APPLICATION NOTE

Protein solubility in potassium hydroxide (KOH) - Dumas

Method based **on ISO 14244:2014** - Oilseed meal - Determination of soluble proteins in potassium hydroxide solution and **VDLUFA Methodenbuch Band III 20.2**

Protein solubility in KOH – A key factor for feed quality and animal welfare

Protein solubility in potassium hydroxide (KOH) is a decisive quality parameter for protein-rich feedstuffs. Particularly in the case of soybeans, a central protein source in animal nutrition, this value provides information on the digestibility and physiological benefits of the protein. As soybeans contain not only high-quality amino acids but also antinutritive factors - natural or synthetic compounds in food or feed that can inhibit or impair the utilization of nutrients in the body - targeted thermal processing is necessary. Processes such as toasting, steaming or extrusion improve the protein structure and reduce antinutritive ingredients. The protein solubility in KOH serves as a measure of the efficiency of these processing methods.

C. Gerhardt instruments:
- N-Realyzer

Additional equipment:
- Rotor mill
- Analytical balance
- Magnetic stirr

KOH protein solubility	Assessment of the protein quality
< 70%	Overheating damage (proteins denature, are less soluble and less digestible)
78-85%	Optimal
> 85%	Possible inadequate heat treatment (antinutritive substances such as trypsin inhibitors are not destroyed, poorer digestibility)

Quelle: Van Eys J.E., Offner A., Bach A. Manual of Quality Analyses for Soybean Products in the Feed Industry. 2nd ed. American Soybean Association; Brussels, Belgium: 2004

The test is based on the determination of the soluble fraction of the protein in a 0.2% KOH solution. Higher values indicate good availability and digestibility, while lower values indicate overheating or inadequate processing. This indicator is particularly important in the animal feed industry to assess the quality of soy extraction meal and other protein sources. Targeted laboratory control can maximize nutrient intake and optimize feed conversion.

Another advantage of laboratory control is that tests on animals can be reduced or even avoided altogether. Laboratory analysis makes it possible to ensure the quality and digestibility of the feed in advance - a significant step towards greater animal welfare and sustainability in feed production.

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However, protein solubility in KOH goes beyond quality control: in compound feed calculation, it serves as a solid basis for ration optimization. Good solubility is directly related to digestibility - a key factor for the growth performance and health of livestock. Combined with indicators such as trypsin inhibitor activity (TIA) or urease activity, it provides a comprehensive picture of protein quality and processing intensity.

Standardized methods such as ISO 14244 and VDULFA Method Book Volume III 20.2 guarantee a high reproducibility of the results. The analysis is usually carried out using the Kjeldahl or Dumas method, which precisely quantify the nitrogen content of the soluble protein components. This means that protein solubility in KOH is not only an indispensable tool for quality assurance, but also makes a significant contribution to optimizing nutrient supply and promoting animal welfare.

The method – Dumas application

Sample preparation

The soy flour is ground through a sieve and then 1.5 g is weighed and placed in a beaker. 75 ml of potassium hydroxide solution is added and the sample is stirred for 20 minutes at minimum speed. The liquid is then centrifuged in a centrifuge tube for 10 minutes at a relative acceleration of 800 g or alternatively filtered - duration between 20 - 60 minutes.

➔ **Application note**: The particle size of the sample influences the analysis result.

➔ **Application note**: If the fat content is more than 5%, the fat must be removed by cold extraction.

Weighing

DumaFoil tin foil is tared and about 20 mg of superabsorbent (ratio 1:10) is added. The balance is tared again and about 200 mg of filtrate is added with a syringe. After the sample has reacted with the superabsorber, the aluminum foil is closed and placed in the transfer tray.

Calibration

The selected calibration must cover the working range. When using a recommended weight of the sample, calibration with a THAM solution of 0.5 % N to 2.5 mg N is sufficient.

Blanke value

To determine the blank value, carry out the analysis (digestion + distillation + titration) using only the specified chemicals and 1 g of sucrose instead of the sample. The consumption of the standard solution must be taken into account in the calculation

Calculation

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

mN. Filtrate analyzed: Nitrogen weight in the analyzed filtrate [g]
Filtrate analyzed: Amount of filtrate analyzed/total amount of filtrate
mOriginal sample for treatment: Weight of the sample for KOH treatment [g]
wN, original sample: Nitrogen content in the original sample [%]

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Analytical results for various sample types from ring test tests (Kjeldahl)

Sample type	Measured value	Result [%]	Mean value ring test [%]	Repeatability s.d.	z-Score
Organic sunflower seeds	Crude protein (N x 6,25)	23,32	23,51	0,73	-0,26
Organic sunflower seeds	Protein solubility in KOH	86,70	93,25	5,69	-1,15
Rapeseed cake	Crude protein (N x 6,25)	33,61	33,63	0,5	-0,04
Rapeseed cake	Protein solubility in KOH	90,00	91,35	3,06	-0,44
Fava bean	Crude protein (N x 6,25)	30,11	29,87	0,52	0,45
Fava bean	Protein solubility in KOH	91,40	88,33	6,8	0,45
Compound feed	Crude protein (N x 6,25)	17,22	17,34	0,66	-0,18
Compound feed	Protein solubility in KOH	79,40	79,54	6,15	-0,02
Raw soybean	Crude protein (N x 6,25)	41,31	41,63	0,6	-0,53
Raw soybean	Protein solubility in KOH	94,40	89,62	7,5	0,64

Conclusion

Determining protein solubility in potassium hydroxide (KOH) is a key parameter for assessing the digestibility and quality of protein-rich feedstuffs such as soybean meal. Using the N-Realyzer, the Dumas method enables precise and reproducible analysis of the soluble nitrogen content in accordance with ISO 14244. This laboratory analysis contributes significantly to quality assurance, ration optimisation and animal welfare – without the need for animal testing.

For detailed information or other applications please contact:

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