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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.224-2016

**National Food Safety Standard - Determination of
trypsin inhibitor activity in soy products**

食品安全国家标准

大豆制品中胰蛋白酶抑制剂活性的测定

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People's Republic of China**

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National Food Safety Standard - Determination of trypsin inhibitor activity in soy products

1 Scope

This Standard specifies determination method for trypsin inhibitor activity (TIA) in soy products.

This Standard is applicable to determination for trypsin inhibitor activity in soy products.

2 Principle

Trypsin can react with benzoyl-L-arginine-p-nitroanilide (L-BAPA) to form p-nitroaniline. This substance has characteristic absorption at 410 nm. Trypsin inhibitor activity in soy products can inhibit this reaction to decrease absorbance value. The degree of decline is proportional to the trypsin inhibitory activity. Use spectrophotometer to determine absorbance values before and after this reaction at 410 nm. Quantitatively analyze trypsin inhibitor activity.

3 Reagents and materials

Unless otherwise stated, reagents used in this method are analytically-pure and water is grade-three water specified in GB/T 6682.

3.1 Reagents

3.1.1 Hydrochloric acid (HCl).

3.1.2 Glacial acetic acid (CH₃COOH).

3.1.3 Sodium hydroxide (NaOH).

3.1.4 Calcium chloride (CaCl₂·2H₂O).

3.1.5 Trypsin: freezing storage at -20°C.

3.1.6 Benzoyl-L-arginine-p-nitroaniline (L-BAPA).

3.1.7 Tris [NH₂C(CH₂OH)₃, Tris].

3.1.8 Dimethyl sulfoxide (C₂H₆OS, DMOS).

4 Instruments and equipment

4.1 Visible spectrophotometer.

4.2 Analytical balance: resolutions are 0.01 g, 0.001 g, 0.0001 g respectively.

4.3 PH meter: precision is 0.05.

4.4 Centrifuge: speed ≥ 4000 r/min.

4.5 Vortex oscillator.

4.6 Constant-temperature water bath: precision is $\pm 0.1^{\circ}\text{C}$.

5 Analysis steps

5.1 Sample preparation

For powder sample, take at least 200 g of representative sample. Fully mix for use. For bulk or granular sample, take at least 200 g of representative sample. Crush to below $425\ \mu\text{m}$. Fully mix for use. Avoid sample is over-heated during crushing process.

5.2 Sample extraction

Weigh $1\text{g} \sim 10\text{g}$ (to the nearest of 0.001 g) of well-prepared sample (5.1) in a 100 mL conical flask. Add 50 mL of 0.01 mol/L sodium hydroxide solution. Mix well. Use 1 mol/L hydrochloric acid solution and 0.1 mol/L hydrochloric acid solution to adjust pH to 9.5 ± 0.1 . Place in a $0^{\circ}\text{C} \sim 4^{\circ}\text{C}$ refrigerator for $15\text{h} \sim 24\text{h}$.

Take sample extract out and place at room temperature. Transfer to a 100 mL volumetric flask. Use water to set volume to scale. Shake well. After 15 min of precipitation, as required, dilute sample extract. Dilution depends on the expected TIA value of sample. Store extract in a $0^{\circ}\text{C} \sim 4^{\circ}\text{C}$ refrigerator. It can be stored for 1 d.

5.3 Dilution of sample extract

Refer to dilution plan in Table A.1 of Annex A. Dilute sample extract into three different dilution concentrations. Ensure that at least determination result of one TIA value in three inhibition percentages is within $40\% \sim 60\%$.

If three determination results are not within this range, it needs to change dilution for re-determination.

5.4 Determination of trypsin use solution activity

L-BAPA solution (3.2.11)	5	5	5	5
Sample dilution solution (5.3)	0	0	1	1
Water	3	3	2	2
Acetic acid solution (3.2.5)	1	0	1	0

Use vortex oscillator (4.5) to mix solutions in centrifuge tubes well. Place in 37°C constant-temperature water bath (4.6). Insulate for 10 min.

Respective add 1 mL of trypsin use solution (3.2.9) into four centrifuge tubes. Use vortex oscillator to mix solutions in tubes well. Put test tubes back into constant-temperature water bath (4.6). Insulate in 37°C water bath for 10min±5s. Add 1 mL of acetic acid solutions (3.2.5) into standard tube and sample tube. Mix well.

Place centrifuge tube in centrifuge (4.4). Perform centrifugation at a speed of 4000 r/min for 10 min.

Use visible spectrophotometer (4.1). At a wavelength of 410 nm, use 10 mm cuvette, use water to perform zeroing. Determine supernatant absorbance.

This solution can remain stable within 2 h.

6 Expression of analysis results

6.1 Inhibition rate of sample extract

Inhibition rate of sample extract is calculated according to formula (1):

$$i = \frac{(A_r - A_{br}) - (A_s - A_{bs})}{(A_r - A_{br})} \times 100\% \quad \dots\dots\dots (1)$$

Where,

i - inhibition rate;

A_r - absorbance of standard solution;

A_{br} - absorbance of standard blank solution;

A_s - absorbance of sample solution;

A_{bs} - absorbance of sample blank solution;

100% - conversion factor.

6.2 Trypsin inhibitor activity

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