The Behavior of Amino Acids in Chromatographic Molasses Desugarization Systems

by

D. Eugene Rearick
and
Cheri McKay

AMALGAMATED RESEARCH INC.
TWIN FALLS, IDAHO

presented at
1996 Conference on Sugar Process Research
New Orleans, LA
April 14-17, 1996

Proceedings of the Conference on Sugar Processing Research
New Orleans, April, 1996

THE BEHAVIOR OF AMINO ACIDS IN CHROMATOGRAPHIC MOLASSES DESUGARIZATION SYSTEMS

D. Eugene Rearick, Cheri McKay
Amalgamated Research Inc.
Twin Falls, Idaho

ABSTRACT

Free amino acids in sugarbeet molasses demonstrate a variety of separation characteristics during molasses desugarization by ion exclusion chromatography. Several amino acids are easily removed with raffinate while others behave more like sucrose and elute in the product fraction. Several amino acids appear to accumulate within simulated moving bed desugarization systems. The relationship between amino acid separation characteristics and ionic properties will be discussed.

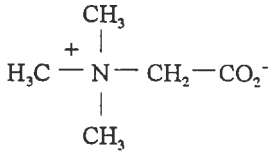
INTRODUCTION

Sugarbeet molasses contains significant quantities of nitrogen compounds including free α-amino acids. Typical levels of α-amino acids and related nitrogen compounds are given in Table 1.

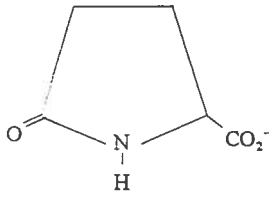
Together the nitrogen compounds listed in Table 1 make up 30-35% of the non-sucrose components of sugarbeet molasses and thus have a significant effect on the desugarization of molasses using simulated moving bed ion exclusion chromatography.

As discussed previously, strongly ionized salts and high molecular weight organic compounds are very efficiently separated from sucrose in the ion exclusion process[1]. The common nitrogen compounds listed in Table 1, however, show a variety of behavior during ion exclusion chromatography, undoubtedly due to the wide variation in ionic properties. Betaine (1), being one of the predominant components of sugarbeet molasses, is naturally of great interest and its separation properties have been mentioned previously[1]. Betaine at neutral to slightly basic pH values exists as the zwitterion shown in (1). This species is ionic but evidently due to its net zero charge behaves somewhat like a small neutral molecule. Betaine is eliminated with raffinate (salts and large molecules) to the extent of approximately 65% in factory systems, as opposed to elimination values of 95% or higher for common inorganic salts. The relatively large fraction (35%) of betaine which elutes with sucrose is probably due to the behavior of betaine like a neutral molecule.

PCA (2-pyrrolidone-5-carboxylic acid) is also a significant component of sugarbeet molasses, arising from the loss of ammonia from glutamine during juice purification. PCA, though, has only one functional group (carboxylic acid) that is easily ionized under chromatographic conditions and exists in neutral solution as the carboxylate anion (2). This species behaves like any other anionic substance and elutes with salts in the raffinate (at 95-98% elimination).

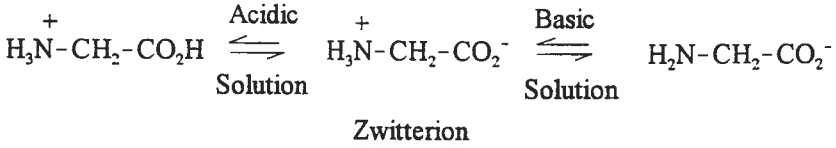


1



2

Unlike betaine which is locked in a zwitterionic structure by the quarternary ammonium salt functional group, and PCA, which behaves like a simple carboxylic acid due to the lack of a basic amine function group, the α-amino acids and γ-aminobutyric acid possess both basic and acidic functional groups and can exist as an anion, cation, or doubly charged zwitterion depending on solution pH. This is illustrated for the simplest α-amino acid, glycine, which can exist as the zwitterion 3. The solution pH at which a particular amino acid exists predominantly as the zwitterion,



3

with a net zero charge, is referred to as the *isoelectric point* of that amino acid. Because the 15 amino acids commonly found at significant levels in sugarbeet molasses possess a variety of structural differences and differing isoelectric points, they demonstrate a full range of separation characteristics in ion exclusion process chromatography. The observed behavior in process-scale systems, which can range from very efficient elimination with raffinate to poor elimination and elution with sucrose, will be the topic of the remainder of this discussion.

DISCUSSION

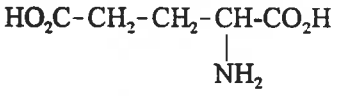
Amino Acid Elimination in Ion Exclusion Chromatography

Typical levels of amino acids in sugarbeet molasses are given in Table 2 which shows values in feed molasses and sucrose eluent stream (extract) for a factory simulated moving bed separation system over an eight hour period. Levels are given in both percent based on refractometric dissolved solids and percent based on non-sucroses. Notice that two amino acids, glutamic acid and aspartic acid, are present at much lower levels (based on solids) in extract than feed while others such as serine, tyrosine. valine, and isoleucine are actually present at significantly higher levels in extract. Amino acid levels calculated based on non-sucroses show these differences even more dramatically. Glutamic and aspartic acid levels based on non-sugars, show some decrease across the separator indicating that they are removed somewhat more efficiently than total non-sucroses (which are removed at levels of about 87% in this example). Most other amino acids, however, are actually present at higher levels, based on non-sucroses, in extract than in feed molasses. Higher levels of

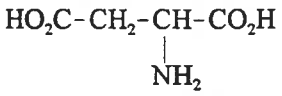
amino acids arise from these substances being more poorly eliminated than other non-sucroses resulting in enrichment of the extract stream non-sucroses in amino acids. Notice that the total amino acids in feed molasses make up approximately 9% of non-sucroses while in the extract stream 31% of the non-sucroses present are amino acids. Notable examples of amino acids that are concentrated with respect to non-sucroses are serine, alanine, tyrosine, valine, and isoleucine all of which go from under 1%/NS in molasses to 3%/NS or higher in separator extract. Level increases for amino acids, based on non-sucroses, are also shown graphically in Figure 1. Material balance calculations for the individual amino acids give the percent elimination values, that is the percent of entering amino acid that does *not* exit with extract, shown in the last column of Table 2 and graphically in Figure 2. Elimination values range from a high of 94% for glutamic acid to a low of 13% for tyrosine.

Amino acids as a group are of great practical interest in ion exclusion chromatographic systems because of their overall poor separation characteristics which result in enrichment of extract in some members of the group. Obviously any such poorly separated constituent has the potential to accumulate in factory systems if separator streams are recycled. In addition individual amino acids may cause specific problems at high levels; tyrosine, for example, is low enough in solubility to crystallize from stored syrups. Another area of interest, and the main topic of this discussion, is the relationship between amino acid properties and separation characteristics.

Amino acid elimination values are somewhat variable but if mean values for three separate tests are plotted versus amino acid isoelectric point the relationship shown in Figure 3 is obtained. Notice that percent elimination is at a minimum for amino acids with isoelectric points in the range of 5.6 to 6.0 while amino acids with isoelectric points outside this range are eliminated more efficiently. This seems to indicate that the ion exclusion system sees amino acids in this range of isoelectric point as small neutral molecules which diffuse into resin beads and travel with sucrose. Composite pH values in the separator are actually higher than this 5.6-6.0 range and amino acids in this isoelectric point range would be expected to be somewhat anionic under separation conditions but there is a pH gradient within such a system and the full effects of pH versus separation characteristics have not been investigated. The amino acids at the extremes of the plot in Figure 3 probably have a higher net ionic charge under separator conditions and are thus excluded from the resin. For example glutamic acid (4) and aspartic acid (5) have low isoelectric points due to the second carboxylic acid group

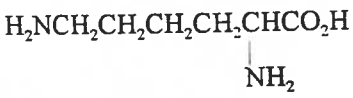


Glutamic Acid
4

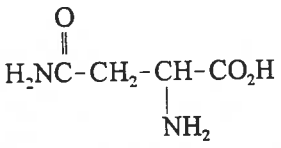


Aspartic Acid
5

present in each molecule. At separator pH levels, far above their isoelectric points, these two species would be predominantly anionic and are always efficiently separated. In contrast, lysine (6) with its high isoelectric point due to a second amine functional group would be expected to be predominantly cationic and has a consistent fairly high elimination value. Asparagine (7) shows a high elimination value even though its isoelectric point is 5.4 but this may be partly due to chemical elimination rather than chromatographic separation. Asparagine can hydrolyze to ammonia and aspartic acid and this may occur to some extent in the separator system. In any event, consistent poor separation of amino acids seems confined to those with isoelectric points between 5.6 and 6.0 probably due to behavior



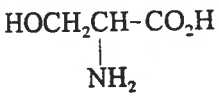
Lysine
6



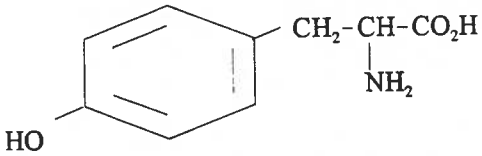
Asparagine
7

like a neutral molecule as discussed above. The possibility that molecular size also affects separation has been considered but does not seem to be related to elimination values. For example two of the least efficiently eliminated amino acids are serine (8), a 3-carbon amino acid with a molecular weight of only 105, and tyrosine (9), one of the largest amino acid molecules with a molecular weight of 181. These two amino acids with much different molecular sizes but nearly the same isoelectric point behave very similarly in an ion exclusion separation system.

Amino Acid Accumulation in Separation Systems



Serine
8



Tyrosine
9

In addition to their separation characteristics as shown in elimination data, the common amino acids demonstrate another interesting behavior, that of accumulation within a simulated moving bed system. The internal inventory of a component in a separation system at equilibrium conditions can be higher than the level in the feed stream provided that during some period of non-equilibrium operation (such as during start-up) the outflow of the component is less than the amount entering. For example, if parameters are set to hold down the amount of sucrose leaving the system, the internal composite sucrose purity can rise to levels above that of the feed stream. In the system from which the data in Table 2 was collected, the purity of a composite representing all material in the internal separation profile was approximately 67 g sucrose/100 g RDS while the feed molasses was 61 purity. This behavior can also be seen with non-sucrose components and, in fact, several of the amino acids accumulate inside the separation system to a greater extent than any other single component investigated. Table 3 and Figure 4 show, again for the same test as data in Table 2 the level of each amino acid in a composite of all material within the separation system. Levels in feed molasses are shown again for comparison. Also given are ratios of the internal composite amino acid level (based on non-sucroses) to the level in feed molasses. Notice that asparagine and threonine are found at levels of approximately four times that of the feed molasses. Serine and alanine are also present at

elevated levels, about twice as high as the feed. Again measured levels vary somewhat from test to test but threonine, serine, and asparagine are consistently greatly elevated in the internal composite while all amino acids except aspartic acid and glutamic acid are consistently elevated to some extent. Total amino acid level in the internal composite is 13.0 g/100 g NS as opposed to 8.9 g/100 NS in feed molasses. Also notice that the level of internal accumulation for a particular amino acid is not necessarily related to percent elimination: tyrosine is eliminated poorly but does not accumulate to extremely high levels while asparagine accumulates but is eliminated well (although chemical reaction is a possible route of partial elimination). Internal accumulation seems to be an indication of how well a component initially fits into the separation profile without totally eluting in either stream rather than a function of which stream it ultimately elutes with. It seems likely, though, that this internal accumulation of particular non-sucrose components must have some effect on separation system performance with respect to system loading and efficiency.

EXPERIMENTAL

All samples were collected over an eight hour period from a factory ion exclusion system processing sugarbeet molasses. Amino acids were converted to phenylthiocarbamyl derivatives by reaction with phenylisothiocyanate[2,3]. Derivatives were analyzed by HPLC using the Waters Pico Tag™ method (triethylamine sodium acetate/60% acetonitrile gradient).

ACKNOWLEDGMENTS

The authors would like to acknowledge the technical support of Stephanie Olmstead and the initial amino acid analysis developmental work of Mary Little.

REFERENCES

1. Rearick, D.E. (1995). Non-sugar removal in the molasses and raw juice chromatographic separation processes. Paper presented at the 28th General Meeting of American Society of Sugar Beet Technologists.

2. Bidlingmeyer, B. A., S. A. Cohen, and T. L. Tarvin. J. Chromatogr., 336, 93 (1984).

3. Inglis, A. S., N. A. Bartone, and J. R. Finlayson. J. Biochem. Biophys. Methods, 15, 249, (1988).

Table 1. Nitrogen Compounds in Sugarbeet Molasses (g/100 g non-sucrose)

Betaine	15-17
Total α-amino acids	7-8
Pyrrolidone carboxylic acid (PCA)	8-9
γ-amino butyric acid (GABA)	0.5-2.0

Table 2. Amino Acid Levels and Separator Elimination

Amino Acid	Amino Acid Level				Elimination
	Feed Molasses	Extract	Feed Molasses	Extract	
	g/100 RDS		g/100 NS		g/100 g entering
Aspartic acid	0.461	0.093	1.18	0.952	90
Glutamic acid	0.598	0.071	1.54	0.727	94
Asparagine	0.203	0.104	0.522	1.06	74
Serine	0.274	0.478	0.704	4.38	20
Glycine	0.075	0.104	0.193	1.06	29
γ-aminobutyric acid	0.243	0.247	0.881	2.53	63
Threonine	0.074	0.093	0.190	0.952	35
Alanine	0.215	0.301	0.552	3.08	28
Proline	0.181	0.110	0.465	1.13	69
Tyrosine	0.302	0.510	0.776	5.22	13
Valine	0.178	0.290	0.457	2.97	16
Isoleucine	0.252	0.384	0.647	3.93	22
Leucine	0.228	0.269	0.586	2.75	39
Phenylalanine	0.044	0.022	0.113	0.225	74
Lysine	0.044	0.022	0.113	0.225	75
Total			***8.92***	***31.2***	

Table 3. Composite Amino Acid Levels in the Separation System

Amino Acid	Feed Molasses Level	Internal Composite Level	Ratio
	g/100 g NS		Internal Composite /Feed
Aspartic acid	1.18	1.40	1.2
Glutamic acid	1.54	1.28	0.83
Asparagine	0.522	1.86	3.6
Serine	0.704	1.27	1.8
Glycine	0.193	0.276	1.4
γ-aminobutyric acid	0.881	1.13	1.3
Threonine	0.190	0.831	4.4
Alanine	0.552	1.28	2.3
Proline	0.465	0.534	1.2
Tyrosine	0.776	0.870	1.1
Valine	0.457	0.456	1.0
Isoleucine	0.647	0.792	1.2
Leucine	0.586	0.792	1.4
Phenylalanine	0.11	0.14	1.2
Lysine	0.11	0.14	1.2
Total	***8.92***	***13.0***	

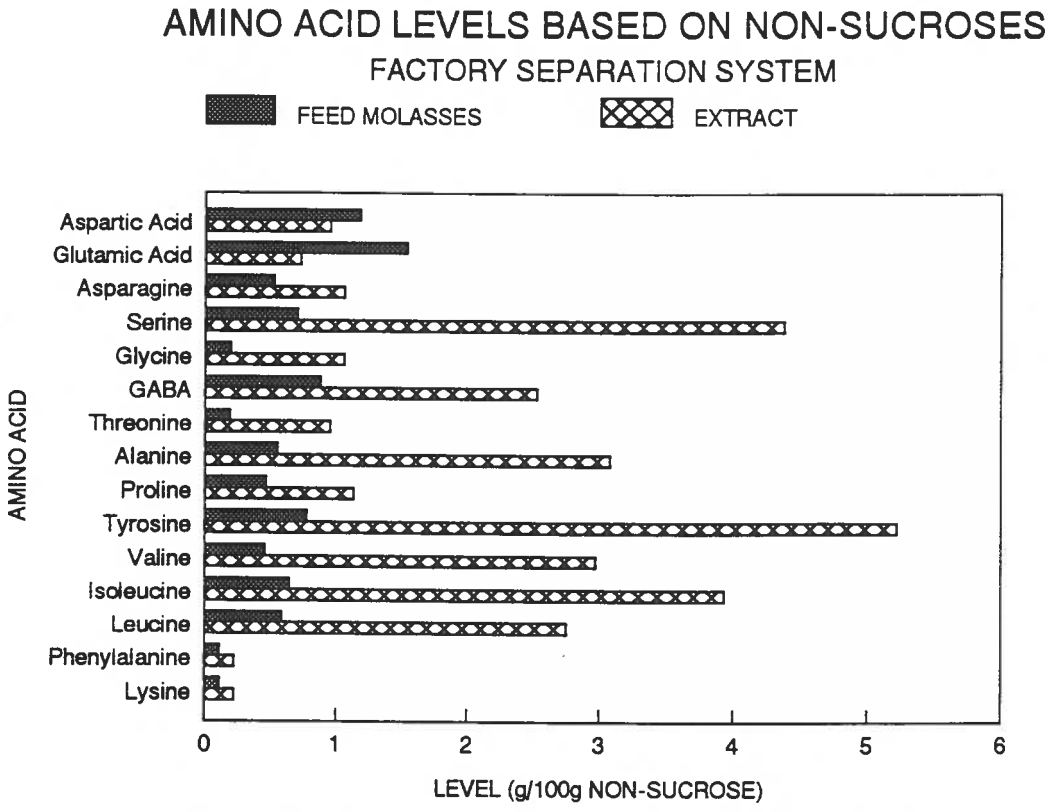


Figure 1. Amino Acid Levels in Molasses and Extract

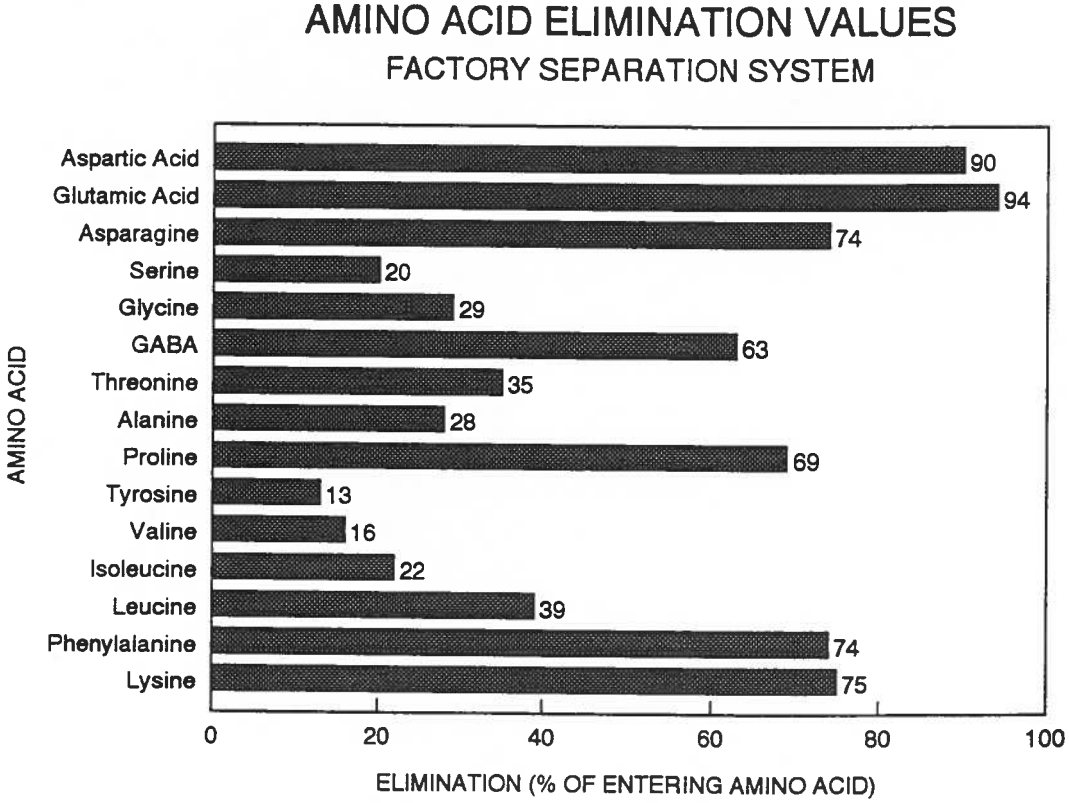


Figure 2. Amino Acid Elimination Values

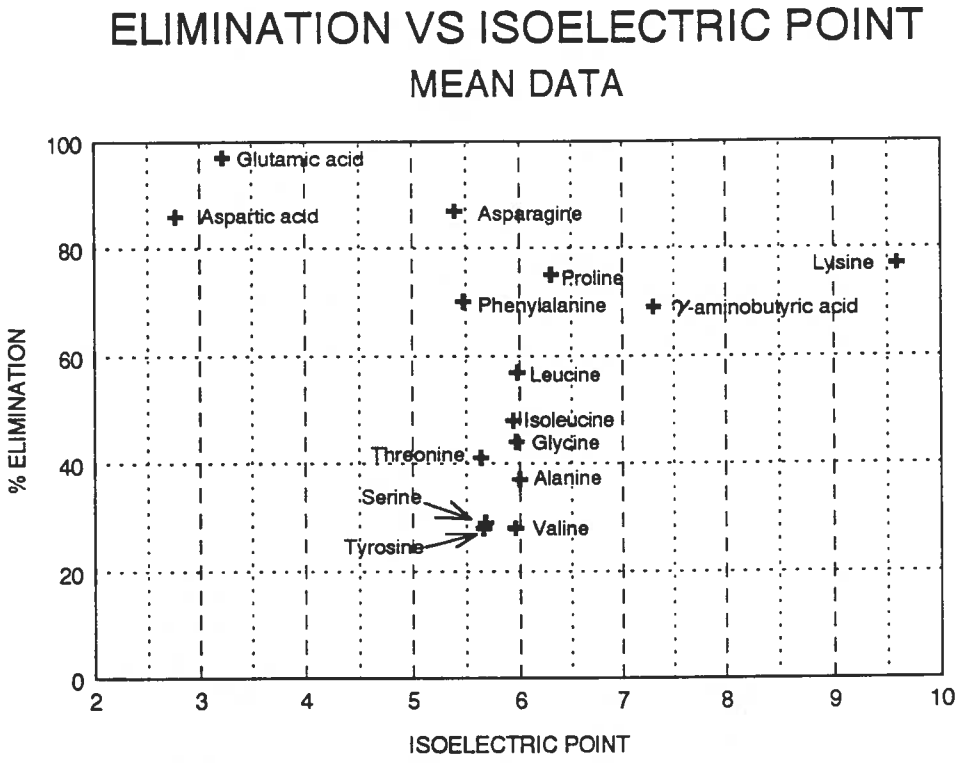


Figure 3. Percent Elimination Versus Isoelectric Point

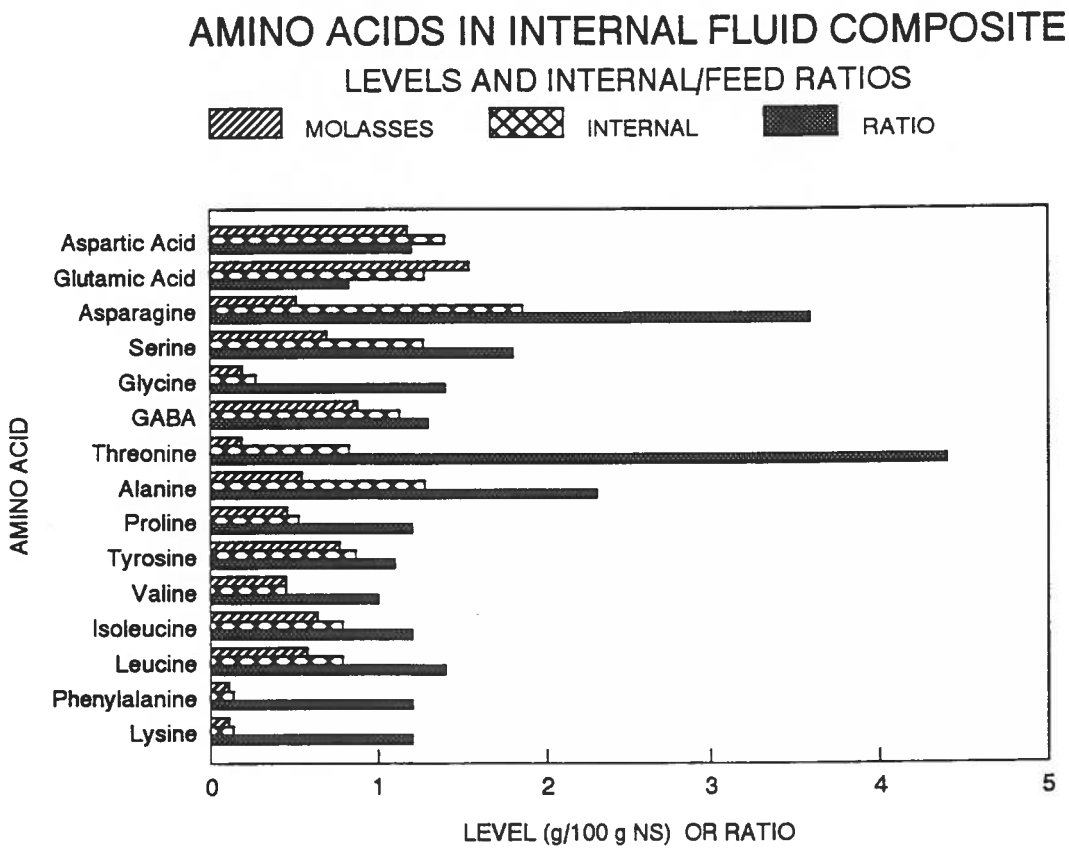


Figure 4. Amino Acid Internal Levels and Ratios