Fractionation of Nitrogen Compounds in Beet Molasses

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For many years the sugar beet industry has been interested in the "harmful nitrogen" content of processing juices. "Harmful nitrogen" is defined here as those nitrogen-containing compounds that are not removed during processing and appear eventually in molasses. These compounds are believed by many sugar beet technologists to be especially deleterious in beet sugar manufacturing operations and to cause the production of more than normal amounts of molasses. Whether or not this is the case, more information on sugar-beet nitrogen compounds seems desirable, since they account for nearly 50 percent of the total weight of non-sugars in molasses.

As part of our molasses composition studies, taken during the 1952 campaign, samples were obtained from 9 factories: 6 operating straight houses—Betteravia, Clarksburg, and Manteca, California; Brighton, Colorado; Carrollton, Michigan; and Moorhead, Minnesota—and 3 operating Steffen houses—Alvarado and Woodland, California; and Toppenish, Washington. Nitrogen determinations were made on the various fractions separated by ion exchange resins. Information is presented relating to some of the known nitrogen compounds as well as the unidentified nitrogen compounds.

The nitrogen compounds of molasses were separated into 3 fractions referred to as basic, acidic, and unadsorbed, depending on their reaction with ion exchange resins under the conditions of this experiment. Diluted molasses was passed in turn through a strongly acidic cation exchange resin in the (H^+) form. Basic nitrogen compounds are defined as those that were adsorbed from a dilute molasses solution by the (H^+-) cation exchange resin. Since this fraction contains many amphoteric compounds, most of the amino acids in addition to more basic compounds such as betaine are present.

The effluent from the cation exchanger mentioned above was then passed through the column of (intermediate strength) anion exchange resin in the chloride-formate form. Only the strongly acidic compounds were retained. Nitrate and pyrrolidone carboxylic acid (PCA) are the principal nitrogenous components of the acidic fraction that are retained on the resin.

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There are traces of at least two unidentified nitrogen-containing acids present.

Unadsorbed nitrogen compounds are defined as those compounds that were not adsorbed from the solution by either resin. Under the conditions of the experiment this fraction contains weakly acidic nitrogen compounds and may contain weakly basic compounds that were not retained by the cation resin and some neutral nitrogen compounds. This fraction also contains sucrose, other soluble carbohydrates, and soluble neutral compounds. If pectin or pectic acid were present in the original molasses they would appear in this fraction. Although these substances are acidic their high molecular weights prevent their adsorption by ion exchange resins.

Experimental Methods

The columns used for separation of the basic fraction were glass tubes 60 cm. long and 4 cm. in diameter. One end of each was stoppered with a one-hole rubber stopper containing an outlet tube which could be closed with a clamp or stopcock. A circle of fine-mesh nylon bolting cloth was used to cover the stopper and outlet tube to prevent loss of resin. The columns were loaded with 400 ml. of commercial-size Dowex-50 (H)[2] (Dow Chemical Company, Midland, Michigan) in a water slurry. As determined by previous trials, this is 5- to 10-fold the amount necessary to remove the basic components.

The columns used for separation of the acidic fraction were glass tubes 60 cm. long and 1.6 cm. in diameter with the type of closure described above. The lower part of the tubes were filled with about 50 ml. of an intermediate-strength anion exchange resin Permutit A (Permutit Corporation, New York, N.Y.) equilibrated with hydrochloric acid to pH 2.5. Fifty ml. of the resin equilibrated with formic acid to pH 2.8 was placed above this. These columns were to fractionate the organic acids of molasses including lactic, glycollic, PCA, citric, and others (1) and to provide a fractionation of nitrogen compounds into the two classes, acidic and unadsorbed.

Figure 1 shows a diagram of the separation procedure. Fifty grams of molasses, diluted to 200 ml. with water, was passed through the column of Dowex-50 (H) cation exchanger at the rate of one liter per hour. The column was washed with water until the pH of the effluent was 3.3. The volume of the acidic effluent (I) including the wash water was about 1300 to 1500 ml. Three liters of 10 percent ammonium hydroxide was passed

[2] Mention of specific products does not constitute endorsement by the Department of Agriculture over others of a similar nature not mentioned.

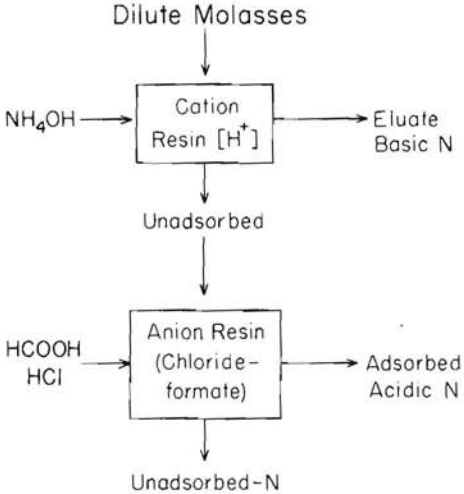

Figure 1.—Separation of nitrogen fractions with ion exchange resins.

through the column of cation resin to elute the basic nitrogen compounds (II). The ammonia was removed by boiling the solution vigorously while the pH was maintained above 7 by additions of sodium hydroxide. The alkaline condition of the ammonium hydroxide eluate of the cation resin degraded most of the amides present to form additional ammonia. This ammonia and the ammonia originally present in the molasses were lost during concentration of this fraction.

An aliquot of the acidic effluent from the cation exchanger (1) containing about 20 milliequivalents of anions, was passed through a column of anion resin (Permutit A) in the chloride-formate form. The column was then washed with 500 ml. of water. The combined effluent and wash form the unadsorbed fraction (III). The anion column was fractionally eluted with a mixture of dilute formic acid hydrochloric acids to obtain the fraction containing PCA (IV) (1).

The methods of analysis used were previously described (2). The original molasses was analyzed for total, amino, amide, and ammonia nitrogen and for betaine. The basic fraction (II) was analyzed for total, amino, amide, and ammonia nitrogen. The acidic effluent (1) from the cation column containing the acidic and neutral substances was analyzed for total and amino nitrogen. Later experiments have shown that the Kjeldahl method used for total nitrogen recovered only 60 to 80 percent of the nitrogen present. As a consequence the total nitrogen values for molasses may be low by four percent and the acidic fraction by eight percent in samples with a high nitrate content. The unadsorbed fraction (III) was analyzed for total nitrogen. The fraction IV eluted from the anion column with dilute formic-hydrochloric acid contains mostly PCA. It was analyzed for total nitrogen to give estimate of the PCA present.

Results

The classification of the nitrogen compounds into basic, acidic, and unadsorbed is arbitrary and depends upon the operating conditions used in the experiment. For example, the anion exchanger in the hydroxyl form would absorb some of the weakly acidic compounds that passed through the resin column when the resin was in the chloride-formate form. Under the conditions of low pH caused by release of mineral acids in the cation exchanger some weakly basic compounds or amphoteric compounds pass through and are found in the acidic or unadsorbed fractions. Most of the nitrogen compounds found in the unadsorbed fraction could have been recovered on an ion exchange resin by recycling the solution through a column of cation resin in the hydrogen form and then passing the effluent through a column of anion resin in the hydroxide form. The known compounds in the basic fraction are mainly betaine and amino acids.

Since only part of the nitrogen compounds were eluted from the anion resin, total nitrogen present in the acidic fraction was calculated by difference between the nitrogen in acidic effluent (I) and the nitrogen in neutral effluent (III). PCA and nitrate comprise most of the acidic nitrogen although there are traces of at least two unidentified nitrogen compounds that were eluted by hydrochloric acid and there are probably others that are eluted only with difficulty if at all.

The unadsorbed fraction is composed mainly of weakly-acidic nitrogen compounds such as uracil and uridine that are not retained by the anion resin. It may also contain some weakly basic compounds that passed through the cation resin as a result of the low pH of the effluent and some nearly neutral nitrogen compounds.

Table 1 shows the amount of molasses nitrogen in the basic, acidic, and unadsorbed fractions. The average distribution shows 66 percent of the nitrogen in molasses as basic, 28% as acidic, and 6 percent as unadsorbed.

Table 2 shows the distribution of basic nitrogen among the known compounds and classes of compounds and also the quantity that has not yet been identified. The basic nitrogen fraction averages 49 percent betaine, 27 percent amino acids,

Table 1.—Basic, Acidic, and Unadsorbed Molasses Nitrogen (g. nitrogen/100 g. non-sucrose solids).

Factory[1]	Basic	Acidic	Unadsorbed
Be	4.55	1.98	0.33
Br	4.33	1.76	.43
CaM	4.42	1.53	.39
Cl	4.52	1.79	.39
Ma	5.05	2.34	.58
Mo	3.94	1.83	.34
Al[2]	4.15	1.99	.42
To[2]	4.91	2.25	.44
Wo[2]	3.24	1.27	.43
Average	4.35	1.86	.42

[1] Abbreviations for the factories are as follows: Al—Alvarado, Be—Betteravia, Br—Brighton, CaM—Carrollton, Cl—Clarksburg, Ma—Manteca, Mo—Moorhead, To—Toppenish, and Wo—Woodland.

[2] Steffen molasses.

Table 2.—Distribution of Basic Nitrogen in Molasses (g nitrogen/100 g. non-sucrose solids).

Factory[1]	Betaine	Van Slyke Amino	Amide	Ammonia	Remainder[3]
Be	2.02	1.34	0.28	0.12	0.66
Br	1.77	1.03	.15	.05	1.20
CaM	2.34	1.04	.17	.04	.70
Cl	2.28	1.06	.20	.09	.74
Ma	2.29	1.40	.25	.10	.88
Mo	2.27	1.00	.20	.05	.29
Al[2]	1.56	1.16	.19	.06	1.05
To[2]	2.43	1.29	.25	.08	.73
Wo[2]	1.65	.81	.20	.07	.38
Average	2.07	1.13	.21	.07	.74

[1] See Table 1 for abbreviations.

[2] Steffen molasses.

[3] Does not include an estimated 0.13 g. purine-pyrimidine nitrogen.

7 percent amide and ammonia, and 17 percent unidentified. Included in the unidentified portion is nitrogen from purines and pyrimidines which are known to be present but were not determined in these samples. On the basis of the analysis of one sample of Manteca molasses (3) for individual purines, pyrimidines, and nucleosides, the N due to these compounds would account for .13 g. of the unidentified .74 g. of N.

The acidic nitrogen distribution is given in Table 3. From the averages, PCA accounts for 55 percent and amino compounds 6 percent of the acidic nitrogen. A large part of the remainder

Table 3.—The Distribution of Acidic Nitrogen in Molasses (g nitrogen/100 g. non-sucrose solids).

Factory[1]	PCA	Van Slyke Amino	Remainder
Be	1.17	0.10	0.71
Br	.90	.13	.73
CaM	.73	.11	.69
Cl	1.02	.09	.68
Ma	1.46	.13	.75
Mo	.98	.14	.71
Al[2]	67	.08	1.24
To[2]	1.58	.10	.57
Wo[2]	.69	.11	.47
Average	1.02	.11	.73

[1] See Table 1 for abbreviations.

[2] Steffen molasses.

is due to nitrate, which averaged 0.55 g. of nitrate nitrogen per 100 g. of impurities for 17 molasses samples from the 1956 campaign. If we accept this as an approximation for the 1952 molasses, however, we must make a correction, since the total N analysis would have measured only 70 percent of this, or 0.38 g. Using this figure, there is still 19 percent of the acidic nitrogen which is unknown. The acidic amino nitrogen is also unknown, but probably includes glutamic and aspartic acids which leaked through the cation resin because of the high acidity, pH 1.5, of the effluent.

Table 4.—Total and Unidentified Molasses Nitrogen (g. nitrogen/100 g. non-sucrose solids).

		Unidentified				
Factory[1]	Total	Basic	Acidic	Unadsorbed	Total	% of Original
Be	7.19	0.66	0.68	0.33	1.67	23
Br	6.09	1.20	.70	.43	2.33	38
CaM	6.40	.70	.66	.39	1.75	27
Cl	6.79	.74	.65	.39	1.78	26
Ma	8.47	.88	.72	.58	2.18	26
Mo	6.44	.29	.68	.34	1.31	21
Al[2]	6.37	1.05	1.21	.42	2.68	42
To[2]	7.61	.73	.54	.44	1.71	22
Wo[2]	5.59	.38	.44	.43	1.25	22
Average	6.75	.74	.70	.42	1.86	28

[1] See Table 1 for abbreviations.

[2] Steffen molasses.

Table 4 gives the relationship between total and unidentified nitrogen in the basic, acidic, and unadsorbed fractions. An average of 28 percent of the total nitrogen appears to be unidentified. If a correction for the nitrate in the acidic fraction is made, based on the average nitrate content of the 1956 campaign molasses samples, 22 percent of the nitrogen remains unidentified. If these unknown compounds average 20 percent nitrogen, about 7.5 percent of the non-sugars in molasses are unidentified nitrogen compounds. If the average nitrogen content of the compounds is as low as 10 percent, then 15 percent of the total non-sugars are made up of this group of unidentified compounds. The true value probably lies between the extremes of 7.5 and 15 percent.

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Summary

A method using ion exchange resins is described that enables the separation of molasses nitrogen compounds into basic, acidic, and unadsorbed classes of nitrogen compounds.

Nitrogen compounds make up about 50% of the nonsucrose molasses solids and their average distribution is 66% basic, 28% acidic, and 6% unadsorbed. The 6% unadsorbed is mainly weakly acidic compounds. Weakly basic, or neutral compounds may also be present.

Forty-nine percent of the basic nitrogen occurs in betaine, 27% in amino acids, 7% as amides and ammonia, and 17% remains unidentified. Acidic nitrogen is distributed as 55% PCA, 6% amino compounds, 20% nitrate, and 19% unidentified. The compounds containing unadsorbed nitrogen have not been identified.

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