AK0005_IFU_EN_V2302

Acetaldehyde, UV method

Catalogue number	Presentation
AK00051	50 tests (manual) / 500 tests (microplate)

Application

This rapid and simple specific enzymatic method is used for the determination of acetaldehyde in foodstuffs such as alcoholic beverages, bread, coffee, cocoa, dairy products, fruit and vegetable products, starter cultures for butter production and biological samples.

Introduction

Acetaldehyde occurs in all living organisms, sometimes in very small amounts, as a product of many metabolic processes. The content of the intermediate acetaldehyde increases considerably when alcoholic fermentation is used for the production of food. Acetaldehyde is an important flavour compound. In wine production, acetaldehyde is bound to sulfite e. g. in order to improve the taste. On the other hand, acetaldehyde is the most common aldehyde found in dairy products (yogurt, cheese, etc.) and is responsible for desirable specific flavours but also for flavour defects. Acetaldehyde in human blood originates from ethanol that has been ingested in foods and beverages. Extreme ethanol consumption can lead to acetaldehyde poisoning following oxidation of the ethanol in the liver by alcohol dehydrogenase.

Principle

$$\text{Acetaldehyde} + NAD^+ + H_2O \xrightarrow{\text{Al-DH}} \text{Acetate} + NADH + H^+$$

The amount of NADH formed through the action of aldehyde dehydrogenase (Al-DH), measured at 340 nm, is stoichiometric with the amount of acetaldehyde in sample volume.

Specificity

Al-DH converts, though with much lower velocity, other aldehydes like propionaldehyde, glycolaldehyde and benzaldehyde. In the presence of these aldehydes acetaldehyde can be measured by extrapolation of A2 to the time of addition of aldehyde dehydrogenase. Under reaction conditions, the oxidation of formaldehyde, crotonaldehyde, glyceraldehyde is so unimportant that its influence on the determination of acetaldehyde can be excluded, even on a high excess.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 AU and a sample volume of 2.00 mL. This corresponds to a acetaldehyde concentration of 0.044 mg/L sample solution when measured at 340 nm. The detection limit of 0.176 mg/L is derived from the absorbance difference of 0.020 (340 nm) and a maximum sample volume of 2.00 mL.

Linearity and precision

Linearity of the determination exists from 0.5 to 20 µg of acetaldehyde per assay (v = 2.00 mL). In a double assay using one sample solution, a difference of 0.005 to 0.010 AU may occur (0.044-0.088 mg/L of acetaldehyde, v = 2.00 mL). The CV is approx. 1 to 3%.

Kit composition

Solution 1. Potassium pyrophosphate buffer (12 mL, 1.5 M, pH 9.0) and sodium azide (0.02% w/v) as a preservative. Store at 2 °C to 8 °C.

Solution 2. NAD^+ (138 mg). Store at 2 °C to 8 °C (Long term storage: -30 °C to -15 °C)

Dissolve in 11 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -30 °C to -15 °C between use and keep cool during use.

Suspension 3. Aldehyde dehydrogenase (Al-DH, EC 1.2.1.3) in 3.2 M ammonium sulphate (1.1 mL). Store at 2 °C to 8 °C. Swirl bottle before use.

Solution 4. Acetaldehyde control (~2 g of acetaldehyde ammonia trimer). Store at 2 °C to 8 °C.

Dissolve approx. 80 mg of acetaldehyde ammonia trimer (~50 mg acetaldehyde) in 1 L of distilled water; store in a well-sealed bottle and use on the day of preparation. This control solution can be used when there is some doubt about the method accuracy.

Protocol (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Note: It is essential to stopper the cuvettes

Temperature: ~25°C

Final volume: 2.52 mL

Sample solution: 0.5-20 µg of acetaldehyde per cuvette (in 0.10-2.0 mL sample volume)

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

PIPETTE INTO CUVETTES (mL)	**BLANK**	**SAMPLE**
Distilled water (at ~25 °C)	2.10	2.00
Sample	-	0.10
Solution 1 (PPi buffer)	0.20	0.20
Solution 2 (NAD^+)	0.20	0.20
Mix, measure the absorbance of the solutions (A1) after ~2 min and start the reaction by addition of		
Suspension 3 (Al-DH)	0.02	0.02
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 4 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 2 min intervals until the reaction ends.

Calculation

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

$$C\ (Acetaldehyde) = 0.1762 \times \Delta A_{Acetaldehyde} \quad [g/L]$$

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

Although compounds in animal extracts do not interfere with the assay, polyphenolics from plant materials may reduce the speed of the reaction. If the conversion of acetaldehyde completes within the time specified in the assay (approx. 4 min), it can be generally concluded that no interference has occurred. However, an internal control should be included during sample analysis if the presence of interfering substances is suspected. A quantitative recovery of this control should be expected. Identification of losses in sample handling and extraction may be identified by performing recovery experiments, i.e., by adding acetaldehyde to the sample in the initial extraction steps.

General information on sample preparation

The amount of acetaldehyde present in the cuvette should range between 0.5 and 20 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield an acetaldehyde concentration between 5 and 200 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water (analyte range from 0.25 to 200 mg/L).

Acetaldehyde is extremely volatile (bp ~20 °C), so all samples and assays must be stored in tightly closed containers. Samples should be analyzed as soon as possible since acetaldehyde is readily oxidized by atmospheric oxygen; control solutions should be used on the day of preparation. The preferred assay control material is acetaldehyde ammonia trimer because it is less volatile than acetaldehyde and it is not readily oxidised.

To implement this assay use clear, colourless 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 pH 8 by adding sodium or potassium hydroxide solution; adjust acid and weakly coloured samples to pH 8 and incubate for approx. 15 min; measure "coloured" samples (if necessary adjusted to pH 8) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

Examples of sample preparation

Determination of acetaldehyde in red wine

It is usually required to decolorize red wine before using in the assay. To do this, in a sealed bottle mix 25 mL of red wine with 0.5 g of activated charcoal, stir for approx. 2 min and then immediately filter an aliquot of the slurry through Whatman GF/A glass fibre filter paper. Collect the resulting mixture into a bottle and seal immediately. Proceed with the assay with the clear, essentially colourless filtrate. In general, no dilution will be required, and a sample volume of 0.2 mL is appropriate.

Determination of acetaldehyde in beer and champagne

It is essential to degass samples, which can be achieved by increasing the pH to approx. 9.0 with 2 M NaOH and gentle stirring in a sealed bottle. In general, no dilution will be required, and a sample volume of 0.2 mL is appropriate.

Determination of acetaldehyde in vegetable products

Vegetable products need to be homogenized in a kitchen blender. Dissolve the homogenised sample vigorously in 100 mL of distilled water and shake. Clarify an aliquot by filtration through Whatman GF/A glass fibre filter 9 paper. Collect into a bottle and seal immediately after collection. Further clarify with Carrez reagents if required. Dilute, if necessary, and use a sample volume of 0.10-0.50 mL. In general, no dilution will be required, and a sample volume of 0.2 mL is appropriate.

References

Beutler, H. O. (1988). Acetaldehyde. In: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VI, pp. 606-613,VCH Publishers (UK) Ltd., Cambridge, UK.

Recommendations

This method is recommended/approved by the:

– Swiss food law;

– Mitteleuropäische Brautechnische Analysen kommission (MEBAK) (Central European Commission for Brewing Technology).

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