ANALYTICAL SOLUTIONS FOR THE ANALYSIS OF BOUND AND FREE AMINO ACIDS IN FOODS AND FEEDS

Antonietta Gledhill[1], Brad Barrett[2], Hillary B. Hewitson[2], Thomas E. Wheat[2]
[1]Waters Corporation, Manchester, United Kingdom; [2]Waters Corporation, Milford, MA, USA

INTRODUCTION

The analysis of amino acids in foods and feeds is one of the most useful characterizations of these sample types. Quantitation of the amino acids released by hydrolysis is one important measure of nutritional value. A free amino acid profile can identify the origin of a food product, and in that way, detect adulteration. Free amino acids are also metabolic indicators that can be used to monitor and optimize processes such as fermentation.

The Waters® UPLC® Amino Acid Analysis Solution is a total system solution that can be used in all of these applications. It combines the well-established AccQ•Tag™ pre-column derivatization with the increased resolution and performance of the ACQUITY UPLC® system to assure accurate and precise qualitative and quantitative results.

The present study focuses on the nutritional analysis of foods and feeds. To confirm the accuracy of the determination, the proportions of amino acids in a pure protein were measured so that the experimental results can be compared to a known true result. The chromatographic method is then evaluated for the typical amino acids encountered in feed analysis. Because of the importance of sulfur-containing amino acids, a sample of chicken feed was analyzed with and without performic acid oxidation. Finally, the robustness of the method was examined in a collaborative study with four different feed types. Precision was assessed for each step in the analysis over a series of five days. The results of these experiments demonstrate the suitability of this analytical solution for assessing the amino acid nutritional value of feed samples.

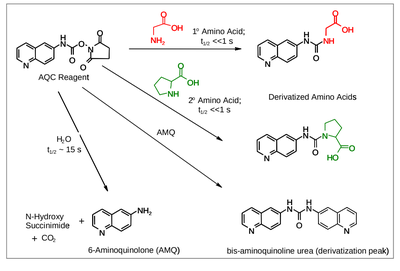

Figure 1. In the AccQ•Tag derivatization, the AQC reagent reacts quickly with unprotonated primary and secondary amino acids in a largely aqueous environment to form stable derivatives that are readily detected with UV. The excess reagent reacts with water on a slower time scale to form byproducts that are easily separated from the amino acids in the analysis.

METHODS

Sample Preparation and Derivatization
Hydrolyzed Bovine Serum Albumin (BSA) was supplied at an estimated concentration of 1.0 mg/mL. Hydrolyzed BSA was diluted 1:10 with 0.1 M HCl prior to derivatization.

Pelletized chicken feed was ground to a fine powder and hydrolyzed with and without performic acid oxidation, according to AOAC Official Method 994.12. Hydrolyzed samples were diluted 1:10 with 0.1 M HCl prior to derivatization.

Swine diet, poultry diet, whole soybean, and soybean meal samples were acid-hydrolyzed in an independent laboratory as part of a collaborative study. The samples were supplied at an estimated concentration of 1.0 mg/mL in 0.1 M HCl and sealed under argon in ampules. The standard was NIST 2389 Amino Acids in 0.1 mol/L HCl Reference Material. The feed samples were diluted 1:16 and the with 0.1 M HCl prior to derivatization.

The standard derivatization protocol was modified to include neutralization of excess acid with 0.1 M NaOH. The samples were derivatized in batches according to Figure 2, and are stable at room temperature for one week when tightly capped.

Pre-column derivatization and analysis conditions are described in detail in the Waters UPLC Amino Acid Analysis Solution System Guide (P/N 71500129702).

Chromatographic Conditions

LC System:	Waters ACQUITY UPLC System
Column:	AccQ·Tag Ultra, 2.1 x 100 mm, 1.7 µm
Column Temp:	55 °C
Sample Temp:	20 °C
Flow Rate:	700 µL/min.
Mobile Phase A:	1:20 Dilution of AccQ•Tag Ultra Eluent A
Mobile Phase B:	AccQ·Tag Ultra Eluent B
Weak Needle Wash:	95:5 Water:Acetonitrile
Strong Needle Wash:	5:95 Water:Acetonitrile
Gradient:	AccQ·Tag Ultra Hydrolysate Method (see UPLC Amino Acid Analysis Solution Guide)
Total run time:	9.5 min
Injection volume:	1 µL, Partial Loop with Needle Overfill
Detection:	UV (TUV), 260nm

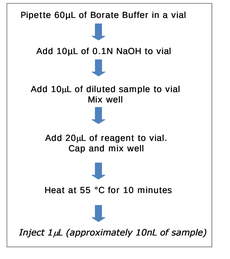

Figure 2. Steps in derivatization for UPLC Amino Acid Analysis

RESULTS

Amino Acid Analysis as a Process Indicator

Free amino acids are metabolic intermediates that can reflect the origin and identity of a food product. The levels of these compounds change during processing. Determination of the amino acid profile of a final food product provides information, therefore, about both the raw materials and the processing.

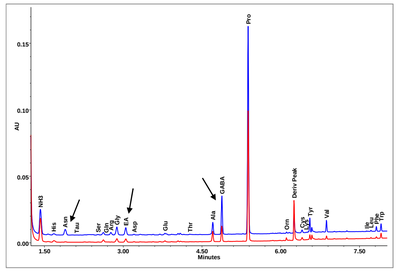

Figure 3. Comparison of two different brands of pale ale beer. Differences in the amino acid profile are seen by the varying amount of such amino acids as asparagine (Asn) and γ-amino butyric acid (GABA). Note especially that the ethanolamine (EA) concentration is different. This compound, that critically affects the flavor profile, is readily separated and quantitated. These differences are the result of both the starting material and the process control for the two brands.

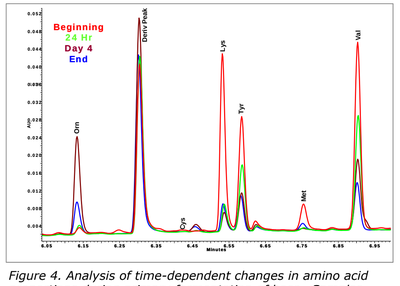

Figure 4. Analysis of time-dependent changes in amino acid proportions during primary fermentation of beer. Samples were taken at intervals during a primary fermentation of a homebrew. Most amino acids are depleted during the fermentation, as shown in this region of the chromatogram. Ornithine (Orn), however, increases to a maximum level on Day 4, and then declines. The amino acid levels reflect the total metabolic processes of the yeast cells that can affect the quality of the final product. By monitoring and measuring these changes, fermentations can be optimized. Timely process control decisions are also possible.

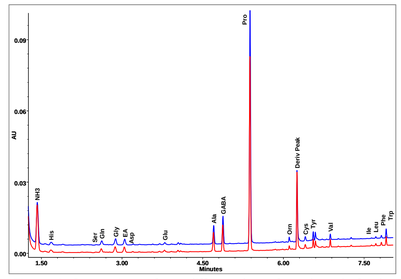

Figure 4. Comparison of two different lots of one brand of pale ale. The lot-to-lot consistency of the amino acid profile provides an indication of process events that affect the quality of the final product.

Amino Acid	Lot 1 nmoles/mL ± Std Dev	Mole %	Lot 2 nmoles/mL ± Std Dev	Mole %
His	81.25 ± 0.85	1.55	91.40 ± 1.35	1.56
Asn	18.83 ± 0.69	0.36	19.18 ± 0.46	0.33
Ser	12.45 ± 0.91	0.24	11.55 ± 0.62	0.20
Arg	5.94 ± 0.46	0.11	4.49 ± 0.26	0.08
Gly	156.58 ± 3.02	2.99	169.21 ± 1.43	2.90
EA	146.13 ± 2.40	2.79	156.45 ± 4.50	2.68
Asp	16.42 ± 1.27	0.31	14.11 ± 0.22	0.24
Glu	49.06 ± 1.47	0.94	49.22 ± 0.40	0.84
Thr	5.58 ± 1.61	0.11	3.54 ± 0.12	0.06
Ala	357.08 ± 6.09	6.81	342.39 ± 1.89	5.86
GABA	475.44 ± 8.68	9.07	487.51 ± 2.22	8.34
Pro	3479.25 ± 42.23	66.40	4091.81 ± 14.83	70.03
Orn	36.70 ± 1.26	0.70	45.19 ± 0.34	0.77
Tyr	100.27 ± 1.99	1.91	99.40 ± 0.39	1.70
Met	13.12 ± 1.64	0.25	12.91 ± 2.13	0.22
Val	101.21 ± 1.73	1.93	73.73 ± 0.76	1.26
Ile	12.87 ± 0.39	0.25	9.49 ± 0.34	0.16
Leu	26.03 ± 0.41	0.50	19.58 ± 0.31	0.34
Phe	41.36 ± 0.55	0.79	34.08 ± 0.29	0.58
Trp	104.12 ± 1.88	1.99	107.50 ± 1.17	1.84
Total	5239.70		5842.74	

Table 1. Quantitative comparison of one brand of pale ale. The total concentration of free amino acids in the two lots differs by approximately 10%. The relative proportions of free amino acids remain fairly constant, shown as Mole % in the table. This consistency indicates a stable, well-controlled production process.

Nutritional Content of Animal Feeds

The amino acid analysis method must give reliable results for a range of sample types that could compromise the derivatizations or the chromatographic analysis. The same result must be obtained for all repeated tests. Four types of hydrolyzed animal feed were provided as part of a collaborative study. The samples were analyzed on each of five days. On each day, each hydrolysate was derivatized five separate times, and each derivatizations was injected three times. Two different columns and five different bottles of eluent were used for the study.

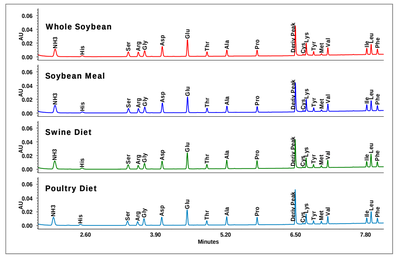

Figure 5. Chromatographic analysis of four animal feed hydrolysates. The different matrices have no effect on retention time and show no interfering peaks.

Amino Acids	Whole Soybean	Soybean Meal	Swine Diet	Poultry Diet
His	1.593 ± 0.027	1.867 ± 0.030	1.966 ± 0.028	1.848 ± 0.030
Ser	3.352 ± 0.016	3.650 ± 0.019	3.508 ± 0.022	3.608 ± 0.019
Arg	5.285 ± 0.025	5.822 ± 0.030	4.693 ± 0.027	5.178 ± 0.026
Gly	2.595 ± 0.016	2.976 ± 0.016	2.814 ± 0.018	3.522 ± 0.017
Asp	8.092 ± 0.037	9.061 ± 0.052	7.097 ± 0.043	7.629 ± 0.039
Glu	13.219 ± 0.059	14.489 ± 0.076	14.145 ± 0.087	14.114 ± 0.063
Thr	2.504 ± 0.013	2.918 ± 0.016	2.732 ± 0.017	2.819 ± 0.014
Ala	2.647 ± 0.013	3.172 ± 0.019	4.057 ± 0.027	4.065 ± 0.018
Pro	3.423 ± 0.022	3.662 ± 0.026	5.111 ± 0.034	5.037 ± 0.029
Cys	0.233 ± 0.004	0.269 ± 0.004	0.235 ± 0.003	0.241 ± 0.003
Lys	4.125 ± 0.027	4.674 ± 0.033	3.784 ± 0.028	3.955 ± 0.026
Tyr	2.525 ± 0.010	2.930 ± 0.016	2.485 ± 0.014	2.593 ± 0.013
Met	0.862 ± 0.020	1.092 ± 0.015	1.331 ± 0.017	1.023 ± 0.012
Val	3.152 ± 0.016	3.663 ± 0.019	3.540 ± 0.023	3.028 ± 0.019
Ile	3.014 ± 0.014	3.446 ± 0.019	2.946 ± 0.018	3.015 ± 0.015
Leu	5.307 ± 0.025	6.122 ± 0.033	7.472 ± 0.044	7.012 ± 0.034
Phe	3.459 ± 0.016	3.919 ± 0.022	3.773 ± 0.024	3.753 ± 0.020

Table 2. Quantitative analysis of four animal feed types, expressed as weight percents. The precision is expressed as absolute standard deviation. The results represent 75 independent determinations. The variability across all amino acids, for all four samples, is well under 1% RSD.

Nutritional Content of Milk and Infant Formula

Human infant formula is a processed food product most often prepared from bovine milk. It must satisfy nutritional requirements that differ from those of the dairy animal.

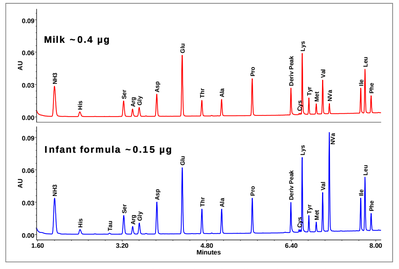

Figure 6. Comparison of hydrolyzed milk and infant formula. The relative amounts of the amino acids are similar, with the addition of the essential Taurine (Tau) to the formula.

Amino Acid	Milk Average	Milk % RSD	Infant Formula Average	Infant Formula % RSD
His	0.602	0.56	0.600	0.24
Tau			0.103	0.92
Ser	1.571	0.59	1.892	1.94
Arg	0.709	0.66	0.724	0.90
Gly	0.883	1.08	1.101	2.36
Asp	2.000	0.29	2.866	1.29
Glu	4.884	0.30	5.303	0.89
Thr	1.176	0.24	1.847	0.40
Ala	1.231	0.42	1.840	1.21
Pro	2.870	0.26	2.756	0.65
Lys	1.936	0.38	2.364	1.28
Tyr	0.850	3.13	0.816	2.71
Met	0.588	1.67	0.579	1.82
Val	1.895	0.08	2.183	0.27
Ile	1.427	0.11	1.879	0.52
Leu	2.546	0.17	3.134	0.55
Phe	1.000		1.000	

Table 3. Quantitative comparison of bovine milk and infant formula, expressed as molar concentration normalized to Phenylalanine (Phe). Normalization shows that the formula is similar to bovine milk, while reflecting adaptation to human nutritional requirements.

CONCLUSIONS

- **The AccQ•Tag Ultra reagent derivatizes primary and secondary amino acids in complex feed samples**
- **Free amino acids can be measured for material and process characterization**
- **A range of complex feed samples can be analyzed consistently in replicate trials over multiple days**
- **Amino acid analysis can be used to assess modification of natural food to meet specific nutritional requirements**
- **UPLC Amino Acid Analysis Solution can be used for the determination of amino acids in foods and feeds**

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS