rFAN Assay

For a fast detection of amino acids in liquid solutions, we used the rFAN assay. This ninhydrin-based assay measures only free alpha amino acids and ammonia. Other nitrogen sources remain undetected [1]. The reagents were prepared as described below.

ninhydrin color reagent:

- 4 g anhydrous Na_2PO_4
- 6 g KH_2PO_4
- 0.5 g ninhydrin
- 0.3 g fructose
- Ad 100 ml dH_2O

Glycine Standard:

- 100 mg glycine
- Ad 100 ml dH_2O

Dilution reagent:

- 2 g KIO_3
- Ad 100 ml dH_2O

Then 400 ml 96% EtOH was added.

Procedure

The assay was performed in 15 x 150mm test tube. Two ml of samples to test and 2 ml dilutions of the glycine standard mix for the standard curve were pipetted into the test tubes. After that 1 ml ninhydrin color reagent was added, and the tubes were heated in boiling water for 30 min. Test tubes were then transferred to a cold-water bath and 5ml Dilution reagent was added. The reaction mixture was briefly mixed, and 1 ml was taken to measure the absorbance at 575nm against a blank containing 2ml of distilled water instead of the sample.

Literature

[1] Analysis of Protein and Total Usable Nitrogen in Beer and Wine Using a Microwell Ninhydrin Assay - Abernathy - 2009 - Journal of the Institute of Brewing - Wiley Online Library. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/j.2050-0416.2009.tb00356.x. (Accessed: 8th October 2019)