APPLICATION NOTE

F&F-D-003-2016/A3

N/Protein Determination in Nuts

according to the Dumas method (He/Ar as Carrier)

Reference: **AOAC 992.23** Crude Protein in Cereal Grains and Oil seeds;
ISO 16634-1:2008 Oilseeds and animal feed stuffs; **AACC 46-30** Crude protein - Combustion method

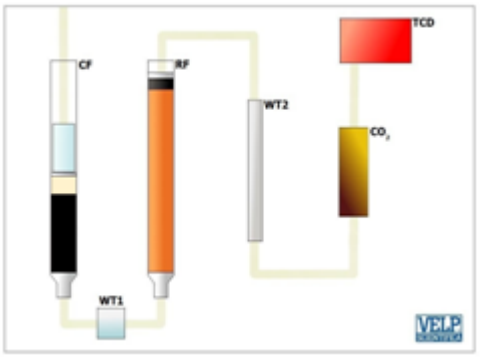
Tested with **VELP Scientifica NDA 702 Dual Carrier Gas Dumas Nitrogen Analyzer** (Code F30800080)

N/PROTEIN DETERMINATION IN NUTS
DUMAS COMBUSTION METHOD

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.

Protein Determination in Nuts



The Dumas method starts with a combustion furnace (CF) to burn the sample, obtaining elemental compounds.
Water is removed by a first physical trap (WT1 - **DriStep™**), placed after the combustion, and a second chemical one (WT2). Between the two, the elemental substances passed through a reduction furnace (RF).
The auto-regenerative CO_2 adsorbers (CO_2) let pass only the elemental nitrogen that is detected by the **LoGas™** innovative Thermal Conductivity Detector (TCD) with no requirement for a reference gas.
The NDA 702 is controlled via PC through the intuitive **DUMASoft™**.

NDA 702 Preliminary Operations (daily)

Follow the operating manual to start the NDA 702 and check that the following parameters are set:
Temperature Combustion reactor (Code A00000158): 1030 °C
Temperature Reduction reactor (Code A00000226): 650 °C
Flow rate MFC1 (He/Ar): 190 ml/min
Flow rate MFC2 (He/Ar): 220 ml/min
Condition the system by testing 2 EDTA standard (Code A00000149) and 3 to 5 empty tin foils (Code A00000153) as Check up.
Verify the calibration curve with one or more tests as Standard by testing the same standard used for the curve creation.

Sample Preparation

Raw almonds	Expected Protein range: 20-25 %
Raw peanuts	Expected Protein range: 20-25 %
Hazelnuts	Expected Protein range: 12-15 %

Samples have been freeze and quickly homogenized by grinding, in order to avoid loss of oil (particle size 0.5 mm).
Using a spatula, put ~ 100 mg of finely grinded sample directly into the tin foil.
Close the tin foil, obtaining a capsule and load the capsule into the autosampler.

Analysis Procedure

Fill the following fields in the database: **Sample name, Weight, Method, Sample type, Calibration number**
The parameters recommended by VELP for the analysis of the different nuts are:

Method name	Almonds	Peanuts	Hazelnuts
Protein factor	5.18	5.46	5.30
O2 flow rate	400 ml/min	400 ml/min	400 ml/min
O2 factor	1.4 ml/mg	1.4 ml/mg	1.6 ml/mg

After settings the parameter press to start the analysis.
Analysis time: from 3 minutes for one run.

N/PROTEIN DETERMINATION IN NUTS
DUMAS COMBUSTION METHOD

Typical Results on Raw almonds, peanuts and hazelnuts

The obtained results are in accordance with the expected value. Results have been obtained with the following calibration curve: in a range of 2 – 8 mg N with 8 measurements of EDTA standard (N% = 9.59) (Code A00000149).
The data obtained are included in the tolerance admitted by the EDTA certificate.
The tables below show the results with Helium and Argon as carrier gases. The sample weight is always the same with both carrier gases.

	HELIUM as carrier gas			ARGON as carrier gas		
Sample	**Sample quantity (mg)**	**Nitrogen %**	**Protein %**	**Sample quantity (mg)**	**Nitrogen %**	**Protein %**
Raw almonds	100,69	4,013	20,787	101,29	4,069	21,077
	100,60	4,059	21,026	100,60	4,032	20,886
	102,07	4,010	20,772	100,52	4,026	20,855
	100,04	4,010	20,772	100,46	4,012	20,782
	101,50	4,036	20,906	100,75	4,058	21,020
	103,68	4,083	21,150	101,25	4,116	21,321
	102,05	4,079	21,129	101,17	4,029	20,870
	102,86	4,089	21,181	102,03	4,072	21,093
	Average ± SD%	4,047 ± 0,034	20,965 ± 0,178	**Average ± SD%**	4,052 ± 0,034	20,988 ± 0,175
	RSD% *	0,848	0,848	**RSD% ***	0,836	0,836
Raw peanuts	102,00	4,360	23,806	101,38	4,322	23,598
	100,33	4,361	23,811	100,11	4,339	23,691
	103,15	4,326	23,620	100,29	4,306	23,511
	100,46	4,286	23,402	101,25	4,387	23,953
	103,05	4,311	23,538	101,82	4,323	23,604
	103,67	4,273	23,331	101,80	4,316	23,565
	103,40	4,270	23,314	101,36	4,298	23,467
	100,65	4,300	23,478	100,77	4,300	23,478
	Average ± SD%	4,311 ± 0,036	23,537 ± 0,196	**Average ± SD%**	4,324 ± 0,029	23,608 ± 0,158
	RSD% *	0,832	0,832	**RSD% ***	0,668	0,668

Protein Expected range: 20-25 %

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

	HELIUM as carrier gas			ARGON as carrier gas		
Sample	**Sample quantity (mg)**	**Nitrogen %**	**Protein %**	**Sample quantity (mg)**	**Nitrogen %**	**Protein %**
Raw hazelnuts	100,53	2,612	13,844	102,40	2,632	13,950
	102,96	2,619	13,881	100,81	2,599	13,775
	101,96	2,612	13,844	102,16	2,606	13,812
	100,91	2,616	13,865	100,60	2,619	13,881
	102,65	2,582	13,685	102,10	2,634	13,960
	102,58	2,611	13,838	101,41	2,633	13,955
	Average ± SD%	2,609± 0,013	13,826 ± 0,007	**Average ± SD%**	2,621 ± 0,015	13,889 ± 0,080
	RSD% *	0,514	0,514	**RSD% ***	0.577	0.577
Protein Expected range: 12-15 %						

Conclusion

Results are extremely reliable and reproducible as demonstrated by the RSD, by using helium or argon as carrier gas, with the same conditions (method and sample weight) since the goal is to obtain < 2.0% relative standard deviation, as requested by official methods.
Helium remains the best choice for premium accuracy but its shortages and interruptions are affecting any related product or instrument, including elemental analyzers. Argon, the best alternative available, has demonstrated to be a valid substitute, ensuring excellent results. VELP Scientifica NDA 702 Dual Carrier Gas Dumas Nitrogen Analyzer is the perfect response to simple, fast and precise nitrogen/protein determination, both with Helium and Argon as carrier gas.