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# N/Protein Determination in Nuts according to the Kjeldahl method

Reference: AOAC 950.48 Protein (Crude) in Nuts and Nut Products

Tested with VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit (Code S30100210) and UDK 169 Automatic Kjeldahl Analyzer with AutoKjel Autosampler (Code S30200160).



## Introduction

Recently there was an archeological dig in Israel where researchers found evidence showing that nuts formed a major part of man's diet 780,000 years ago. Seven varieties of nuts along with stone tools to crack open the nuts were found buried deep in a bog. Even then man was aware of the health benefits connected to the consumption of nuts. In fact nuts are an excellent source of protein and healthy fats; the amounts vary depending on the type of nut.

Peanuts provide a good source of B vitamins essential for energy, protein metabolism, and the synthesis of red blood cells. Their protein content is about 29 %. Peanuts can be eaten raw, dry roasted and salted. Popular confections made from peanuts include salted peanuts, peanut butter (sandwiches, peanut candy bars, peanut butter cookies, and cups), peanut brittle, and shelled nuts (plain/roasted). Almonds contain about 20 % of protein. Almonds are often eaten on their own, either raw or toasted, and are also a component of various dishes. Almonds are available in many forms, such as whole, flaked, slivered and ground. Almonds yield almond oil and can also be made into almond butter or almond milk. These products can be used in both sweet and savoury dishes.

## Nitrogen Determination in Nuts according to the Kjeldahl Method

Thanks to the high level of precision and reproducibility and to its simple application, Kjeldahl is nowadays the most used method for determining nitrogen and protein contents in the food and feed industry. It also has several other applications in environmental control (phenols and nitrogen in water, sludge, soil and lubricants) and in the chemical and pharmaceutical industry according to official AOAC, EPA, DIN e ISO procedures.

The modern Kjeldahl method consists in a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salt at digestion temperature higher than 400 °C. During the process the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the digested solution liberates ammonia which is quantitatively steam distilled and determined by titration.

## Samples

Raw almonds	Expected Protein range: 15-25 %
Raw peanuts	Expected Protein range: 20-30 %

## Sample Digestion

Samples have been freeze and quickly homogenized by grinding, in order to avoid loss of oil (particle size 0.5 mm). Weigh about 0.500 g of sample into a nitrogen-free weighing boat (code CM0486000) and place it into a 250 ml test tube. For each sample, add in the test tube:

- 2 catalyst tablets Kjtabs VCM (code A00000274; 3.5 g K<sub>2</sub>SO<sub>4</sub>, 0.1 g CuSO<sub>4</sub>\*5H<sub>2</sub>O Missouri)
- 2 antifoam tablets VS (code A00000283)
- 20 ml concentrate sulphuric acid (96-98%)
- 5 ml hydrogen peroxide (30-32 %)

Prepare some blanks with all chemicals and without the sample.

Connect the Digestion Unit to a proper Aspiration Pump (JP code F30620198) and a Fume Neutralization System (SMS Scrubber code F307C0199) to neutralize the acid fumes created during digestion phase.

Digest the samples for 30 minutes at 300 °C and 60 minutes at 420 °C, according to the method "almonds, nuts, hazelnuts" or "peanuts and Brazil nuts" (respectively n° 2 and 4 on DKL 20).

## Distillation and Titration

Let the test tubes cool down to 50-60 °C. Condition the **UDK 169 with AutoKjel Autosampler** unit by performing the Automatic Check-up and Wash-down in the Menu-System.

Distill the samples according to the following parameters (pre-defined method n° 2 for almond and n° 4 for peanuts):

- H<sub>2</sub>O (dilution water): 50 ml
- NaOH (32 %): 70 ml
- H<sub>2</sub>SO<sub>4</sub> (0.1 N) as titrant solution
- H<sub>3</sub>BO<sub>3</sub> (4 % with indicators): 30 ml
- Protein factor: 5.18 for almonds and 5.46 for peanuts

Distillation & Titration analysis time: from 4 minutes for one test.

### Typical Results on Nuts

The results are automatically calculated by UDK 169 as percentage of nitrogen and percentage of protein.

Sample	Sample quantity (g)	Nitrogen %	Protein %
<b>Raw almonds</b>	0.496	3.807	19.718
	0.500	3.785	19.604
	0.499	3.816	19.769
	0.502	3.829	19.834
	0.502	3.817	19.774
	0.500	3.826	19.718
	<b>Average ± SD%</b>		3.813 ± 0.016
	<b>RSD% *</b>	0.418	0.425
<b>Raw peanuts</b>	0.500	4.196	22.910
	0.502	4.144	22.626
	0.502	4.212	22.998
	0.505	4.189	22.872
	0.508	4.131	22.555
	0.509	4.172	22.779
	<b>Average ± SD%</b>		4.192 ± 0.033
	<b>RSD% *</b>	0.780	0.751

Protein Factor: 5.18 for almonds and 5.46 for peanuts

\* RSD% = (Standard Deviation \* 100) / Average

The complete procedure was verified by using 5 ml of glycine standard solution (3%) containing 28 mg of nitrogen as reference substance. The obtained recovery falls into the expected range: between 98 % and 102 %.

### Conclusions

The obtained results are reliable and reproducible in accordance with the expected values: all data fulfill the expected range.

Benefits of Kjeldahl method by using DKL 20 and UDK 169 with AutoKjel Autosampler are:

- High level of precision and reproducibility
- Maximum productivity and full automation
- Worldwide official method
- Reliable and easy method
- Time saving
- Moderate running costs