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**Determination of urease activity in soya bean products for
feeds**

饲料用大豆制品中尿素酶活性的测定

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Table of Contents

Foreword.....	3
1 Scope	4
2 Normative references	4
3 Terms and definitions	4
4 Principles.....	5
5 Instruments and equipment	5
6 Reagents and solutions	5
7 Preparation of specimens	6
8 Measurement steps.....	6
9 Calculation of results.....	7

Determination of urease activity in soya bean products for feeds

1 Scope

This standard specifies the determination of urease activity in soybean products and their by-products.

This standard applies to the determination of urease activity in soybeans, soybean products, and by-products. By this method, it can understand the degree of moist heat treatment of soybean products.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For the dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Standard; however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 601 Chemical reagent - Preparations of standard volumetric solutions

GB/T 6682 Water for laboratory use - Specifications (neq ISO 3696)

3 Terms and definitions

The following terms and definitions apply to this standard.

3.1

Urease activity in soya bean products

The mass of amino nitrogen, which is released by the decomposition of urea per minute per gram of soybean product, at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and pH7.

Note: Expressed in urease activity unit per gram (U/g).

Weigh 0.1 g of methyl red. Dissolve it in 95% of ethanol. Dilute it to 100 mL. Then weigh 0.5 g of bromocresol green. Dissolve it in 95% ethanol and dilute to 100 mL. Mix the two solutions in equal volumes. Store in a brown bottle.

7 Preparation of specimens

Use a pulverizer (5.1), to pulverize a representative sample, so that it all passes through the sample sieve (5.2). For special samples (samples that cannot be crushed due to their high moisture or volatile content), it shall be pre-dried at the laboratory temperature before crushing. The weight loss on dryness shall be included in the calculation of the results.

8 Measurement steps

Weigh about 0.2 g of the prepared specimen (Chapter 7), accurate to 0.1 mg, into a glass test tube (if the activity is very high, it can weight 0.05 g of specimen). Add 10 mL of urea buffer (6.1). Immediately cover the test tube cap and shake vigorously. Immediately place the test tube in a constant temperature water bath (5.4), at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Perform timekeeping for $30 \text{ min} \pm 10 \text{ s}$. It is required that the time interval for adding urea buffer solution to each specimen shall be consistent. When the reaction is stopped, add 10 mL of hydrochloric acid solution (6.2), at the same time interval. Shake and cool to 20°C quickly. Transfer all the contents of the test tube into a small beaker. Use 20 mL of water to rinse the test tube several times. Use a sodium hydroxide standard solution (6.3), to titrate it to pH 4.70, through an acidity meter (5.6). If it chooses to use an indicator, transfer all the contents of the test tube into a 250 mL conical flask. Add 8 ~ 10 drops of the mixed indicator (6.4). Use sodium hydroxide standard solution (6.3), to titrate, until the solution turns blue-green.

Take another test tube for blank test. Weigh about 0.2 g of the prepared specimen (Chapter 7), accurate to 0.1 mg. Put it in a glass test tube (if the activity is very high, it may weigh 0.05 g of specimen). Add 10 mL of hydrochloric acid solution (6.2). After shaking, add 10 mL of urea buffer solution (6.1). Immediately cover the test tube and shake vigorously. Immediately place the test tube in a constant temperature water bath (5.4), at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Perform the timekeeping for $30 \text{ min} \pm 10 \text{ s}$. Cool the test tube rapidly to 20°C , when the reaction is stopped. Transfer all the contents of the test tube into a small beaker. Use 20 mL of water to rinse the test tube for several times. Use a sodium hydroxide standard solution (6.3) to titrate to pH 4.70, through an acidity meter (5.6). If it chooses to use an indicator, then transfer all the contents of the test tube into a 250 mL conical flask. Add 8 ~ 10 drops of mixed indicator (6.4). Use sodium hydroxide standard solution (6.3) to titrate, until the solution turns blue-green.

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