Non-Sugars in Beet Juices Removable by Ion Exchange

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THE ACTUAL introducing of ion exchange into sugar beet processing brings compelling reasons for further studying and indentifying the non-sugars present in the juices. The potency of ion exchange for purifying sugar beet juice has been appreciated for several years. However, far less is known concerning what compounds constitute the non-sugars and how these behave individually in relation to ion-exchange columns.

More knowledge concerning the nature and ion-exchange behavior of non-sugars would be helpful in integrating the process of an ion-exchange unit to the sugar factory as a whole. That is, while an ion-exchange station itself can now be engineered with confidence, just how a factory should be operated to make the *most* of this revolutionary process is something which remains to be settled.

There is also another, and perhaps even more attractive reason for identifying and studying the non-sugars removable by ion-exchange. Several of these impurities, although most noxious if left in the juice, are capable of becoming valuable and profit-increasing by-products when isolated. Ion-exchange can offer some of them in a highly segregated form, separated cleanly from the sugars which constitute the bulk of the solids in the juice, and in such condition that further by-product purification is relatively simple.

Information given by ordinary sugar juice analyses is not suitable for predicting de-ionization behavior, and is not detailed enough to be of much value in surveying the by-product possibilities. Also, since juices vary from locality to locality, from season to season, and from day to day, a great number of juices must be analyzed before any generalizations can be made. In our early investigations of by-product possibilities, samples from but a few sources were used. In order to broaden the information on the composition of non-sugars, thick juice samples were requested this season from factories in various beet sugar districts of the country, thick juice samples being chosen because of their high concentration and keeping qualities. Sufficient data were also requested to permit working the thick juice analyses back to beets. The samples kindly supplied from the various factories are now being analyzed in The Dorr Company's Westport Laboratories. The analyses are far from complete, but the data collected thus far are here presented, in the nature of a progress report.

Previous work in our laboratory has shown that with a thoroughly regenerated cation bed (upflow, 900 percent HCL) ionized ash is 99.9 percent removed up to a certain percentage of the total capacity of the bed.

Simple amino acids, including betaine, are just as completely removed, however, to a somewhat lower percentage of the total capacity of the bed.

Therefore, if a volume of feed is chosen which is insufficient to give an amino acid breakthrough, any nitrogen leakage from the cation bed is almost assuredly other than simple amino acids. Thus both ash cations and simple amino acids are collected by the cation cell, and may be recovered from it for analysis.

The cation bed can also remove some peptides, but capacity for these decreases rapidly as they become more complex, and in tests on soluble peptide mixtures, 100 percent removal was not obtained even at the very start of a cycle.

A fully regenerated anion exchange resin can now remove from the decationized sample essentially all anions whose acids have an ionization constant greater than about 10^{-7}, the capacity of the resin being the greater for anions of the stronger acids. This category includes the mineral acid anions such as $SO_4^{=}$, Cl^-, NO_3^- and NO_2^-, and also apparently most of the organic non-sugars which had not been taken out in the cation cell. In fact more than half of the anions present in the beet sugar juices we have tested seem to correspond to organic acids with an average pKa in the range of 5 to 3.

Nitrogen compounds which are not removed from solution upon passing through the cation cell either must have no appreciable basic properties at all, or else like peptides must be tied up in molecules too large to diffuse into the cation resin structure with appreciable rapidity. Examples of such compounds are pyrrolidone carboxylic acid, browning reaction products, nitrates or nitrites, polypeptides, and perhaps also some nitrogenous organic compounds with neither acid nor basic properties.

Of these, pyrrolidone carboxylic acid has appeared to be the principle constituent, and tests have shown it to be removed quantitatively by the anion cell. Browning reaction products also must be completely absorbed, because the final effluent juice has been absolutely colorless. Inorganic nitrate and nitrite would be picked up, if present, but would not show up in our work because they are excluded by the Kjeldahl method used for nitrogen analysis. On the other hand, part of the peptides passing the cation cell would most probably also pass unabsorbed through the anion cell, and indeed, it would seem logical to guess that most of the nitrogen passing into the anion effluent will be in this form.

After passing the juice sample through a pair of exchanger columns, then, the ash cations, simple amino acids, some peptides and nitrogenous organic bases such as betaine remain adsorbed on the cation exchanger. The anions of the juice, including certain nitrogenous compounds, remain adsorbed on the anion exchanger. The sugars themselves, together with other essentially non-ionizable compounds and extremely weak acids, escape into the effluent.

Further fractionations of the adsorbed impurities are now possible, most important being separate elution of the nitrogen compounds from the cation exchanger. As published by Freudenburg (1)[2], the nitrogen compounds may be eluted by means of a weak base, such as pyridine or ammonium hydroxide. Weak alkali unloading of a cation exchange column also causes some downward displacement of the adsorbed ash in the column, but if part of the column at the downstream end has remained free of ash cations up to the start of unloading operations, then ash is displaced into this portion of the column, and does not escape into the effluent with the nitrogen.

In brief outline then, the procedures used for preliminary separation of the constituents of juice comprise the following steps: First the capacity of the cation resin with respect to the juice in question is determined in a 100 cc. column. Then a quantity of diluted juice calculated as insufficient to exhaust the exchangers is passed in series through one-liter beds of thoroughly regenerated cation and anion resins. With the effluent is collected the water from both sweetening-on and a substantial sweetening-off rinse. Analyses are made on feed and effluent juice samples without delay, in order to avoid spoilage.

Next, amino acids and related nitrogenous materials are eluted or "unloaded" from the cation bed by passing through it 9 liters of one normal ammonia solution followed by 1 liter of de-ionized rinse water, all at fixed low feed rate. The unloading effluent is boiled down to a small volume, made strongly ammoniacal to prevent spoiling, and is stored for subsequent analyses.

The cation cell after unloading is regenerated upflow with 9 liters of one normal hydrochloric acid, followed by 1 liter of rinse water. The spent regenerant is concentrated and stored for analysis.

The anion cell is regenerated with 3 liters of one normal ammonia solution followed by 1 liter of rinse water. This spent regenerant also is concentrated, made strongly ammonical, and stored for analysis.

The affect of this procedure upon the juice samples is shown in part by table 1. As will be seen, the apparent purities are increased by de-ionization to above 99 percent, at which level the exact significance of apparent purity becomes questionable. Nitrogen removals of 94 percent and up were obtained. Ash was not determined on the treated juice, but from flame photometer analyses, the removal of sodium plus potassium was in the range of 99.9 percent. In addition, there was essentially 100 percent removal of color in all cases; the effluent juices being water white.

It may be asked why the purification obtained in treating these samples is greater than that realized in existing commercial operations. The answer lies in the thoroughness with which the beds are regenerated. The relationship between the removal of impurities and regeneration has been previously reported by Thompson and Roberts (3). It will be sufficient

[2]The numbers in parentheses refer to literature cited.

here to recount that it is not considered economical to regenerate for such high removals in commercial operation, particularly so, when using conventional downflow regeneration.

In the course of the above described ion-exchange separations, certain resin capacity figures were collected. These include values for the total removable ash cations in the juices, the total anions to be removed, and an actual measurement of the amounts of juice which could be treated per cycle by a cation exchange column.

Table 1.—Ash and nitrogen removals from thick juice samples.

Factory location	Sample taken	Ash content % in D.S.	Apparent Purities Sample	DI Effluent	Nitrogen in sample % N_2 on D.S.	% Removal of nitrogen
A California	Early	3.4	88.0	99.7	0.473	96.5
	Mid-campaign	4.2	90.0	99.1		
B Washington	Early	2.7	93.0	99.1	0.295	96.9
	Mid-campaign	1.94	95.8	99.6	0.405	95.8
C. Ohio	Early	3.58	91.4	99.4	0.348	94.6
D Montana	Early	2.85	92.5	99.5	0.490	98.0
E Colorado	Early	2.72	93.4	99.5	0.134	94.6
F Montana	Mid-campaign	3.4	94.0	99.8		

The total normality of the anions in a juice sample is obtained fairly directly by an ion-exchange technique. The juice, diluted to some suitable brix, if necessary, is passed through a thoroughly regenerated column of hydrogen exchange resin, whereby cations are replaced in the juice by hydrogen, and acids corresponding to the anions are created in the effluent. Successive samples of the effluent are titrated up to pH 7 until a constant titration for successive samples indicates that the column has been fully sweetened-on. Since some CO_2 may be lost from the acid effluent, if present, CO_2-free air should be blown through the effluent samples before titration to eliminate all of it. The value of the constant titration found shows the normality of the anions in the juice and is commonly termed cation acidity.

A second aliquot of the samples is titrated to pH 7, at which point most of the amino acids present will be in their iso-electric form. That is, they will be present in neither a cationic nor an anionic form. This gives the original alkalinity (or acidity) of the juice. The aliquot is then titrated on down to some low pH, the CO_2 blown off, and the sample titrated back to pH 7. The difference in titrations gives a value for the bicarbonate which was present in the juice at pH 7.

The normality of the total cations originally present in the sample is taken as the sum of the cation acidity, the original alkalinity, and the bicarbonate normality. The value will, of course, be in error if any amino acids were originally present which were not in their iso-electric form at pH 7, by whatever amount they affected the original alkalinity titration.

Table 2 shows the results of such determinations. In order to relate the original alkalinity, cation acidity, and bicarbonate normality to dry

solids, each was expressed in terms of equivalents per 1,000 grams of dry solids. This is a convenient form for the data, because when such figures are multiplied by the percentage solids in the juice (or brix) and the specific gravity of the juice, the result comes out directly in normality.

Table 2. Cation exchange data. (Equivalents per 1,000 grams dry substance.)

Factory	Sample taken	Apparent purity	Alkalinity	Cation acidity	CO_2	Removable cations	Cation cycle[1] Bed volumes 10 bx juice	Cation Cell Ash loading Meqs/cc Exchanger
A	Early	88.0	0.016	0.538		0.55	13[2]	0.71[2]
	Midseason	90.0	0.022	0.54	0.021	0.58	6	0.32
B	Early	93.0	0.015	0.261	----	0.28	18[2]	0.51[2]
	Midseason	95.8	0.009	0.313	Trace	0.32	7	0.22
C	Early	91.4	0.117	0.435	0.002	0.55	9	0.48
	Midseason		0.123	0.287	0.017	0.43	8	0.33
D	Early	92.5	0.01	0.423	0.01	0.42	9	0.36
E	Early	93.4	0.015	0.438	0.004	0.46	10	0.45
F	Early	94.0	0.031	0.397	0.003	--	--	---
	Midseason		0.028	0.414	0.002	0.44	10	0.43
G	Early		0.004	0.376	---	0.38	8	0.29
	Midseason		0.005	0.342	-	0.35	11	0.58

[1]Cation cycle represents the volume of sample diluted to 10 brix which can be fed to a cation exchange column before there is a pH breakdown in the effluent. Samples were fed through a 100 cc. column at 20 cc. per minute with the exception of those marked [2], which were fed at very low rates.

The cation cycle data shown in table 2 were obtained by feeding samples, diluted to about 10 brix, through a 100 cc. column of C-3 exchanger. In all cases but two, the rate of feed was 20 cc. per minute. The effluent from the column was collected in 100 cc. portions, pH and acidity titration determined on each sample, until an upward break in the pH indicated that buffering bases were starting to leak through the column. In table 2 is recorded the nearest whole number of samples of juice which could be collected before pH break-through after correcting the data to the basis of a 10 brix feed.

The ash cation content of columns at the end of each test cycle was determined from the quantity of juice samples which could be handled per cycle, corrected for the water originally present in the voids of the exchanger columns, and from the value determined for removable cations. As seen in the final column of table 2, the value varies widely even if the two slow run values are disregarded. While the capacity data was not taken with great precision, still the wide variation in net ash capacity of the bed to pH breakthrough indicates that the value for removable ash cations in a juice does not constitute in itself an accurate basis for calculating the amount of juice which can be treated per cycle. Also, since most of the values obtained are much lower than analogous values obtained in the de-ionization of water, it appears that a substantial portion of the cation cell capacity is being here employed to remove nitrogenous materials.

When nitrogen has been determined on more of the cation unloading samples, it will be interesting to see what sort of correlation can be worked

out between actual measured cycle length on one hand, and some combination of removable ash cation and nitrogen on the other hand.

Thus far, we have dealt with the purification of the juice effected by the de-ionization treatment, and the relationship, or lack of it, between certain juice titrations and the cation cycle length. Now we will return to the impurities which we left adsorbed on the cation cell.

As has been stated, the nitrogenous compounds are selectively eluted from the cation exchanger by means of an ammonia solution. This produces an effluent which we call amino liquor.

The unloading procedure used makes a fairly sharp separation between the ash and the nitrogen. For example, the total of sodium plus potassium contained in the amino liquor amounts to less than ten parts per million, on an original thick juice basis, while the corresponding concentration in the spent acid regenerant is of the order of magnitude of ten thousand ppm. The discrimination in the case of nitrogen compounds is not so sharp, but is still quite satisfactory, as shown in table 3. This gives the overall organic nitrogen balance obtained from two juices for which the necessary analyses have been completed. All values are expressed in terms of normality in the original thick juice samples. Ammoniacal nitrogen was expelled from all samples before analysis.

Table 3. Nitrogen balance.

	Factory A	Percent	Factory B	Percent
Nitrogen in amino liquor	.170 equivs/liter	61.1	.104 equivs/liter	58.4
Nitrogen in spent cation regenerant	.004 equivs/liter	1.4	.004 equivs/liter	2.2
Nitrogen in spent anion regenerant	.089 equivs/liter	32.0	.062 equivs/liter	34.8
	.273	98.1	.176	98.5
Nitrogen in original juice	.278		.178	

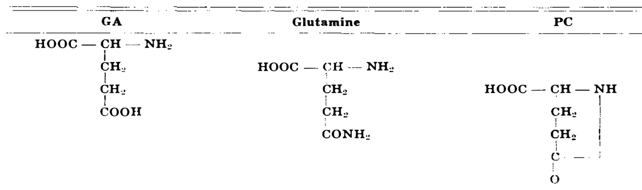
It can be seen that in the case of Factory B only about 4 percent of the nitrogen removed from the cation cell went with the acid regenerant, and in the case of Factory A, the fraction was substantially less. It is also apparent that a large fraction of the nitrogen is in anionic form.

The nitrogen compounds are prime candidates for by-product recovery. One of them, namely glutamic acid, is already being recovered from Steffens filtrate, and a large market has been developed for it. Thus, at this time, we are most interested in the content and distribution of glutamic materials in the juices.

The glutamic acid picture is complicated by the fact that potential glutamic acid may exist in three different forms in the juice, namely, as glutamic acid, glutamine and pyrrolidone carboxylic acid. In order to spare our tongues and save time, we use the abbreviations GA and PC for glutamic and pyrrolidone carboxylic acid, respectively.

Figure 1 shows the structure of these compounds.

Figure 1.—Potential glutamic compounds.



As will be seen, glutamic acid has both an acid carboxyl group and a basic amino group attached to the top carbon atom. Through zwitterion formation, the acid and basic strengths of these two groups tend more or less to cancel or mask each other.

As a result, the top group does not have sufficient residual acid strength to permit adsorption on an anion exchange resin through this group. The residual basic strength is, however, still sufficient to permit basic ionization and adsorption in a cation exchanger. Also, there is another carboxyl group in the bottom, or distal position, which is not appreciably weakened by the alpha amino group, and through which the molecule may be held on an anion exchanger. Thus, GA itself can be held on either a cation exchanger or on an anion exchanger.

In glutamine, on the other hand, the distal group is an acid amide, with insufficient acid strength to permit adsorption in an anion exchanger. Therefore, glutamine is held by a cation exchanger only.

In PC the distal group has swung around and reacted with the amino group. The basic properties exhibited by the amino group are neutralized, and PC is not adsorbed in a cation exchanger. At the same time, the latent acidity of the alpha carboxyl group is unmasked, and the molecule can be adsorbed in an anion exchanger through this group.

Thus, if we pass a mixture containing all three compounds through a pair of exchange cells, the GA and glutamine will stop in the cation exchanger, while PC will pass through the cation cell to be caught in the anion exchanger.

Glutamine is very unstable, and converts easily to PC upon heating to boiling. There is an equilibrium between PC and glutamic acid, which has been reported in considerable detail by Wilson and Cannon (2). At very high or very low pH's, the equilibrium is shifted far in favor of GA. In the intermediate range the equilibrium favors PC, but between about pH 4 and pH 10, the conversion reaction velocities are too slow to be of much help in either analytical or process work. Use is made of both conversion and ion-exchanger behavior in the analytical methods used.

Analysis for glutamic compounds has been carried through on only two of the amino liquors to date. This is done by autoclaving samples which have been adjusted to an initial pH of 3.2 for 6 hours at 20 pounds

steam pressure, which breaks down all the glutamine present and would give at equilibrium something like 94 percent PC and 6 percent GA. The autoclaved sample is passed through a cation exchange column to remove all nitrogen compounds but the PC. The effluent is then analyzed for nitrogen. As a check, a portion of the effluent is hydrolyzed with HCl to convert the PC back to GA, and a formol titration made on the hydrolysate. The results so far obtained are as follows: The glutamine plus GA in

Factory A=.023 normal in thick juice
Factory F=.009 normal in thick juice.

Actually, all these two figures amount to is sort of a teaser. No conclusions can be drawn until many of the stored amino liquors are analyzed in much greater detail. This will be done during the coming months.

With respect to the potential glutamic acid in the juice, however, it may be said that whatever portion of it is collected on the cation exchanger can be isolated and recovered fairly simply. Recovery of glutamic acid from the PC picked up on the anion exchanger has not been found so expedient.

Another consideration concerning nitrogen compounds is as follows: If the ammoniacal spent regenerants from a D-1 house are used as fertilizer, which seems a promising prospect, then it is probable that the relative amino acid content of the beets so fertilized will increase. This would be disadvantageous if nitrogen compounds were not being recovered, but might be very attractive if they constituted a valuable by-product.

Returning now to the original analytical separations, it will be remembered that after the nitrogen compounds are unloaded from the cation cell, it is regenerated. The spent regenerants are being analyzed for the most important cationic ash constituents, and the available results are shown in table 4. Here sodium and potassium are analyzed in a flame photometer, while Ca and Mg are analyzed by standard methods.

Table 4.—Ash constituents.

Factory	Sample taken	Equivalents per 1,000 grams dry solids			
		Na	K	Ca	Mg
A	Early	0.15	0.26	0.02	0.002
	Mid-campaign	0.23	0.26	----	----
B	Early	0.045	0.24	0.029	0.0016
	Mid-campaign	0.058	0.24	0.029	0.0025
C	Early	0.25	0.34	0.005	0.0007
D	Early	0.094	0.37	0.041	----
E	Early	0.140	0.370	0.062	----
F	Mid-campaign	0.183	0.388	0.097	----
G	Early	0.083	0.230	0.017	0.0001

We have done very little work as yet with the spent anion regenerant from the series of thick juice samples. However, using canned first fillmass as a working material, we found in the past that over 90 percent of the anionic nitrogen adsorbed in that case would be recovered as alpha amino nitrogen by acid hydrolysis, and presumably was PC although

that will require further checking. Anions adsorbed from this material have been fractioned chromatographically in ion-exchange resins, to yield weak acid fractions containing substantially more than half the total anions originally present in the juice. It is hoped that among these weak acids can be identified further compounds with by-product possibilities.

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