Nitrogenous Compounds in Sugarbeet Juices[1]

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Introduction

The sugarbeet (*Beta vulgaris* L.) is composed of 1% or more of nitrogen-containing compounds (12)[3]. The percentage varies with sugarbeet variety, type of fertilization, and general growing conditions. Technically, knowledge of the nitrogen components is important in sugarbeet processing because, except for the proteins and some ammonia, most of them are not removed during standard juice purification.

Nitrogen, from various sources, is metabolized in the sugarbeet plant to form a variety of compounds of biological significance. Identification of all the nitrogen in its respective constituents has been a major problen even though numerous studies have been made of the non-sucrose components in sugarbeet juices (3,4,5,7,8,11,17,18). Nitrogenous compounds, especially those containing amino nitrogen, have a highly deleterious effect on juice purification and sucrose crystalization (3,4,5,12,15,17,18). Amino acids act as buffers to lower the effective alkalinity of the carbonated juices; some of the amino acids and the amides, through chemical changes, also produce products which cause additional lowering of the effective alkalinity (3,4). Low effective alkalinity during factory liming and carbonation processes results in incomplete precipitation of some other nonsugars which later interfere also with sucrose crystallization (3,4). The resultant effect of these nitrogen components during processing can be understood only when it is known what and how much of each type of constituent is present in the sugarbeet juices and the changes, if any, they undergo during processing.

This experiment was designed to quantitatively determine the individual nitrogenous compounds in sugarbeet juices, and the effect of nitrogen fertilization on each nitrogen constituent.

Materials and Methods

One sugarbeet cultivar was used for this experiment. The open-pollinated former commercial cultivar 'GW 359-52R' was one of 20

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[3]Numbers in parentheses refer to literature cited.

in a large experiment grown under irrigation at Fort Collins, Colorado in 1969 at three levels of applied nitrogen (0, 57, and 113 kg N/acre). Single-row plots, 21 feet long, were used in a randomized complete block design with six replications. Each plot was bordered by a medium vigor common competitor row. Plot weight and sucrose content were determined on the roots at harvest. Pressed juice was obtained from standard tare laboratory root-brei samples of each plot. Aliquots of the pressed juice samples were used to prepare phosphated thin juice (2,4). Thin juice purity was determined and recoverable sucrose per plot was calculated. The refractive dry substance (RDS) was obtained on each pressed and thin juice sample.

To determine possible changes in amino acids and amides during preparation of the phosphated thin juice, individual amino acid analyses were made on the pressed and thin juice samples using a Technicon Amino Acid AutoAnalyzer[4] by the method of Payne *et al.* (14). The pressed juice was treated first with sulfosalicylic acid (0.1 mg/ml) to deproteinize the samples and deactivate the enzyme systems (1).

Thin juice samples were analyzed a second time, after basic hydrolysis, to determine the quantity of the amides, glutamine and asparagine, and of pyrrolidone carboxylic acid (PCA), a deamination product of glutamine (3,4).

The ammonium ion is ninhydrin positive, therefore, it also was measured during the amino acid analysis. This did not include that which escaped as ammonia gas during thin juice preparation and basic hydrolysis.

Duplicate thin juice samples were analyzed also, before and after hydrolyzation, for the other nitrogen components by the following methods:

1. Total nitrogen (N) — Modified Kjeldahl Method by Maag (10).
2. Nitrate N (NO_3-N) — The specific ion meter, Orion Model 404[4], was used with the nitrate ion activity electrode, Orion No. 92-07, and the single junction reference electrode, Orion No. 90-01. Method by McCaslin *et al.* (11).
3. Ammonium N — Calculated from ammonium ion determination during automated analysis.
4. Amino N — Modified Moore and Stein method (13). Hydrazine sulfate was used to reduce some of the ninhydrin to hydrindantin in place of adding hydrindantin directly to the buffered ninhydrin solution (19). More reproducible results were obtained by this modified method which determined all ninhydrin positive nitrogen including the amino N in the amino acids and amides, NH_4-N, and possibly some amine

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N (16). The amino N values used in this study were obtained by subtracting the NH_4-N (obtained from the amino acid analyzer analysis) from the total ninhydrin positive value resulting from this procedure.

5. Betaine N — Procedure described by Focht *et al.* (5).
6. Amino acid N — Since some amino acids and the amides contain N in addition to the α-amino N determined above, the N in each was calculated from the individual amino acid values obtained from the automated analysis.

All thin and pressed juice N components were calculated in mg per 100 RDS. Statistical analysis of variance was made on the data from which Duncan's multiple range test was computed.

Results and Discussion

Nonsignificant differences among the root yield and recoverable surcrose of sugarbeets grown at three N fertility levels indicated a high residual N in the experimental field (Table 1). Significant differences, however, were obtained in % sucrose and % purity at some N fertility levels.

Table 1.—Root and purity means of sugarbeet cultivar 'GW 359-52R' at three N fertility levels.

N Treatment	Root yield Kg/plot	Sucrose %	Purity %	Recoverable sucrose Kg/plot
O Kg/acre	17.8a[1]	16.0a	95.7a	2.6a
57 Kg/acre	20.6a	14.lb	94.1a	2.6a
113 Kg/acre	19.2a	13.5b	91.8b	2.2a

[1]Means followed by the same letter do not differ significantly at the 5% level according to Duncan's multiple range test; means for six replications.

Twenty-one pressed and thin juice amino acids and amides were quantitatively determined (Tables 2 and 3). Also present were several unidentified amino acids or ninhydrin positive analogs, some of which we have recently identified as α-aminoadipic acid, pipecolic acid, α-amino-n-butyric acid, α-aminoisobutyric acid, and possibly isovaline. Homoserine, sarcosine, and citrulline, also recently identified by us, were found only in hydrolyzed thin juice. Other unknowns, including two which give moderately large peaks on the chromatograms, have not been identified. Cystine was present only in the thin juice, and 3,4-dihydroxyphenylalanine (Dopa) was found only in the pressed juice.

The amides, which formed occluded (not separated) peaks with serine in the pressed and thin juice analyses, were converted to glutamic and aspartic acids during the basic thin juice hydrolysis (3,4,8,12,17). These occluded peaks could not be separated at the optimum temperature, pH, and buffer concentrations for best separation of the other sugarbeet amino acids (14). The occluded peak area was calculated using serine as the standard since serine was the amino acid in the Technicon Amino Acid standard mixture which eluted in that area on the chromatogram. Paper chromatography

and analyzer analysis of hydrolyzed juice samples in our laboratory showed the approximate average ratio of serine:glutamine:asparagine to be 1:3:0.5, however, this can vary with genotype, N fertility, and growth conditions. This ratio was used, with respective formula weights, to convert from micromoles of serine to mg of the serine, glutamine, asparagine combination.

PCA is not ninhydrin positive and, therefore, produced no peak on the amino acid chromatograms; nor was it included in the modified Moore and Stein amino N determination. The PCA nitrogen was included in the total N value, however. The PCA was converted to glutamic acid during the basic thin juice hydrolysis. Therefore, the glutamic acid peak, from the hydrolyzed samples, represented the original glutamic acid plus that converted from PCA and glutamine. The aspartic acid peak, after hydrolysis, represented the original aspartic acid plus that converted from asparagine, and the serine peak, after hydrolysis, was only serine.

Table 2.—Pressed juice amino acids for sugarbeet cultivar 'GW 359-52R' grown at three N fertility levels.

Amino acids	0 Kg N		57 Kg N		113 Kg N	
(A.A.)	mg A.A.	mg N	mg A.A.	mg N	mg A.A.	mg N
1.1 ASP[1]	98.4b[2]	10.3	126.1ab	13.3	155.5a	16.3
2. THR	9.8b	1.1	12.1a	1.4	occ.[4]	occ.
3. SER[3]	188.1c	34.0	282.5b	51.1	383.0a	69.3
4. GLU	118.3b	11.3	157.6a	15.0	187.0a	17.8
5. PRO	11.4	1.4	occ.	occ.	occ.	occ.
6. GLY	2.2b	0.4	3.3b	0.6	4.8a	0.9
7. ALA	12.6b	2.0	20.8b	3.3	30.2a	4.7
8. VAL	10.8b	1.3	16.7ab	2.0	22.7a	2.7
9. CYS	0.0	0.0	0.0	0.0	0.0	0.0
10. MET	2.8b	0.3	4.3ab	0.4	6.0a	0.6
11. ILE	22.8b	2.4	32.0ab	3.4	42.8a	4.5
12. LEU	19.8b	2.1	30.2ab	3.2	41.2a	4.3
13. DOPA	7.3b	0.6	10.9ab	0.8	13.3a	0.9
14. TYR	20.1b	1.5	46.4a	3.6	71.9a	5.6
15. PHE	1.9b	0.2	2.4ab	0.2	3.0a	0.3
16. GABA	44.3b	6.0	52.1ab	7.1	57.4a	7.7
17. ORN	0.2a	0.0[5]	0.2a	0.0	0.2a	0.0
18. LYS	3.4b	0.6	4.2b	0.8	5.7a	1.1
19. HIS	3.5b	0.9	5.4ab	1.4	7.0a	1.9
20. TRY	6.9b	0.9	8.9ab	1.2	11.2a	1.5
21. ARG	4.0b	1.3	7.2ab	2.3	9.2a	2.9
TOTAL	588.6	78.6	823.3	111.1	1052.1	143.0

[1]1. aspartic 2. threonine 3. serine 4. glutamic 5. proline 6. glycine 7. alanine 8. valine 9. cystine 10. methionine 11. isoleucine 12. leucine 13. 3,4-dihydroxyphenylalanine 14. tyrosine 15. phenyalanine 16. gama-aminobutyric acid 17. ornithine 18. lysine 19. histidine 20. tryptophan 21. arginine.

[2]Means within the same row across the table followed by the same letter do not differ significantly at the 5% level according to Duncan's multiple range test; means for six replications in mg/100 RDS.

[3]Combination of serine, glutamine, and asparagine.

[4]Occluded peaks.

[5]Less than 0.05 mg N.

In pressed juice, from 32 to 36% of the total measured amino acids existed in the serine, glutamine, asparagine combination (Table 2), depending upon N fertility level. Glutamic acid ranked second quantitatively (20%) and aspartic acid third (15%). Since each amide molecule in the serine, glutamine, asparagine combination contained one amino and one amide N, the combination contained from 43 to 48% of the N in the measured amino acids. The serine, glutamine, asparagine combination showed a significant increase with each increase in N fertility. Threonine, glutamic acid, and tyrosine showed a significant increase between the O and 57 Kg N fertility treatment only, while glycine, alanine, and lysine showed a significant increase only between the 57 and 113 Kg N treatments. All others showed a significant increase only between the 0 and 113 Kg N fertility levels, except ornithine, which showed no significant difference among the three N treatments.

Each amino acid quantity was greater in the phosphated thin juice than in the pressed juice, except for DOPA (Tables 2 and 3). This was possibly due to the hydrolyzation of some peptide molecules during preparation of the thin juice, and some amino acids may have absorbed on the sulfosalicylic acid precipitate formed in the pressed juice clarification before analysis. DOPA is not a peptide constituent and, therefore, it was not increased by the peptide hydrolysis; actually DOPA disappeared due to a separate reaction in which it was converted, possibly to dopamine or a quinone (9). The thin juice preparation process apparently altered some amino acids more than others. Evidence of this was shown in the change in relative quantities of some of the amino acids and changes in the significant differences between N treatments (Tables 2 and 3). The serine, glutamine, asparagine combination accounted for only 26 to 28% of the total measured amino acids in the thin juice, depending upon N fertility, while glutamic acid accounted for 22 to 25%. Glutamic acid showed the greater increase quantitatively due to some glutamine deamination and PCA conversion to glutamic acid. Aspartic acid also increased because of some asparagine deamination. Glycine and phenylalanine more than doubled quantitatively during the thin juice preparation. Glycine was the only thin juice amino acid which showed a significant difference with each N fertility increase (Table 3). Glutamic acid, tyrosine, and tryptophan showed a significant increase between the 0 and 57 Kg N treatment only. All other thin juice amino acids showed significant differences only between the O and 113 Kg N treatments, except threonine, proline, cystine, phenylalanine, ornithine, and arginine which showed no significant differences among N treatments.

Some thin juice amino acids showed large increases after basic hydrolysis (Table 4). Glutamic acid increased by 300 to 400%, depending upon N fertility; consequently, 62-63% of the total measured amino acids, after hydrolysis, was glutamic acid. Aspartic acid increased by 75 to 100%, and the combination of serine, glutamine,

Table 3.—Thin juice amino acids, from cultivar 'GW 359-52R' sugarbeets, grown at three N fertility levels, and mg N in each.

Amino acids (A.A.)	0 Kg N		57 Kg N		133 Kg N	
	mg A.A.	mg N	mg A.A.	mg N	mg A.A.	mg N
1. ASP[1]	99.2b[2]	10.4	141.1ab	14.8	178.6a	18.8
2. THR	19.1a	2.2	20.0a	2.3	64.1a	7.6
3. SER[3]	212.3b	38.4	330.9ab	59.9	400.3a	72.5
4. GLU	202.5b	19.3	277.3a	26.4	327.8a	31.2
5. PRO	16.5a	2.0	23.7a	2.9	23.4a	2.8
6. GLY	5.2c	1.0	8.1b	1.5	11.5a	2.1
7. ALA	20.3b	3.2	37.0ab	5.8	50.4a	7.9
8. VAL	19.0b	2.3	30.9ab	3.7	39.7a	4.7
9. CYS	1.4a	0.2	1.4a	0.2	1.9a	0.2
10. MET	5.4b	0.5	8.1ab	0.8	9.9a	1.0
11. ILE	38.8b	4.1	57.2ab	6.1	71.6a	7.6
12. LEU	33.1b	3.6	50.7ab	5.4	68.2a	7.3
13. DOPA	0.0	0.0	0.0	0.0	0.0	0.0
14. TYR	34.5b	2.7	81.3a	6.3	115.7a	8.9
15. PHE	4.6a	0.4	6.0a	0.5	5.8a	0.5
16. GABA	68.3b	9.2	84.8ab	11.5	101.2a	13.7
17. ORN	0.5a	0.1	0.5a	0.1	0.8a	0.2
18. LYS	5.5b	1.1	7.2ab	1.4	9.1a	1.7
19. HIS	4.0b	1.1	5.7ab	1.5	7.6a	2.0
20. TRY	9.7b	1.3	13.1a	1.7	16.3a	2.2
21. ARG	6.7a	2.1	12.3a	4.0	14.0a	4.4
TOTAL	806.6	105.2	1197.3	156.8	1517.9	197.3

[1]See Table 2.
[2]Thin juice A.A. means within the same row across the table followed by the same letter do not differ significantly at 5% according to Duncan's multiple range test; means for six replications in mg/100 KDS.
[3]Serine values are a combination of serine, glutamine, and asparagine.

and asparagine, which represented serine only after hydrolysis, decreased to 20 to 25% of the thin juice values. Glycine also increased during thin juice hydrolysis, possibly from some conversion of allantoin, a nitrogenous base in sugarbeet juices (12). Allantoin, with high pH and heat, will convert to urea and glyoxylic acid. Glyoxylic acid or the glyoxylate salt can then form glycine through a transamination reaction (6,12). GABA, the decarboxylation product of glutamic acid, decreased during hydrolysis. Arginine was converted completely, possibly to ornithine, which showed some increase, or to ornithine and citrulline (6). The latter was present only in the hydrolyzed thin juice.

Analysis of other thin juice N components indicated that each increase in N fertility caused a significant increase in total N and amino N (Table 5). Both NO_3-N and NH_4-N were significantly higher between the 0 and 113 Kg N treatments only. There was no significant difference in betaine N among the fertilizer treatments.

Table 4.—Hydrolyzed thin juice amino acids from cultivar 'GW 359-52R' sugarbeets, grown at three N fertility levels, and mg N in each.

Amino acids (A.A.)	0 Kg N		57 Kg N		113 Kg N	
	mg A.A.	mg N	mg A.A.	mg N	mg A.A.	mg N
1. ASP[1]	169.1[2]	17.7	198.5	20.9	283.0	29.8
2. THR	14.2	1.6	17.0	2.0	24.7	2.9
3. SER	55.4	7.3	69.9	9.3	101.8	15.6
4. GLU	850.0	80.9	1144.0	108.9	1492.7	142.1
5. PRO	occ[3]	occ.	occ.	occ.	occ.	occ.
6. GLY	17.1	3.2	18.9	3.5	26.7	5.0
7. ALA	24.1	3.8	34.0	5.3	51.4	8.0
8. VAL	20.3	2.4	28.5	3.4	39.3	4.6
9. CYS	0.0	0.0	0.0	0.0	0.0	0.0
10. MET	5.9	0.6	7.9	0.8	11.0	1.1
11. ILE	40.9	4.3	53.4	5.7	72.4	7.7
12. LEU	37.4	4.0	51.3	5.5	71.6	7.6
13. DOPA	0.0	0.0	0.0	0.0	0.0	0.0
14. TYR	36.4	2.8	78.0	6.0	119.8	9.3
15. PHE	6.4	0.6	6.7	0.6	7.7	0.7
16. GABA	61.7	8.4	67.1	9.1	78.3	10.6
17. ORN	2.1	0.5	3.2	0.7	3.9	0.8
18. LYS	5.8	1.1	7.2	1.4	9.7	1.8
19. HIS	2.4	0.7	2.5	0.7	2.3	0.7
20. TRY	9.9	1.4	22.2	3.1	15.2	2.1
21. ARG	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL	1359.1	141.3	1810.3	186.9	2411.5	248.4

[1]See Table 2.
[2]Means for six replications in mg/100 RDS
[3]Occluded peaks.

Table 5.—Thin juice N, before and after hydrolysis, for four N sources, the sum of N from the four sources, and the Kjeldahl total N for sugarbeet cultivar 'GW 359-52R' at three N fertility levels.

N Source	0 Kg N		57 Kg N		113 KgN	
	Thin juice	Hyd[1] T.J.	Thin juice	Hyd T.J.	Thin juice	Hyd T.J.
NO_3-N	31.1b[2]	32.4	92.4ab	97.1	115.5a	130.5
NH_4-N	22.0b	6.3	37.2ab	6.0	43.9a	7.0
Betaine N	140.1a	140.5	148.8a	147.6	146.2a	147.4
Amino N	120.5c	141.9	184.3b	203.6	235.2a	254.4
Sum (N Sources)	313.7	321.1	462.7	454.3	540.8	539.3
Kjeldahl Total N	389.9c	342.4	563.5b	481.8	694.3a	561.6

[1]Hydrolyzed thin juice
[2]Means within the same row across the table for thin juice values followed by the same letter do not differ significantly at the 5% level according to Duncan's multiple range test; means for six replications in mg/100 RDS.

The Kjeldahl total N was greater than the sum of the N from the four N sources at each fertility level (Table 5). This indicated that some N existed in compounds not determined in this study, including the N in the unmeasured and unknown amino acids and analogs. The amino N, given in Table 5, was also greater, at each N level, than the total thin juice amino acid N shown in Table 3, because of the amino N in the unmeasured amino acids. Some of the ammo N differences,in Tables 3 and 5, may have been due to an incorrect estimation of the serine, glutamine, asparagine combination amino N quantity. The difference in the two amino N values after hydrolysis was much less, possibly because the amino acid N, after hydrolysis, was more accurately measured; also the PCA nitrogen was measured in both determinations as amino N. The NO_3-N showed some increase after hydrolysis especially at the 57 and 113 Kg N fertility levels. The NH_4-N decreased because of the ammonia gas lost during hydrolysis. The N loss due to ammonia gas was greater than indicated by the difference between the two measured NH_4-N quantities because considerable ammonia gas, formed from the hydrolyzation of the amides, was immediately expelled from the hot alkaline solution. Some ammonia N loss possibly resulted, also, from some decomposition of the nitrogenous bases, choline and allantoin, and some amines, which may have been present in minor quantities (12). Betaine, the stable nitrogenous base, apparently was unaffected by the hydrolysis.

If we use the thin juice Kjeldahl total N as 100%, NO_3-N accounted for 8.0% of the total N before hydrolysis at the O Kg N level, 16.4% at the 57 Kg N level, and 16.6% at the 113 Kg N level. Likewise, NH_4-N accounted for 5.6%, 6.6%, and 6.3%; betaine N accounted for 35.9%, 26.4%, and 21.1%; and amino N accounted for 30.9%, 32.7%, and 33.9%, respectively. Before hydrolysis, the nitrogen in the PCA was included in the total N but not in the amino N. After PCA was hydrolyzed to glutamic acid, its N was included in the amino N, and the amino N then contributed 36.4%, 36.1%, and 36.6% of the original thin juice Kjeldahl total N depending upon the N fertility level. The N from the four N sources accounted for 80.5%, 82.1%, and 77.9%, respectively, of the Kjeldahl total N before hydrolysis. Again the N in the PCA was not included in the sum from the four N sources. After hydrolysis, the N from the four N sources accounted for 93.8%, 94.3%, and 96.0%, respectively, of the hydrolyzed thin juice Kjeldahl total N. The hydrolyzed thin juice Kjeldahl total N was 87.8%, 85.5%, and 80.9%, respectively, of the thin juice Kjeldahl total N before hydrolysis. This difference apparently indicated the N lost as ammonia gas during hydrolysis. This seems high, but it also emphasizes the apparently large amount of amides and PCA which exist in the sugarbeet juices.

If we assume that the difference between the thin juice Kjeldahl total N values, before and after hydrolysis, was due to the N lost in the ammonia gas, from 4.0 to 6.3% of the total N is not accounted for in the hydrolyzed juice. But, if we use only the unhydrolyzed

thin juice values, from 22.1% to 17.9% of the total N was unaccounted for, however, some of this missing N was in the PCA. Stark *et al.* (18) reported the presence of some purines, pyrimidines, and nucleosides in sugarbeet juices which could account for part of the missing N. McGinnis (12) states that the purine (xanthine) bases account for 1 to 2% of the sugarbeet juice nitrogenous compounds. The amino purines reported present by McGinnis (12) were adenine, guanine, xanthine, and hypoxanthine. Grouped with these were the pyrimidines (uracil and cytosine), the ribosides (guanosine, uridine, and cytidine), and the desoxyriboside (thymidine). All are nitrogenous compounds, and none, apparently, are removed during juice purification (12). Allantoin and choline, mentioned previously, are nitrogenous bases present usually in minor quantities with betaine, the important stable nitrogenous base which was determined in this study.

This study accounted for and emphasized the importance of the N constituents in the sugarbeet juices. In phosphated thin juice the measured amino acids accounted for 0.8 to 1.5% of the RDS, depending upon N fertility (Table 3). After deamination of the amides and conversion by basic hydrolysis, 1.4 to 2.5% of the RDS was due to the measured amino acids (Table 4). Betaine, the second important N constituent, averaged 1212.5 mg/100 RDS or 1.2% in the thin juice. The RDS percentage due to N components would be increased somewhat if the nitrate and ammonium constituents, the other nitrogenous bases, and the purines, pyrimidines, and nucleosides were included.

Summary

Pressed and phosphated thin juices, from sugarbeets grown at three nitrogen fertility levels, were analyzed for nitrogenous constituents. The relative quantity of the total N in each type of compound, the effect of nitrogen fertilization on these compounds, and the chemical changes involving the nitrogen constituents were assessed. Twenty-one individual amino acids and amides were determined by automated analysis. Some unknown amino acids and analogs present were not determined; however, some were later identified. Amino, ammonium, betaine, nitrate, and total N were also measured.

The following results were obtained:

1. In pressed juice, the serine, glutamine, asparagine combination showed the greatest relative differences between nitrogen fertility levels. In phosphated thin juice, glycine showed the greatest relative differences among nitrogen fertility levels, although several other amino acids showed larger quantitative differences.
2. Thin juice total N and amino N increased significantly with increasing N fertility. Betaine N showed no significant difference among N treatments.
3. All measured amino acids, except DOPA, increased when pressed juice was processed to make phosphated thin juice, but all did not increase in the same proportion.

4. In pressed juice, the serine, glutamine, asparagine combination accounted for about 34%, glutamic acid 20%, aspartic acid 15% and GABA 7% of the total measured amino acids, with some difference among the N fertility levels.
5. In phosphated thin juice, the serine, glutamine, asparagine combination accounted for about 27%, glutamic acid 24%, aspartic 12%, and GABA 7% of the total measured amino acids.
6. After the amides and PCA were converted to their respective amino acids by basic hydrolysis, glutamic acid accounted for almost 63%, aspartic 12%, serine 4%, and GABA 4% of the total amino acids measured.
7. Amino N accounted for between 31 to 34% of the total N in thin juice; betaine N accounted for 36% at low N fertility, and 21% at high N fertility.
8. From 18 to 20% of the thin juice total N was not accounted for, but part of the missing N was in the PCA.
9. The thin juice total N, after basis hydrolysis, was 88% of the total N before hydrolysis, at the low N fertility level, and 81% at the highest N level. The difference apparently indicated the N lost as ammonia gas during the basic hydrolysis.
10. From 4 to 7% of the hydrolyzed thin juice total N was not identified.

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