

Nonsucrose Constituents of Beet Sugar Processing Liquors of the Rocky Mountain Area

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The ratios of types of nonsucrose constituents in the beet sugar processing liquors vary widely from season to season, but the compositions of the individual groups tend to remain fairly constant. The ratio of the quantity of mineral group to nitrogenous group has tended to decrease with the increasing use of nitrogenous fertilizers. While different straight beet house liquors exhibit widely varying rates of crystallization of sugar, analyses of nonsucrose components do not reveal the causes of the variations, though some indirect effects have been observed. Steffen factory operations are hindered by the presence of large quantities of carbohydrate nonsucrose compounds and aided by the same compounds in producing final molasses of lower purity. Steffen and barium saccharate "waste waters" are valuable sources of nitrogenous concentrates, in the preparation of stock feeds.

BEET SUGAR CHEMISTS for many years have been investigating the nonsucrose constituents of the processing liquors and, with varying degrees of success, attempting to correlate the nonsucrose constituents with processing problems. Much of the work has been done on liquors from beets grown in humid areas. This discussion presents data on liquors from beets grown on the irrigated mineral soils of the Rocky Mountain area.

It treats only of those nonsucrose components which pass through first carbonation. As they undergo relatively minor changes during crystallization, it is assumed that the nonsucrose components of molasses satisfactorily represent those in earlier stages of the process.

The nonsucrose constituents may conveniently be classed into three groups: (1) mineral, (2) nitrogenous, and (3) carbohydrate. The mineral group is arbitrarily defined to include the inorganic bases and the associated inorganic and nonnitrogenous organic acids. The nitrogenous group includes all nitrogenous compounds other than nitrates. Processes for desugarizing molasses separate the mineral and nitrogenous constituents fairly completely, but carry varying quantities of the carbohydrate nonsucrose compounds along with the sucrose. The waste waters from the molasses desugarizing processes carry the mineral and nitrogenous nonsugars in essentially the ratios they originally bore in the straight beet house liquors, but the amount and composition of the carbohydrate constituents show marked variations. The calcium saccharate process normally produces a liquor which carries essen-

tially all of the raffinose and much of the other carbohydrate nonsucrose compounds of the molasses worked. Therefore, the nonsucrose components of Steffen factory liquors show a high and variable percentage of carbohydrates, but the ratio of mineral to nitrogenous components remains essentially the same as in the original straight beet house liquor.

Processing Liquors

In 1933 this laboratory published analyses of nonsucrose compounds in a variety of non-Steffen and Steffen molasses (3). Table I presents the average of the 1933 analyses of non-Steffen molasses, also analyses of products of fairly recent date.

Straight Beet House Liquors

Analyses of molasses since 1933 have shown a trend toward an increase in the nitrogenous group and a decrease in the carbohydrate group. The normal compositions of the three groups of components in straight beet house liquors of today's production are given in Table II.

The quantity of organic acids has been estimated from the analyses, by Stark, Goodban, and Owens (10), of Idaho and Montana beet molasses, which, because of its similarity to the molasses of the eastern slope of the Rockies in other respects, is assumed to be similar in organic acid content. Deducting from their values for total acidity that due to pyrrolidone carboxylic acid and to mineral acids (here it was necessary to estimate the nitric acid content), the residual acidity is that of

the nonnitrogenous organic acids. Assuming a combining weight of 75 for these acids, they amount to about 15% on nonsucrose compounds.

The quantities of the three groups of nonsucrose constituents are estimated by use of factors developed from analyses of many samples. The mineral group is found by multiplying ash by the factor 1.07. If sulfated ash is used, the factor becomes 0.89, as analyses of a great many samples from the Rocky Mountain area has shown that sulfated ash is invariably very near 1.2 times carbonated ash.

The nitrogenous group is estimated at $9.09 \times$ (total nitrogen minus nitric nitrogen). Various analyses of the nitrogenous fraction, recovered from Steffen waste water by ion exchanger techniques, have shown that this group commonly contains 11% total nitrogen on solids. The sum of the mineral and nitrogenous groups deducted from the total yields the carbohydrate group, by difference.

The applicability of the factors for conversion of ash and nitrogen to complete groups is demonstrated by the observation that when these factors are applied to ideally prepared Steffen waste water, which contains very little carbohydrate matter, the sum of the estimates of the two groups is very close to 100, regardless of the ratio of the two groups present.

The analyses given in Table II are normal for today's liquors. However, the ratios of the three groups of components vary from year to year, and except in a very abnormal season, the ranges of the three groups are expected to be: mineral 45 to 50%; nitrogenous 40 to 45%; and carbohydrate 8 to 12%.

Table I. Composition of Nonsucrose Components of Beet Sugar Factory Processing Liquors

	Individual Components, % on Total Nonsucrose					
	Beet House Liquors			Steffen House		Barium saccharate waste water
	Straight		Steffen	Sirup	Waste water	
	1	2	3	4	5	6
Ash (carbonated)	44.6	42.6	39.2	10.0	45.4	42.5
K ₂ O	19.6	18.6	17.5	2.7
Na ₂ O	6.2	3.5	5.2	0.5
CaO	...	0.3	0.5	4.5
Cl	5.0	4.2	7.6	0.6
SO ₃	5.7	2.5	2.9	0.0
N ₂ O ₅	2.7	1.8	0.7
P ₂ O ₅	...	Trace	0.12	0.0	...	0.0
Total organic acids (see text)	15.0	14.5	8.0
Total N	4.75	5.25	4.03	0.6	6.05	4.52
Organic N (total N-N ₂ O ₅ N)	4.05	4.80	3.86	...	5.46	4.13
N ₂ O ₅ N	0.70	0.45	0.17	...	0.59	0.39
NH ₃ N	0.02	0.05
Amide N	0.08	0.09
Amino N (Van Slyke)	...	1.84	1.24	...	2.43	1.58
Betaine N	...	2.16	1.37	...	2.27	1.63
Other N, pptd. by iodine	...	0.11	0.13	...	0.07	0.08
Undetermined N	...	0.69	1.02	...	0.59	0.70
Carbohydrates (calcd. by formula)	15.7	10.9	22.8	77.0	7.0 ^a	17.8 ^a
Raffinose (by paper chromatography)	4.6 ^b	2.9	8.0	Ca. 30
Galactinol ^c (by paper chromatography)	...	1.1	2.0	Present
Inositol (by paper chromatography)	...	1.9	5.0	Present
Undetermined	...	5.0	7.8	Present

^a Determined, not calculated.

^b Analysis by double enzyme hydrolysis.

^c An α-galactoside of inositol.

Column

1. Average of 1933 non-Steffen data.
2. Composite of non-Steffen molasses which represented full 1949-50 campaign.
3. Single sample of Steffen molasses produced during 1950-51 campaign.
4. Single sample of Steffen saccharate cake.
5. Factory production of concentrated Steffen filtrate (C.S.F.) representing a few days during 1953-54 campaign.
6. Barium saccharate filtrate (C.J.F.) representing one day's production during 1953-54 campaign.

This laboratory published data on the composition of laboratory preparations of Steffen waste waters in 1951 (2). A sample of molasses representing one week's operation of one factory during 1948 was found in which the mineral components were 178% on nitrogenous nonsucrose constituents. The average of the analyses reported in 1933 showed 130%. During the past few years the average has been 110 to 115%. The constantly increasing use of nitrogen in the production of the beet crop is being reflected in a higher ratio of nitrogenous components. The high mineral content of the 1948 nonsucrose constituents was the result of an obscure climatic condition which disturbed development of various crops west of the Mississippi River.

The carbohydrates appear to be lower today than they were 20 years ago. In 1933 (3) it is estimated that carbohydrate nonsucrose compounds constituted 19% of the total nonsucrose constituents. Today, they amount to about 10%. At least part of this decrease results from elimination of inversion of sucrose during processing, by proper pH control.

Mineral Constituents

Within a single group, the components vary widely. The sodium, the chloride, and the nitrate contents of the mineral group decrease as the beet approaches maturity at harvest time. The sodium contents of today's liquors are generally lower than those of earlier years because of hereditary characteristics of the domestic beet seed used today. Potassium normally holds fairly constant. The lime content of the nonsucrose compounds in ideally carbonated liquors from undeteriorated beets is less than 0.1%, but ideal conditions are rarely met in the factory.

The sulfate ion does not remain constant throughout processing. Beet molasses is commonly a supersaturated solution of potassium sulfate, the solubility of potassium sulfate being slightly less than the equivalent of 2.5% on nonsucrose constituents; 6% or more sulfate on nonsucrose constituents was commonly observed in earlier years. Molasses of this type quickly precipitates copious quantities of potassium sulfate on dilution of 60 to 65% total solids. The lower values for sulfate in today's

liquors result from changes in processing methods, the introduction of the three-boiling practice being an important factor. In addition to sulfates, a very small amount of sulfite is present. Sulfate and sulfite account for from 35% to 45% of the total sulfur. The form of the residual sulfur has not been determined.

Nitrogenous Constituents

The composition of the nitrogenous fraction varies greatly. Table III reproduces some results from the 1951 publication (2), and also includes more recent data.

No attempts have been made in the writer's laboratory to break down the nitrogenous fraction further than was presented in the first publication (2). However, the content of various amino acids has been reported by the Western Regional Research Laboratory (7, 10, 11). The major amino acid is shown to be glutamic. In this discussion glutamic acid and its anhydride (pyrrolidone carboxylic acid) are considered as one. Some of the amino acids (phenylalanine and threonine), which

are present in the raw juice, seem to disappear during processing, while others decrease in quantity.

The amino acid nitrogen (Van Slyke nitrogen) constitutes almost 40% of the total organic nitrogen in the liquors from the Rocky Mountain area, equivalent to about 18% amino acids on nonsucrose matter. From the data of Stark, Goodban, and Owens (7, 10), it is estimated that amino acid content is as given in Table IV.

The figure of 7.7% glutamic acid on 18.0% total amino acids, or 43%, agrees well with the value of 45% previously reported (2).

Except for relatively minor changes, as shown by Goodban, Stark, and Owens (7), the amino acids pass through the processing operations unchanged until the barium saccharate process is reached, where some amino acid nitrogen appears to be lost.

Carbohydrate Constituents

Only recently has much progress been made in analysis of the carbohydrate group. Table II presents the data available to date concerning the composition of this group. By means of paper chromatography the presence of significant quantities of three carbohydrates has been demonstrated: raffinose, galactinol (an α -galactoside of inositol), and inositol. As only relatively recently have techniques been developed which provide a satisfactory degree of accuracy for the determination of galactinol and inositol (4, 9), it is too early to state the "normal" content of these carbohydrates. The average results on five samples of non-Steffen molasses produced in 1953 provided the values shown in Table II, which were obtained by paper chromatography (9). The total of the three compounds accounts for somewhat over half the expected carbohydrate matter. In chromatographic studies the presence of small quantities of other unidentified carbohydrates has been observed. Similar studies have indicated the presence of a significant quantity of very slightly acidic substance, too weakly acidic to be bound by mixed bed ion exchange, and not exhibiting its presence by color reactions on paper chromatograms. The presence of a significant quantity of unidentified carbohydrate matter is commonly demonstrated in Steffen saccharate sirup, where the sum of raffinose, galactinol, and inositol is often much less than the known total of determinable carbohydrate-type nonsucrose matter; 3 to 5% of undetermined carbohydrate nonsucrose compounds appears to be a very reasonable value.

The foregoing relates primarily to liquors from normal beets. Variations in the various constituents may be very

Table II. Composition of Nonsucrose Components of Normal Beet Liquors of Rocky Mountain Area

Individual Components, % on Total Nonsucrose					
Mineral	Nitrogenous Organic		Carbohydrate		
K ₂ O	19	Betaine	19	Raffinose	3
Na ₂ O	4	Amino acids	19	Galactinol	1
CaO	0.25	Undetermined	4	Inositol	2
Cl	4.5			Undetermined	4
SO ₃	2.5				
N ₂ O ₅	2.0				
Organic acids	15				
Total group (by factor)	45-50		40-45		8-12

Table III. Composition of Nitrogenous Constituents

Nitrogenous components	% on Total Nitrogen					
	Non-Steffen Molasses		C.S.F., ^a		C.J.F., ^b	
	One factory, one week, 1948	1949 averages	One Factory, One Week, 1952	1953	Johnstown Factory, One Day, 1952	1953
NH ₃ N	1.4	1.2	0.4	0.4	1.1	1.2
Amide N ^c	1.2	1.2	1.1	2.3
NO ₃ N	26.9	8.7	12.4	9.8	7.0	8.6
Betaine N	29.4	40.4	36.3	37.5	35.9	36.2
Other N pptd. by iodine	3.2	2.9	1.7	1.1	2.6	1.7
Amino acid N	29.2	35.2	39.2	40.2	34.1	35.1
Undetermined N	10.0	12.0	8.8	9.8	18.2	14.9

^a Factory production of calcium saccharate waste water.

^b Factory production of barium saccharate waste water.

^c Probably not amide N, but N released during standard amide hydrolysis.

great. On one occasion, defecated liquor from long stored beets grown on a very fertile field contained over 12% raffinose on nonsucrose constituents.

Processing Liquors Other Than Straight Beet House Liquors

Steffen factory liquors are a mixture of straight beet liquors and calcium saccharate. The Steffen process eliminates most of the mineral and nitrogenous components, present in the molasses worked, and retains most of the carbohydrate matter. The composition of the nonsucrose constituents in the Steffen factory liquors depends primarily on the ratio of those introduced in beets sliced and those of the carbohydrate type introduced in molasses worked. The percentage of carbohydrate nonsucrose components on total can vary greatly in Steffen house liquors.

The composition of the nonsucrose constituents in Steffen saccharate sirup is fairly constant, and is mostly carbohydrate, but for various reasons the total quantity present in the saccharate sirup varies through an extremely wide range.

If the saccharate cake has been well washed, the sirup contains essentially no nitrogenous compounds and a very small amount of mineral components, equivalent to about 4 to 6% calcium oxide on nonsucrose matter. The writer is unacquainted with any investigation of the nature of the organic acids involved. Lime salts are commonly considered as a definite group of com-

pounds. It has recently been demonstrated that, within limits, the lime salt content of a saccharate sirup increases as the washing of the saccharate cake progresses, and that while the lime salts are increasing the potash content of the sirup is decreasing. Apparently the insoluble salts of organic acids originally precipitated are salts of both calcium and potassium. As washing the cake reduces the concentration of soluble potassium surrounding the precipitated salt, the potassium is displaced from the double salt through ion exchange reactions, and replaced by calcium. This causes an increase in the measured lime salts in the saccharate sirup, but does not necessarily effect a change in the equivalent quantity of salts of organic acids present.

Factory production of saccharate sirup commonly carries 0.5 to 2.0% soluble nonsucrose solids on total solids because of incomplete washing of the saccharate cake.

For the purpose of the discussion a saccharate purity of 89.0 or 11% nonsucrose matter is assumed. Of this, 1.0% is to be considered as precipitated salts and 1.5% as soluble waste water solids. This accounts for 2.5% of the total solids or 23% of the nonsucrose matter. The remainder, 77% of the total, are the carbohydrates, composed of raffinose, galactinol, inositol, and other undetermined matter. Because of the influence of galactinol on the analysis, inversion methods generally show more raffinose to be present than actually exists. Average results on

10 saccharate sirups by inversion methods showed the presence of 25% more raffinose than was actually demonstrated to be present by paper chromatography. The analysis of saccharate sirups by inversion methods, acid or double enzyme, generally shows raffinose to compose about 50% of the carbohydrate nonsucrose matter. On the basis of available data it appears that 40% is nearer correct. The composition of the remaining carbohydrate matter is undetermined. Since the combined waste and wash water carries little raffinose but significant quantities of galactinol and inositol, it follows that the carbohydrate nonsucrose components of saccharate sirup contain lower percentages of these two components than does the straight beet liquor.

A brief attempt was made to identify the nonsucrose carbohydrates from California beets (sample supplied through kindness of F. M. Sabine, American Crystal Sugar Co.). These liquors are notably low in raffinose. The examination revealed the presence of some raffinose and inositol, a very small amount of galactinol, and traces of unidentified carbohydrates. No ideas were gained as to the composition of the major portion of the nonsucrose carbohydrates.

Table IV. Per Cent Amino Acids on Nonsucrose Components of Beet Liquors

Total amino acids	Ca. 18.0
Aspartic acid	2.4
Glycine	0.3
Serine	1.2
Alanine	1.0
γ -Amino butyric acid	3.0
Valine	0.8
Leucines	1.6
Glutamic acid (by difference)	7.7

No analysis of nonsucrose components in the barium saccharate processing liquors was given in Table I. Their composition is of little interest. They consist of 60 to 75% raffinose, a slight amount of the mineral and nitrogenous groups which were not completely washed from the barium saccharate cake, and carbohydrate matter that has not been investigated.

Steffen and Barium Waste Water

Numerous analyses of ideally prepared Steffen waste waters have been made over the past years. These waste waters are so prepared as to contain a minimum of carbohydrates, and show zero sugar by polarization. Calculating the ratios of ash to mineral nonsugars and organic nitrogen to nitrogenous nonsugars, by the factors previously given, the compositions of the nonsucrose contents of Steffen waste waters for four recent years are as shown in Table V.

In spite of wide variations of ratios of mineral and nitrogenous components, the consistently low calculated values for the carbohydrate group give evidence of satisfactory accuracy of the factors used for conversion of ash and organic nitrogen contents to groups represented.

Table V. Nonsucrose Constituents of Steffen Waste Water

Type of Constituent, %	Season Averages			
	1947	1948	1949	1950
Mineral	55.3	61.1	54.6	54.3
Nitrogenous	42.2	38.1	43.8	46.0
Carbohydrate	2.5	0.8	1.6	-0.3

The waste waters contain much less carbohydrate matter than do factory productions. The factory operation rarely produces complete precipitation and a significant portion of the precipitated carbohydrate nonsucrose matter is taken up in the saccharate cake wash.

Table VI presents the composition of the nonsucrose constituents of representative factory productions of Steffen waste water and barium saccharate waste water.

The carbohydrate components were determined by separation by column chromatography applied to a mixed bed of ion exchanger. Sucrose was deducted from the total carbohydrate matter. No further attempt was made to determine the composition of the carbohydrate group.

The sum of the three types of nonsucrose constituents of the Steffen waste water is too far above 100 to be satisfactory, showing that the factors for conversion of ash and nitrogen to groups of nonsucrose can fail. The results on the barium saccharate waste water are perfectly satisfactory.

The principal difference between Steffen and barium waste waters lies in their relative carbohydrate content. The barium saccharate waste water contains almost half the raffinose and almost all of the other carbohydrate nonsucrose constituents which were present in the molasses worked. Another difference is the apparent disappearance of some amino acid nitrogen during the hot alkaline barium saccharate processing. As was shown in Table III, the percentage of amino acid nitrogen on total is down somewhat and the undetermined nitrogen is correspondingly increased. The loss of amino nitrogen conceivably results from imide formation.

The Steffen and barium saccharate waste waters are of interest because of the ease with which they can be prepared to provide valuable nitrogenous concentrates for stock feed. The barium saccharate waste water is also of interest because of its relatively high

galactinol content, from which galactinol has been recovered on a pilot plant scale.

Relation of Nonsucrose Components to Processing

Much has been written concerning the melassigenic character of various components of beet liquors. The author's experience has given little positive information. While sodium, nitrate, and chloride decrease as the beet matures, solubility investigations (3) have demonstrated little effect of variations in these components on the solubility of sugar in molasses. However, Hungerford (8) demonstrated that the crystallization rate of sugar from supersaturated solutions started at a low point at the beginning of campaign, rose as the degree of maturity of beets increased, and dropped off again when the factory was working piled beets. This might be interpreted as a melassigenic action of sodium nitrate and chloride, although the actual solubility of sugar was not markedly affected. A minor change in solubility of sugar in liquors on the low raw side causes a fairly small change in the concentration of the liquor at a given degree of supersaturation, but in the vicinity of that concentration minor changes produce excessive changes in viscosity of the liquor (7), and viscosity is an important factor in crystallization rates in raw side operations.

Hungerford (8) also demonstrated variations in crystallization rates of sucrose from supersaturated solutions, which could not be correlated with the variations in nonsucrose components, as found by analysis.

Table VI. Composition of Nonsucrose Components of Factory Waste Waters

Component, %	Type of Waste Water	
	Steffen	Barium saccharate
Mineral	48.4	45.2
Nitrogenous	49.7	37.6
Carbohydrate	7.0	17.8

Dahlberg and Bennett (6), by employment of improved techniques for preparation of products with high and low ratios of the mineral and nitrogenous groups, demonstrated that the mineral group shows only slightly greater melassigenic capacity than does the nitrogenous group.

The season of 1948 was one of abnormally high ratio of the mineral to the nitrogenous group. The purity of molasses produced during that campaign did not reflect marked increase in the melassigenic properties of the mineral components.

The effects of the carbohydrate nonsucrose compounds on processing are

well recognized. To some extent, they replace sugar in saturated solution. Therefore, Steffen molasses is produced at a purity lower than non-Steffen. But, because they increase viscosity and, in the case of raffinose, interfere with crystal development (8), the carbohydrate nonsucrose components decrease the crystallization rate. Specific effects of the members of the carbohydrate group, other than raffinose, have not been investigated, but there is evidence that they also replace sugar in saturated solutions of low purity.

The carbohydrate content of a liquor is the primary factor in determining its viscosity. Therefore, carbohydrate compounds may indirectly cause a depression of crystallization rates, but if the sugar end has sufficient capacity their presence will result in a reduced molasses purity.

That different liquors present different processing problems cannot be questioned. A reduction in the raffinose content of the beet would be advantageous. It is anticipated that the beet

breeders will make progress in this line in the near future. But it appears that the major improvement will be made by reduction of the total nonsucrose components in the carbonated juice. With the introduction of a method for determining the purity of carbonated juice yielded by individual mother beets (5), it is hoped that the beet breeders will be able to provide juices containing less of all types of nonsucrose components. However, a reduction of quantity of nonsucrose compounds in the beets worked, which will reduce the quantity of molasses produced, will do little to simplify the problem of obtaining molasses of minimum purity, because it will not make the crystallization of sucrose, from low purity liquors, more rapid.

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BEET SUGAR TECHNOLOGY

Application of Compositional Knowledge to Beet Sugar Technology

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Investigations of the composition of beets and their processing liquors have been under way for about 5 years. Results of these studies and their significance in beet technology are reviewed.

STUDIES OF THE COMPOSITION OF SUGAR BEETS and beet-processing liquors in recent years have provided much necessary fundamental information on changes in composition effected by processing, and on the nature of melassigenic compounds and agents which have deleterious effects on processing. At this laboratory, a general study of the composition of beets and their processing liquors has been under way during the past 5 years. This paper reviews the findings and suggests what they mean to beet technology.

Marc Analysis

The sugar beet contains about 95% juice and 5% marc, the water-insoluble portion. Because only a small amount of information is available on the marc, it was analyzed in 80 different varieties of beets and found to vary from 2.7 to 6%

of the weight of the beet (2, 13). Of the 80 samples of marc, 15 were analyzed for anhydrouronic acid (a measure of pectin), araban, and galactan. Results are given for some of the samples in Table I. There is a positive correlation between sucrose and marc, and sucrose and araban. Anhydrouronic acid appears in almost constant percentage in the marc (13). No conclusions can be drawn concerning galactan because the analytical method was not sufficiently accurate. These compounds are mentioned again in the discussion on alcohol-insoluble constituents.

Preparation of Juice Samples

Cosettes were collected at four different factories located in various parts of the country. The cosettes were immediately placed in boiling 70% iso-

propyl alcohol. After 15 minutes' heating, the mixture was cooled and subsequently shipped to this laboratory, where the alcohol was removed by evaporation. The cosettes were then extracted three times in boiling water. The extracts were concentrated to about 10% sugar concentration.

Factory diffusion juice from similar cosettes was collected, concentrated in vacuo, and sent to this laboratory. Molasses samples were also collected, but they probably were not representative of the same beets as those sampled for cosettes and diffusion juice.

Although data were obtained on juices from these four factories and later on diffusion juice and molasses from 13 factories, for purposes of simplification only those from Moorhead, Minn., are presented. The results on juices from various factories generally disagreed in amount, but the nature of constituents