Some Substances Adsorbed on Granular Carbon From Beet Thick Juice

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During a study of nonsucrose carbohydrates of beet molasses, it occurred to us that these materials might accumulate selectively on the activated carbons used by beet processors. Several sugar companies kindly supplied samples of their granular carbon (Pittsburgh CAL type) spent in continuous adsorption purification of beet thick juice. Analysis of the materials eluted from the carbon samples showed no unusual accumulation of rare carbohydrate materials, but did reveal that a variety of sugars and nonsugars are adsorbed by the carbon. We believe that it would be worthwhile to report some qualitative and quantitative estimates of the adsorbed materials.

Materials and Methods

One sample, #1, was received from a Colorado source in 1964. Two more, #2, #3, were obtained from two California factories operating during the spring of 1965. All samples were sweetened off at the factory and air dried under conditions wherein no bacterial action was probable. All three had about the same moisture content ($\sim 10\%$) and apparent bulk density (.606); however, sample #2 had more visible gray particles of magnesite.

No method seems to be available for complete and nondestructive removal of materials adsorbed on granular carbons (2)[2]. Combustion regenerates the carbons very nicely but does not yield individual components for analysis. Our analytical results are based on elution, and describe only what actually came off the carbon samples. An air-dry batch of each of the three carbons (200 g) was poured into a glass column ($\sim$ 6.5-cm I.D.) and eluted at room temperature with 10 volumes (about 3 liters) of 50% ethanol-water mixture over several hours. The eluates were evaporated to 50-75 ml, filtered through medium-porosity sintered glass, and made up to 250 ml with water. A trace of phenyl-mercuric nitrate was added as preservative. In other experiments, small samples of each carbon were extracted repeatedly with boiling 80% ethanol; the analytical results for

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[2] Numbers in parentheses refer to literature cited.

sucrose and reducing sugar were essentially the same as for the cold 50%-ethanol treatment.

In general, analyses were performed by standard methods: sucrose, invert sugar, total solids, moisture, total nitrogen (1); saponin (8); total anions (5); raffinose and kestose by a paper chromatographic method (6); and chloride by automatic titration. Alcohol insolubles were defined as material that precipitated when 3 volumes of absolute ethanol were added to 1 volume of aqueous solution; color was calculated from absorbance at 420 mμ minus absorbance at 720 mμ, 1-cm path length, 10% solids, at pH 7.

Results and Discussion

Tables 1 and 2 give the results of the analyses of the eluates from the three granular carbons. Evidently real quantitative differences exist in the composition of adsorbed impurities in the samples. Whether these differences depend solely on the composition of the thick juice being worked or also on the column operating conditions we could not determine because suitable factory information was not available.

Table 1.—Physical properties of the granular carbon eluates.

	Carbon sample		
	1	2	3
pH	6.4	7.8	7.6
Color[1]	0.45	4.85	5.13
$[\alpha]_d^{25}$	+2.156	—0.204	—0.285

[1] Absorbance at 420 mμ - absorbance at 720mμ.

Table 2.—Constituents eluted from granular carbon samples.

Constituent[1]	Carbon sample		
	1	2	3
Total solids	21.8	13.6	14.0
Sucrose	10.07 (46.2)	3.68 (27.1)	2.99 (21-4)
Invert sugar	0.39 (1.8)	0.22 (1.6)	0.25 (1.8)
Raffinose	2.51 (11.5)	0.18 (1.3)	0.24 (1.7)
Kestose	0.93 (4.2)	0.09 (0.7)	0.07 (0.5)
Alcohol insoluble	0.63 (2.9)	1.05 (7.7)	2.53 (18.1)
Saponin	0.003	0.305 (2.2)	0.011
Acid (meq)[2]	0.0229	0.0190	0.0198
Acid (mg)[3]	2.06 (9.4)	1.71 (12.6)	1.79 (12.8)
Total N	0.44	0.38	0.78
Protein[4]	2.74 (12.6)	2.35 (17.3)	4.85 (34.6)
Chloride	0.01	0.02	0.01

[1] Numerical value shows mg constituent per gram of dry carbon. Parenthetic values are weight percent of total solids for individual constituents.

[2] Milliequivalents per gram dry carbon.

[3] Equivalent weight of 90 assumed.

[4] Total N × 6.25.

The sample eluates, as made up for analysis, were clear, yellowish, approximately neutral solutions. Samples 2 and 3 had about 10 times as much color as sample 1--the continuous absorption curves for all from 720 to 420 mμ were similar but not completely superimposable. Only sample 1 (containing the most sucrose) had a small positive rotation; samples 2 and 3 had a slight negative rotation. We conclude that any sucrose analysis of carbon eluates by pol alone is not reliable.

Of the simple sugars adsorbed, sucrose is the major component, but in samples 2 and 3 is only about 25% of the total weight. The relative amounts of kestose (2.5%) and raffinose (5%) are higher than would be expected in normal thick juices from the geographical areas supplying samples 2 and 3. Evidently in factory operation the trisaccharides behave as they do in the laboratory--that is, they adsorb onto granular carbon more readily than does sucrose (9). Paper chromatograms of all samples show that glucose is the predominent reducing sugar, and almost no fructose is present.

In each sample acidic material accounts for around 10% of the total weight of eluted product if we assume an average equivalent weight of 90 for the thick juice acids, a figure which appears reasonable judging from work of Stark of this laboratory (7). From the low chloride figure and the known inability of granular carbon to remove ash (4), we presume that most of the acidic fraction is organic rather than inorganic acids.

Each sample contained appreciable nitrogen, as determined by the Kjeldahl method. Only sample 3 gave a positive qualitative ninhydrin test before hydrolysis. When sample 2 was hydrolyzed overnight in 6 *N* hydrochloric acid at 110° C, amino acids could then be detected in the hydrolysate by thin layer chromatography. Further examination of the hydrolyzed material on an amino acid analyzer showed that the amino acids came from natural proteinaceous material. It would be erroneous to conclude, however, that all the nitrogen was associated with protein in the sample, because some is undoubtedly tied up in the color bodies adsorbed from thick juice. Evidence for this view was found in the hydrolyzed sample where the free ammonia content was higher than normal for a pure protein. The nitrogen present as cationic ammonium ion must have been insignificant because no ammonia could be detected when the solutions were made strongly alkaline at room temperature with potassium hydroxide.

Contrary to our expectations, only sample 2 retained a large amount of saponin from thick juice. The saponin analysis results agreed qualitatively with a visual floc test carried out on the eluates by acidifying them—sample 2 produced an immediate precipitate, sample 3 gave a small precipitate after heating and long standing, and sample 1 failed to produce visible floc under any circumstances.

Granular carbon treatment of thick juice also removed an "alcohol insoluble" fraction. That portion of the aqueous eluate precipitating when 3 volumes of absolute ethanol were added, was examined carefully in the case of sample 3. The low-ash product so obtained, soluble in water but insoluble in 75% ethanol, made us suspect the presence of a carbohydrate polymer larger than a two- or three-unit oligosaccharide. Its specific rotation, −35.5°, changed to +23.8, after one hour's hydrolysis in 1 *N* sulfuric acid so that the carbohydrate could not have been a levan which would have had a final rotation of about −90° (3), or a dextran which would have had an initial rotation of about +200° (10). The rotations closely resemble those of a mixed arbo-, galacto-, gluco-, galacturono-polymer isolated from certain molasses samples in this laboratory. Paper chromatograms of hydrolyzed polymeric product from the carbon sample show the presence of a number of simple sugars, and are strikingly similar to chromatograms of molasses polymer hydrolysate. We conclude that at least part of the "alcohol insolubles" removed by granular carbon is material derived from arabans and/or pectins in the thick juice, and that removal of nonsucrose carbohydrate polymer may be an effective part of the carbon treatment.

Summary

Analysis of material eluted from granular carbons used to treat beet thick juice shows that a variety of sugars and nonsugars are removed by the treatment. Considering the relative amounts present, nonsucrose oligo- and polysaccharides appear to be adsorbed more strongly than sucrose. The carbon also retains nitrogenous compounds. both colored and uncolored, and organic acids.

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