Application Note
No. 395/2020

Protein determination in plant-based meat alternatives

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-377:
Nitrogen and protein determination in plant-based meat alternatives according to the Kjeldahl method

1. Introduction

Plant-based meat alternatives are gaining popularity as a result of growing health and climate concerns. Being based on proteins derived from plants instead of traditional protein sources of animal origin, these products are being critically evaluated not only for their taste and texture but also for their nutritional value. An easy and reliable Kjeldahl method for the determination of total nitrogen and protein in plant-based meat alternatives, is presented below. In this method, samples are digested using the KjelDigester K-449. Distillation and boric acid titration are performed with the KjelMaster K-375 and KjelSampler K-377. A combination of the KjelDigester K-449 and KjelMaster system K-375/K-377 offers high sample throughput.

2. Equipment

- KjelDigester K-449
- Scrubber K-415 TripleScrub ECO
- KjelMaster K-375 with KjelSampler K-377
- Mixer, B-400
- Analytical balance (accuracy ± 0.1 mg)

3. Chemicals and Materials

Chemicals:

- Sulfuric acid conc 98 %, VWR (19I064018)
- BUCHI Kjeldahl Tablets Titanium, BUCHI (110370347)
- Weighing boat, nitrogen free, BUCHI (11060522)
- Sodium hydroxide 32 %, VWR (19L204010)
- Boric acid 4 % pH 4.65, BUCHI (11064973)
- Sulfuric acid 0.1 mol/L, VWR (19K254011)
- Neutralization solution for the Scrubber: 600 g sodium carbonate, calcined, technical, Synopharm (0179420) about 2 mL ethanol and a spatula tip of bromothymol blue, Fluka (18460) diluted to 3 L with distilled water
- Glycine, assay 99.7 %, Merck (1.04201.0100)

Please refer to MSDS of respective chemicals for safe handelling.

Table 1: Sample information

Sample	Source of Protein	Protein [g /100 g]
Vegan cold cuts	pea and soya	6.5
Vegan fishsticks	wheat and vegetable	12.6
Vegan sausages	wheat and soya	17
Vegan burger	pea	18
Vegan steak	wheat and soya	20
Soya schnitzel	soya flour	49

The samples were purchased at a local supermarket. There are no commercial certified reference materials available yet.

4. Procedure

The determination of nitrogen and protein in plant-based meat alternatives includes the following steps:

- Homogenization of each sample by grinding and pounding depending on the matrix
- Digestion of the sample, using the block digester K-449
- Distillation and titration of the sample, using KjelMaster system K-375/K-377

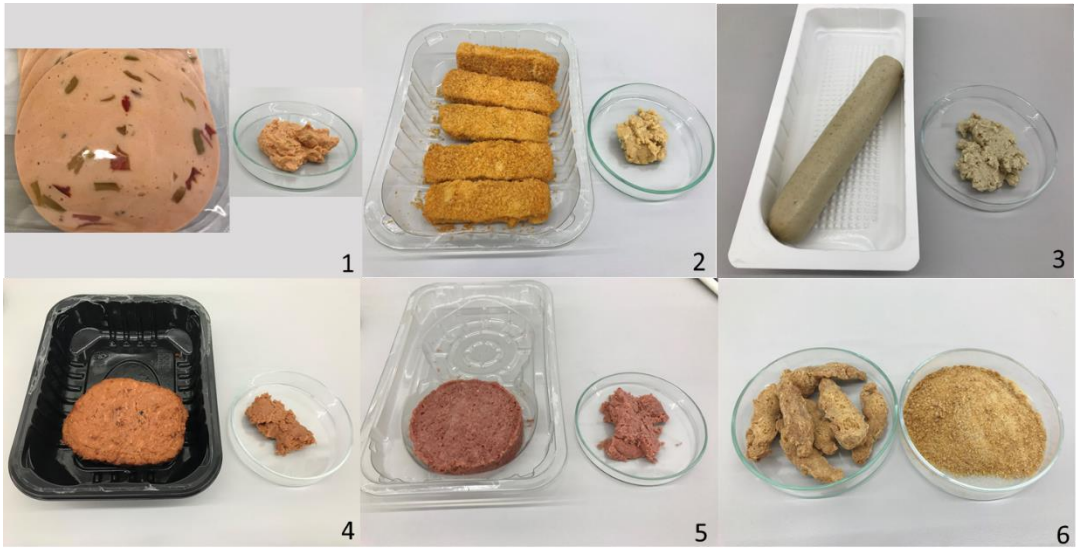

Figure 1: Plant based meat alternatives Vegan cold cuts (1), Vegan fish sticks (2), Vegan sausages (3), Vegan steak (4), Vegan burger (5), Soya schnitzel (6).

4.1 Digestion method – Glycine (verification of the method)

1. Start the KjelDigester K-449 according to the programme as described in Table 3
2. Place around 0.2 g glycine in a nitrogen free weighing boat and transfer it (with the boat) in a 300 mL sample tube
3. Add 2 Titanium Tablets and 15 mL of sulfuric acid (conc. 98 %).
4. Prepare additional blanks, chemicals without the sample
5. Connect the Scrubber K-415 to the K-449 for absorbing the acid fumes generated during the digestion process
6. Insert the rack with the samples into the cooling position and mount the suction module onto the samples, immediately start the digestion according to the parameters listed in Table 3
7. A cooling step of around 35 min in the digestion programme is usually enough to cool the sample down to 50-70 °C. If necessary, samples can be further cooled to room temperature before distillation

4.2 Digestion method – samples

8. Start the KjelDigester K-449 according to the parameters listed in Table 3
9. Weigh each sample in a nitrogen free weighing boat and place in a 300 mL sample tube as described in Table 2

Table 2: Optimized sample weight for digestion

Sample	Weight [g]
Vegan cold cuts	1.0
Vegan fish sticks	1.0
Vegan sausages	1.0
Vegan burger	1.0
Vegan steak	1.0
Soya schnitzel	0.5

10. Add 2 Titanium Tablets and 15 mL of sulfuric acid (conc. 98%) to each tube
11. Prepare additional blanks, chemicals without the sample
12. Connect the Scrubber K-415 to the K-449 for absorbing acid fumes generated during the digestion process
13. Insert the rack with the samples into the lift and immediately start the digestion according to the parameters listed in Table 3

Table 3: Temperature programme for digestion on the K-449

Step	Temperature [°C]	Time [min]
1	320	0
2	420	120
Cooling	–	35

NOTE: If the liquid inside the sample tube is not clear and blue-green, digest for additional 15 min at 420° C

14. Let the samples cool down and start the distillation according to Table 4

4.3 Distillation and titration

Table 4: Parameters for distillation and titration with the KjelMaster system K-375/K-377

Method parameters KjelMaster K-375

H_2O volume	40 mL	Titration solution	H_2SO_4 0.1 mol/L
NaOH volume	63 mL	Sensor type	Potentiometric
Reaction time	5 s	Titration mode	Online
Distillation mode	Fixed time	Titration start time	90 s
Distillation time	240 s	Measuring mode	Endpoint pH
Stirrer speed distillation	5	Endpoint pH	4.65
Steam output	100 %	Stirrer speed titration	7
Titration type	Boric acid	Titration start volume	0 mL
Receiving solution vol.	60 mL	Titration algorithm	Optimal

4.4 Calculation

The results are calculated in terms of percent nitrogen. In order to calculate the protein content of the sample, the nitrogen content is multiplied with a sample-specific protein factor. The following equations (1), (2), and (3) are used to calculate the results. For the reference substance, the purity of the glycine is considered in equation (4).

$$w_N = \frac{(V_{Sample} - V_{Blank}) \cdot z \cdot c \cdot f \cdot M_N}{m_{Sample} \cdot 1000} \quad (1)$$

$$\%N = w_N \cdot 100\ \% \quad (2)$$

$$\%P = w_N \cdot PF \cdot 100\ \% \quad (3)$$

$$\%N_{Gly} = \frac{\%N \cdot 100}{P} \quad (4)$$

w_N : weight fraction of nitrogen
V_{Sample} : volume of titrant for the sample [mL]
V_{Blank} : mean volume of titrant for the blank [mL]
Z : molar valence factor (1 for HCl, 2 for H_2SO_4)
C : titrant concentration [mol/L]
F : titrant factor (for commercial solutions please refer on the label)
M_N : molecular weight of nitrogen (14.007 g/mol)
m_{Sample}: sample weight [g]
1000 : conversion factor [mL/L]
%N : percent weight of nitrogen
$\%N_{Gly}$: purity corrected percentage weight of the reference substance glycine [%]
%P : percent weight of protein
P : purity of the reference substance glycine [%]
PF : sample-specific protein factor (6.25 or 5.71 for soya products)

5. Results

5.1 Recovery of glycine

The results for nitrogen determination and recovery for glycine (assay > 99.7 %) are presented in Table 5. The nitrogen content of glycine is 18.60% (assay corrected). The recoveries are within the specification of 98-102%.

Table 5: Results of the recovery of nitrogen in glycine

Glycine	m_{Sample} [g]	V_{Sample} [mL]	%N	Recovery [%]
Sample 1	0.2138	14.246	18.32	98.50
Sample 2	0.2117	14.272	18.54	99.66
Sample 3	0.2048	13.844	18.58	99.87
Average	–	–	18.48	99.34
Rsd [%]	–	–	0.74	–

The mean blank volume (V_{Blank}) was 0.264 mL (n = 5).

5.2 Protein determination in plant-based meat alternatives

The results of the determination of nitrogen and protein contents in plant-based meat products are presented in Tables 6 to 11.

Table 6: Results of the determination of nitrogen and protein in vegan cold cuts (declared protein content 6.5 g /100 g)

Vegan cold cuts	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	1.0243	4.295	1.10	6.89
Sample 2	1.0233	4.275	1.10	6.86
Sample 3	1.0329	4.413	1.13	7.03
Average	–	–	1.11	6.93
SD	–	–	0.01	0.09
Rsd [%]	–	–	1.32	1.32

The mean blank volume (V_{Blank}) was 0.264 mL (n = 5). 6.25 was considered as a protein factor (PF) as the product contains proteins from various plant-based sources.

Table 7: Results of the determination of nitrogen and protein in vegan fish sticks (declared protein content 12.6 g/100 g)

Vegan fishsticks	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	1.0015	8.059	2.18	13.63
Sample 2	1.0063	7.369	1.98	12.36
Sample 3	1.0234	7.445	1.97	12.29
Average	–	–	2.04	12.76
SD			0.12	0.75
Rsd [%]	–	–	5.91	5.91

The mean blank volume (V_{Blank}) was 0.264 mL (n = 5). Grubb's test was performed for outlier detection and it was found that Sample 1 is furthest from the rest but not a significant outlier (P > 0.05). 6.25 was considered as a protein factor (PF) as the product contains proteins from various plant-based sources.

Table 8: Results of the determination of nitrogen and protein in vegan sausage (declared protein content 17 g/100 g)

Vegan sausage	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	1.0128	9.783	2.63	16.46
Sample 2	1.0156	9.798	2.63	16.44
Sample 3	0.9909	9.557	2.63	16.42
Average	–	–	2.63	16.44
SD	–	–	0.00	0.02
Rsd [%]	–	–	0.11	0.11

The mean blank volume (V_{Blank}) was 0.264 mL (n = 5). 6.25 was considered as a protein factor (PF) as the product contains proteins from various plant-based sources.recovery and increases repeatability for those sample types and is necessary for a reliable fat determination.

Table 9: Results of the determination of nitrogen and protein in vegan burger (declared protein content 18 g/100 g)

Vegan burger	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	1.0550	10.821	2.80	17.52
Sample 2	1.1104	11.803	2.91	18.20
Sample 3	1.1002	11.321	2.82	17.60
Average	–	–	2.84	17.77
SD	–	–	0.06	0.37

Rsd [%]	–	–	2.08	2.08

The mean blank volume (V_{Blank}) was 0.264 mL (n = 5). 6.25 was considered as a protein factor (PF) as the product contains proteins from various plant-based sources.

Table 10: Results of the determination of nitrogen and protein in vegan steak (declared protein content 20 g/100 g)

Vegan steak	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.9505	10.899	3.13	19.59
Sample 2	1.04	12.084	3.18	19.90
Sample 3	1.0187	11.646	3.13	19.56
Average	–	–	3.15	19.68
SD	–	–	0.03	0.19
Rsd [%]	–	–	0.95	0.95

The mean blank volume (V_{Blank}) was 0.264 mL (n = 5). 6.25 was considered as a protein factor (PF) as the product contains proteins from various plant-based sources.

Table 11: Results of the determination of nitrogen and protein in soya schnitzel (declared protein content 49 g/100 g)

Soya schnitzel	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.468	14.126	8.30	47.38
Sample 2	0.4699	14.192	8.30	47.41
Sample 3	0.5038	15.186	8.30	47.38
Average	–	–	8.30	47.39
SD	–	–	0.00	0.02
Rsd [%]	–	–	0.04	0.04

The mean blank volume (V_{Blank}) was 0.264 mL (n = 5). 5.71 was considered as a protein factor (PF) as the product contains proteins of soya origin.

6. Comparison to Standard Methods

This application is compared to the standard method AOAC 920.87.

Table 12: Comparison to AOAC 920.87

	Application note	AOAC 920.87	Notes/Impact
Catalyst	2 × 3.7 g Tablets Composition 94.4 % K_2SO_4 2.8 % TiO_2 2.8 % $CuSO_4.5H_2O$	15 g K_2SO_4 + 0.7 g HgO	Easy to handle for routine analysis. No toxic Hg. Amount of catalyst saved is ~50%
Sulfuric acid	15 mL	25 mL	No impact. Substantially, less sulfuric acid in turn less sodium hydroxide for alkalization.
Water	40 mL	200 mL	No impact. Steam is generated in a separate vessel in K-375. 5 times less amount of water consumption per sample.
Titration	Boric acid titration	Back titration	No impact
Protein factor	6.25 for mixed protein sources, 5.7 for exclusively soy-based proteins	5.7	A general protein factor (6.25) is considered for mixed protein sources and protein factor 5.7 was used for soy-based proteins as the use of factor 6.25 is still debatable.

7. Conclusion

The determination of nitrogen and protein in plant-based meat alternatives using the KjelDigester K-449 and KjelMaster system K-375/K-377 provides reliable and reproducible results. These results correspond well to the labelled values of the products with low relative standard deviations (rsd) except for vegan fish sticks where the sample matrix requires through homogenization pertaining to the different parts of the product with different nutritional composition and physical characteristics. Recovery of reference substance glycine was 99.34 % (rsd = 0.74 %), which was within the specification of 98-102%.
With the KjelDigester K-449 the digestion process (including preheating, digestion and cooling) is very fast and fully automated. The KjelMaster system K-375/K-377 allows unattended analysis with significantly reduced determination time with the online mode.

8. Reference

AOAC 920.87
Nitrogen and protein content measurement and nitrogen to protein conversion factors for dairy and soy protein-based foods: a systematic review and modelling analysis (https://www.who.int/publications/i/item/9789241516983)
KjeldahOptimizer App
Operation Manual of KjelDigester K-446/K-449
Operation Manual of Scrubber K-415
Operation Manual of KjelMaster system K-375/K377