NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.183-2025

Replacing GB 5413.31-2013

National food safety standard - Determination of urease in food

食品安全国家标准 食品中脲酶的测定

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Relationship to previous standards

This document replaces GB 5413.31-2013, GB/T 5009.183-2003 and GB/T 5009.186-2003 in full, and replaces Annex A of GB/T 30885-2014 and Annex A of GB 20371-2016, which remain in force for their other provisions.

1 Scope

1 The standard lays down methods for the determination of urease activity in food. The first method, Nessler reagent colour development, applies to the qualitative determination of urease activity in beverages containing soybean ingredients, infant formula foods, supplementary foods for infants and young children, dairy products, formula foods for special medical purposes, soy protein peptides and soybean products. The second method, titration, applies to the quantitative determination of urease activity in soy protein, soybean flour and soy milk powder.

2 First method - Nessler reagent colour development

2 At pH 7.0 and 40 °C urease catalyses the conversion of urea into ammonium carbonate, which under alkaline conditions gives ammonium hydroxide; this reacts with the potassium mercuric iodide double salt of the Nessler reagent to give brown dimercuric ammonium iodide, and the urease activity of the sample is judged qualitatively from the colour that develops.

3 Unless otherwise stated the reagents are analytically pure and the water is grade three water to GB/T 6682. The reagents are urea, sodium tungstate dihydrate, potassium sodium tartrate tetrahydrate, sulfuric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, red mercuric iodide, potassium iodide, sodium hydroxide, and a graphitised carbon black solid phase extraction cartridge of 300 mg per 6 mL.

3.2 The solutions are prepared as follows. Urea solution at 10 g/L: 5 g of urea, weighed to 0.01 g, is dissolved in water and made up to 500 mL, mixed, kept in a brown bottle in the dark at 2 °C to 8 °C and used within 2 weeks. Sodium tungstate solution at 89.0 g/L: 50.0 g of sodium tungstate dihydrate is dissolved in water and made up to 500 mL. Potassium sodium tartrate solution at 14.9 g/L: 10.0 g of the tetrahydrate is dissolved in water and made up to 500 mL. Sulfuric acid solution (5+95): 25 mL of sulfuric acid is added slowly to 475 mL of water with stirring and mixed after cooling. Buffer solution at pH 7.0: 5.79 g of disodium hydrogen phosphate is dissolved in 200 mL of water, 3.53 g of potassium dihydrogen phosphate is dissolved in a further 200 mL of water, the second solution is added to the first and the whole is made up to 1000 mL. Nessler reagent: 14.4 g of sodium hydroxide is dissolved fully in 50 mL of water and cooled; 5.5 g of red mercuric iodide and 4.125 g of potassium iodide are dissolved in 25 mL of water and this solution is transferred slowly, a little at a time, into the sodium hydroxide solution with continuous shaking; the whole is made up to 100 mL with water, mixed, transferred to a brown bottle, allowed to stand and the supernatant used. It is kept at 2 °C to 8 °C and used within 1 month. A commercial Nessler reagent meeting the requirement may be used instead.

4 The equipment consists of electronic balances reading to 0.01 g and 0.001 g, a vortex mixer, a constant temperature water bath controllable to 40 °C +/-1 °C and 25 mL stoppered colorimetric tubes.

5.1 Liquid samples are shaken until homogeneous, solid samples are ground until homogeneous and semi-solid samples are stirred until homogeneous.

5.2 For liquid and semi-solid samples, portions each equivalent to 0.10 g of solids or dry matter, weighed to 0.001 g according to the solids content of the sample, are placed in two 25 mL colorimetric tubes A and B, 1 mL of water is added to each and the tubes are shaken for 30 s, after which 1 mL of buffer solution is added to each. 1 mL of urea solution is added to tube A, the sample tube, and 1 mL of water to tube B, the sample blank tube. After mixing, the tubes are held in the water bath at 40 °C +/-1 °C for 20 min. They are then taken

out, 4 mL of water is added to each and mixed, followed by 1 mL of sodium tungstate solution with mixing and 1 mL of sulfuric acid solution with mixing, and the contents are filtered through medium speed qualitative filter paper, the filtrate being kept. 2 mL of the filtrate is taken into each of two 25 mL stoppered colorimetric tubes, 15 mL of water, 1 mL of potassium sodium tartrate solution and 2 mL of Nessler reagent are added to each, the tubes are made up to 25 mL with water and mixed, and the result is read within 5 min. For solid samples, 0.10 g of the sample, weighed to 0.001 g, is placed in each of the two tubes with 1 mL of water, shaken for 30 s and treated in the same way. Note 1: the solids or dry matter content is determined in accordance with Annex A. Note 2: where the sample solution is coloured, 6 mL of the solution is passed through a graphitised carbon black solid phase extraction cartridge after the sulfuric acid solution has been added and mixed, the first 2 mL of eluate being discarded and 2 mL of the middle fraction collected before the colour is developed.

6 The result is judged from Table 1, which relates the urease activity, the symbol used for it and the colour observed. Strong positive, four plus signs: the sample tube is darker than the blank and shows a brick red turbid or clear liquid. Second strong positive, three plus signs: the sample tube is darker than the blank and shows an orange red clear liquid. Positive, two plus signs: the sample tube is darker than the blank and shows a deep golden yellow or yellow clear liquid. Weak positive, one plus sign: the sample tube is darker than the blank and shows a pale yellow or faintly yellow clear liquid. Negative, a dash: the sample tube is the same colour as the blank or lighter. A footnote to the table defines urease activity as the milligrams of amino nitrogen released per minute per gram of sample from the decomposition of urea under the given conditions.

7 The method is qualitative; with a test portion of 0.10 g, or an amount equivalent to 0.10 g of solids, the limit of detection is 0.005 U/g.

8 Second method - titration

8 At pH 7.0 and 30 °C urease catalyses the conversion of urea into ammonium carbonate, which is neutralised by an excess of hydrochloric acid and back titrated with standard sodium hydroxide solution, the urease content being calculated from the volume of sodium hydroxide solution consumed.

9 Unless otherwise stated the reagents are analytically pure and the water is grade three water to GB/T 6682. The reagents are urea, sodium hydroxide, hydrochloric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, methyl red, bromocresol green and 95 % ethanol.

9.2 The solutions are prepared as follows. Urea buffer solution at pH 7.0 +/-0.1: 3.55 g of disodium hydrogen phosphate and 3.40 g of potassium dihydrogen phosphate are dissolved in water and made up to 1000 mL; 30 g of urea is dissolved in that buffer, mixed, transferred to a brown bottle and kept in the dark at 2 °C to 8 °C for up to 1 month.

Hydrochloric acid solution at 0.1 mol/L: 8.3 mL of hydrochloric acid is diluted to 1000 mL with water and mixed. Standard sodium hydroxide solution at 0.05 mol/L: a 0.1 mol/L standard solution is prepared by the method of GB/T 601 and diluted to 0.05 mol/L by the method of GB/T 601. Mixed methyl red and bromocresol green ethanolic solution: 0.1 g of methyl red is dissolved in 95 % ethanol and made up to 100 mL, 0.5 g of bromocresol green is dissolved in 95 % ethanol and made up to 100 mL, and equal volumes of the two are mixed and kept in a brown bottle for up to 1 month.

10 The equipment consists of electronic balances reading to 0.01 g and 0.0001 g, a pH meter of 0.02 precision fitted with a magnetic stirrer and a titration device, a constant temperature water bath controllable to 30 °C +/-0.5 °C, a mill that does not generate strong heat, a timer, a 25 mL burette, a 100 mL beaker, a 10 mL single-mark pipette and a thermometer covering 0 °C to 100 °C.

11.1 The sample is ground and mixed with the mill, the temperature during grinding being kept below 70 °C.

11.2 About 0.2 g of the sample, weighed to 0.001 g, is placed in a glass test tube and 10.0 mL of urea buffer solution is added to each sample at the same time interval; the tube is stoppered at once, shaken vigorously and held in the water bath at 30 °C +/-0.5 °C for 30 min +/-10 s. The tube is then taken out and 10.0 mL of hydrochloric acid solution is added at once at the same time interval; after shaking, the contents are cooled to 20 °C. The whole content of the tube is transferred to a 100 mL beaker, the tube being rinsed several times with 20 mL of water, and titrated with the standard sodium hydroxide solution to pH 4.70 as shown by the pH meter. Where an indicator is used instead, the whole content is transferred to a 250 mL conical flask, 8 to 10 drops of the mixed methyl red and bromocresol green indicator are added and the titration is carried out with the standard sodium hydroxide solution until the solution turns blue-green and holds that colour for 30 s. A blank is run on a further tube with the same mass of sample, 10.0 mL of hydrochloric acid solution being added at the same time interval, followed after shaking by 10 mL of urea buffer solution; the tube is stoppered at once, shaken vigorously and held in the water bath at 30 °C +/-0.5 °C for 30 min +/-10 s, then cooled to 20 °C and determined by the same procedure. Note: where the pH meter reads 4.70 or above before titration, or the solution shows blue-green after the indicator has been added, a 0.05 g test portion may be taken instead.

12 The urease activity is expressed as the milligrams of nitrogen produced from urea per minute per gram of sample and is calculated from the equation given in the standard. The symbols are the urease activity of the sample, in activity units per gram; the concentration of the standard sodium hydroxide titration solution, in moles per litre; the volume of standard sodium hydroxide titration solution consumed by the blank, in millilitres; the volume consumed by the sample, in millilitres; the reaction time of 30, in minutes; the mass of the test portion, in grams; and the molar mass of nitrogen, 14, in grams per mole. The