AK0009_IFU_EN_V2302

Ammonia, UV method

Catalogue number	Presentation
AK00091	96 tests (manual) / 960 tests (microplate)

Application

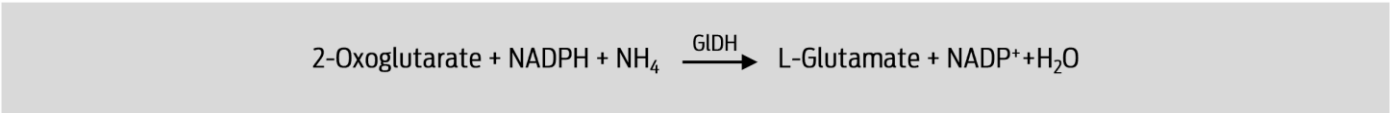
This rapid and simple specific enzymatic method is used for the determination of ammonia (ammonium ions) in foodstuffs such as wine, fruit juice, bakery products, dairy products, egg products, meat, seafood and dietetic food, as well as in paper, fertilizer, pharmaceuticals, cosmetics, water and biological samples.

Introduction

Ammonia is mainly produced by microbial protein catabolism of organic material. Therefore, high concentrations of ammonia can indicate the decomposition of substances like milk, meat and seafood, being ammonia a major component of the off-flavours and odours. Ammonia also indicates the presence of faeces, urine and microorganisms in water.

In the wine industry, ammonia determination is important in the calculation of yeast available nitrogen (YAN). This kit uses a glutamate dehydrogenase that is not inhibited by tannins found in grape juice and wine. However, YAN comprises three components: free ammonium ions, amino nitrogen from free amino acids and from the side chain of L-arginine. Thus, for the most accurate determination of YAN, all three components should be quantified, which is possible by using NZYtech's L-Arginine/Urea/Ammonia kit.

Principle



The amount of NADPH consumed through the action of glutamate dehydrogenase (GlDH), measured at 340 nm, is stoichiometric with the amount of ammonia in sample volume.

Specificity

The determination is specific for ammonia.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 absorbance units and a sample volume v = 2.00 mL. This corresponds to an ammonia concentration of 0.02 mg/l sample solution when measured at 340 nm. The detection limit of 0.07 mg/L is derived from the absorbance difference of 0.020 (340 nm) and a maximum sample volume v = 2.00 mL.

Linearity and precision

Linearity of the determination exists from 0.2 µg to 7 µg ammonia/assay. In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. The relative standard deviation ("coefficient of variation") is approx. 1 to 2 % in the measuring range.

Kit composition

Solution 1 (2x). TEA buffer (24 mL, 0.5 M, pH 8.0) plus imidazole (200 mM), 2-oxoglutarate (40 mM) and sodium azide (0.02 % w/v) as a preservative. Store at 2 °C to 8 °C.

Tablets 2. 192 tablets of NADPH supplied in a plastic vial containing desiccant. Allow this container to warm to room temperature (in the presence of a desiccant if possible) before opening to remove tablets. Store desiccated at 2 °C to 8 °C (Long term storage: -30 °C to -15 °C)

Add 2 tablets and 0.5 mL of solution 1 per assay, including blank reaction, to a test tube and stir intermittently over 2-3 min (Solution 1+2).

Suspension 3. Glutamate dehydrogenase (GlDH) is provided in 2.5 M lithium sulphate (EC 1.4.1.2; 2.2 mL). Store at 2 °C to 8 °C. Swirl bottle before use.

Solution 4. Ammonia standard solution (5 mL, 0.04 mg/mL) in 0.02 % (w/v) sodium azide. Store at 2 °C to 8 °C. This standard should be used when there is doubt about the method accuracy.

Protocol (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~25 ºC

Final volume: 2.62 mL

Sample solution: 0.2 – 7 µg of ammonia per cuvette (in 0.1 – 2.0 mL sample volume)

Read absorbances against air (without cuvette in the light path), or against water

PIPETTE INTO CUVETTES (mL)	**BLANK**	**SAMPLE**
Distilled water	2.10	2.00
Sample	-	0.10
Solution 1+2 (NADPH/TEA buffer)	0.50	0.50
Mix, measure the absorbance of the solutions (A1) after ~2 min and start the reaction by addition of		
Suspension 3 (GlDH)	0.02	0.02
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 3 min)*		

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

* if necessary, continue to read the absorbances at 1 min intervals until the reaction ends.

Calculation

Determine the absorbance difference for both blank and sample (A1-A2). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{Ammonia}$. The concentration (C) of ammonia (g/L), based on the ε of NADH at 340 nm (6300 L×mol^{-1}×cm^{-1}), is calculated as follows:

$$C\ (Ammonia) = 0.07082 \times \Delta A_{Ammonia} \quad [g/L]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the “Alternative Procedures”, available on the NZYtech website.

Interferences

If the conversion of ammonia has been completed within the time specified in the assay (approx. 3 min), it can be generally concluded that no interference has occurred. However, this can be further checked by adding ammonia (approx. 4 µg in 0.1 mL) to the cuvette on completion of the reaction. A significant decrease in the absorbance should be observed. Interfering substances in the sample being analysed can be identified by including an internal standard. Although tannins in fruit juice can lead to the inhibition of some GlDH, the enzyme used in this kit does not have this limitation.

General information on sample preparation

The amount of ammonia present in the cuvette should range between 0.2 and 7 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield an ammonia concentration between 2 and 70 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water (analyte range from 0.10 to 70 mg/L).

To implement this assay use clear, slightly coloured and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to approx. pH 8 by adding 2 M NaOH; measure "coloured" samples (if necessary adjusted to approx. pH 8) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with 0.2 g PVPP/10 mL of sample; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water at 60 °C.

Examples of sample preparation

Determination of ammonia in grape must and wine

The concentration of ammonia in white and red grape juice/must and wine can be determined usually without any sample treatment, except filtration and dilution, if necessary. For red wine, it may be necessary to remove some of the colour by the addition of 0.2 g of PVPP per 10 mL of sample. Shake the tube vigorously for 5 min and filter through Whatman No. 1 filter paper. No dilution is usually required and a sample volume of 25-50 µL is generally satisfactory.

Determination of ammonia in fruit juices

Adjust 25 mL of filtered sample to a pH of approx. 8 using 2 M NaOH. Quantitatively transfer the solution to a 50 mL volumetric flask and adjust to volume with distilled water. If the solution is highly coloured, it may be necessary to add 0.2 g of PVPP/10 mL of sample. Shake the tube vigorously for 5 min and then filter through Whatman No. 1 filter paper. No dilution is usually required and a sample volume of 0.1 mL is generally satisfactory.

Determination of ammonia in milk

In a test tube, mix 1 mL of milk with 3 mL of 0.3 M trichloroacetic acid. Incubate at room temperature for 5 min to ensure complete precipitation of protein and then centrifuge at room temperature for 3 min at 2,000 g. Decant supernatant and neutralise with 10 M KOH. Filter, and use the clear supernatant directly for the assay. Typically, no further dilution is required and sample volumes up to 2.0 mL are satisfactory.

Determination of ammonia in meat

Weigh approx. 5 g of meat or meat product into a 100 mL bottle. Add 20 mL of 1 M perchloric acid and homogenise for 2 min. Transfer to a 40 mL beaker and adjust the pH to approx. 8.0 using 2 M KOH. Transfer to a 100 mL volumetric flask and adjust to the mark with distilled water. Store on ice for 20 min to allow the precipitation of potassium perchlorate and fat separation. Filter, discarding the first 3-5 mL, and use the clear filtrate for the assay. Usually, no further dilution is required and a sample volume of 0.5 mL is satisfactory.

References

Bergmeyer, H. U. & Beutler, H. -O. (1990). Ammonia. In Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VIII, pp. 454-461, VCH Publishers (UK) Ltd., Cambridge, UK.

Recommendations

A method with less interferences than chemical methods (e. g. Nessler's reagent or Berthelot with phenol/hypochlorite), recommended/approved by the:

- German food law;

- Recommended by the Mitteleuropäische Brautechnische Analysenkommission (MEBAK) (Central European Commission for Brewing Technology).

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications