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Fast and simple determination of free amino acids in beer

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Introduction

With an annual consumption of approximately 200 million kiloliters, beer is one of the most popular beverages world-wide. It comes in a huge variety of styles and flavors, with or without alcohol, pure or as a mixed drink, as ale, lager, pils, wheat-beer, Kölsch or Alt, just to name a few. For regular consumers beer adds up to an important part of their daily diet. Apart from the risks of overindulging in alcoholic beverages beer is not all-bad. It is easily digestible and offers an important source of vitamins, as well as potassium, magnesium, phosphor and other trace elements, proteins as well as free amino acids [1].

The composition of beer wort is largely dependant on the raw materials and the style of brewing used in the production. The contribution of specific amino-acids in the formation of off-flavors as well as a positive effect of others on fullness and drinkability has been established [2, 3]. Also the significance of amino acids as a source of nitrogen for fermentation is indisputable. Therefore, determination of changes in the composition and content of amino acids is an important measure in ensuring the consistency in quality of the end-product.

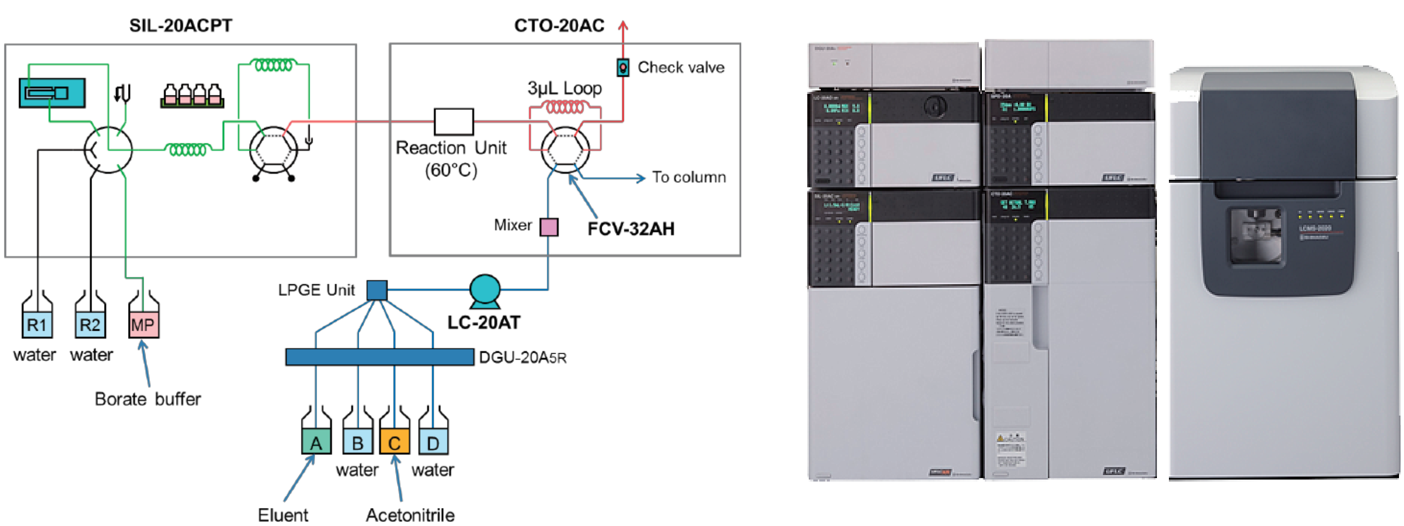

Figure 1: Flow diagram for derivatization and injection in the UF-Amino system

Materials and Methods

There are many possibilities for analyzing amino acids. In this study we used the Shimadzu UF-Amino station for automated pre-column derivatization and on-line reversed-phase LC separation with MS detection using an LCMS 2020 single quadrupole MS.

Chemicals used:

- amino acid analysis reagent (AminoTag®) (Wako Chemicals GmbH)
- amino acids mixture standard solution (Wako Chemicals GmbH)
- amino acids internal standard mixture solution (Wako Chemicals GmbH)
- AminoTag Eluent (Wako Chemicals GmbH)
- MS grade Acetonitrile (mobile phase B) (Promochem)

A series of calibration standards with the concentration of 500, 300, 50, 10 and 2.5 µmol/L was prepared.

Five beer samples were used as test probes: non-alcoholic pils and wheat beer, alcoholic pils and wheat beer and an Alt beer from the Düsseldorf, Germany region.

Sample preparation: 50 µl probe (water for the blank), 50 µl of internal standard solution (water for the blank) and 100 µl of acetonitrile were filled in deep well plate tubes and thoroughly mixed.

Pretreatment is automatically performed in the autosampler, where the probe is combined with borate buffer solution and the AminoTag® reagent and injected. Derivatization is completed in the reaction unit at 60 °C before forwarding the obtained amino acid derivatives onto the analytical column (Shimpack UF-Amino 2.0 µm, 100 x 2.1 mm) and subsequent detection using the LCMS-2020 single-quadrupole mass spectrometer.

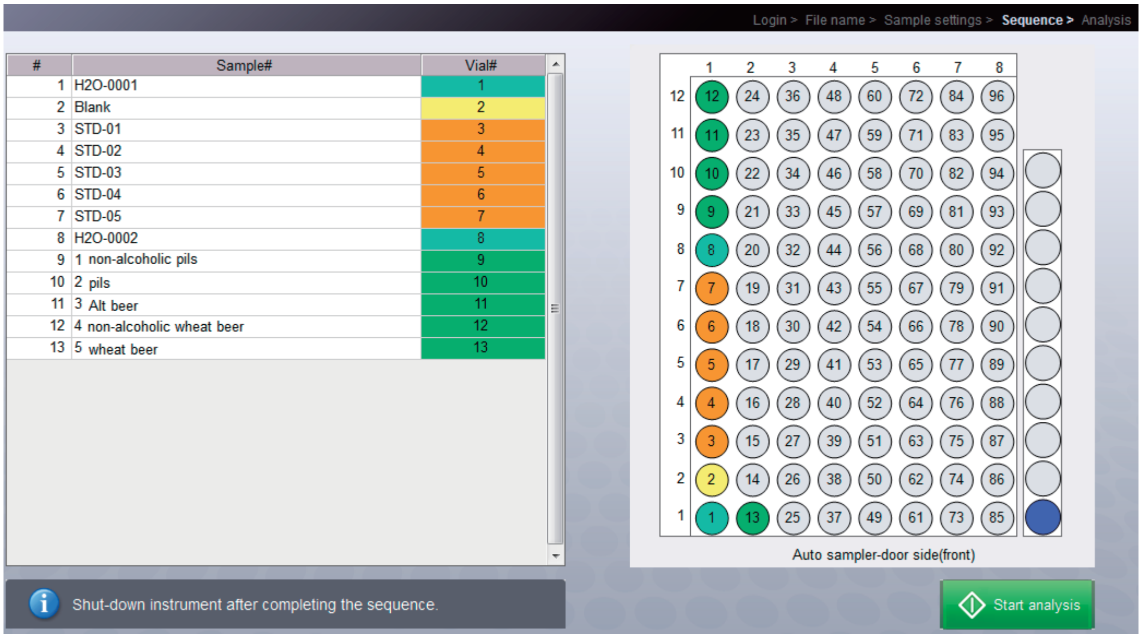

Figure 2: Analysis – Color highlighting of the individual sample positions in the batch

Using the overlap-injection feature, derivatization of a second sample is performed during analysis of the first probe, resulting in considerable time-saving during the course of the experiment.
Programming of the analysis and evaluation of the resulting data was carried out using the AmiNavi software. After entering the sample names, a batch with the desired number of standards, blank injections and samples was created automatically and illustrated via color highlighting of the positions of the individual substances in the deep-well plate (see figure 2).

Results

Calibration curves for each analyte of interest were created automatically according to the preset reprocessing method. Integration of the individual compounds was verified and manually corrected if necessary. Results of the quantification of the 38 amino acids included in the analytical method in the five beer samples is given in an overview table as well as a display of individual chromatographic data.

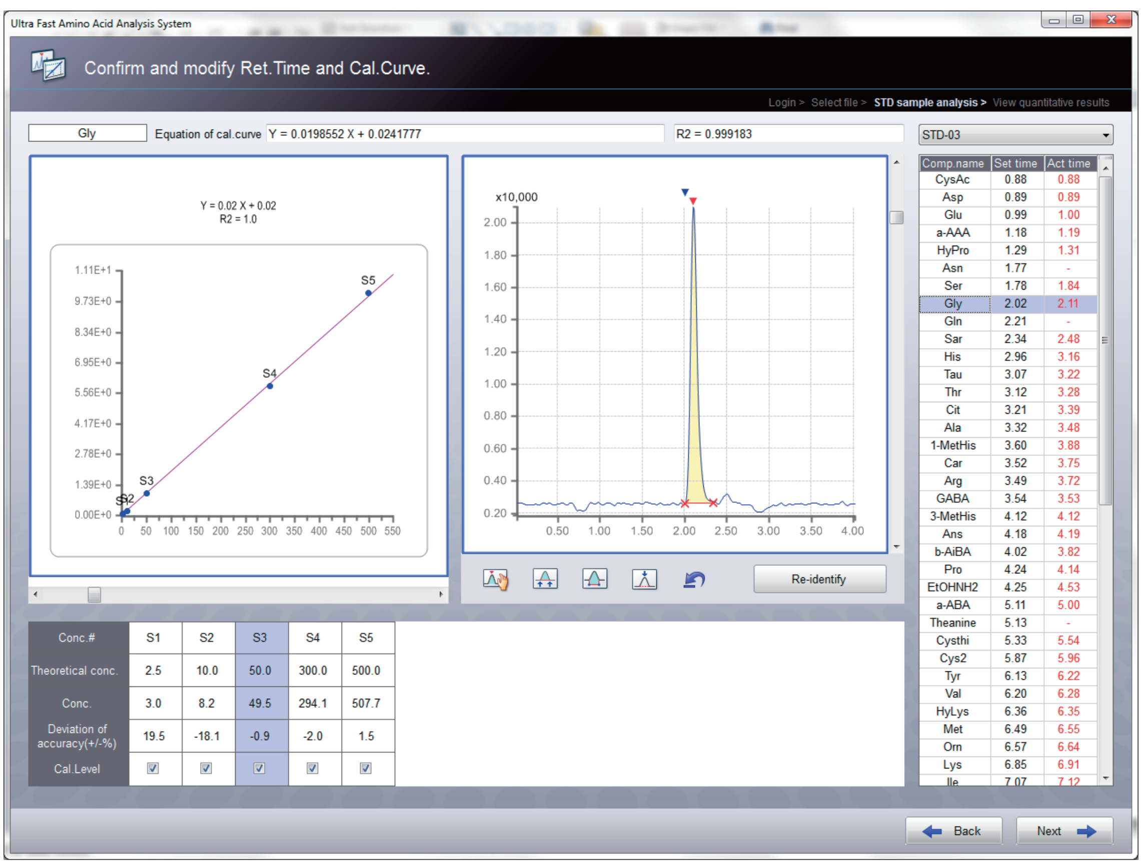

Figure 3: Processing – Calibration curve of the individual substances

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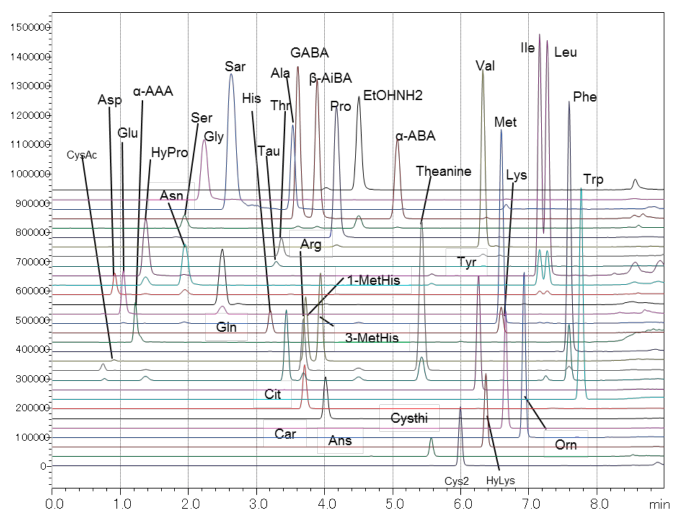
Amino acid content of five beer samples from different manufacturers of different make and variety was determined and is displayed in figure 4. Figure 4 also shows a typical HPLC/MS chromatogram of an amino acid mixed standard solution. The beers differed in amino acid pattern. The highest total amino acid content was found in non-alcoholic beers (8074 µmol/L and 7117.6 µmol/L), followed by the pils (6910 µmol/L). The smallest amount of free amino acids with 5238 µmol/L was present in wheat beer. From the 38 analytes measured, concentration of eleven amino acids was significantly higher in non-alcoholic wheat beer than in the other samples tested. They can be separated into three distinctive taste groups: bitter tasting amino acids (leucine, isoleucine, lysine, arginine, valine, methionine), sweet amino acids (serine, threonine, alanine) and sour, umami-taste amino acids (aspartic acid, glutamic acid) [4]. The most abundant amino acids in all five beer samples were alanine (242 - 744 µmol/L) and proline (1943 – 3402 µmol/L), which is in accordance with the finding that proline is not absorbed by yeast during the process of fermentation.



	non-alcoholic Pils	Pils	Alt beer	non-alcoholic Wheat beer	Wheat beer
Aspartic acid	28.6	-	31.4	187.9	43.9
Glutamic acid	144.2	54.6	43.1	193.8	59.7
Hydroxyproline	6.4	8.5	6.2	4.7	-
Serine	-	9.6	28.1	258.4	39.8
Glycine	208.3	193.2	234.2	316.2	251.3
Histidine	124.1	109.5	63.6	156.7	127.7
Threonine	-	-	20.9	163.3	21.5
Citruline	15.6	8.3	10.7	38.0	28.1
Alanine	566.1	434.6	242.3	744.4	399.2
Arginine	249.1	223.3	50.7	404.7	50.5
GABA	1315.6	1245.6	1331.5	961.6	890.4
3-Methylhistidine	5.2	-	-	-	-
beta-Aminoisobutyric acid	-	6.3	7.0	-	-
Proline	3214.7	3402.3	2987.7	1942.7	1959.7
Ethanolamine	147.2	182.3	167.3	116.7	139.4
alpha-Aminobutyric acid	15.6	30.5	-	6.1	-
Tyrosine	227.1	283.2	132.5	289.2	216.5
Valine	295.3	252.8	49.3	513.7	290.2
Methionine	-	0.8	12.2	99.4	20.9
Ornithine	14.7	16.5	20.7	68.4	75.1
Lysine	11.2	10.8	37.7	213.0	30.2
Isoleucine	50.7	27.9	30.1	250.7	55.8
Leucine	121.1	57.9	53.8	570.3	176.6
Phenylalanine	214.1	203.1	35.1	395.9	213.7
Tryptophan	142.9	148.2	55.5	178.4	148.1
Total amino acid content	7117.6	6910.0	5651.7	8074.3	5238.4

Figure 4: SIM chromatograms of the determination of 38 derivatized amino acids and table of results of the determination of amino acids in beer samples (µmol/L)

Conclusion

A rapid and reliable HPLC-MS method for the determination of up to 38 amino acids in 9 minutes (12 minutes total run time), using an automated pre-column derivatization approach has been established. The assay showed good reproducibility of the derivatization procedure, as can be seen from the 5 point calibration curves, that all produced a linearity of $R^2 \geq 0.99$. The amount of amino acids in beer depends on the materials and technology used for beer preparation and is partially responsible for nutritional value, quality and stability of beer. The total content of amino acids in the five beer samples tested varied between 5238 – 8074 µmol/L, with significant variability in the amino acid distribution in the different types of beer.

References

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