DETERMINATION OF AMINO ACIDS IN BEERS USING THE UPLC AMINO ACID ANALYSIS SOLUTION

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INTRODUCTION

Beer is a complex matrix consisting of over 100 components. Water, ethanol and carbohydrates are the major constituents of beers and ales. However, there are many minor compounds, some of which are critical for proper taste and quality. One class of compounds, amino acids is metabolized by yeast during fermentation, leading to the formation of critical flavor components. Therefore, the monitoring of amino acids is essential to demonstrate product consistency and ensure customer satisfaction.

Current HPLC methods for amino acids require run times that exceed 30 minutes with poor resolution between many analytes. Here we illustrate the ability of the Waters® UPLC® Amino Acid Analysis Solution to resolve 27 amino acids and an internal standard in less than 10 minutes and apply this capability to amino acid analysis of several imported and domestic beer and ale samples.

METHODS

Conditions :

System: ACQUITY UPLC® with Tunable UV Detector
Method: Cell Culture[1]
Column: AccQ Tag™ Ultra, 2.1 X 100 mm
Temperature: 60º C
Injection Volume: 1.0 µl
Detection: UV @ 260 nm
Data: Empower™ Software

Standard Preparation:
A stock 1000 pmol / µl stock mixed amino acid standard was prepared per the Cell Culture Method [1,2,3]. An intermediate 100 pmol / µl mixture was prepared by mixing 100 µl of stock with 900 µl water. The working derivatized standard was prepared by adding 10 µl of the 100 pmol/µl mixture to 70 µl borate buffer followed by 20 µl AQC derivatization reagent in a total recovery vial and mixing well. The mixture was heated for 10 minutes at 55°C, cooled to room temperature then injected. The concentration is 10 pmol/µl for the target analytes except Cystein (Cys) which is 5 pmol/µl.

Sample Preparation:
The 14 samples of beer and ale are commercial products. These include domestic and imported, regular, lite, non-alcoholic, and dark. Approximately 100 ml of each beer was sonnicated to remove carbonation. If the sample appeared excessively cloudy or turbid, it was filtered through a 0.45 micron hydrophilic filter. 200 µl of each beer and ale were mixed thoroughly with 160 µl water and 40 µl of a 1000 pmol / µl Norvaline (Nva– internal standard) solution. The preparation of the internal standard is described in the Cell Culture Method [1,2,3]. This resulted in a 1:2 dilution (400 µl total volume) of the beer, made 100 pmol / µl in internal standard.

10 µl of this mixture was then mixed with 70 µl of borate buffer and 20 µl of AQC derivatization reagent and heated as described in the standard preparation section. This working sample mixture, now a 20 fold dilution of the beer, made 10 pmol / µl in internal standard (similar to the working standard) was injected.

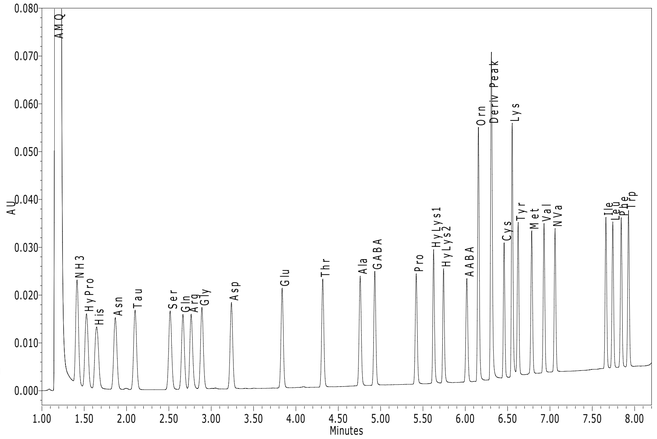

Figure 1. Chromatogram of 10 pmol / µL Amino Acid Standard

Analyte	RT (% RSD)	Area (% RSD)	Analyte	RT (% RSD)	Area (% RSD)
Hypro	0.522	0.736	HyLys1	0.013	0.874
His	0.581	0.743	HyLys2	0.014	0.860
Asn	0.417	0.853	AABA	0.017	0.856
Tau	0.329	0.798	Orn	0.017	0.846
Ser	0.236	0.708	Cys	0.014	0.885
Gln	0.219	0.725	Lys	0.014	0.841
Arg	0.217	0.762	Tyr	0.013	0.846
Gly	0.167	0.754	Met	0.012	0.809
Asp	0.126	0.817	Val	0.012	0.822
Glu	0.074	0.413	Nva	0.012	0.413
Thr	0.050	1.029	Ile	0.010	0.964
Ala	0.035	0.903	Leu	0.010	0.983
GABA	0.030	0.816	Phe	0.010	0.857
Pro	0.019	0.845	Trp	0.010	0.900

Table 1. Reproducibility Data for amino acid standard, (% RSD) Retention Time (RT) and (% RSD) Area, 5 injections

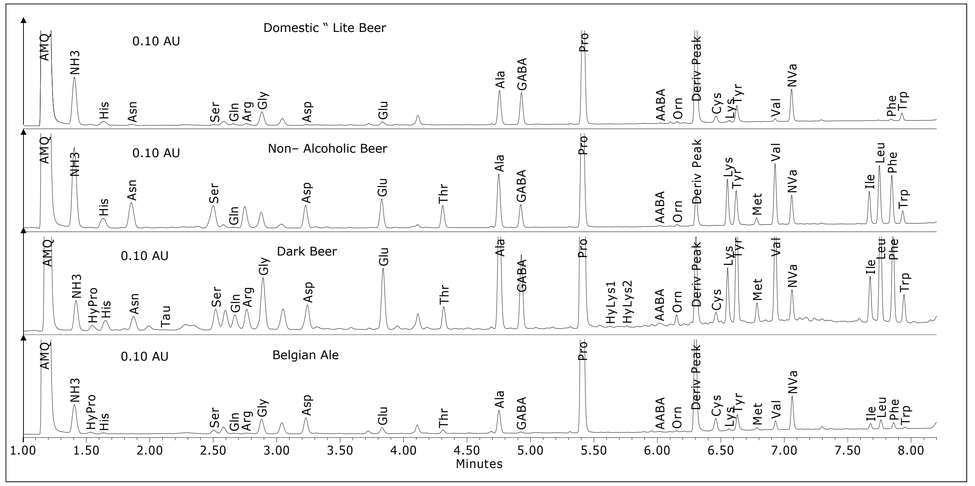

Figure 2. Chromatographic Profiles of Amino Acid Content for Various Beer Types

RESULTS AND DISCUSSION

Figure 1 is a chromatogram of the cell culture standard. Table 1 is reproducibility data (RSD) for retention time and area for 5 injections of this standard. An overlay of the chromatograms of several of the beers analyzed is found in Figure 2.

The differences in amino acid content, both qualitative and quantitative, for the samples tested are quite evident. Proline (Pro) was found in all samples tested and at a high level, not surprising given the fact that beer yeast can not ferment proline. On the other hand, Taurine (Tau) and Hydroxy-L– Lysine (HyLys) were absent or at a very low level. In general, the darker beers had higher amino acid content than light beers.

Also note that in Figure 2 there are many unidentified peaks, possibly amino acids not included in the standard mixture, or other compounds that contain an amino group that would react with the derivatization reagent. Since the methodology is fully compatible with mass spectrometry detection, it is possible to positively identify these additional compounds, which may also be of critical importance to product consistency.

CONCLUSION:

Waters UPLC® Amino Acid Analysis (AAA) System can be used to determine the amino acids found in beers and ales. This method illustrates excellent resolution of all sample components with a 10 minute cycle time. Simple sample preparation and analysis times that are approximately three times faster than traditional HPLC methods make the UPLC® AAA solution ideal for demonstrating consistency of beer production.

References

1. T. Wheat, E. Grumbach, and J. Mazzeo etal. " A new Amino Acid Analysis Application Solution". Waters Corporation, 2006 720001683EN
2." UPLC® Amino Acid Analysis Application Solution System Guide", Sections 4,6 waters Corporation, 2006 720001565EN
3. Paula Hong, Private Communication

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