Dumas Application

A.3.1.4.3 Soluble Proteins in KOH Solution – Soybean Flour

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1 Principle

Potassium hydroxide solubility test is used to evaluate the quality of protein of soybean meal. The sample is dispersed in a solution of potassium hydroxide of approximately 12,5 pH, stirred and centrifuged. Then, the nitrogen content of the clarified liquid is determined according to the Dumas method and compared with the value of crude protein of the original sample.

The analysis according to the Dumas method is performed as follow. The nitrogen contained in the sample is oxidized to nitrogen oxides in an oxygen atmosphere, at high temperatures and in the presence of a catalyst. The nitrogen oxides are then reduced to nitrogen with the help of copper. The side products, water and carbon dioxide, are separated in specific traps. Finally, the nitrogen is detected by a thermal conductivity detector (TCD) and its quantity is determined on the basis of a previously performed calibration by analysing a suitable substance with a known nitrogen content.

2 Methods

This application note is meant to be a guideline for the operation of your C. Gerhardt analysis system and has to be adapted to your sample matrix and the local circumstances in your laboratory.

This document is based on

- ISO 14244:2014, Oilseed meals – Determination of soluble proteins in potassium hydroxide solution.

3 Gases and Consumables

The following items and gases are required for the operation of N-Realyzer:

3.1 Helium cylinder gas, quality grade min. 5.0
3.2 Oxygen cylinder gas, quality grade min. 5.0
3.3 Compressed air, class 3 as per ISO 8573-1
Alternative to compressed air: nitrogen cylinder gas, quality grade 2.6 (99.6 %, oil and water-free)
3.4 DumaFoil, tin foil for sample wrapping (Order number 14-0017) or DumaFoil XL, tin foil specially designed for weighing in larger samples (Order number 14-0417)
3.5 DumaCollect, ash insert with bottom (Order number 14-0015)
3.6 DumaReact, prepacked combustion reactor filled with HT- and LT-catalyst (Order number 14-0244)
3.7 DumaTube, quartz tube for reactor (Order number 14-0203), DumaPad, wool pads for reduction reactor (Order number 14-0225), DumaCop, copper for reduction (Order number 14-0007)
3.8 Water trap filled with adsorbent silica gel and wool (Order number 14-0217,14-0219, 14-0243)
Note: A mixture of 10 g silica gel (Order number 14-0219) and 30 g magnesium perchlorate (CAS 10034-81-8, ThermoFisher Scientific, 99% wasserfrei, ACS, 011636.36) can be prepared and used for the water trap to extend its lifetime. For further details, refer to the operating instructions of N-Realyzer, chapter 10.4.
3.9 REAL-N Spiral adsorber for CO_2 (Order number 14-4085)
3.10 DumaEDTA, calibration standard, purity > 99 % (Order number 14-0032)
3.11 THAM, Tris(hydroxymethyl)aminomethane, purity > 99 %
3.12 Potassium hydroxide solution c(KOH) = 0,036 mol/l

4 Instruments

- Rotor mill
- Analytical balance (accuracy at least 0.1 mg, preferably 0.01 mg)
- Magnetic stirrer and stir bars
- Beakers
- Pipettes
- Centrifuge or filter paper, nitrogen free
- N-Realyzer basic unit, with starter kit and consumables, Order number 14-4000

5 Procedure

5.1 Sample preparation and weighing

The soy flour is grinded through a 250 µm sieve.

The particle size of the sample affects the final result.

If the fat content is higher than 5%, it shall be defatted by cold extraction.

1,5 g of the soy flour is weighted and placed in a beaker. 75 ml of potassium hydroxide solution (3.12) are added, and the sample is stirred at minimum speed for 20 minutes to maintain all the solids in suspension.

Stirring of the solution for 20 minutes

The totality of the liquid is transferred to a centrifugal tube and centrifugated for 10 min or is filtered.

No particle should be in suspension in the filtrate.

The DumaFoil (Art. 14-0017) is tared and about 20 mg of Super-absorber (ratio 1:10) (Art. 14-0295) are added. The balance is tared again and about 200 mg (or µl) of filtrate are added using a syringe. The first stable weight is either noted or automatically transferred from the balance into the software REAL-OS. After having waited to let the sample react with the Super-absorber, the tin foil is closed and placed in the transfer tray.

Note: The determination of the nitrogen content according to Dumas is performed in parallel with the same sample as described in the Application A.3.1.4.2.

5.2 Daily Routine

Before the analysis, perform the quality assurance described in the operating instructions of N-Realyzer (Check-up consumables, Check-up leak test, Check-up blank value, Check-up standard).

Check-up consumables	For further details about the handling of consumables, refer to the operating instructions of N-Realyzer, chapter 8.1.2, chapter 10.2 (reactors), chapter 10.3 (crucibles), chapter 10.4 (water trap) and chapter 10.5 (CO_2 adsorbers).
Check-up leak test	For further details about the leak test, refer to the operating instructions of N-Realyzer, chapter 8.1.3.
Check-up blank value	For further details about the blanks, refer to the operating instructions of N-Realyzer, chapter 8.1.4.
Check-up standard	For further details about the standards, refer to the operating instructions of N-Realyzer, chapter 8.1.4.

5.3 Combustion of the sample

For the combustion, we recommend the following settings:

Parameter	Setting
Combustion method	C 0.6 (with 0.6 ml O_2 / mg sample and a dosing speed of 200 ml/min)
Combustion temperature	With DumaReact (3.6): 980 °C
Reduction temperature	With DumaReact (3.6): 650 °C

Note: For further information about the optimization of the combustion method, refer to the operating instructions of N-Realyzer, chapter 13.2.

5.4 Calibration

The selected calibration must cover the working range. Using a sample weight as recommended, a calibration performed with a THAM solution 0,5%N till 2,5 mgN is sufficient.

Note: A THAM solution 0.5%N is prepared by weighing 4.324 g of THAM (tris(hydroxymethyl)aminomethane, $H_2NC(CH_2OH)_3$) (3.11) in 100 ml of distilled water.

The minimum requirement for the correlation factor R2 is a value ≥ 0.999.

Note: For further information about the calibration, refer to the operating instructions of N-Realyzer, chapter 8.2 and 13.1.

5.5 Calculation

$$KOH\ Protein\ Solubility\ [\%] = \frac{\frac{m_{N,filtrate\ analyzed}}{Portion\ of\ filtrate\ analyzed \times m_{orig.sample\ for\ treatment}} \times 100}{w_{N,orig.sample}} \times 100$$

With:

$m_{N.filtrate\ analyzed}$: nitrogen weight in filtrate analyzed [g]
Portion of filtrate analyzed: amount of filtrate analyzed/total amount of filtrate
$m_{orig.\ sample\ for\ treatment}$: weight of sample for KOH treatment [g]
$w_{N,orig.sample}$: nitrogen content in original sample [%]

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- Fat in food and feed
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- Trace metal in soil and sludge
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