucan with approximately 75% alpha-1,4 and 25% alpha-1,6 bonds arranged as a polymer of maltotetraose and maltotriose.[23-25] It has some similarity to pullulans, but is not a pullulan. Recently, Blake and Littlemore[5, 6] examined the soluble polysaccharides in cane that had been allowed to stand over from one crop year to the next and isolated a glucan which appeared to be similar to sarkaran. Intensive structural analysis by proton and carbon n.m.r., methylation analysis and enzymic analysis, led Blake to conclude that the glucan in standover cane is indeed sarkaran. Blake found sarkaran in molasses from refractory syrups from several factories crushing standover cane, verifying its structure by proton n.m.r., periodate oxidation and enzyme analysis. He described the specific arrangement of maltotetraose and maltotriose in the molecule.

Sarkaran is a problem in processing because it increases viscosity of molasses and syrups, and appears to lower crystalization rate and cause frothing.[6] Sarkaran, with a rotation of +160° to +170°, can increase juice polarization, as it is apparently not removed by lead acetate clarification.[11]

Blake found sarkaran to be present at up to 0,13% on cane, in standover cane in poor condition. In speculation on the origin of sarkaran, which increases in concentration as cane is stored after harvest, he proposes that sarkaran is a product of a microorganism, perhaps a yeast, rather than a product of plant response to change, because of the varied structural conformation, and long induction period for the appearance, of sarkaran.[11]

Blake also[10] published an assay, using pullulanase digestion and subsequent colorimetric reaction of the reducing sugars produced, for sarkaran. He found errors from interference by reducing sugars and starch fragments. There may also be error caused by transglycosylation activity of the enzyme.

Roberts' Glucan

Very recently, Roberts of the S.P.R.I. group reported the isolation of a small glucan from fresh cane.[12] This glucan travels with I.S.P. in isolation procedures, and had heretofore been thought to be a low molecular weight component of I.S.P. This glucan is found in all cane, not only in stale or standover cane. It is low in molecular weight (< 50,000 daltons), and has a specific rotation of +120°. Structural investigation by methylation, periodate oxidation, enzyme analysis and proton nmr (280 mHz) showed a backbone of alpha-1,4 linkages, with about 12% branching as alpha-1,6 linkages. The glucan appears similar in structure to amylopectin, but is extremely water soluble, while amylopectin is quite insoluble. The glucan products a red-purple colour reaction with iodine (similar to the colour produced by amylopectin) but under polarized light and magnification does not show the Maltese cross pattern characteristic of amylopectin.

This glucan is probably a type of storage polysaccharide in the food chain of the cane plant, and may be an intermediate in the biosynthesis of sucrose, or the product of an alternate biosynthetic pathway. Roberts proposes that it is a plant glycogen. It is present in quite small amounts (< 0,01 % on cane) but the ratio of quantities of glucan to I.S.P. varies with age and variety of cane. Studies are underway to explain this observation, with the aim of increasing knowledge of the maturing process of the plant.

This glucan does not increase viscosity of syrups[12] and has little effect on polarization so it does not appear to have any deleterious effect on processing. It does not produce acid beverage floc, and is near the lower limit of the molecular weight range to produce alcohol floc in cordials.

Galactomannan from Japan (CP)

In studying raw sugars that, when refined, produced beverage floc in carbonated beverages, Miki *et al.* At the Japan Sugar Refiners' Association, isolated polysaccharide material that showed positive floc-forming potential.[13, 26] The polysaccharide material, named CP, contained galactose, glucose and mannose in varying ratios. Subsequent work[14] on structural analysis of the purified polysaccharide from CP, by gel filtration, methylation, periodate oxidation and enzymic analysis, showed that it is a galactomannan, with two possible structures: (a) The galactomannan has a main chain consisting of a alpha-1,6 and alpha-1,2 linked D-mannopyranose residues. Single alpha-D-galactopyranose residues

and an alpha-D-mannopyranose residue are attached in the ratio of 3,0 : 1,0 to 86% of the alpha-1,6 linked D-mannopyranose residues in the main chain through alpha-1,2 links or (b) the main chain of the galactomannan consists of only alpha-1,6 linked D-alpha-1,6 mannopyranose residues, 86% of which are branched at 0-2. D-mannopyranose alpha-1,2 residues are alpha-1,2 linked in side chains that are terminated by non-reducing alpha-D-mannopyranosyl and/or alpha-D-galactopyranosyl groups. In this possibility, most or all of the alpha-D-galactopyranose and some non-reducing alpha-D-mannopyranose residues are considered to be attached through C-2 to alpha-D-mannopyranose residues of the main chain.[14]

No indication is given of the source of this polysaccharide.

Polysaccharide from Cuban molasses

In 1980, Cremata and Orozco[15] isolated a previously unreported polysaccharide from molasses of high viscosity, by separation with CTAB and gel filtration. Carbon nmr, GLC and mass spectrometry show a backbone of 1,3 linked glucopyranose residues. branching at C-6 galactose with arabinofuranosyl terminal groups, all in alpha-linkage.

No indication is given of the source of this polysaccharide, but it may be another aspect of the hemicellulose complex with storage glucans.

This laboratory, and other Cuban research workers, in work on the influence of polysaccharides as crystal habit modifiers, have identified low molecular weight (< 10,000 daltons) polysaccharides as among responsible factors for crystal elongation. No definite indication of structure or origin of these large oligosaccharides is given.

Summary

A brief review has been presented of non-starch soluble polysaccharides of sugarcane. Among the compounds discussed with regard to structure, origin and role in processing are sarkaran, I.S.P. and other arabinogalactans, Roberts' glucan, a galactomannan and a copolymer of glucose with arabinose and galactose.

Appendix A. S.P.R.I. test for estimation of total soluble polysaccharides

This procedure determines total soluble polysaccharides: dextran, starch, mannans, I.S.P. (arabinogalactans) and any other soluble polysaccharides in sugar or cane juice. The soluble polysaccharides are those of special concern to the processor and refiner because they remain in solution throughout processing, and go into final products.

This procedure is based upon the precipitation of the polysaccharides from a sugar solution by alcohol. The precipitated polysaccharides are filtered off and filter is washed with 80% V/V alcohol until free of sugar. The polysaccharides are dissolved out of the filter by boiling in 1% V/V sulfuric acid. The polysaccharide solution thus obtained is adjusted to a definite volume, filtered, and the mg of polysaccharides per ml of solution is determined colorimetrically.

Apparatus and Materials

Apparatus

Millipore filter - 300 ml millipore filter holder with fritted glass bottom. Millipore filter - Type LS Teflon filter paper, 47 mm in diameter and pore size of 5 microns. Filter aid - Celite analytical filter aid. Volumetric flask - several volumetric flasks of 200 ml and 250 ml. Flasks - Several 250 ml Erlenmeyer flasks. Funnels - Several short stem funnels 70 mm in diameter. Filter paper - Whatman No. 42 ashless filter paper 12,5 cm diameter, and Whatman No. 1 or No. 2 coarse filter paper. Solutions 1% V/V sulfuric acid - dissolve 5 ml of concentrated sulfuric in 495 ml of deionized water. 5% W/V phenol solution - place 5 g of phenol in a 100 ml flask and add deionized water to the mark, and shake until dissolved. 80% V/V alcohol - measure 400 ml of absolute ethanol in a 500 ml flask, add 100 ml of water and stir. Alcohol - absolute ethanol. Concentrated sulfuric acid.

Preparation of sample solution

Dissolve 100 g of the sugar to be analyzed in 150 ml of deionized water and adjust the volume to 250 ml in a volumetric flask and let stand undisturbed for 30 minutes. Filter about 40 ml of solution through coarse filter paper in a 70 mm funnel. For cane juice: filter 40 ml through coarse filter paper in a 70 mm funnel.

Precipitation of polysaccharides

Withdraw 10 ml of the solution with a pipet and place in a 100 ml beaker, add 0,5 g of celite analytical filter aid, stir, and add 40 ml of absolute ethanol. Filter the solution with suction on a Millipore filter using a type LS Teflon filter paper, 47 mm in diameter, and pore size of 5 microns. When the liquid has disappeared from the surface of the filter aid, wash the filter with 150 ml of 80% V/V alcohol to remove sugars. This is conveniently done with the 80% alcohol contained in a plastic wash bottle. The 80% alcohol should be carefully applied down the inside walls of the funnel in 20-25 ml portions, allowing each portion to disappear from the surface of the filter aid before adding the next.

Separation of polysaccharides

Quantitatively transfer the filter aid and filter paper to a 400 ml beaker and add 150 ml of 1% V/V sulfuric acid solution. Boil the mixture for 5 minutes. Remove the filter paper and rinse with water, allowing the rinse water to go into the beaker. Quantitatively transfer the contents of the beaker to a 200 ml flask, cool to room temperature and dilute to the mark with deionized water. The volume of the filter aid is insignificant. Filter the solution through a Whatman No. 42 filter paper by gravity, discarding the first 10-15 ml of filtrate. The next 10-15 ml may be used for the determination. The soluble polysaccharides are in the filtrate. It is not necessary to filter the entire solution.

Development of color (phenol-sulfuric acid test)

Pipet a 2 ml aliquot of the polysaccharide solution into a 20 mm × 150 mm test tube and add 1 ml of a 5% aqueous solution of phenol. Ten (10) ml of concentrated sulfuric acid is then added at one time from a pipet with a large opening, preferably an automatic pipet. After the solution has cooled to room temperature (about 30 min.) the color is read on a spectrophotometer at 485 nm against a blank prepared in the same way as the sample except that 2 ml of water is used instead of the polysaccharide solution. The color determinations and blanks should be done in duplicate. If the percent transmission in the duplicates varies more than 2%, both should be repeated. The mg of glucose per ml of solution corresponding to the color reading is then determined from the standard curve. Take 90% of the glucose value to convert it to polysaccharide value, as polyglucoside.

Preparation of standard curve

Place 100 mg of pure glucose in a 1 000 ml volumetric flask and make up to the mark with deionized water. For each point on the curve dilute this stock solution as follows:

ml stock solution	dilute to	mg glucose/ml
10	100 ml	0,01
20	100 ml	0,02
30	100 ml	0,03
40	100 ml	0,04
50	100 ml	0,05
60	100 ml	0,06
70	100 ml	0,07
80	100 ml	0,08
90	100 ml	0,09
100	—	0,10

Place 2 ml of each solution in a 20 mm × 150 mm test tube, and add 1 ml of a 5% aqueous phenol solution to each tube. Then 10 ml of concentrated sulfuric acid is added all at once from a pipet with a large opening, preferably an automatic pipet. When the solutions have cooled to room temperature the color is read on a spectrophotometer at 485 nm against a blank prepared in the same manner except that 2 ml of water is used instead of the glucose solution. The color readings are then plotted on graph paper. If the color is read as percent transmission it must be plotted on semi-log paper of one cycle. If the color is read as optical density the values are plotted on a square paper. The curve is used to determine the polysaccharides corresponding to the color measurement in an unknown solution.

Interferences and Sources of Error

The phenol-sulfuric acid reaction is extremely sensitive to all carbohydrate material including cellulose and starch. Every precaution must be taken to make sure that all glass apparatus is free from dust particles, pieces of tissue, etc. which may render the results erroneous. All glassware should be washed with deionized water immediately before use. The 5% phenol solution should be prepared fresh about every ten days. It is important that every detail of the procedure be followed, including use of the specified filter paper.

The teflon Millipore filters may be reused a number of times, until they become plugged or develop a hole.

In this method the polysaccharides are precipitated by making the solution 80% V/V with alcohol. This precipitates almost all of the polysaccharides which are filtered off, and washed free of sugar; precipitated polysaccharides are then partially hydrolyzed before the color forming reagents are added. In this way the high molecular weight polysaccharides are included. The phenol-sulfuric acid color reaction has been well established as a reliable method for carbohydrates.

The method is simple to perform and is rapid. It should be well suited for factory control work.

Possible problems lie in non-specific order of precipitation of the various polysaccharides, and the necessity for precise and reproducible performance of the procedure.

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