Application HYDROTHERM

B.1.9.1.b.HT. Total fat determination in potato flakes (mashed potatoes)

Analysis according to Weibull-Stoldt

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1 Principle

The sample is heated up with hydrochloric acid to digest protein and free bound lipids. The digestion solution is filtered. After the drying process, the fat remaining in the filter is extracted with petroleum ether. The solvent is distilled and the dried residue is weighed. The fat content is calculated from the difference between initial sample weight and the extract.

2 Method

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 peculiarities in your laboratory.

This document is based on the following official methods:

- § 64 LFGB (formerly § 35 LMBG) L 17.00-4 Determination of the total fat content in bread including biscuits made from bread doughs, May 1982

3 Chemicals and Accessories

Quality p. a.

1. Water: demineralised or distilled
2. Hydrochloric acid HCl 15 %
3. Petroleum ether, boiling range 40 to 60 °C
4. pH indicator paper
5. HT Weighing paper for Hydrotherm, Art.1004939
6. Folded filter FF240, Art. 1004092
7. Extraction thimble 33 x 80 mm, Art. No. 13-0054
8. Extraction thimble 33 x 94 mm, Art. No. 13-0057
9. Cotton wool, chemically pure and degreased

4 Instruments

- Analytical balance (reading accuracy 0.1 mg)
- Desiccator, with a desiccant, e.g.: Silica gel orange / Sorbsil C 2.5
- HYDROTHERM Hydrolysis System HT 6
- Extraction unit SOXTHERM Micro/Macro with MULTISTAT, Art. 13-0011 or SOXTHERM Manager, Art. 13-0012
- Drying cabinet, electrically heated, with natural ventilation and automatic temperature control

5 Sample Type and Preparation

The hydrolysis beakers are loaded with 3 - 5 boiling stones, dried for 1 h in the drying oven at 100 ± 2°C, cooled to room temperature in the desiccator and then weighed to 1 mg on point.
The sample is to be stored sealed at 2 - 6 °C in such a way that spoilage and changes in its composition are prevented. Before weighing, mix the sample with a spoon so that an even distribution of the fat is achieved.
In most cases, the sample does not have to be grinded down, so it can be used directly for the hydrolysis.

Fig.1: Potato flakes for the production of mashed potatoes

5.1 Hydrolysis

Depending on the expected fat content (the value on the balance should be between 0.5 and 1.5 g) 10-15 g of sample are weighed into weighing paper (see 3.5.) with an accuracy of ± 0.1 mg. The weighing paper and then sample are then transferred into the digestion glass.

Alternatively, the sample can also be weighed into a small beaker and then transferred quantitatively into the HYDROTHERM hydrolysis beaker. The hydrolysis beakerwith the sample is then inserted into the HYDROTHERM and locked.

The HYDROTHERM unit is taken into operation according to the operating instructions. A dry folded filter (3.6.) is inserted into the respective position. The apparatus is closed and the program can be started. At first, the automatic addition of **150** ml hydrochloric acid (see 3.2.) takes place. The liquid is quickly heated up to boiling temperature and with reduced heating power kept at a low boiling point for approx. 1 h. When the hydrolysis is complete, the digestion solution is diluted to 250 ml with hot water and immediately filtered through a folded filter which has been automatically moistened by the apparatus before. The hydrolysis beaker, cooler and filtration device are rinsed several times with hot water. The filter is washed out with hot water until the rinsing water reacts neutrally. HYDROTHERM carries out all these operations automatically.

Table 1: Recommended parameters and settings: HYDROTHERM Best Practice

Parameter	Setting	Scale unit	Note
Filling levels			
HCl amount	100	ml	
H2O amount / dilution	50	ml	
Heating / cooling phases			
Heat-up phase	12	min	
Boiling phase 1	20	min	Power 50 %
Boiling phase 2	30	min	Power 70 %
Cooling down phase - duration	15	min	
Filter moisture			
Number of moisture cycles	3		
Moisture amount per cycle	30	ml	
Filter phase			
Filter wait time	5	s	
Rinsing cycles	20		
Pipe opening time	200	ms	
Sample rinse time	10	s	
Sample shower - amount	30	ml	
Condenser shower - amount	30	ml	
Filter shower - amount	50	ml	

***In this sample, 150 ml of hydrochloric acid is added, as the sample absorbs a large part of the liquid.**

At the end of the programme, the filters are each placed on a watch glass and dried for approx. 1.5 h at 103 ± 2 °C in the drying cabinet. Since moisture can affect the results, the drying time must be extended if necessary. Alternatively, the filters can also be dried overnight in the ambient air.

5.2 Extraction

After cooling down, the filter is placed in an extraction thimble (3.7.) and covered with cotton wool (3.9.). Any traces of grease on the watch glass have to be absorbed with another piece of cotton wool (3.9.), which is soaked with the extraction solvent and placed in the hydrolysis beaker as well.
After addition of 150 ml (SOXTHERM macro) or 100 ml (SOXTHERM micro) extraction solvent (3.3.) the sample is extracted in the SOXTHERM with the following program:

Table 2: Instrument configuration SOXTHERM

Parameter	Note / Cat.-no.
Solvent	Petroleum ether
Boiling point/range	40-60 °C
Solvent amount	100 ml SOXTHERM micro
	150 ml SOXTHERM macro
Sealing type	Viton / 1000578
Extraction thimbles	33 x 80 mm /13-0054 33 x 94 mm /13-0057
Thimble holders	SHK2 /13-0062
Boiling stones	13-0047
Compressor / Connection compressed air	13-0010, minimum 4.5 bar
Water connection / Chiller	minimum 0.5 bar

Table 3: SOXTHERM Program

Program step	Parameter	Note
T-classification	200 °C	
Extraction temperature	150 °C	
Reduction interval	4 min.	
Reduction pulse	2 s	
Hot extraction	30 min	Sample must be immersed completely
Evaporation A	4-5 x interval	After phase A the solvent level should be at least 10 mm below the thimble
Rinsing time	60 min.	
Evaporation B	3-4 x Intervall	After B the extraction beaker should be widely free of extraction agent.
Evaporation C	4 min.	

At the end of the program, the hydrolysis beakers are dried for 30 minutes at 103 ± 2°C in the drying cabinet. The hydrolysis beakers are then placed in the desiccator, cooled down to room temperature and weighed as precisely as possible to 1 mg. To check the weight consistency, the sample is dried again for 30 minutes and weighed again after cooling down.
This procedure is repeated until two successive weighings do not differ more than 1 mg. In case of an increase in weight, the previous low value is to be taken.
Extraction, drying and weighing must follow each other immediately.

Note: At extraction temperatures above 150 °C, the hydrolysis beakers should be cooled down slowly, e.g. in a drying cabinet. That is an important step to avoid extreme thermal stress, especially for the bottom of the hydrolysis beaker, and an associated formation of fine glass cracks.

6 Calculation

The crude fat content w in g/100 g (equivalent to %) of the sample is calculated according to the following equation:

$$w = \frac{(m_2 - m_1)\ 100}{m_0}$$

m_1: Mass of the empty hydrolysis beaker with boiling stones [g].
m_2: Mass of the extraction cup with fat after drying [g].
m_0: Weighing-in [g]

The result is rounded to one decimal place.

6.1 Repeatability

r = 0.20 g/100 g s(r) = ± 0.07

6.2 Reproducibility

R = 0.40 g/100 g s(R) = ± 0.15 %

7 Sample

Mashed potatoes with 0.5 % fat according to packaging

Sample name	Weighing g	Fat %
Mashed potatoes	10.2433	0.73
Mashed potatoes	15.0144	0.71
Mashed potatoes	10.4127	0.71
Mashed potatoes	15.5715	0.69

8 Troubleshooting Hydrolysis

Cause	Remedy
Sample with hydrochloric acid is not boiling well, sample won´t be hydrolyzed completely →recovery too low	Increase boiling power; be sure that the hydrolysis beaker is well placed at the hotplate
No boiling bubbles ascending at the end of the hydrolysis beaker overflow, no continuous boiling →recovery too low	The screw connect (screw cap GL 18) is not tight; screw it tight, place the hydrolysis beaker plane on the hotplate
Due to delays in boiling fat is sticking to the lid which won´t be transferred → recovery too low	Reduce boiling power and/or sample weight
Fat is remaining in the hydrolysis beaker→ Recovery is too low during the fat extraction	Increase rinsing cycles; add 0,2g +/- 0,05 of Silica to the hydrolysis beaker to get itclean after filtration
Fat is burnt in the hydrolysis beaker	Reduce boiling power. If the cool down duration was reduced it´s absolutely necessary to set it again to 15 minutes.
Fat is lost to the waste tank → recovery is too low during the fat extraction	During the filtration the whole fat isn´t collected completely in the folded filter; reduce sample weight and/or take a double folded filter
Hydrolysis beaker is dirty → recovery too high	Clean hydrolysis beaker before starting hydrolysis; control the beaker if it is clean before starting hydrolysis

9 Reasons for Failure

9.1 Result too high

Cause	Remedy
Water in the sample, water drops swim on the fat surface, fat can't be concentrated within the usual drying time	Filter or sample must be dry when they are put in
Too high temperature in the drying oven, fat is oxygenized and mass increases	Check temperature of the drying oven
Drying time too long, fat is oxygenized and mass increases	Observe needed drying time
Extraction beaker is dirty	Work as clean as possible in drying, weighing and cooling off process
Solvent is used several times; it has taken up fat which is detected, too.	Check blank value of the solvent
Parts of the thimble or sample residue are left in the extraction beaker	Check whether the fat film is clear Check whether the thimble is porous Check whether the grinding of the sample is too fine. Use a second filter Thimble is too impermeable, sample spills over

9.2 Result too low

Cause	Remedy
Incomplete extraction	Extract thimble again
Incomplete extraction	Flowrate cannot be achieved because of too low permeability of the thimble. Change thimble
Incomplete extraction	Sample sticks together, enlarge the surface e.g. by using sodium sulphate
Cooling off time too short	Follow correct weighing procedure
Boiling delay during concentration process, fat splashes can be found in the thimble	Wrong or missing boiling stones, glass beads cannot be used. Heating power too high, reduce the extraction temperature

9.3 Result fluctuates

Cause	Remedy
Uneven extraction due to solvent losses	Check for leakages in the apparatus at O-rings and connectors
Delayed boiling during extraction, sample residues may stick to the teflon connector	Use boiling stones
Delayed boiling because of usage of glass beads	Use standard boiling stones
Filtration problems, paper filter was not evenly wetted, fat losses because of fat running through. Can be seen from fat residues in the washing solution	Make sure filter in HYDROTHERM is wetted evenly

COMPREHENSIVE APPLICATION DATA BASE

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- 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
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