Free Amino acid analysis in beverages using the AccQ•Tag™ Ultra Derivatization Kit with UPLC™ UV detection

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INTRODUCTION & OBJECTIVE

Beverages, especially those produced by fermentation, can widely vary in their amino acid profiles, which can contribute to taste and therefore impact on consumer acceptance. Research into wine and beer production includes the analysis of chemical analytes for characterization such as the presence of amino acids. In beer brewing, amino acids have been measured to support taste profiling (1, 2). Different strains of winery yeast (3) and grape varieties (4) have a strong impact on free amino acid profiles in wines. Nitrogen availability and utilization by wine yeast (Saccharomyces cerevisiae) significantly influence fermentation kinetics and the production of volatile compounds important for wine aroma (5). Reliable monitoring of those profiles during the complex process fermentation can provide a valuable tool in product development and characterization in beverage research. Different strains of microorganisms lead to differences in flavor profiles, rooted in changes in amino acid profiles at during the fermentation process. Furthermore, strains of microorganisms have specific preferences on nitrogen sources which impact metabolite production like volatiles e.g. ethylacetate, propanol, acetaldehyde for example in tequila fermentation (6). Plant pathogenic fungi have been shown to produce altered levels of mycotoxins depending on available nitrogen sources and amino acids (7). Fungal products like gibberellic acid, penicillin, cephalosporin C and toxins like patulin and aflatoxins that are regulated by nitrogen availability (8) can have impacts on food safety in beverage manufacturing.

Other beverage manufacturing sectors such as sports nutrition and protein supplements also need to characterize production batches to source ingredient suppliers and ensure consistent quality. The analysis of free amino acids can be lengthy with traditional HPLC methods or amino acid analyzers. The AccQ•Tag Ultra Derivatization Kit combined with analysis on a UPLC System with optical detection provides a fast and reliable quality control application as well as a tool for food research to correlate metabolite production with amino acid presence.

METHODS

Sample code	Sample type	Dilution in water
A	Coconut water	1 in 10
B	Cola (Global brand)	no dilution required
C	Energy drink (Global brand)	1 in 100
D	Energy drink (Local brand)	no dilution required
E	Kombucha (Raspberry)	no dilution required
F	Cider (Alcohol-free, Mixed fruit)	no dilution required
G	Cloudy apple juice	1 in 100
H	Pale Ale	1 in 10
J	White wine	1 in 20
K	Vitamin water (Apple & Elderberry)	1 in 20
L	Vitamin water (Orange, Mango & Passionfruit)	1 in 20
BCAA	Drinks powder of branched-chain amino acids (Watermelon flavor)	1 in 400 (of 6.55 mg in 100 ml)

Table 1: Recommended dilutions that result in all or most of the amino acids of a sample type being within the rage of the calibration line.

Beverage samples were derivatized according to the AccQ•Tag Ultra Derivatization Kit Care and Use Manual (715001331EN) with the modification of the borate buffer containing internal standard (Norvaline) (Figure 1). A calibration line was prepared from the Amino acid food and feed standard kit (Part number: 186009299) which contains 21 amino acids. Samples were run using bracketed calibration curves for quantitation. We recommend derivatizing each liquid sample undiluted and as a 1:10, 1:20 and 1:100 dilution. The dilutions that enable quantification of most amino acids in the given beverage samples are given in Table 1.
The ACQUITY™ UPLC H-Class System Plus was set up with established Empower™ CDS software methods without any modifications required for this analysis.

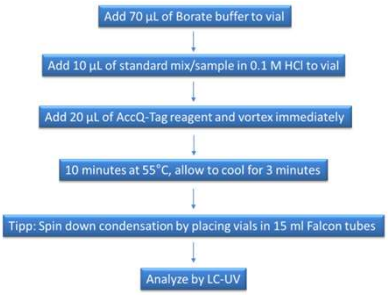

Figure 1: Derivatization workflow for liquid samples. Internal standard has been added to Borate buffer beforehand.

RESULTS

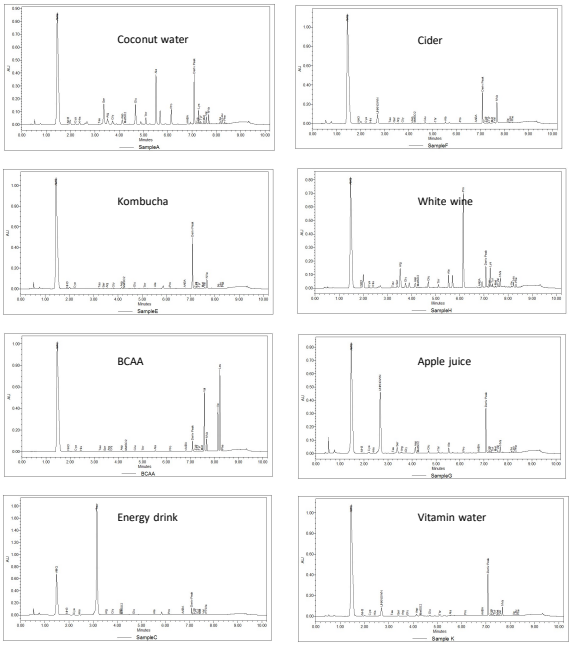

Figure 2: Typical chromatograms of derivatized, undiluted beverage samples.

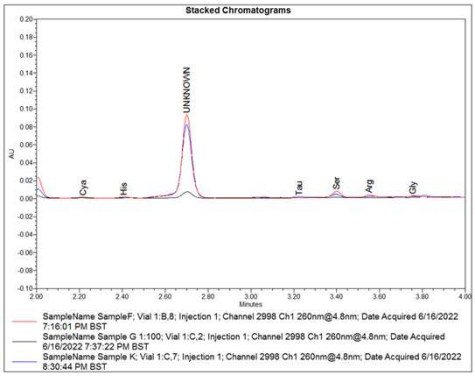

Figure 3: Overlay of unknown peak found in products containing apple. Sample F-Cider, Sample G-Cloudy apple juice, Sample K-Vitamin water Apple & Elderberry.

Seven-point, bracketed calibration curves were run with all sample sets. R^2 ranged from 0.984 ($MetSO_2$) to 0.9999 (Val, Ile, Leu, Ser, Thr, Pro). Figure 2 shows typical chromatograms of undiluted beverages to provide an overview of compounds present in the investigated products. Samples were quantified with the provided Empower CDS software methods sets but due to the lack of reference materials only the quantification of taurine in energy drink is reported as an example. The calculated taurine content was 0.44 g/100 mL which closely matches the stated amount on the product label of 0.4 g/100 mL.

Three analyzed products containing apple (apple juice, cider, apple & elderberry vitamin water) showed a high intensity, common unidentified peak eluting between Histidine and Taurine (Figure 3). L-Asparagine, L-aspartic acid and L-glutamine were previously identified with the AccQ•Tag Ultra Derivatization Kit as the principal amino acids observed in most apple juices (9). Based on the elution time the unknown peak was suspected to be asparagine. This could be confirmed by an injection of a single standard derivatization of asparagine with the AccQ•Tag Kit (data not shown). A common issue with derivatization using the AccQ•Tag Kit is the presence of several amine groups in a reference standard which can lead to double-peaks (for example asparagine). To investigate peak identities, it is recommended to perform the incubation in concentration ranges within the calibration curve to achieve an excess ratio of derivatization agent compared to the sample as well as to bring the reference standard incubation close to the sample pH.

CONCLUSIONS

- Straight-forward application with all necessary methods and minimal sample preparation
- A range of beverage samples were tested, and linearity of the detection was investigated in seven-point calibration curves
- AccQ•Tag Ultra Derivatization and separation via an UPLC System ensure a reproducible and reliable analytical solution for busy laboratories in quality control and fermented beverage research.

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