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Report Highlights:

On May 10, 2023, the People's Republic of China (PRC) Food Safety Standards Monitoring and Evaluation Department of the National Health Commission (NHC) published an announcement soliciting domestic public comments on several draft national food safety standards. This report contains an unofficial translation of the revised National Food Safety Standard for Protein Determination, which is open for comments till June 30, 2023. Commodities involved include items like cereal and coarse grains, oilseeds, tree nuts, and eggs. The PRC will likely notify this standard to the WTO after completion of this domestic notice and comment procedure.

THIS REPORT CONTAINS ASSESSMENTS OF COMMODITY AND TRADE ISSUES MADE BY USDA STAFF AND NOT NECESSARILY STATEMENTS OF OFFICIAL U.S. GOVERNMENT POLICY

Report Summary:
On May 10, 2023, the NHC released an "Announcement from the Secretariat of the National Food Safety Standard Review Committee on Soliciting Public Comments of Five National Food Safety Standards" (link in Chinese). The announcement included a catalog of national food safety standards for food nutrition fortifiers, food additives, protein testing, and standard for dairy powder and modified dairy powder. Written comments (in Mandarin) must be submitted by June 30, 2023 from within Mainland China to the Secretariat via the national food safety standards management information system (https://sppt.cfsa.net.cn:8086/cfsa_aiguo).

This report contains an unofficial translation of the revised National Food Safety Standard for Protein Determination for comments.

Organizations and exporters of U.S. food and agricultural products can email the Office of Agricultural Affairs at AgBeijing@usda.gov with any questions or concerns.

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National Food Safety Standard
Determination of Protein in Foods
(Open for Comments)

Foreword

This standard replaces GB 5009.5-2016 "National Food Safety Standard for Determination of Protein in Foods".

Compared with GB 5009.5-2016, the main changes of this standard are as follows:
- Modified sampling volume and standard titration solution concentration of the Kjeldahl method in the first method;
- Added the storage conditions and time for standard solution and chromogenic reagent in the second method;
- Modified the applicable scope and detection limit of combustion method in the third method;
- Added the calibration curve of combustion method in Appendix B;
- Modified the expression of analysis results and the protein conversion factors table in Appendix C.

National Food Safety Standard
Determination of Protein in Foods

First Method Kjeldahl Method

1. **Scope**

This standard specifies the methods for determination of protein in foods.
This standard applies to the determination of protein in foods.
This standard does not apply to the determination of protein in foods with added non-protein nitrogenous substances.

2. **Theory**

The protein in foods is decomposed under catalytic heating conditions, and the ammonia and sulfuric acid produced are combined to form ammonium sulfate. Alkalized distillation makes ammonia free, which absorb boric acid and titrate with sulfuric acid or hydrochloric acid standard titration solution. The protein content is calculated by the content of nitrogen according to the consumption of sulfuric acid or hydrochloric acid, then multiplied by the conversion factor.

3. **Testing Reagents and Materials**

3.1 Reagents

Unless otherwise specified, reagents used in this method are of analytical purity grade, and the water used is third-grade water as specified in GB/T 6682.

3.1.1 Copper sulfate ($CuSO_4 \cdot 5H_2O$).

3.1.2 Potassium sulfate (K_2SO_4).

3.1.3 Sodium hydroxide (NaOH).

3.1.4 Sulfuric acid (H_2SO_4): 98%.

3.1.5 Boric acid (H_3BO_3).

3.1.6 Ethanol (C_2H_5OH): 95%.

3.1.7 Methyl red indicator ($C_{15}H_{15}N_3O_2$).

3.1.8 Bromocresol green indicator ($C_{21}H_{14}Br_4O_5S$).

3.1.9 Methylene blue indicator ($C_{16}H_{18}ClN_3S \cdot 3H_2O$).

3.1.10 pH test paper: the measuring range is 0 to 14.

3.2 Preparation of reagents

3.2.1 Boric acid solution (20 g/L): Weigh 20 g of boric acid and add water to dissolve and dilute to 1000 mL.

3.2.2 Sodium hydroxide solution (400 g/L): Weigh 40 g of sodium hydroxide and add water to dissolve, let it cool, and dilute to 100 mL.

3.2.3 Sulfuric acid standard titration solution [$c(\frac{1}{2}H_2SO_4)$] or hydrochloric acid standard titration solution [c(HCl)] 0.10 mol/L. Prepare and titrate according to requirements of GB/T 5009.1, or the standard titration solution certified by the state and granted with standard substance certificate.

3.2.4 Sulfuric acid standard titration solution [c($\frac{1}{2}H_2SO_4$)] or hydrochloric acid standard titration solution [c(HCl)] 0.05 mol/L: pipet 50 mL of 0.10 mol/L sulfuric acid standard titration solution [c($\frac{1}{2}H_2SO_4$)] or hydrochloric acid standard titration solution [c(HCl)] to the volumetric flask, dilute to 100 mL with water, prepare before use, and recalibrate if necessary.

3.2.5 Methyl red ethanol solution (1 g/L): weigh 0.1 g of methyl red, dissolve in 95% ethanol, dilute to 100 mL, and prepare before use.

3.2.6 Methylene blue ethanol solution (1 g/L): weigh 0.1 g of methylene blue, dissolve in 95% ethanol, dilute to 100 mL, and prepare before use.

3.2.7 Bromocresol green ethanol solution (1 g/L): weigh 0.1 g bromocresol green, dissolve in 95% ethanol, dilute to 100 mL, and prepare before use.

3.2.8 Mixed indicator solution A: take 2 portions of methyl red ethanol solution and 1 portion of methylene blue ethanol solution and mix before use.

3.2.9 Mixed indicator solution B: take 1 portion of methyl red ethanol solution and 5 portions of bromocresol green ethanol solution and mix before use.

4. Instrument and Equipment

4.1 Analytical balance: the sensitivity is 1 mg.

4.2 Nitrogen distillation device: as shown in Appendix A.

4.3 Pipettes: 10 mL, 25 mL and 50 mL.

4.4 Digestion furnace: ≥ 420 °C.

4.5 Semi-automatic Kjeldahl nitrogen analyzer or automatic Kjeldahl nitrogen analyzer.

4.6 Homogenizer.

4.7 Blender.

5. Analysis Steps

5.1 Kjeldahl method

5.1.1 Sample preparation
Shake liquid samples well, and protein beverages containing gas or volatile additives should be degassed with ultrasonic waves before weighing and measuring. The semi-solid and powder samples with homogeneous matrix can be directly weighed and other types of samples need to be homogenized or blended evenly before weighing. Prepared samples should be stored at 0°C to 5°C and measured as soon as possible.

5.1.2 Water content determination
If the sample result is calculated on a dry basis, the water content shall be determined according to the method specified in GB 5009.3.

5.1.3 Sample handling
Weigh 0.2 g to 2 g of solid sample, 2 g to 5 g of semi-solid sample (accurate to 0.001 g), and 10 g to 25 g or 10 mL to 25 mL (accurate to 0.001 g/0.01 mL) (approximately 30 mg to 40 mg nitrogen) of liquid sample respectively, pipette into dry nitrogen bottle of 100 mL, 250 mL or 500 mL, add 0.4 g of copper sulfate, 6 g of potassium sulfate, and 20 mL of sulfuric acid, shake gently and put a small funnel at the bottle mouth, and place the bottle obliquely at a 45-degree angle on an asbestos mesh with small holes. Slowly heat the bottle until the contents are all carbonized. After foam stops completely, increase the heating power and keep the liquid in the bottle boiling slightly until the liquid turns blue-green and clear, then continue to heat for 0.5 to 1 hour. Remove the nitrogen bottle and cool to room temperature, carefully add 20 mL of water, and transfer the liquid to a 100 mL volumetric flask. Wash the inner wall of the nitrogen bottle with a small amount of water, put the washing solution into the volumetric flask, add water to the mark, and mix well for use. At the same time, conduct reagent control test.

5.1.4 Testing
Install the nitrogen distillation device according to Appendix A, fill the steam generator with water to 2/3 of the bottle. Add a few glass beads, add a few drops of methyl red ethanol solution and several milliliters of sulfuric acid until the methyl red solution turns red to keep the water acidic, boil water in the vapor generator, and keep it boiling.

Add 10.0 mL of boric acid solution and 3 to 4 drops of mixed indicator liquid A or mixed indicator liquid B into the receiving bottle, insert the lower end of the condensation tube under the liquid surface, and accurately absorb 2.0 mL to 10.0 mL of the sample treatment solution according to the nitrogen content in the sample into reaction chamber via the small glass. Wash the small glass with 10 mL of water and let it flow into the reaction chamber, then tighten the rod-shaped glass stopper. Pour 10.0 mL of sodium hydroxide solution into the small glass, lift the glass stopper to let the liquid slowly flow into the reaction chamber, put on the glass stopper immediately, and seal it with water. Turn the screw clamp and start the distillation. After distilling for 15 minutes, move the distillate receiving bottle until the lower end of the condensation tube is away from the liquid, distill for another 1 minute, and a pH paper test shows that the distillate is neutral. Then rinse the outside of the lower end of the condensation tube with a small amount of water and remove the distillation receiving bottle. Titrate with 0.0500 mol/L sulfuric acid or hydrochloric acid standard titration solution to the end point, if mixed indicator solution A is used, the end point color is purple, and if mixed indicator solution B is used, the end point color is light grayish red. At the same time, reagent control test should be conducted.

5.2 Automatic Kjeldahl method

Weigh 0.2 g to 2 g of solid sample, 2 g to 5 g of semi-solid sample (accurate to 0.001 g), and 10 g to 25 g or 10 mL to 25 mL (accurate to 0.001 g/0.01 mL) (approximately 30 mg to 40 mg nitrogen) of liquid sample, then add 0.4 g of copper sulfate, 6 g of potassium sulfate, and 20 mL

of sulfuric acid into the digestion furnace. When the temperature of digestion furnace reaches 420 °C, continue to digest for at least 1 hour, at this time, the liquid in the digestion tube is green and transparent, take it out and cool it to room temperature, add 50 mL of water, and put it in the automatic or semi-automatic Kjeldahl nitrogen analyzer (optimize the analysis parameters according to different instruments before use, add sodium hydroxide solution, hydrochloric acid or sulfuric acid standard solution, and boric acid solution containing mixed indicator A or B) to realize automatic liquid addition, distillation, titration, and recording of titration data. When the protein content is ≤1 g/100 g or 1 g/100 mL, it is recommended to use 0.0500 mol/L of standard titration solution for titration. When the protein content is >1 g/100 g or 1 g/100 mL, it is recommended to use 0.1000 mol/L of standard titrant for titration.

Note: When the protein content is too low and the titration volume is less than 1 mL or the protein content is too high and the titration volume is larger than the titration tube in the automatic Kjeldahl nitrogen analyzer, the sample volume can be adjusted to reduce the titration errors. When the fat content of the sample is too high or the sugar content is too high, the sample volume can be adjusted to reduce or avoid bumping, splashing, and overflowing during the digestion.

6. Expression of Analysis Results

The protein content in the sample is calculated according to formula (1):

$$X = \frac{(V_1-V_2) * c * 0.0140}{m * V_3/V_4} * F * 100 \quad \text{............................ (1)}$$

In the formula:
X: protein content in the sample, in grams per hundred grams or grams per hundred milliliters (g/100 g or g/100 mL),
V_1: the volume of sulfuric acid or hydrochloric acid standard titration solution consumed by the sample solution, in milliliters (mL),
V_2: the volume of sulfuric acid or hydrochloric acid standard titration solution consumed by the reagent, in milliliters (mL),
c: concentration of standard titration solution of sulfuric acid or hydrochloric acid, in moles per liter (mol/L),
0.0140: mass of nitrogen equivalent to the titration solution of 1.0 mL sulfuric acid [$c(\frac{1}{2}H_2SO_4)$] =1.000 mol/L] or hydrochloric acid [c (HCl) =1.000 mol/L], in kilograms per mole (kg/moL),
m: mass of the sample, in grams or milliliters (g or mL),
V_3: volume of digestion solution absorbed, in milliliters (mL),
F: protein conversion factor, is 6.25 unless specified in Appendix C or corresponding product standards,
V_4: constant volume of the digestion solution, in milliliters (mL),
100: conversion factor from g/g to g/100 g.

When the protein content is ≥1 g/100 g or 1 g/100 mL, the result shall retain three valid digits; when the protein content is <1 g/100 g or 1 g/100 mL, the result shall retain two valid digits.

Note: When the analysis result is expressed in terms of nitrogen content, it is not necessary to multiply the protein conversion factor F. When the analysis result is expressed in terms of protein content, the protein conversion factor should be reported at the same time.

When using semi-automatic or automatic Kjeldahl nitrogen analyzer, V_3=100.

When calculating on a dry basis, protein content needs to be converted based on the water content of the sample.

7. Accuracy

The absolute difference between two independent determination results obtained under repeated conditions shall not exceed 10% of the arithmetic mean.

8. Other

When the sample weight is 5.0 g or 5.0 mL, the maximum limit for nitrogen when using this method is 8 mg/100 g or 8 mg/100 mL.

Second Method Spectrophotometry

9. Theory

The protein in foods is decomposed under catalytic heating conditions, the ammonia and sulfuric acid produced by the decomposition combined to form ammonium sulfate, which reacts with acetylacetone and formaldehyde in pH 4.8 sodium acetate-acetic acid buffer solution to form yellow 3,5-diacetyl- 2,6-dimethyl-1,4-dihydropyridine compound. The protein content is the result of conversion factor multiplied by the absorbance value measured at a wavelength of 400 nm and compared with the standard curve for quantification.

10. Testing Reagents and Materials

10.1 Testing reagents

Unless otherwise specified, reagents used in this method are of analytical purity grade and water is third-grade water specified in GB/T 6682.

10.1.1 Copper sulfate ($CuSO_4 \cdot 5H_2O$).

10.1.2 Potassium sulfate (K_2SO_4).

10.1.3 Sodium hydroxide (NaOH).

10.1.4 p-Nitrophenol ($C_6H_5NO_3$).

10.1.5 Sodium acetate ($CH_3COONa \cdot 3H_2O$).

10.1.6 Anhydrous sodium acetate (CH_3COONa).

10.1.7 Sulfuric acid (H_2SO_4): 98%.

10.1.8 Acetic acid (CH_3COOH).

10.1.9 Formaldehyde (HCHO): 37%.

10.1.10 Acetylacetone ($C_5H_8O_2$).

10.1.11 Ammonium sulfate ($(NH_4)_2SO_4$): premium grade.

10.2 Preparation of reagents

10.2.1 Sodium hydroxide solution (300 g/L): weigh 30 g of sodium hydroxide and add water to dissolve, let it cool, and dilute to 100 mL.

10.2.2 p-nitrophenol indicator solution (1 g/L): weigh 0.1 g of p-nitrophenol indicator solution, dissolve in 20 mL of 95% ethanol, and dilute to 100 mL with water.

10.2.3 Acetic acid solution (1 mol/L): measure 5.8 mL of acetic acid and dilute with water to 100 mL.

10.2.4 Sodium acetate solution (1 mol/L): measure 41 g of anhydrous sodium acetate or 68 g of sodium acetate, dissolve in water, and dilute to 500 mL.

10.2.5 Sodium acetate-acetic acid buffer solution: measure 60 mL of sodium acetate solution, mix with 40 mL of acetic acid solution, and pH of the solution is 4.8.

10.2.6 Chromogenic agent: mix 15 mL of formaldehyde with 7.8 mL of acetylacetone, dilute with water to 100 mL, shake vigorously, and mix well. Stored in a brown bottle at 4°C for 3 days.

10.3 Standard substance

Ammonium sulfate standard stock solution (calculated as nitrogen) (1.0 g/L), or a standard product that has been certified by the state and granted a standard substance certificate.

10.4 Standard solution preparation

10.4.1 Ammonium sulfate standard stock solution (calculated as nitrogen) (1.0 g/L): weigh 0.4720 g of ammonium sulfate dried at 105 °C for 2 hours, dissolve with water, dilute to 100 mL, and mix well. Each milliliter of the solution is equivalent to 1.0 mg of nitrogen. The solution can be stored at 4°C for 1 month.

10.4.2 Ammonium sulfate standard working solution (0.10 g/L): pipette 10.00 mL of ammonium sulfate standard stock solution into a 100 mL volumetric flask, add water to dilute to the mark,

and mix well. Each milliliter of the solution is equivalent to 0.1 mg of nitrogen. Prepare the solution before use.

11. Instrument and Equipment

11.1 Spectrophotometer.

11.2 Electric constant temperature water bath pot: 100 ℃ ± 1 ℃.

11.3 pH meter: accurate to 0.01.

11.4 10 mL glass colorimetric tube with stopper.

11.5 Analytical balance: sensitivity is 1 mg and 0.1 mg.

11.6 Homogenizer.

11.7 Blender.

12. Analysis Steps

12.1 Specimen preparation

The steps are the same as 5.1.1.

12.2 Moisture determination

The steps are the same as 5.1.2.

12.3 Sample digestion

Weigh 0.1 g to 0.5 g of solid sample, 0.2 g to 1 g of semi-solid sample (accurate to 0.001 g) or 1 g to 5 g or 1 mL to 5 mL (accurate to 0.001 g or 0.01 mL) of liquid sample, transfer to a dry 100 mL or 250 mL nitrogen bottle, add 0.1 g of copper sulfate, 1 g of potassium sulfate and 5 mL of sulfuric acid, shake well, put a small funnel on the mouth of the bottle, and place the nitrogen bottle at an angle of 45 degrees on an asbestos mesh with small holes. Heat slowly, until all the contents are carbonized, and the foam stops completely. Increase the heating power and keep the liquid in the bottle boiling slightly until the liquid turns blue-green and clear, then continue heating for 0.5 to 1 hour. Take off the nitrogen bottle, cool to room temperature, slowly add 20 mL of water, then transfer the solution into a 100 mL volumetric flask. Wash the inner wall of the nitrogen bottle with a small amount of water and put the washing solution into the volumetric flask. Add water to the mark, mix well, and put aside for future steps. At the same time, conduct reagent control test.

12.4 Preparation of sample solution

Measure 2.0 mL to 5.0 mL (accurate to 0.01 mL) of sample or reagent digestion solution into 50 mL or 100 mL volumetric flask, add 1 drop to 2 drops of p-nitrophenol indicator solution, shake well, add sodium hydroxide solution to neutralize until the solution turns yellow, then add

dropwise acetic acid to dissolve solution until the solution is colorless, dilute to the mark with water, and mix well.

12.5 Preparation of standard curve

Pipette 0.00 mL, 0.05 mL, 0.10 mL, 0.20 mL, 0.40 mL, 0.60 mL, 0.80 mL and 1.00 mL of sulfur ammonium acid standard solution (equivalent to 0.00 μg, 5.00 μg, 10.0 μg, 20.0 μg, 40.0 μg, 60.0 μg, 80.0 μg and 100.0 μg nitrogen) respectively, place in 10 mL of colorimetric tubes. Add 4.0 mL of sodium acetate-acetic acid buffer solution and 4.0 mL of chromogen, add water to dilute to the mark, and mix well. Place in a water bath at 100 °C for 15 minutes. After cooling to room temperature with water, transfer to 1 cm of colorimetric cup, take the tube 0 as a reference, measure the absorbance value at a wavelength of 400 nm, draw the scale according to the absorbance value at each point of the standard curve, or calculate a linear regression equation.

12.6 Determination of samples

Pipette 0.50 mL to 2.00 mL (approximately equivalent to nitrogen <100 μg) sample solution and the same amount of reagent solution respectively and place it into a 10 mL of colorimetric tube. Add 4.0 mL of sodium acetate-acetic acid buffer solution and 4.0 mL of chromogenic reagent, dilute with water to the mark, and mix well. Place in a water bath at 100 °C for 15 minutes. Take it out and cool it to room temperature with water, then transfer it into a 1 cm of colorimetric cup, take the tube 0 as reference, measure the absorbance value at a wavelength of 400 nm, and find the concentration of the test solution on the standard curve.

13. Expression of Analysis Results

The protein content in the sample is calculated according to formula (2):

$$X = \frac{(c-c_0) * V_1 * V_3}{m * V_2 * V_4 * 1000 * 1000} * 100 * F \qquad (2)$$

In the formula:

X: protein content in the sample, in grams per hundred grams or grams per hundred milliliters (g/100 g or g/100 mL),
c: the content of nitrogen in the test sample liquid, in micrograms (μg),
c_0: nitrogen content in the reagent measurement solution, in micrograms (μg),
V_1: constant volume of the sample digestion solution, in milliliters (mL),
V_2: volume of digestion solution used to prepare the sample solution, in milliliters (mL),
m: mass of the sample, in grams or milliliters (g or mL),
V_3: total volume of the sample solution, in milliliters (mL),
V_4: volume of the sample solution for measurement, in milliliters (mL),
1000: conversion factor from μg to mg,
1000: conversion factor from mg to g,

100: conversion factor from g/g to g/100 g,
F: is 6.25 unless specified in Appendix C or corresponding product standards.

When the protein content is ≥1 g/100 g, the result retains three valid digits, and when the protein content is <1 g/100 g, the result retains two significant digits.

Note: When the analysis result is expressed in terms of nitrogen content, it is not necessary to multiply the protein conversion factor F. When the analysis result is expressed in terms of protein content then the protein conversion factor should be reported at the same time.

When calculating on a dry basis, protein content needs to be converted based on the water content of the sample.

14. Accuracy

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

15. Other

When the sample weight was 5.0 g or 5.0 mL, the maximum limit for nitrogen when using this method is 0.1 mg/100 g or 0.1 mg/100 mL.

Third Method Combustion Method

16. Theory

The sample is burned at a high temperature of 900 °C to 1200 °C and a mixed gas is generated during the combustion process. The interfering gases such as carbon, sulfur, and salts are absorbed by the absorption tube and the nitrogen oxides are all restored to nitrogen. The formed nitrogen gas flows through the Thermal Conductivity Detector (TCD) for detection.

17. Testing Reagents and Materials

17.1 Oxygen (O_2) gas: purity ≥99.995%.

17.2 Carrier gas (CO_2, H_2, Ar_2 gas, etc.): purity ≥99.995%.

17.3 Nitrogen-containing standard substances: aspartic acid ($C_4H_7NO_4$), urea (CH_4N_2O), purity ≥ 99%. Or nitrogen-containing standards substances that have been certified by the state and granted a certificate for standard substance.

18. Instrument and Equipment

18.1 Nitrogen/protein analyzer: equipped with thermal conductivity detector, measuring range is 0.1 mg to 200 mg of nitrogen content with automatic calibration function.

18.2 Analytical balance: the sensitivity is 0.1 mg.

18.3 Homogenizer.

18.4 Blender.

19. Sample Preparation

The steps are the same as 5.1.1.

20. Water Content Determination

The steps are the same as 5.1.2.

21. Testing Steps

21.1 Instrument working conditions

21.1.1 Combustion temperature
900°C~1200°C.

21.1.2 Oxygen flux
Check the optimal oxygen flow according to different instruments before use. The amount of oxygen should be controlled after the sample is burned and 2% to 8% should be reserved for residual oxygen.

21.1.3 Oxygen time
Adjust the oxygen time to ensure the complete combustion of the sample according to the difference in the amount of the sample and the difficulty of burning the sample.

21.1.4 Instrument calibration
After the instrument is stabilized after starting up, use the optimized instrument parameters to measure the standard substance whose nitrogen content is slightly higher than that of the sample to be tested.

The daily calibration factor (f) was obtained by repeating the tests for 3 times. If the daily calibration factor is greater than 1.1 or less than 0.9, or after the replacement of the thermal conductivity detector, the calibration curve should be redrawn (see Appendix B). After the daily calibration factor is within the range of 0.9 to 1.1, the sample should be re-tested.

$$f = \left(\frac{c}{c_1} + \frac{c}{c_2} + \frac{c}{c_3} \right) * \frac{1}{3} \qquad \text{(3)}$$

f: daily calibration factor,
c: theoretical nitrogen content of the standard substance, in grams per hectogram (g/100 g),
c_1, c_2, c_3: nitrogen content of three repeated tests of the standard substance, in grams per hectogram (g/100 g).

21.2 Tests

Weigh 0.1 g to 1.0 g of a well-mixed solid sample (accurate to 0.001 g) according to the requirements of the instrument manual, or pipette 0.1 g to 1.0 g or 0.1 mL to 1.0 mL of fully mixed liquid sample (accurate to 0.001 g/0.01 mL), wrapped in tin foil or weighed in a stainless-steel crucible, and place it on a sample tray. Measure the content after the sample is fully burned.

22. Expression of Analysis Results

The protein content in the sample is calculated according to formula (4):

$$X = c * F \quad \text{.........................} (4)$$

In the formula:
X: the protein content in the sample, in grams per hundred grams or grams per hundred milliliters (g/100 g or g/100 mL),
c: nitrogen content in the sample, in grams per hundred grams (g/100 g),
F: protein conversion factor, is 6.25 unless specified in Appendix C or corresponding product standards.

When the protein content is ≥1 g/100 g, the result retains three valid digits, when the protein content is <1 g/100 g, the result retains two valid digits.

Note: When the analysis result is expressed in terms of nitrogen content, it is not necessary to multiply the protein conversion factor F. When the analysis result is expressed in terms of protein content, the protein conversion factor should be reported at the same time.

When calculating on a dry basis, protein content needs to be converted based on the water content of the sample.

23. Accuracy

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

24. Other

When the sample weight was 0.2 g or 0.2 mL, the maximum limit when using this method is 0.50 g/100 g or 0.50 g/100 mL.

Appendix A

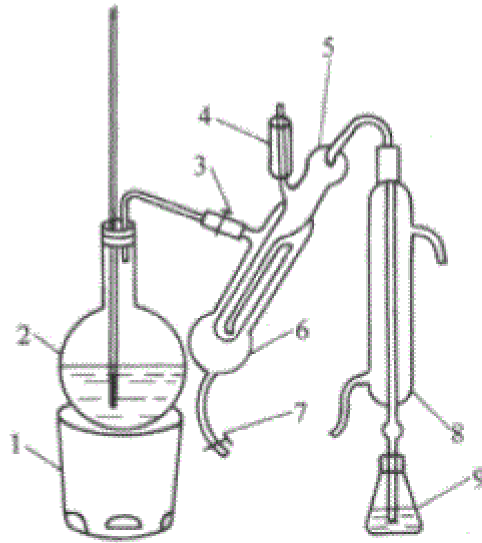

Figure A.1: Diagram of Nitrogen Distillation Device

1: Electronic Heater
2: Vapor Generator (2L flask)
3: Screw Clamp
4: Small Glass with stopper
5: Reactor Chamber
6: Outer Layer of Reactor Chamber
7: Rubber Tube and Screw Clamp
8: Condensation Tube
9: Receiver of Distillate

Appendix B

Calibration Curve of Combustion Method

B. 1 Drawing of the Calibration Curve

The appropriate quantification of calibration curve should be selected according to the built-in calibration curve model of the instrument and the corresponding determination level of the nitrogen content. The nitrogen content determined by the sample should be within the range of the curve, and the result of quality control sample for the quantity calibrated curve should be within the satisfactory range.

Under the optimized instrument conditions, aspartic acid (nitrogen content: 10.52%) or urea (nitrogen content: 46.65%) is used as the standard substance to draw the calibration curve of the instrument.

Calibration curve drawing example 1: suitable for samples with nitrogen content of about 0.07 % to 2.6 %. Weigh aspartic acid standard substance, the mass is 7 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg and 250 mg respectively, a calibration curve is drawn with the peak area measured by the TCD and the absolute nitrogen content in the standard substance as a coordinate.

Calibration curve drawing example 2: suitable for samples with nitrogen content of about 2.6 % to 20 %. Weigh urea standard sample, the mass is 60 mg, 80 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 250 mg, 300 mg, 350 mg and 429 mg respectively, a calibration curve is drawn with the peak area measured by the TCD and the absolute nitrogen content in the standard substance as a coordinate.

Appendix C

Conversion Factors of Converting Nitrogen into Protein in Foods

See Table C.1 for conversion factors of converting nitrogen into protein in common foods.

Table C.1 Conversion Factors of Protein

Foods	Conversion Factors
Whole wheat flour	5.83
Wheat embryo	5.80
Wheat embryo flour, Rye, Wheat, Flour	5.70
Oat, Barley	5.83
Millet, Green rye	5.83
Corn, Triticale, Sorghum	6.25
Rice	5.95
Sesame, Cottonseed, Sunflower, Castor, Safflower	5.30
Rapeseed	5.53

Other oil seeds	6.25
Brazil nuts	5.46
Peanut	5.46
Almond	5.18
Walnuts, Hazelnuts, Coconuts	5.30
Bean and its products	6.25
Meat and its products	6.25
Pure dairy and its products	6.38
Eggs (whole) (adjust position by category in table)	6.25
Egg yolk (adjust position by category in table)	6.12
Egg white (adjust position by category in table)	6.32
Animal gelatin	5.55
Casein	6.40
Collagen	5.79

Note: If the protein conversion factor has been clearly stipulated in the product standard, it shall be implemented according to the product standard. Pure dairy and pure dairy products refer to dairy and dairy products that have no ingredient containing non-dairy protein nitrogen added during production and processing, so that dairy protein in the product is the only source of nitrogen.

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Attachments:

No Attachments.