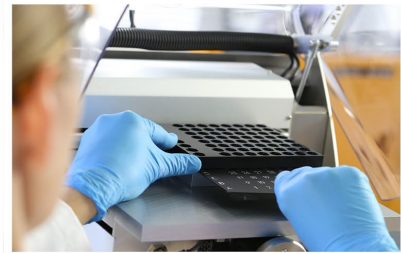
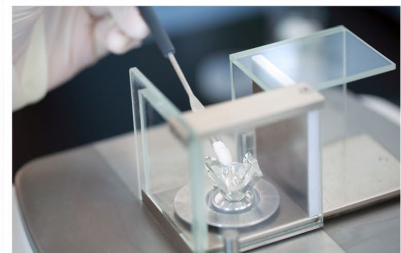


Dumas Application

A.2.1.7 Nitrogen Determination in Yoghurt



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1 Principle

The nitrogen contained in a sample is oxidized to nitrogen oxide at high temperatures in a pure oxygen stream and in the presence of a catalyst. Then nitrogen oxide is reduced to nitrogen with the help of copper and the side products water and carbon dioxide are separated in specific traps. Finally, the amount of nitrogen is detected by a thermal conductivity detector (TCD). The calibration of the TCD is performed with a suitable substance with 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.

The document is based on

- DIN EN ISO 14891, identisch mit IDF 185, *Nitrogen/Protein determination in milk and milk products according to the Dumas combustion principle.*
- § 35, V 02.00.24, Amtliche Sammlung von Untersuchungsverfahren nach LMBG, *Bestimmung des Stickstoffgehaltes von Milchprodukten, Verfahren nach Dumas.*

3 Gases and Consumables

The following gases and consumables are needed for the operation of DUMATHERM:

- Helium cylinder gas, quality grade min. 5.0
- Oxygen cylinder gas, quality grade min. 5.0
- Nitrogen or air cylinder gas, quality min. 2.6, resp. quality grade 1 acc. ISO 8573-1. (free from moisture, particles or oil)
- DumaReact, combustion reactor, packed, filled with HT and LT catalyst, 14-0244
- DumaCollect, ash insert with bottom, 14-0015
- DumaTube, quartz glass for reactor, 14-0203
- DumaPad, quartz wool pads for reduction reactor filling, 14-0225
- DumaFoil, tin foil for sample wrapping, 14-0017
- DumaCop, copper for reduction, 14-0046
- DumaSorb, absorber for liquid samples, 25g, 14-0022 or DumaSorb_{eco}, absorber for liquid samples, 50g, 14-0295
- DumaEDTA, calibration standard, purity >99 %, 14-0032
- THAM, Tris(hydroxymethyl)aminomethan, purity >99%

4 Instruments

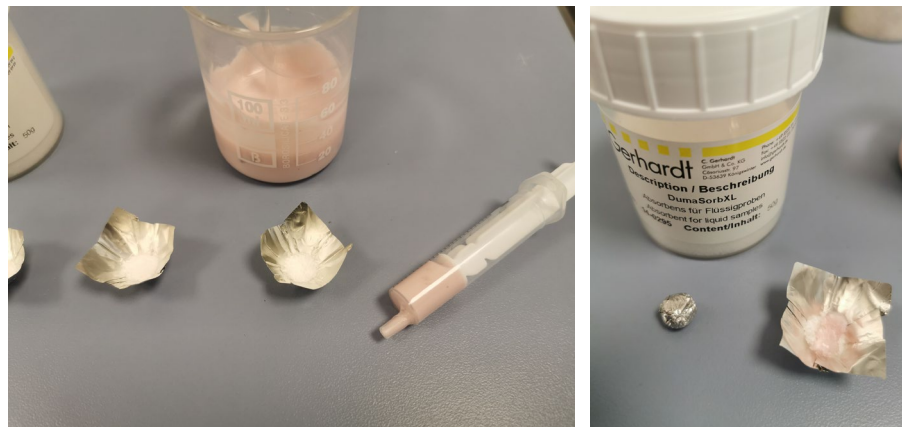
- Analytical balance (accuracy at least 0.1 mg, preferably 0.01 mg)
- 14-0400, DUMATHERM DT N Pro, with starter kit and consumables
- Homogenizer, e.g. Kinematica Polytron PT1200E, disperser
- Magnetic stirrer

5 Procedure

5.1 Sample preparation and weighing

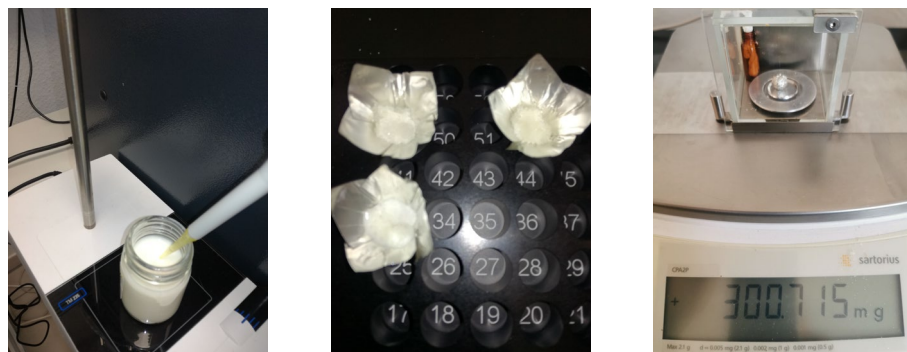
- Open the commercially available yoghurt, transfer it into a glass beaker and homogenize it at room temperature with the help of a disperser or stir the yoghurt thoroughly with a magnetic stirrer
- Prepare the tin foils with approx. 100 mg DumaSorb_{eco} (Picture 2)
- While stirring the yoghurt is taken with single-use syringe and added to the tin foil

- Press tare on the balance and fill yoghurt dropwise with the syringe into the tin foils. Take note of the first stable weight (Picture 3)
- If the sample has not been absorbed completely (you can recognize the wet surface), the foil can be closed later.
- In case the absorption is not enough, add another spatula of DumaSorb_{eco}
- Transfer the weighing data automatically into the software DUMATHERM-Manager and store the tin foils with the samples in the sample transfer magazine. Wait until samples have been absorbed completely by the absorber (dry surface) and close the tin foils.



Picture 1: Joghurt is taken with a syringe and added to a tin foil, prepared with appr. 100 mg DumaSorb_{eco}, the weight is directly taken and the foil can be put aside for drying until the absorption process is complete (surface dry). Then close the foil.

With more liquid yoghurt the product can be stirred on a magnetic stirrer at room temperature and weighed into tin foil with DumaSorb_{eco}.



Picture 2: Series of pictures with more liquid products. Homogenizing with a magnetic stirrer and weighing into tin foil prepared with DumaSorb_{eco}.

The weighing data can be transferred automatically to the device software after the foil is closed. The samples are stored in the transfer tray until the analysis.

5.2 Analysis

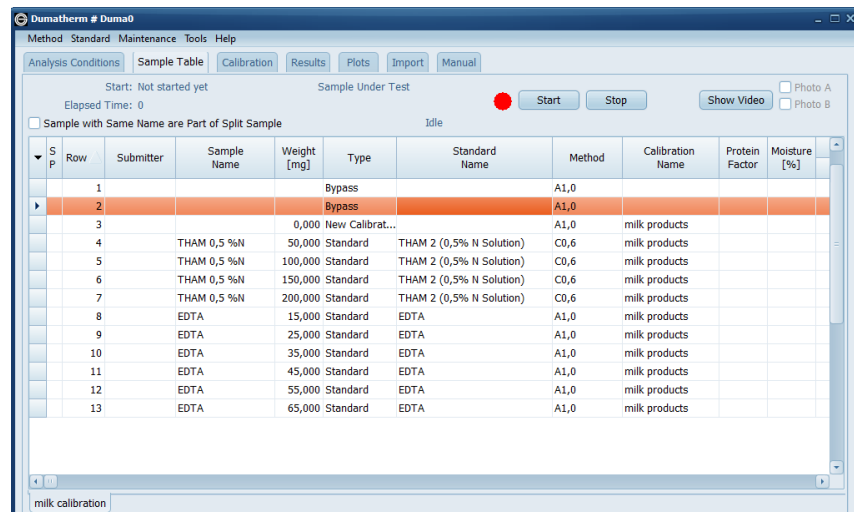
Before the analysis, activate DUMATHERM and perform the quality assurance described in the operating instructions of the instrument (Check-up maintenance, Check-up leak-test, Check-up blank value, Check-up standard). For the combustion analysis of yoghurt samples we recommend the following settings:

Parameter	Setting	
Sample weight	250 mg +/- 10 mg (add with disposable syringe)	
Sample packing	Tin foil with 2 x spatula tips of DumaSorb _{eco} (approx. 100 mg)	
Combustion Method	D 1,4 (with 1.4 ml O ₂ / mg sample dosing speed 100 ml/min), for fat contents < 1.5 % less oxygen can be used.	
Protein factor	6.38	
Combustion temperature	With DumaReact 14-0244: 980 °C	With DumaReact 14-0245: 1030 °C
Reduction temperature	With DumaReact 14-0244: 650 °C	With DumaReact 14-0245: 750 °C

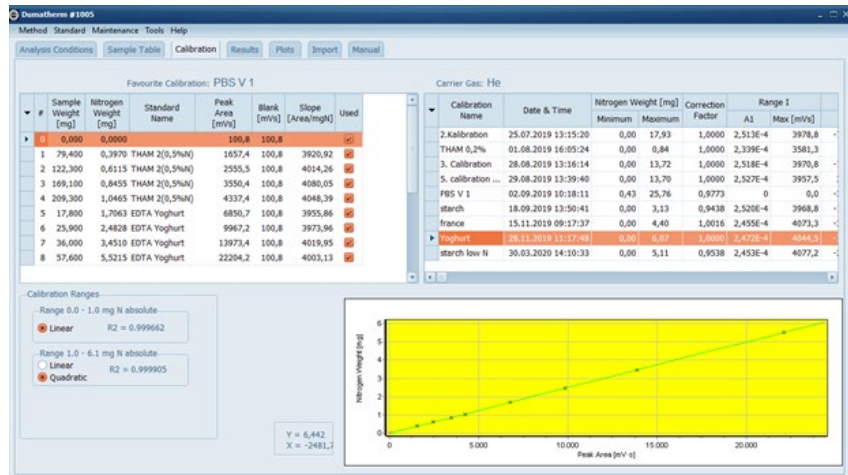
The challenge in analysing dairy samples is their low nitrogen content and the very high requirements from the ISO norm regarding repeatability (e.g. 0.080 % for yoghurt with a nitrogen content of approx. 0.730 %N).

A special calibration for the low nitrogen samples is required: a series of EDTA standards is extended by a series of a liquid nitrogen standard for the low N range. For this purpose, THAM (Tris(hydroxymethyl)aminomethane) has proven to be a suitable and can be easily stored for a long time without disintegration. For getting a solution with 0,5 % N, weigh 4.324 g THAM and fill up the flasks to 100 ml with distilled water.

The following picture shows a calibration series with EDTA and THAM standards:



The minimum requirement for the correlation factor R2 is a value > 0,9999xx for all measuring ranges as shown in the following example:

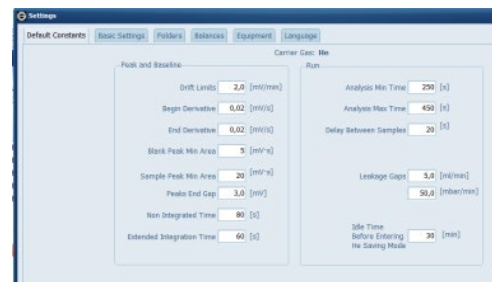


With this procedure we achieve a long-term stability in the calibration ensuring high-precision and repeatable results for samples with low nitrogen contents. For further information refer to C. Gerhardt „Guideline DUMATHERM N Pro Calibration“.

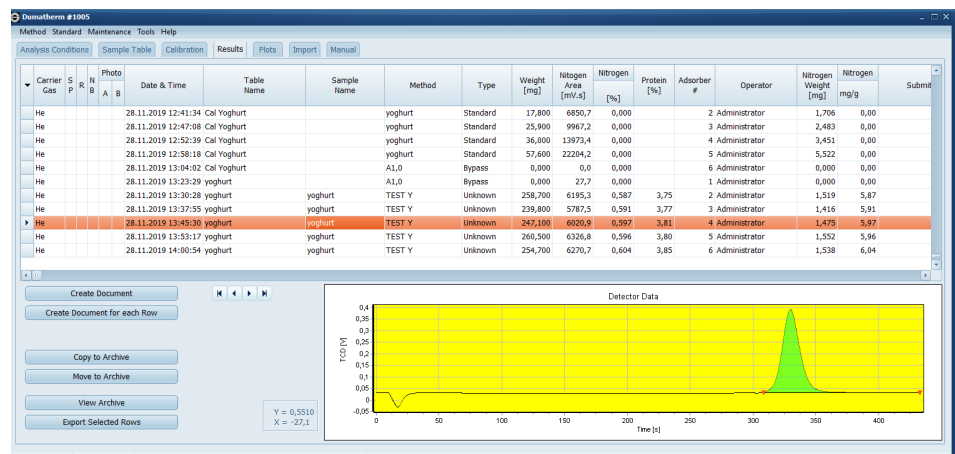
The slow combustion requires a change in the standard settings. Change ANALYSIS MIN TIME from 130 s to 250 s and ANALYSIS MAX TIME from 400 s to 450 s. Confirm changes SAVE CHANGES and OK:

Default constants

... changed settings for dairy analysis



The signal reaches the detector after 5-6 minutes.



6 Results

6.1 Example results for yoghurt samples

Using 250 mg weight and the slow combustion during combustion we achieve the following results:

Gerhardt									
Dumatherm Nitrogen / Protein Analyser									
Serial Number :			1005			Submitter : slow combustion 100 ml O2/min, Factor 1,4			
Software Version:			DUMATHERM MANAGER V8.09			Operator : Wemer Kueppers			
Date	Time	Sample name	Weight [mg]	Protein factor	Nitrogen Peak Area [mV*s]	N Weight [mg]	Nitrogen [%]	Protein [%]	
28.11.2019	13:30:28	yoghurt	258,700	6,38	6,195E+03	1,519	0,587	3,75	
28.11.2019	13:37:55	yoghurt	239,800	6,38	5,788E+03	1,416	0,591	3,77	
28.11.2019	13:45:30	yoghurt	247,100	6,38	6,021E+03	1,475	0,597	3,81	
28.11.2019	13:53:17	yoghurt	260,500	6,38	6,327E+03	1,552	0,596	3,80	
28.11.2019	14:00:54	yoghurt	254,700	6,38	6,271E+03	1,538	0,604	3,85	
28.11.2019	14:08:20	yoghurt	238,800	6,38	5,760E+03	1,409	0,590	3,76	
28.11.2019	14:16:02	yoghurt	258,600	6,38	6,132E+03	1,503	0,581	3,71	
Calibration number and standard name :							Average		3,78
for N Yoghurt (L-Q) THAM 2(0,5%N); EDTA Yoghurt							Standard Deviation		0,05
Method :							RSD [%]		1,25
Sample Table :									
Temperatures:		Flow Rates:		Carrier Gas: He		Times:			
Combustion Reactor	1029 °C			He(FC1):	195,0 ml/min	Sample Delay	5 s		
Reduction Reactor	749 °C			He(FC2):	200,0 ml/min	Sample Stop	9 s		
Degassing Oven	300 °C			Oxygen	100,0 ml/min	Run Time	Auto		

Analyses with 250 mg initial sample weights will lead to peak areas of approximately 6000 mVs. This corresponds to approx. 1.5 mg nitrogen absolute. For the calibration we recommend to perform a series of 12 standard analyses: 6 EDTA solid samples with increasing weights in steps of 10 mg (starting with 5 mg EDTA up to 55 mg EDTA) and 6 THAM liquid standard samples (2 x 50 mg, 2 x 100 mg, 2 x 150 mg).

According to DIN ISO 14891 a repeatability of $s_d < 0.080$ is requested for the % N value. AOAC 992.23 allows a tolerance of $s_r < 0.15$ for the protein value in %. The analysis results achieved with DUMATHERM are compliant and fulfill even higher requirements.



COMPREHENSIVE APPLICATION DATA BASE

C. Gerhardt offers a wide range of application notes for many methods and procedures. Please contact our application lab team via application@gerhardt.de for deeper information on:

- Nitrogen in food and feed samples according to Kjeldahl and Dumas
- Crude fibre, ADF and NDF in feed
- Fat in food and feed
- Alcohol determination
- Total cyanide in water
- Trace metal in soil and sludge
- COD determination in water
- Total nitrogen determination in water, soil and plants
- Many more application notes on request.

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HYDROTHERM – automatic acid hydrolysis system for fat determination according to Weibull-Stoldt. When combined with SOXTHERM, HYDROTHERM is an ideal system solution for total fat determination.

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