Harmful Constituents of the Beet—Factors Which Influence the Harmful Nitrogen

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From the manufacturing point of view the non-sugar compounds of the beet are not of equal importance. Substances which remain in the pulp after the sugar extraction naturally have no disturbing effect. Substances in the diffusion juice, which are precipitated with lime and the protein which coagulates when lime is added at a certain pH are not involved in the subsequent process of the manufacturing of sugar. The non-protein nitrogen compounds such as amino acids (asparagin, glycocoll, leucin) and betain, allantoin, etc., cannot be precipitated and therefore are carried over into the thin juice. These are harmful because they prevent the crystallization of the sugar, thereby forming molasses. Some of the soluble ash, such as sodium, potassium chloride, and organic potassium salt, are also molasses forming. Our present study is devoted entirely to the harmful nitrogen.

Chemical Methods for Determination of Harmful Nitrogen

There are several methods which can be used for the determination of the harmful nitrogen.

The Stutzer-Barnstein Andrlik (1) method determines the harmful nitrogen indirectly by subtracting the protein, the ammonia and one-half of the amid from the total nitrogen.[2] This method is exact but tedious. In the factory during the campaign and in the breeding station for genetical work only methods which are rapid are practical.

In response to this need, I attempted in 1932 and 1933 to perfect a method for quick determination of harmful nitrogen. The experiments showed that when we treat the sugar solutions with copper sulfate and sodium hydroxide we always have a positive correlation between the blue color and the analytically determined harmful nitrogen. (In other words the darker the color the higher the amount of harmful nitrogen.) The solutions were measured in Stammer Colorimeter (2) and for comparison we used auramin and water blue in a gelatin mixture. But because the shade of the blue filtrate was sometimes greenish (3) not all the readings could be made from these plates.

One year later in The Institute for Sugar Industry in Prague a colorimetric method based on the same principle was worked out by Stanek and Pavlas (4). They found the color of the mixture of the inorganic reagents cobalt-ammonium-sulfate and copper sulfate is comparable to the sugar-beet solutions to which copper nitrate and sodium acetate has been added. A standard series was made from

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[2]Figures in parentheses refer to Literature Cited.

the above reagents and it was recommended in the factory laboratory that the comparison be made in flasks with blue light on a white table. But as in the case of all colorimetric methods the reading with the naked eye is too subjective. In order to obtain satisfactory results we used the photocolormeter.

However, the colorimetric method does not give any indication of the presence of some of the compounds which are shown to be present by the long quantitative method. As my artificial molasses experiment showed (5) glycocoll, asparagine, glutamin, leucine, leucine Ca gave a blue color (and the results of the colorimetric reading were quite close to the results of the analytical method), whereas betain, glutiminic acid, etc. belonging also to the harmful nitrogen, did not give a blue color. All the compounds of the harmful nitrogen which do not give a blue color are present only in small quantity, except betain which was found to be about one-third of the total harmful nitrogen. But as indicated by the above mentioned molasses experiment and as also confirmed by Sorgato (6), and Stanek (7), betain has the smallest molasses-forming quality. What seems to be more important is the fact that betain is always in positive correlation with the other compounds of harmful nitrogen, which give a blue color.

There are other methods for determining the harmful nitrogen. In the method developed by Vondrak (8), mercurous acetate and soda are used.

All amino-acid determinations which are not specific to certain amino groups (like the ninhydrin reaction to "a" amino group) can be used for harmful N.

The Van Slyke (9) method is based on the reaction between aliphatic amino groups and nitrous acid whereby N is liberated as nitrogen gas.

In the Engeland (10) exhaustive methylation, the amino acids are methylated in alkaline solution with dimethylsulfate and the methyl-product can be isolated and weighed as Hg double salt. The principle of the Sorensen (11) method is, that when formaldehyde is added the basic character of the amino group is destroyed and the free carboxylic acid can be titrated with $Ba(OH)_2$.

The Janke-Holota (12) macro and micro electrometric titration with glass electrode is based on the same principle as Sorensen, but is more exact. All the salts, the acids of which are dissociated between pH 7-9 such as carbonate and phosphate are precipitated with $BaCl_2$ and $Ba(OH)_2$ the surplus of barium is removed with acidified Na_2SO_4 and centrifuged. The supernatant liquid is titrated to pH 7, formaldehyde is added and titrated with n/10 NaOH to pH 9.

These methods are also limited to certain compounds of harmful nitrogen and are not so well adapted to mass analysis as the colorimetric method,

Molasses Quantity Calculated from the Harmful Nitrogen

It is possible to calculate the probable amount of molasses quantity from the harmful nitrogen content by the Andrlik formula (13).

$$\text{Molasses} = \frac{\text{Harmful N} \times 0.9 \times 25}{\text{Molasses polarization}}$$

This formula is valid only if we use the ''long'' method of Stutzer and Barnstein. If the colorimetric method is used the factor 25 should be corrected because in this method not all of the harmful nitrogen is included. The factor 25 has to be changed. The new factor was derived empirically (14) from our data of the campaigns of 4 years as follows:

1935	57.3
1936	55.3
1937	52.7
1938	57
Average	55

In order to make this factor generally applicable it is necessary to collect statistical data from many regions where sugar beets are grown.

Factors Influencing the Harmful Nitrogen

Since the harmful nitrogen is an important characteristic of the quality of the beet it is necessary to investigate all of the factors which may influence it.

These factors are: Climate, variety, soil, fertilizer, and harvesting time.

Influence of Climate

The climate has great influence on the harmful nitrogen. Beets grown in a hot, dry climate contain more harmful nitrogen than those grown in moderately cool climate with plenty of rain. Beets grown in Russia near the Dnieper River, in South Hungary, South Yugoslavia, and in Italy have higher harmful N than those grown in any other part of Europe. Sorgato (15) compared the Italian beets with those from other countries and came to the conclusion that because of the climate the Italian beets have high harmful N.

Influence of Variety

Like other qualities of the beet the amount of harmful nitrogen varies with the variety. However, variations exist within the same variety: When 300 individual beets from the same variety were examined for harmful nitrogen 48 percent were found to contain 30 mg. per 100 grams of beet, 37 percent between 30 to 60 mg., and 15 percent above 60 mg. Experiments completed with 11 varieties showed an average (16) as follows:

Year	Lowest Harmful nitrogen mg. per 100 gm. of beet	Highest Harmful nitrogen mg. per 100 gin. of beet
1935	20.0	53.5
1936	20.0	33,9
1937	13.8	24.0
1938	23.6	30.9

This range is much greater when the varieties are planted in different soils. According to a recent communication sent to me by the Hungarian Breeding Station "Beta" (17) in 1940 the beets showed a range between 9 to 100 mg. per 100 gm. beets. Urban (18) has established the fact that nitrogen in beet is hereditary. If the same can be proved for harmful nitrogen, as it is reasonable to believe then the breeder has great possibilities of selecting varieties for low harmful N, as suggested by the wide range shown above. The difficulties in selection are recognized, namely to bring the harmful nitrogen in "harmony" with other important qualities of the beet. Rasmusson (19) of Sweden observed that the well-shaped beets often had a higher, harmful, nitrogen content. According to Sorgato (20) of Italy the same strain which seemed to have a hereditary tendency for high sugar had likewise the same tendency for high, harmful nitrogen. In other words, there is no one indirect correlation between sugar content and harmful nitrogen. However, Munerati (21) believes that it is possible to obtain through careful selection, certain biotypes with consistently low, harmful nitrogen and fair sugar. Thus, in our selection we should think of the technical value of the beet in terms of the ratio of harmful nitrogen to sugar content.

Influence of Soil

The soil has also an influence on the harmful N, especially the nitrogen of the soil. In one of the factory districts of the company with which I was connected, the beets grown by all the farmers contained a normal amount of harmful nitrogen, except at the State Farm where horse manure was heavily used. The N content of the soil became 1 percent. The beets from this soil had 130 to 140 mg. harmful N per 100 gm. of beet (22).

On the company's own farm many investigations were carried out in order to study the influence of the nitrogen content of the soil on the harmful N. Data are represented from about 600 acres for the year 1933 in table 1 and show the results when no fertilizer was used. Table 2 shows data from about 3,000 acres for the year 1937, when nitrogen fertilizer was applied:

Table 1: The highest, harmful N content corresponds to the highest soil N content.

Table 1.—Nitrogen content of the soil and harmful nitrogen of the beet.

Location	Nitrogen mg. per 100 gm. of soil	Harmful nitrogen mg. per 100 gm. of beet
R. IAI	129	63
G. VI. B	135	68
B. II.	138	57
K. K I.	139	41
S. 25	150	43
T. Z XII	154	40
G. K III	159	71
A. WI Ny.	167	51
F. G.	168	67
S. K. V	276	130

Discussion of table 2: The soils are grouped according to the nitrogen content ranging from low to high. The higher N content of the soil did not produce higher harmful N, because where the soil nitrogen was low, more fertilizer was applied, and where the nitrogen content was higher, less or no fertilizer was given. This shows that a well-balanced fertilization applied according to the soil test can keep the harmful N about the same level.

Influence of Fertilizer

Fertilizer experiments were conducted using 8 replications with increasing amount of potash, phosphate, and nitrogen. It was done with E yield strain and Z sugar strain in order to study the effect of nutrition on the quantity and form of the nitrogen on both strains simultaneously. The results are given in tables 3, 4, and 5. The abbreviations are N = Cal. Nitro with 17 percent N, P = Superphosphate with 18 percent $P_2 0_5$ and potassium chloride with 40 percent $K_2 . O$

Since this study is concerned with harmful nitrogen, the yields are not given. The sugar content is included because we cannot discuss the beet from any point of view without knowing the most important characteristic of it, namely the sugar content. In order to get a true picture of the technical value of the beet, we present the ratio of harmful nitrogen to sugar content. We also give the relationship between harmful nitrogen and total nitrogen as additional information.

Potassium.—Table 3 shows the influence of potassium fertilizer on the nitrogen content of the beet.

The effect of K on the total nitrogen was found to be significant in only one case. But the effect on both harmful nitrogen and sugar content was significant in all cases with the B type and in two cases with the Z type.

As the amount of potassium in the fertilizer is increased, the sugar content goes up progressively from 14.76 to 16.07 for E strain and from 17.42 to 18.66 for Z strain. Expressed in percentage, this increase in sugar is 8.9 percent for the E type and 6.6 percent for the Z type. Thus we see that the E type response to potash is better than the Z type. We found in previous years in many experiments that the sugar content was increased by potassium (23).

The ratio between total N and sugar decreased as the potassium is increased, but this is due only to an increase of sugar content effected by the potassium. The ratio between harmful N and sugar represents a change in both constituents. Potassium decreased the harmful N sugar ratio with 41 percent for the E type and 25 percent for the Z type. Furthermore, the ratio between harmful N and total N is influenced by the potash, as shown in the last column of table 3. Since the total N is practically constant, and decrease in harmful N means an increase in protein (total N-protein==harmful N). In other words, the potassium exerted an influence on the molecular N formation in the plant. Similar observation was reported by Rauterberg Loofman (24) with barley and grass.

Phosphate.—The experiment with phosphate was carried out according to the same plan as described above for potassium, using increasing amounts of superphosphate.

The results show the following: Phosphate decreased the total N only in one case; the harmful N in three cases, but only slightly. The decrease in ratio harmful N to sugar is due more to the sugar increase. However, the effect was far less significant than with potassium, despite the fact that for each experiment we selected soil which was known by chemical test and previous field experiment to have phosphorus and potassium deficiency, respectively.

Nitrogen.—To study thoroughly the effect of nitrogen fertilizer on the harmful N, variously arranged experiments were conducted:

1. Beets fertilized with nitrogen were compared with those without any fertilizer.

2. Beets with complete fertilizer P, K, N, were compared with P, K as standard.

Both experimental series 1 and 2 were carried out in two different fields: In one, comparing E yield type with Z sugar type, and in the other comparing E with ZZ, extreme sugar type.

In another arrangement of the experiment, N was applied in increasing amounts up to very high quantities. This was done *in* order to find out to what extent the harmful N increases by adding a high amount of nitrogen fertilizer. The results are given *in* tables 5 and 6.

Table 4.—Influence of phosphate fertilizer on the nitrogen content of the beet.

Variety	Fertilizer (lb. per acre) N	P	K	Percentage of sucrose	Difference	Total nitrogen (mg. per 100 gm. of beet)	Difference	Harmful nitrogen (mg. per 100 gm. of beet)	Difference	Total nitrogen (mg. per 100 gm. of sucrose)	Harmful nitrogen (mg. per 100 gm. of sucrose)	Ratio (Harmful N per total N) 100
	107	...	125	15.57±0.23	...	147± 8	...	24.7±1	...	944	159	16.8
E	107	94	125	15.71±0.11	+0.14	146± 5	— 1	19.8±0.3	— 4.9**	929	126	13.6
Yield type	107	156	125	15.74±0.22	+0.17	140±11	— 7	21.5±2	— 3.2	889	137	15.3
	107	220	125	15.91±0.18	+0.34	137±13	—10	21.2±2	— 3.5	861	133	15.5
	107	250	125	16.06±0.19	+0.49*	145± 8	— 2	21.0±1	— 3.7**	903	131	14.5
	107	...	125	17.32±0.18	...	176± 5	...	30.7±2.6	...	1000	175	17.4
Z	107	94	125	17.60±0.13	+0.28*	176± 5	0	31.5±3	+ 0.8	983	176	17.9
Sugar type	107	156	125	18.40±0.26	+0.88*	177± 8	+ 1	25.7±2.2	— 5.0**	962	140	14.5
	107	220	125	18.09±0.07	+0.57*	185±11	+ 9	30.2±2.8	— 0.5	1020	167	16.3
	107	250	125	18.25±0.02	+0.73*	196± 9	+20*	30.7±3.4	0	1074	168	15.6

*Increase is significant from N, K (without P).

**Decrease is significant from N, K (without P).

Table 5.—Influence of nitrogen fertilizer on the nitrogen content of the beet.

Location	Variety	Fertilizer (lb. per acre) N	P	K	Percentage of sucrose	Difference	Total nitrogen (mg. per 100 gm. of beet)	Difference	Harmful nitrogen (mg. per 100 gm. of beet)	Difference	Total nitrogen (mg. per 100 gm. of sucrose)	Harmful nitrogen (mg. per 100 gm. of sucrose)	Ratio (Harmful N per total N) 100
		....	....	....	15.32±0.11		148± 5		25.1±1.4		965	163	16.9
	E	110	....	....	15.20±0.20	—0.12	159± 9	+11	20.8±1.5	— 4.3	1046	136	13.1
K. C. S.	Yield type	....	156	125	16.09±0.38		134±10		21.0±3.1		832	131	15.6
		110	156	125	15.74±0.22	—0.25	140±11	± 6	21.5±2.2	+ 0.5	890	137	15.3
		....	....	....	17.40±0.14		180± 4		30.8±1.1		1034	177	17.1
	Z	110	....	....	17.23±0.11	—0.17	202±16	+22	31.7±2.9	+ 0.9	1172	183	15.7
	Sugar type	....	156	125	18.01±0.04		174±10		28.0±4.7		966	154	15.9
		110	156	125	18.40±0.25	+0.39	177± 8	— 3	25.7±2.2	— 2.3	962	139	14.5
		....	....	....	14.91±0.09		171± 4		33.8±1.4		1147	227	19.7
	E	125	....	....	14.71±0.16	—0.20	163±10	— 8	35.5±4.5	+ 1.7	1108	241	21.8
K. T. 2	Yield type	....	156	156	18.01±0.04		163± 7		32.7±3.5		1058	212	20.1
		125	156	156	18.40±0.25	+0.20	179± 5	+16	27.7±1.4	— 5.0	1147	177	15.4
		....	....	....	17.51±0.06		215± 4	— 1	45.1±0.9		1227	258	20.9
	ZZ	125	....	....	17.19±0.13	—0.32	214±11		45.5±2.5	+ 0.4	1245	267	21.2
	High sugar	....	156	156	17.50±0.26		197± 4		37.0±2.7		1125	211	18.8
	type	125	156	156	17.72±0.13	+0.22	200± 7	+ 3	38.5±1.5	+ 1.5	1129	217	19.2

Table 6.—Influence of nitrogen fertilizer on the nitrogen content of the beet.

Variety	Fertilizer (Calnitro lb. per acre)	Percentage of sucrose		Total nitrogen (mg. per 100 grm. of beet)		Harmful nitrogen (mg. per 100 grm. of beet)		Total nitrogen (mg. per 100 grm. of sucrose)	Harmful nitrogen (mg. per 100 grm. of sucrose)	Ratio (Harmful N per total N) 100
	0	14.42±0.09	....	172± 6	....	21.7±0.8	....	1193	150	12.6
	157	14.12±0.13	—0.30*	177±10	+ 5	25.0±1.7	+ 3.3**	1253	177	14.1
E	314	14.24±0.31	—0.18	194±15	+22	27.7±2.2	± 6.0**	1362	194	14.3
type	471	14.26±0.36	—0.16	205± 4	+33**	29.0±2.0	+ 7.3**	1437	203	14.1
	628	14.12±0.20	—0.30	199± 9	+27**	35.0±2.6	+13.3**	1409	248	17.5

•Decrease is significant from without fertilizer.

••Increase is significant from without fertilizer.

Table 5: The total nitrogen per sugar of the nitrogen-fertilized beets was higher than those without fertilizer in three cases and only in one case was it slightly lower. The harmful N per sugar was higher in three cases and lower in one case. In other words the quality is better without any fertilizer than with N application alone. Comparing N, P, K with P, K the total N per sugar was slightly higher in three cases while the more important harmful N per sugar was about the same in two cases and lower in two. Thus we see that when nitrogen is applied with P and K the quality is not inferior. To improve the quality, nitrogen should not be applied without P and K.

Table 6: Nitrogen was given in increasing amounts up to 628 pounds, which resulted in an increase in harmful nitrogen of 61 percent. The maximum of the total nitrogen did not correspond to the maximum amount of fertilizer given, but to a smaller amount. After reaching an increase of 19 percent, the total N remains at about the same level. It is interesting to note that when the total N reached this limit the yield reached the highest point too, and still larger amounts of N fertilizer actually decreased the yield of the beet, but increased the leaves and tops. It is possible that if the vegetation time were longer, the translocation of N could occur resulting in a higher yield.

Influence of Harvest Time

In order to study the effect of the harvest time on the nitrogen forms, samples were taken from several fields on September 1 and again on October 30.

The results are given in table 7. (The lower total nitrogen in the October beets is explained by the fact, that between the sampling there was 162 mm. of rainfall. The beets absorbed water, subsequently the dry substance became lower, likewise the total N.) More harmful nitrogen and higher ratio of it to total nitrogen were found in the September samples, than in the October ones. Two hypotheses can be given to explain why the harmful N was higher and consequently the protein N lower.

1. In unripe beets the amino acids are not yet built up to protein. However, Professor Roemer (25) maintains that in younger beets there is less harmful N and more protein than in the older.

2. That dry weather is responsible for a high harmful N to total N ratio in the earlier beets; later the rain changed this ratio.

As we see, the harmful nitrogen is more or less influenced by all growing conditions of the beet. Since the reduction of its quantity in the beet is important for the improvement of the technical value of the beet, we have to include the harmful-nitrogen tests in all our investigations. Beets, from experiments on variety, fertilizer, cultivation, etc., should be tested for harmful N. Furthermore, for geneti-

Table 7.—Influence of the harvest time on the nitrogen content of the beet.

Location	Harvested in September: Total nitrogen (mg. per 100 gm. of beet)	Harmful nitrogen (mg. per 100 gm. of beet)	Ratio (Harmful N per total N) 100	Harvested in October: Total nitrogen (mg. per 100 gm. of beet)	Harmful nitrogen (mg. per 100 gm. of beet)	Ratio (Harmful N per total N) 100	Difference in ratio between Sept. and Oct. beets
R. Y. A.I.	227	127	53.3	184	63	34.2	19.1
AI	200	76	38.0	100	51	26.8	12.2
T. Z. X15	215	76	35.4	168	40	23.8	11.5
F. Go.	248	88	35.5	201	67	33.3	2.2
G. YL. E	243	111	45.7	215	98	31.6	14.1
B. II.	201	104	51.7	183	57	31.1	20.6
K. K. I	222	114	51.3	173	41	23.7	27.6
G. K. III	287	150	52.3	215	71	33.0	19.3
S'. K. V.	224	190	S4.8	212	130	61.3	23.5
S. 25	154	96	62.3	150	43	28.6	33.7
		nverage: 51.0			average: 32		

cal work in breeding stations the harmful N should be considered in addition to sugar, purity, and soluble ash.

A few years ago this recommendation could not be adapted because the methods were not adequate for mass analyses. Today we can simplify the procedure by supplementing the Bachler "One solution method" (26) with the eolorimetric test in the following way: Extract one half of the normal weight of the beet with warm water and leave it for 30 minutes in water bath without adding lead acetate to the solution. After cooling, filter and determine the dry substance by direct reading with Bachler-Zeiss dip refractometer. Continue using the same solution in the eonduetometer for the determination of the soluble ash. Then add Home's dry lead, filter, and use one part for polarization and another for determination of harmful N. Add 5 cc. of Stanek reagent to 50 cc. aliquot; shake and read immediately in photocolorimeter.

For speed we modified the Lange photocolorimeter to make possible a continuous flow of the samples. I am sure this modification for time saving can be done with the available photocolorimeter.

Quite another proposition would be to approach the problem of harmful nitrogen from the technical angle, by attempting to remove the harmful N in the factory during the purification process. A. R. Nees (27) believes it is technically possible to eliminate the harmful N with an operation following the lime and carbonate addition. May I suggest that this should be done during the lime and carbonation process, since the amino acids combine with carbonic acid and calcium to form the calcium salt of the carhaminoacid (28). Unfortunately for the sugar technologists, these salts are soluble in water, though

insoluble in alcohol. Of course to precipitate with alcohol would make the manufacturing of sugar too expensive. There might be other means of precipitating these salts such as radical changes of the temperature, in the pH or with some inexpensive precipitant.

If we can thus solve this problem, we shall soon forget that once we called the nitrogen harmful.

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