Gerhardt
Analytical Systems

Application Fibre

G.0.FibreBag Total Dietary Fibre in Food

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analítica Salvadoreña

1 Principle

The sample is subjected to a multi-stage enzymatic process in duplicate.
Apart from the insoluble dietary fibre, all other components are dissolved. The dissolved dietary fibre is precipitated with ethanol. The precipitate is separated out using FibreBag technology. The precipitate from one sample is then subjected to nitrogen determination to determine the remaining proteins and from the second sample the ash is determined. A difference calculation results in the total dietary fibre.

FibreBags make it easier to handle the dissolving and filtering of the components.

2 Method

The method is based on the specification:

- On the basis of the German Food Law LFGB § 64 L 00.00-18 Determining dietary fibre in foodstuffs
- On the basis of AOAC 991.43 Total, soluble and insoluble dietary fiber in foods

This application document is intended to be a guide to assist users in the initial use of C. Gerhardt analytical equipment. It is not a definitive method. Users may have to adapt this method to the ambient conditions and to suit their own analytical requirements.

3 Chemicals and Accessories

Quality p. a.

1. Ethanol 95 %
2. Ethanol 78 %: 179 ml water is topped up with ethanol 95% to 1000 ml
3. Acetone p.a.
4. Sodium hydroxide 6 mol/l: Dissolve 24 g NaOH in 70 ml water and top up to 100 ml
5. Sodium hydroxide 5 %
6. Hydrochloric acid 0.56 mol/l: 93.5 ml hydrochloric acid (c = 6 mol/l) are topped up to 1000 ml with water
7. Hydrochloric acid 5 %
8. MES/TRIS buffer 0.05 mol/l, pH 8.3:
   19.52 g MES (N-morpholino ethanesulfonic acid monohydrate) and 12.2 g TRIS (tris(hydroxymethyl)aminomethane) are dissolved in 1.7 l water, adjust to 8.3 pH with NaOH 6 mol/l and top up to 2 l
9. Enzyme Kit: e.g. Megazyme Total Dietary Fibre (200 determinations per kit), art. no. 1609473 (from Coring), consisting of amylase-, protease- and amyloglucosidase suspension.
10. Petroleum Ether, Boiling Range 40 to 60 °C
11. Glass Spacer for FibreBags, to open and fix the bags, Package with 6 pieces, Order No.: 10-0124
12. FibreBags S, bag with 100 pcs, cat. no. 10-0142
13. Incineration Module for FibreBags, 12-place, complete with handle and 12 quartz glass crucibles, Order No.: 13-0092
14. Concentrated sulphuric acid H_2SO_4, min. 96 – 98%
15. Catalyst tablets KJELCAT Cu 5,0 g K_2SO_4 + 0,5 g $CuSO_4$ + $5H_2O$ (Art. 12-0328) or comparable
16. Caustic soda NaOH 32 % p.a.
17. Boric acid H_3BO_3 2 %
18. Indicator solution M5 (Merck) or comparable
19. Volumetric Standard solution: Hydrochloric acid c(HCl)= 0.1 mol/l or sulphuric acid $c(H_2SO_4)$ = 0.05 mol/l
20. Acetanilid
21. Saccharose, nitrogen free

22. Ammonium sulphate, to be dried for at least 2 hours at a temperature of 102° C ± 2°C immediately before usage and subsequently to be kept in a desiccator for cooling to room temperature
23. Weighing paper WP250 (Art. 1004939) or paper weighing boats

4 Instruments

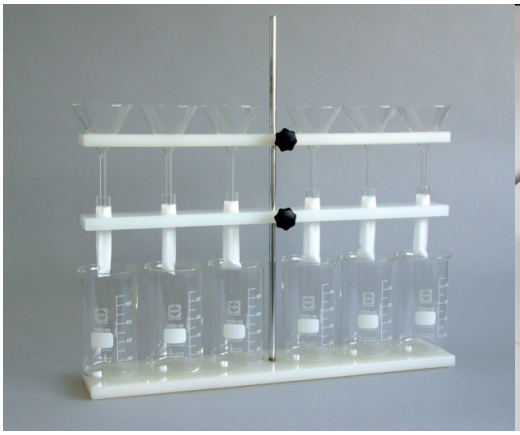


Fig.1: FibreBag Filtering Frame

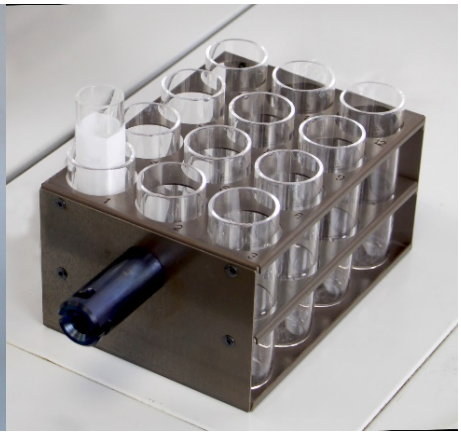


Fig.2: Incinerating Module

Article	No.	Quantity
Insert rack with intermediate plate	1004781	1
Funnel	1001526	6
Spacer	10-0124	1 case (= 6 units)
Glass beaker	10-0121	6
FibreBags S	10-0142	1
Incinerating module, 12-place, with crucibles	13-0092	1

- Water bath
- Balance
- For sample preparation: mill or cutter or homogeniser
- Glass beaker, 400 or 800 ml
- Desiccator with a drying agent as silica gel
- Drying chamber, electric driven, temperature 100°C +/- 5°C
- Muffle Furnace with thermostat, temperature 550°C +/- 25°C
- Timer or alarm clock
- Fume cabinet
- Kjeldahl digestion system KJELDATHERM, TURBOTHERM or flask heater for Kjeldahl flasks with enlarged neck
- Fume Scrubber TURBOSOG, alternatively VACUSOG or water jet pump
- VAPODEST Steam distillation system, models 200 to 450 without titrator, titration has to be performed by means of a manual burette (class A, according to ISO 385), 50 ml nominal volume, with volume scale in 0.05 ml steps or a Titrator, or instead of indicator solution with a pH meter with a combination electrode. The titration is performed automatically in case of VAPODEST 450 with external titrator or VAPODEST 500/500c with integrated titrator.

5 Sample Type, Preparation and Performance

A representative sample quantity of the foodstuff to be analysed is – after any predrying necessary – ground to a particle size of ≤ 5mm without remainder in a laboratory mill or homogeniser and mixed thoroughly.

Degreasing
Alternatives:
a. Samples with a fat content of more than 10 % should be degreased cold after weighing and before adding detergents. The degreasing process involves dousing them 2 to 3 times with petroleum spirit that is decanted carefully after each deposition. Allow the residual solvent to evaporate.

b. Twice weigh about 1 g sample into a rough folded filter and rinse with acetone from a wash bottle (3 to 4 times), then dry for about 15 minutes in a drying cabinet.
(The dietary fibre content is then specified in the grease-free dry matter.)

c. Depending on the properties of the sample, we recommend placing a larger quantity of the sample in a glass beaker and dousing it with petroleum ether. Rotate several times to completely dissolve the fats from the sample in the solvent. Then filter through a folded filter. Dry out the residue and then use this to determine the initial weights for the two dietary fibre methods.
(The dietary fibre content is then specified in the grease-free dry matter.)

Enzymatic decomposition
Transfer the dried sample (twice about 1 g initial weight) to a 400 ml glass beaker making sure that the entire sample is transferred (depending on the prior degreasing). Make a note of the precise initial weights M_1 and M_2 for the weighing log.
For blank value determination, 2 empty 400 ml glass beakers are set with the same reagents and subjected to the same procedure.

➢ Addition of 40 ml MES/TRIS buffer and small magnetic stirring rod

🕐 Stir for approx. 5 minutes

➢ Addition of 50 µl α Amylase suspension (Megazyme kit)
Cover the glass beakers with aluminium foil (tightly) + watch glass + weight ring

🕐 30 minutes in 99 °C water bath (gentle simmer at 99 °C), cover closed, rotating slowly several times;
remove from water bath, allow to cool at a room temperature to 60 °C (takes a few minutes)

➢ Addition of 100 µl protease solution, briefly stir on a magnetic stirrer

🕐 30 minutes in 60 °C water bath (cooling at 60 °C), slight rotating every 10 minutes

➢ Next, add ~ 5 ml hydrochloric acid solution (c = 0.56 mol/l) to all samples (outside the water bath), adjust pH to 4.0 to 4.7

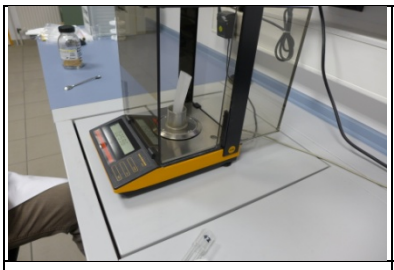
➢ Then add the 200 µl Amyloglucosidase suspension, next and without delay
⇩

30 minutes in 60 °C water bath (cooling at 60 °C), slight rotating every 10 minutes

Precipitation
Remove samples from water bath and add 220 ml 95 % ethanol (warmed up to 60 °C, at least 4 times the quantity of ethanol in relation to the liquid volume present)

Leave for at least 1 hour at room temperature; for samples to which "modified" dietary fibre content was added, the precipitation time should be at least 4 hours. For specific samples, the precipitation should be left overnight.

Filtration
Preparing the FibreBags for filtration and subsequent drying, incineration and protein determination

	The weight of the empty FibreBag gives the value M_{FB} for the weighing log.
	The glass spacer is inserted into the FibreBag.

The FibreBag and spacer are now inserted into the filtering frame.

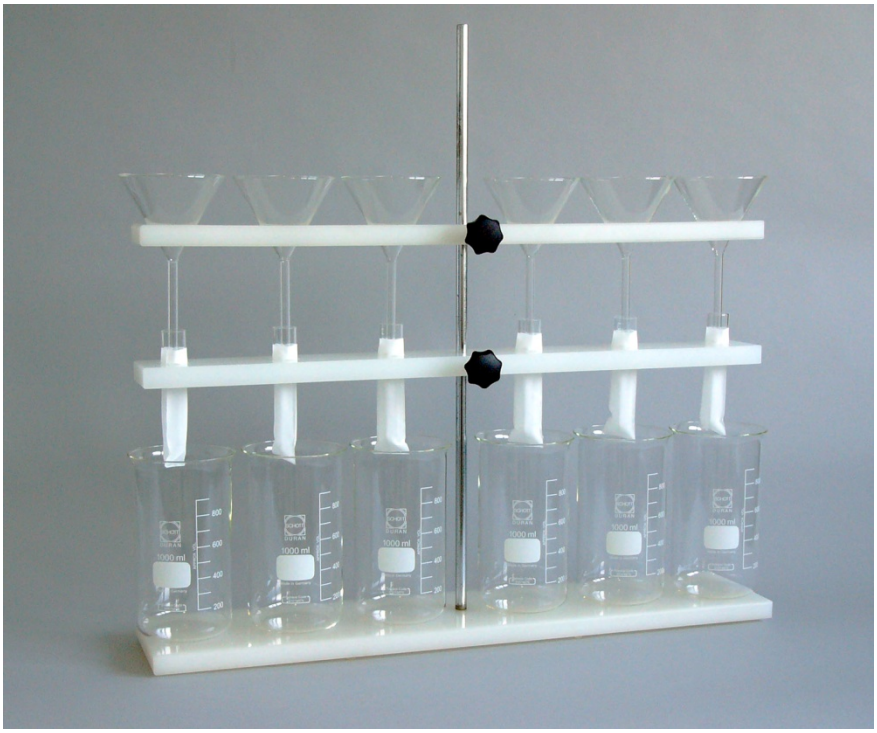


Fig.3: FibreBag Filtering Frame

Filter liquid through the FibreBags, first allowing as much liquid as possible without solids to flow through; stir up the precipitate with as little liquid as possible in the glass beaker and pour it into the FibreBags little by little; only fill the FibreBags by a third and no more than by half.
Immediately after transferring the sample, add about 10 ml of 78% ethanol to the sample glass beaker, tilt out any sample waste and pour this into the FibreBag. Rinse out the funnel immediately with 78% ethanol from a wash bottle.
Add 20 ml of 78% ethanol into a 25 ml glass beaker for each bag and rinse out the FibreBags again with this.

Drying the samples for determining mass of precipitated fraction
After all the ethanol has drained away, rinse the FibreBags (without funnel) directly on the spacers with acetone to transfer any remaining residue into the bag; repeat until all the residue has been removed from the spacer.

- Dry out the incineration crucible with FibreBags, sample residue and spacer for about 4 hours at about 100 °C, then cool down in desiccator

Drying samples for protein determination
After the complete filtration, separate the FibreBag from the spacer by gently and intermittently pulling down on it. Rinse the spacer without the FibreBag with acetone, thereby removing any sample residue that is still adhering to it; briefly dry the FibreBags and then perform the protein determination using the Kjeldahl method. The FibreBags are nitrogen-free.

Alternatively:
Place FibreBags with sample residue in a weighed glass beaker and dry out overnight in a drying cabinet at about 100 °C, then allow to cool in a desiccator;

Incinerating the samples
Incineration of sample 1 and blank 1; at least 5 hours at 550 °C until white residue remains.

The mass of ash contained therein results in M_A for the weighing log.

Protein determination
Kjeldahl determination of sample 2 and blank 2; the FibreBags can be digested without any problems in Kjeldahl digestion;
The mass of the proteins contained therein results in M_{Pr} for the weighing log.

Determination of the nitrogen content (Protein)
The dried FibreBags with the dry residue are transferred to the Kjeldahl digestion tubes and the chemicals (see below: Chemicals 3.15 and 3.16) are added.

Digestion

Chemicals	Amount per sample
Sulphuric acid	20 ml
KJELCAT	2

Digestions with KJELDATHERM, time-optimised (differs from reference digestion time, however leads to comparable results in nearly all cases)

For digestions with a KJELDATHERM system with 250 ml KJELDATHERM digestion tubes, we recommend the following program parameters:

Phase	Step	hh:mm	Temp. [°C]	Power [%]	Lift	Suc	Cool Vent	Cool Water
Digestion	1/2	01:30	410			☑	☐	☐
Cooling	2/2	00:30	-	-		☑	☑	☐
Done		-	-	-		☐	☐	☐

If your digester does not have an automatic lift system, take out the insert rack after digestion manually and leave the samples for cooling.

Tip: Shorten the digestion time by placing the samples in a pre-heated digester.

For digestions with a TURBOTHERM system with 12 x 250 ml KJELDATHERM digestion tubes, we recommend the following program parameters:

Note	Step	hh:mm	Power [%]	Suc
Heat-up of the system until boiling of the digestion solution	1/3	00:15	100	☑
After 20 – 30 minutes the digestion solution should be clear	2/3	01:15	75	☑

Sample cooling	3/3	00:30	0	☑
Digestion done		-	-	

Digestions with TURBOTHERM TTs and foaming samples

For digestions with a TURBOTHERM system with 12 x 250 ml KJELDATHERM digestions tubes, we recommend the following program parameters:

Note	Step	hh:mm	Power [%]	Suc
Heat-Up of the system until boiling of the digestion solution	1/7	00:05	100	☑
	2/7	00:05	0	☑
	3/7	00:05	100	☑
	4/7	00:05	0	☑
	5/7	00:05	100	☑
After 20 – 30 minutes the digestion solution should be clear	6/7	01:30	75	☑
Sample cooling	7/7	00:30	-	☑
Digestion done		-	-	-

Choose the method from the method library or program an older TURBOTHERM unit following the method „Foaming Samples“.

Digestions with a classic flask heater

For digestions with classic flask heaters in Kjeldahl flasks of 500 ml or 750 ml volume with enlarged neck, we recommend the following program parameters:

Time [min]	Power level	Note
20	3	Heating and evaporation until the digestion solution boils and white foams occur
60	1,5	Boiling of digestion solution
30	-	Cooling down samples

Suction of the digestion fumes

During the digestion, a fume scrubber (TURBOSOG or VACUSOG) must be activated. For the washing bottle we recommend to fill approx. 1.200 ml of caustic soda (concentration approx. 15 %). The suction power is adjusted correctly when no fumes come out of the tubes.

You can check if the caustic soda is still usable by adding an indicator and checking the pH value.
Allow 30 minutes for cooling down after taking out the insert rack or after deactivating the heating. Leave the fume scrubber activated during this time.

Tip: You can shorten the cooling down time of your samples by half with a KJELDATHERM ECO KIT.

Distillation with VAPODEST
After cooling down the samples, a steam distillation is performed with the following program:

	Method Food / Feed TKN	VAP 200	VAP 300	VAP 400	VAP 450	VAP 500 / 500c
H_2O Addition	100 ml	•	✓	✓	✓	✓
NaOH Addition	80 ml	✓	✓	✓	✓	✓
Reaction time	0 s	✓	✓	✓	✓	✓
Distillation time	240 s	✓	✓	✓	✓	✓
Steam power	100 %	✓	✓	✓	✓	✓
Sample suction	30 s	-	✓	✓	✓	✓
H_3BO_3 Addition	80 ml	•	•	✓	✓	✓
Suction receiver solution	30 s	-	-	-	✓	✓
Titration	-	•	•	•	✓	✓
Calculation	-	•	•	•	•	✓
Reading pH value, fixed endpoint or automatic endpoint	-	-	-	-	✓	✓
Titration online	-	-	-	-	-	✓

✓ = automatic • = manual - = not applicable

Choose the method from the method library or program an older VAPODEST unit following the method „Food / Feed TKN“.
Note: If you use a different amount of sulfuric acid for digestion, also the addition of water and caustic soda during distillation has to be adjusted accordingly.
A guideline for the proportions is: „1 part acid : 5 parts water : 4 parts caustic soda“ .

Titration
Add 3-4 drops of mixed indicator M5 to the receiver solution (3.11) and titrate with standard solution (3.20) until the colour changes from green to violet.
If you determine the endpoint with a pH meter or titrator, you do not have to add the mixed indicator M5.

Blank value
For blank value determination, perform the analysis (digestion + distillation + titration) just with the indicated chemicals and 1 g saccharose (3.14) instead of the sample. The chemical consumption has to be taken into account for the calculation.

Performance check
To check the analytical performance of your water steam distillation system, perform a distillation and titration of 0.12 g ammonium sulphate (3.15). The percentage of the recovered nitrogen must be between 99.0 and 100.0 % taking the purity of the standard solution into account a recovery rate up to 101 % is still acceptable in sporadic cases.

6 Calculation

$$TDF\ \% = \frac{\frac{M_{R1} + M_{R2}}{2} - M_{Pr} - M_A - M_{Bl}}{\frac{M_1 + M_2}{2}} \cdot 100\ \%$$

M_{R1} = Mass of residue of sample 1 in mg ($M_{before} - M_{after}$)

M_{R2} = Mass of residue of sample 2 in mg ($M_{before} - M_{after}$)

M_{Pr} = Mass of proteins (f = 6.25) in mg (from sample 2) residue R2

M_A = Mass of mineral compounds (ash) in mg (from sample 1) residue R1

M_{Bl} = Mass of blind value in mg *

M_1 = Mass of sample in mg for protein determination

M_2 = Mass of sample in mg for ash determination

TDF % = Total dietary fibre content in %

* = Mass formula for blank value M_{Bl}

$$M_{Bl} = \frac{M_{R1Bl} + M_{R2Bl}}{2} - M_{PrBl} - M_{ABl}$$

Example:

M_{R1} = 149.32 mg M_{R2} = 152.11 mg M_{Pr} = 31.26 mg M_A = 14.52 mg
M_{Bl} = 1.1 mg M_1 = 1002.18 mg M_2 = 1019.07 mg

TDF % = 10.38

Calculation table

Sample Name	M [mg]	M_{FB} [g]	M_{before} [g]	M_{after} [g]	M_{R1} [mg]	M_{R2} [mg]	M_{Pr} [mg]	M_A [mg]	TDF [%]

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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