Total Dietary Fiber in Corn Flakes
according to AOAC 985.29

Reference: **AOAC 985.29 Total Dietary Fiber in Foods**

Tested with **VELP Scientifica GDE Enzymatic Digester** (Code F30400209) and **CSF6 Filtration Unit** (Code F30420210). Protein determination was performed with **DK 6 Kjeldahl Digestion Unit** (Code F30100182) and **UDK 159 Automatic Kjeldahl Distillation & Titration System** (Code F30200150)

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Introduction

Dietary fiber is a mixture of complex organic substances, including hydrophilic compounds such as soluble and insoluble polysaccharides and non-digestible oligosaccharides as well as a range of nonswellable, more or less hydrophobic compounds such as cutins, suberins and lignins.
Dietary fiber is not digested from human stomach and it represent a crucial nutrient in human diet for its ability to enhance satiety, to regulate intestinal functions and to modulate the nutrients absorption.
Thanks to its benefits, the fiber is increasingly used in functional foods, and food manufacturers declare the fiber content on the package as part of nutritional labeling.

Total Dietary Fiber (TDF) in corn flakes sample

Total dietary fiber is determined using a combination of enzymatic and gravimetric methods.
Duplicate of samples of dried, fat-free foods are gelatinized with heat-stable alpha-amylase and digested using amyloglucosidase to remove starch, and protease to remove proteins.

The soluble dietary fiber is precipitated with ethanol. The residue is then filtered, washed with ethanol and acetone, dried and weighed. One of the duplicate samples is assayed for indigested proteins and the other is ashed.
The total dietary fiber is the weight of the enzymatic digestion residue less the weight of the undigested protein and ash.

Equimpment

- 6 glass beakers 400 ml (code A00000999)
- Magnetic stirring bars, 6 x 35 mm (code A00001056)
- Pool balls (code A00000241)
- Fan oven
- Muffle furnace
- Desiccator
- Analytical balance 0.1 mg accuracy
- pH-meter standardized at pH 4.0 and 7.0
- Glass crucibles P2 (code A00000140)
- Automated Pipettor 1 ml, 100 µl, 200 µl
- Aluminum foils

Chemicals

- Petroleum ether 40-60 °C, analytical grade
- Ethanol 95 % v/v,
- Ethanol 78 % v/v - 207 ml of distilled water diluted to 1 L with 95 % ethanol. Mix and if necessary, dilute again to volume with 95 % ethanol
- Acetone, analytical grade
- Phosphate buffer, 0.08 M, pH 6.0 - 1.400 g of sodium phosphate dibasic anhydrous (Na_2HPO_4) and 8.400 g of sodium phosphate monobasic anhydrous (NaH_2PO_4) dissolved in 1 L distilled water. Check pH with pH-meter. Adjust to pH 6.0 if necessary using either sodium hydroxide solution or phosphoric acid solution
- Alpha-amylase, heat-stable, solution
- Protease solution - 50 mg in 1 ml phosphate buffer
- Amyloglucosidase solution
- Sodium hydroxide solution, 1.0 N, analytical grade
- Sodium hydroxide solution 0.275 N - 275 ml of 1.0 N sodium hydroxide diluted to 1 L with distilled water
- Hydrochloric acid solution, 1.0 N, analytical grade
- Hydrochloric acid solution 0.325 N - 325 ml of 1.0 N hydrochloric acid diluted to 1 L with distilled water
- Celite, acid washed

Sample

Corn flakes

Fiber labeled value: 3 %

Sample Preparation

Homogenize test sample and dry over night in oven at 105 °C.
Cool in desiccator and grind test sample to 0.3–0.5 mm mesh.

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Enzymatic Digestion and Filtration Procedure

Run 2 blanks through entire procedure to control possible contributions from reagents to final result.

1. Mix 1 g of sample, weigh 0.1 mg, with 50 ml of phosphate buffer pH 6.0 in a 400 ml beaker. Add a magnetic stirring bar.
2. Add 100 µl of heat stable alpha-amylase solution. Mix thoroughly. Cover the beakers with aluminum foil and place in GDE at 95-100 °C for 30 min. Incubation with sample temperature reaching 95-100 °C for 15 minutes is needed under continuous stirring.
3. Remove beaker from boiling water bath and let cool to room temperature.
4. Remove aluminum foils and rinse flask inner walls with 10 ml of distilled water.
   Adjust pH to 7.5 ± 0.2 with ~10 ml of sodium hydroxide solution 0.275 N.
5. Set the temperature of GDE to 60 °C adding cool distilled water to lower temperature from 95-100 °C.
6. Add 100 µl of protease solution.
   Incubate the beakers covered with aluminium foils for 30 minutes at 60 °C under continuous stirring.
7. Cool to room temperature.
   Adjust pH to 4.5 ± 0.2 with ~10 ml of hydrochloric acid solution 0.325 N. Control pH by a pH meter.
8. Add 200 µl of amyloglucosidase solution. Incubate the beakers covered with aluminium foils for 30 minutes at 60 °C under continuous stirring. Start timing when the temperature reaches 60 °C.
9. Remove magnetic stirring bar. Add 280 ml of 95 % ethanol preheated to 60 °C (measure volume before heating).
   Let precipitate form during 60 minutes at room temperature.
10. Glass crucibles are to be thoroughly cleaned and about 1 g of celite is added, placed in muffle at 550 °C during 5 hours, cooled at room temperature in a dessiccator up to constant weight, and weighed to nearest 0.1 mg (Crucible tare).
11. Wet the crucibles containing celite with some ml of 78 % EtOH, getting a uniform celite bed, without holes.
12. Set the crucibles in CSF 6 filtration equipment. Apply vacuum.
    Transfer quantitatively enzymatic digested to crucible through funnel. Start the filtration.
13. After the filtration rinse the residue in crucible with three 20 ml volumes of 78 % ethanol, two 10 ml volumes of 95 % ethanol and two 10 ml volumes of acetone. If a gummy film on residue impairs filtration, retaining liquid, break the surface with a slight air flow operating the air valve on filtration unit.
14. Dry crucible with residue and celite overnight in an air oven at 105 °C (70 °C in a vacuum oven).
    Let cool in a desiccator and weigh to nearest 0.1 mg.
    The weight of residue is obtained by subtracting the weight of crucible tare from the final weight.
15. One of the duplicate residues is to be analyzed by Kjeldahl method for undigested protein content determination (N x 6.25). Second duplicate residue is to be incinerated for the ash content determination.
16. Kjeldahl Protein Determination: transfer all the content of 3 crucibles in 3 Kjeldahl test tubes and proceed as in Application Note “N/Protein Determination in Feed products”
17. Ash Determination: second duplicate residue is to be incinerated in a muffle at 550 °C during 5 hours. In order to avoid thermal shocks it is suggested to perform a gradual heating. Let crucible cool in a dessicator and weigh to nearest 0.1 mg.
18. Correct the result for undigested proteins and ash content determined separately.

To reach temperatures of 95-100 °C with GDE, floating balls use is recommended (code A00000241).

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Typical Results

Average Blank residue (ABR) = average blank residues (g) (step 14)
Blank protein residue (BPR) = protein in blank (g) (step 16)
Blank ash residue (BAR) = ash in blank (g) (step 17)
Corrected blank (CB) = ABR-BPR-BAR (g)
Sample residue (SR) = sample residue (g) (step 14)
Sample protein residue (SPR) = average of protein in sample (g) (step 16)
Sample ash residue (SAR) = average of ash in sample (g) (step 17)
Corrected sample residue (CSR) = SR-SPR-SAR-CB (g)

$$\%\ TDF\ d.m.(dry\ matter) = \frac{CSR}{sample\ weight\ (g)} * 100$$

$$\%\ TDF = \frac{\%\ TDF\ d.m. * \%\ d.m.}{100}$$

If the defatting of sample was done during sample preparation step, correct the final average % TDF.

Crucible	Sample ID	Crucible Tare (g)	Sample Weight (g)	Crucibles Weight (g)	Total Residue (g)	CSR (g)	TDF % d.m.	TDF %
1	BLANK a	31.0831	-	31.1275	0.0444			
2	BLANK p	31.0481	-	31.0915	0.0434			
3	1p	30.6026	1.0000	30.7151	0.1125	0.0279	2.8	**2,7**
4	2p	31.1503	1.0003	31.2641	0.1138	0.0292	2.9	**2.8**
5	1a	31.0548	0.9999	31.1701	0.1153	0.0307	3.1	**2.9**
6	2a	30.8120	0.9999	30.9241	0.1121	0.0275	2.8	**2.6**
					Average ± SD%		2.9 ± 0.1	**2.7 ± 0.1**

Samples **a** are used for ash content calculation; sample **p** are used for protein content calculation

ABR = 0.0439 g
BPR = 0.0259 g
BAR = 0.0000 g
CB = 0.0180 g
SPR = 0.0659 g
SAR = 0.0006 g
Moisture = 4.833%
Dry matter = 95.167%

Conclusion

This makes the GDE and CSF6 system suitable for TDF determination and able to provide precise and reliable results.

Benefits of GDE and CSF6 units are:

- Precise temperature control during critical enzymatic digestions
- Integrated Digital Timer (GDE)
- Filter and wash up to 6 samples in only 20 minutes (CSF6)