

Determining the amino acid profile of beer using post column derivatization with ninhydrin



Introduction

Beer is brewed after liquid gelatinization and saccharification, and then liquid fermentation. The main raw materials of beer are wheat malt and barley malt, and hops is also added during the process. Beer has low alcohol content and high nutritional value. The ingredients include water, carbon dioxide, a variety of amino acids, vitamins, low molecular sugars, inorganic salts and various enzymes. These nutrients are easily absorbed and utilized by the body. The low molecular sugars and amino acids in beer are easily digested and absorbed, and generate a lot of heat in the body, so beer is often called "liquid bread". 1L of 12 % beer can produce 3344 kJ of calories, which is equivalent to the calories produced by 3-5 eggs or 210 g of bread. A light manual worker can obtain one-third of the calories he needs, if he drink 1L of beer a day.

The composition of the amino acids that are created during the brewing process plays an important role in the characteristic taste of the beer. They also strongly influence the smell, appearance and foam. Therefore, the analysis of central, process-relevant amino acids is an important quality criterion in beer production. From a nutritional-physiological point of view -

anyway. The amino acids in beer are produced by the decomposition of protein contained in raw barley through the action of enzymes. Its content is very rich, and it exists in a dissolved state, and is indispensable for human nutrition. 17 kinds of amino acids have been analyzed in beer. 7 kinds of them cannot be synthesized by the human body, and they are indispensable. If they are lacking, many diseases will be easily caused. Beer contains peptides and amino acids that balance human nutrition. Each liter of beer contains about 3.5 g of peptides and amino acids, hydrolysis of the protein, which can be almost 100% digested, absorbed and utilized by the human body.

Sample Preparation and Analysis

For the analysis of the amino acid content in beer, a sample of beer in commercial packaging and a sterile-filled sample of the corresponding farm wort were used. Farm wort is obtained by boiling hops and is an important ingredient in the process of yeast fermentation, known as "yeast recovery".

400 μL of the sample were mixed with 100 μL precipitation buffer and deposit in the refrigerator for 20 min for the protein precipitation. The sample was centrifuged for 5 min at 14000 rpm. Afterwards the supernatant was filtered with a membraSpin by centrifugation at 14000 rpm for five minutes.

Beer:

200 μL of the particle free solution were diluted with 50 μL sample dilution buffer and 205 μL sample dilution buffer (including internal standard norleucin 200 nmol/mL)

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Farm worth:

100 µL of the particle free solution were diluted with 150 µL sample dilution buffer and 250 µL sample dilution buffer (including internal standard norleucin 200 nmol/mL)

The samples were analyzed in a double run with determination of a mean value and the RSD by the Amino Acid Analyzer ARACUS, manufactured and distributed by membraPure GmbH worldwide. ARACUS is using the classic routine analysis of amino acids by post-column derivatization with ninhydrin and the detection at 440 nm and 570 nm.



Figure 1: Amino Acid Analyzer ARACUS

Table 1: Mean values of the determined amino acid concentration as well as the RSD in the sample "beer".

Amino Acid	Beer nmol/mL	RSD %
Asp	34.1	0.47
Thr	9.1	0.36
Ser	15.4	0.69
Glu	150.9	0.69
Pro	5.549.5	1.98
Gly	220.2	0.88
Ala	661.7	0.94
Val	146.7	0.75
Met	5.9	1.06

Ile	21.8	1.16
Leu	37.1	1.26
Tyr	185.8	0.50
Phe	84.7	0.63
g-ABA	786.2	0.33
His	149.2	0.44
Lys	3.4	0.47
NH4	434.2	0.65
Arg	280.6	0.32

Table 2: Mean values of the determined amino acid concentration as well as the RSD in the sample "farm worth".

Amino Acid	Farm worth nmol/mL	RSD %
Asp	332.1	0.24
Thr	399.9	0.61
Ser	465.1	0.98
Glu	545.6	0.77
Pro	3.958.4	0.31
Gly	313.3	0.23
Ala	987.0	0.27
Val	766.7	0.77
Met	149.6	0.57
Ile	390.0	0.19
Leu	912.3	0.19
Tyr	442.8	0.54
Phe	672.9	0.48
g-ABA	876.2	0.75
His	334.5	0.08
Lys	528.5	0.25
NH4	1.257.5	0.06
Arg	717.0	0.27

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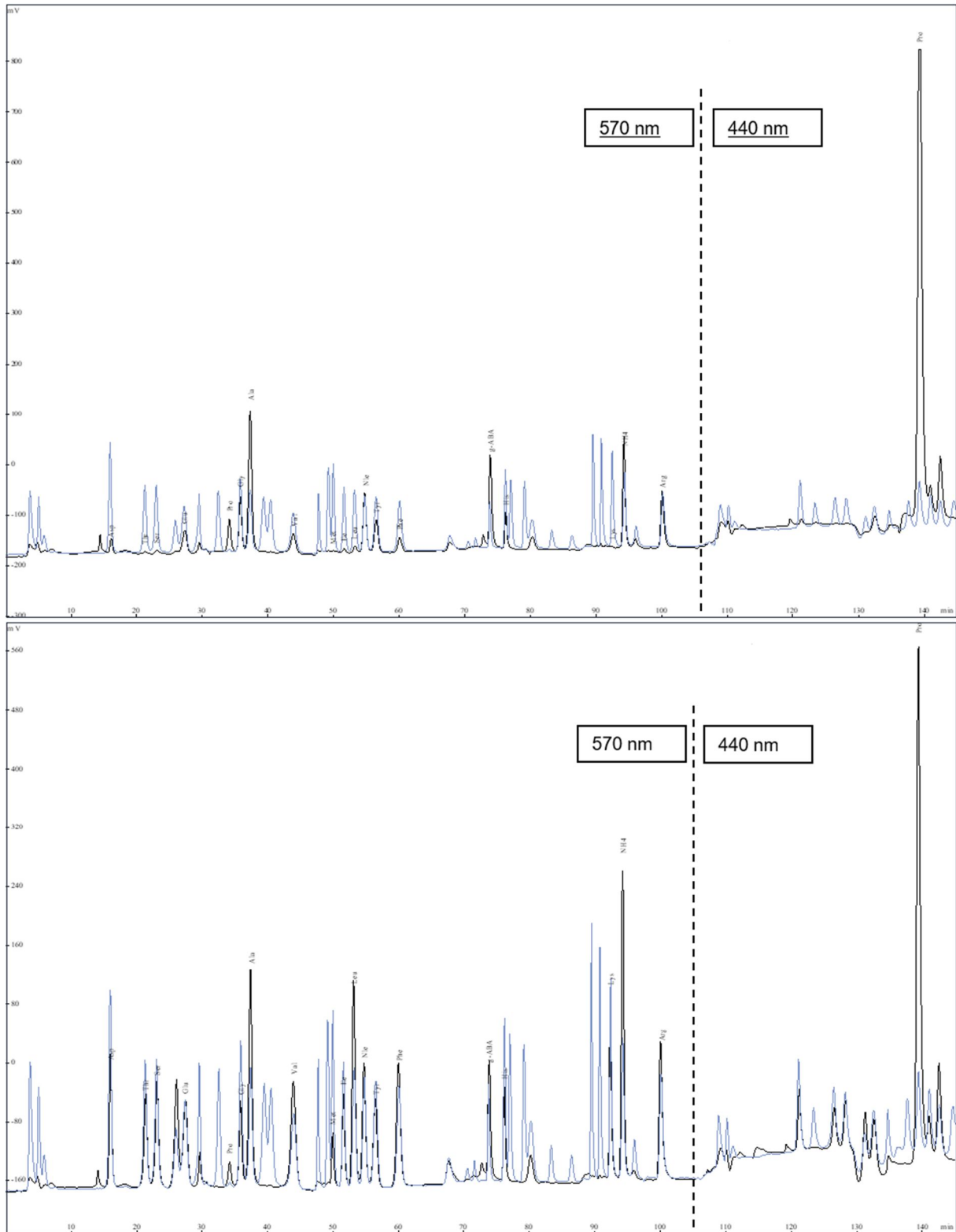


Figure 2: Comparison of a physiological amino acid standard (blue) with “beer” sample (black, top image) and “farm worth” sample (black, bottom image). The detection was performed at 440 nm and 570 nm. The concentrations of the individual amino acids were determined using a known concentration of the standard amino acid mixture.

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