Application HYDROTHERM

B.1.13.HT.Total Fat in Plant Based Drinks (milk-substitutes)

C. Gerhardt GmbH & Co. KG
Cäsariusstraße 97
53639 Königswinter, Germany
☎+49 (0)2223 2999-0
✉ info@gerhardt.de
✉ application@gerhardt.de
@ www.gerhardt.de

1 Introduction

Plant based drinks became very popular over the last few years. They taste good, are healthy and are a good alternative for people who do not like animal milk or do not want to support the way animals are kept. But plant-based drinks are not only a replacement for animal milk. Also, they are available in different flavors including amaranth and cedarnut. Soy drinks are the most common among the milk substitutes used in coffee. The drinks consist of soft boiled and squeezed soybeans which are very rich in protein. Therefore it´s particularly suitable for frothing. Frequently the drink is enriched with calcium and vitamins, therefore it´s an appropriate aliment for vegans and vegetarians.
Oat drinks consist of fermented grain or flour and belong - just as soy drinks - to the low-cost milk substitutes.
During the process of the total fat determination, it´s important to take care of some details which are described in this application.

	
Organic Soy Drink, Lab.Nr.: 8409	Organic Oat Drink, Lab.Nr.: 8410
Fat: 1.9g/100ml (nutritional values on package)	Fat: 1.2g/100ml (nutritional values on package)
Corresponds to **Fat 1.88 %**	Corresponds to **Fat 1.19 %**

2 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.

3 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 peculiarities in your laboratory.

This document is based on the following official methods:

- Official Collection of Methods of Examination according to § 64 LFGB (formerly § 35 LMBG), L 01.00- 20 Determination of the fat content of milk and milk products, method according to Weibull, May 1988
- AOAC 989.04: Fat in raw milk
- AOAC 989.05: Fat in milk

4 Chemicals and material

Quality p. a.

1. Water: demineralized or distilled
2. Hydrochloric acid HCl, 15 %
3. Petroleum ether, boiling range 40 to 60 °C
4. pH indicator paper
5. DumaSorb order number 14-0022, 25 g absorbent for liquid samples
6. Disposable syringe, 10 ml
7. Folded filter FF240, order number 1004092
8. Extraction thimbles 33 x 80 mm, order number 13-0054
9. Extraction thimbles 33 x 94 mm, order number 13-0057
10. Cotton wool, chemically pure and defatted

5 Instruments

- Analytical balance, precision 0.1 mg
- Water bath
- Desiccator with drying agent, e.g. silica gel blue
- HYDROTHERM HT 6 hydrolysis system
- SOXTHERM extraction system micro/macro with control unit MULTISTAT order number 13-0011 or SOXTHERM-Manager order number 13-0012
- Drying oven, heated electrically, with natural aeration and automatic temperature control

6 Procedure

6.1 Sample preparation

Fill 3-5 boiling stones into each extraction beaker and leave the beakers in the drying oven at 100 ± 2 °C for 1 hour. After that, allow the beakers to cool down to room temperature in the desiccator and weigh them with an accuracy of 1 mg.
Store the sample closed at 2 - 6 ° C to prevent any spoilage or alteration of its composition. Before weighing, stir the sample thoroughly to achieve an even fat distribution. Place the sample in a beaker glass and warm it up to 30°C in a water bath. Keep the sample at this temperature for a few minutes and carefully stir from time to time. Allow cooling down to room temperature and weigh with samples using a disposable syringe.

6.2. Hydrolysis

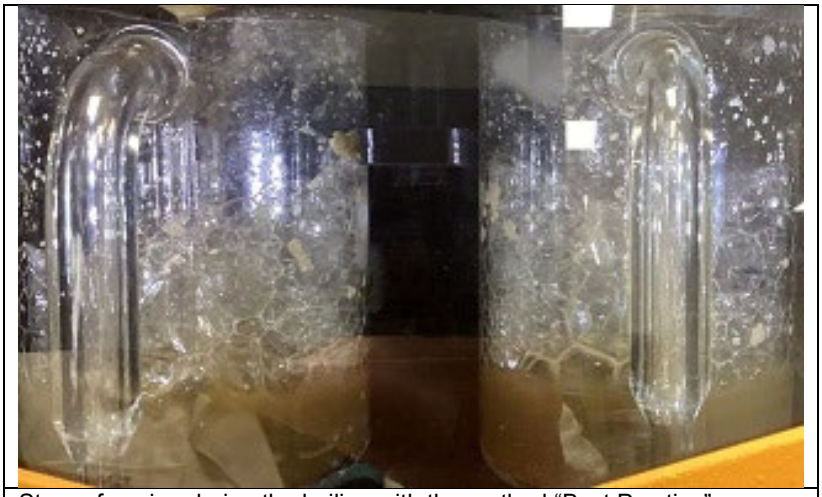
The sample size depends on the expected fat content. The amount of extracted fat should be around 0.5 – 1.5 g.
Thus, up to 30 g sample is weighed directly into the hydrolysis beaker (± 0.1 mg precision). The beaker containing the sample is inserted in the HYDROTHERM and is locked. Detailed information concerning the sample weight you may gather in chapter 8. Sample Details.
To avoid too low recovery of the fat 0.2 – 0.3g Celite is added. During the hydrolysis the samples are foaming a lot as expected. Therefore, the Method “Best Practice Soft” instead of “Best Practise” is preferred.

Start use of the HYDROTHERM unit following the instruction manual.
A dry folded filter (3.7.) is placed into the instrument. Then the apparatus is closed, and the method can be started.
The hydrochloric acid (3.2.) is added automatically. The liquid is slowly brought to boil and simmered with reduced heating capacity for about 1 h. At the end of the hydrolysis the digestion mixture is diluted with hot water and is then immediately filtered through a folded filter, which has been moistened automatically by the system with hot water. The beaker, the condenser and the filtration components are rinsed several times with hot water. The filter is rinsed with hot water till the backwash water has a neutral reaction.
HYDROTHERM performs all these steps automatically.

Table 1: Recommended parameters and settings: HYDROTHERM Best Practice Soft

Parameter	Setting	Scale unit	Note
Filling levels			
HCl amount	100	ml	
H_2O amount / dilution	50	ml	
Heating / cooling phases			
Heat-up phase	**8**	min	reduced from 12 min
Boiling phase 1	**25**	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	

After the program is completed, place the wet filters on a watch glass and leave them to dry at room temperature overnight. Alternatively dry the filters approximately 1.5 h at 103 ± 2°C in a drying oven. A higher temperature could lead to a loss of easy volatile fatty acids. Extract the folded filters immediately after drying or store them in a desiccator before the extraction. As moisture could eventually affect the result, add sodium sulphate (approx. a half teaspoon) to the sample into the extraction thimble.
The values given in the attachment can be used as orientation for the sample weight.



Strong foaming during the boiling with the method “Best Practise”
(Sample Soy Drink and Oat Drink)

Simmering of the samples with the method “Best Practise Soft”

6.3. Extraction

After cooling, place the filter in the extraction thimble and cover him with cotton wool. In case any fat traces remain on the watch glass, moisten cotton wool with the extraction solvent to absorb the fat and place the wet cotton wool inside the thimble.
Add 150 ml solvent (SOXTHERM macro) or 100 ml solvent (SOXTHERM micro) and extract samples with the following program:

Table 2: Instrument configuration SOXTHERM

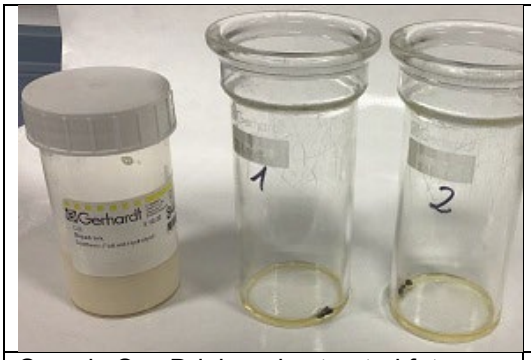
Parameter	Note / Order number
Solvent	Petroleum ether
Boiling point/range	40-60 °C
Solvent amount	100 ml SOXTHERM micro
	150 ml SOXTHERM macro
Sealing type	Viton / 1000578
Extraction thimbles	33 x 80 mm /13-0054 33 x 94 mm /13-0057
Thimble holders	SHK2 /13-0062
Boiling stones	13-0047
Compressor / Connection compressed air	13-0010, minimum 4.5 bar
Water connection / Chiller	minimum 0.5 bar

Table 3: SOXTHERM Program Fat

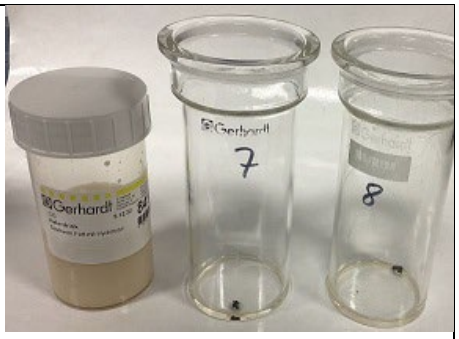
Program step	Parameter	Note
T-classification	200 °C	
Extraction temperature	150 °C	
Reduction interval	4 min.	
Reduction pulse	2 s	
Hot extraction	30 min	Sample must be immersed completely
Evaporation A	4-5 x interval	After phase A the solvent level should be at least 10 mm below the thimble
Rinsing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

After the extraction program is finished, leave extraction beakers in the drying oven for 30 minutes at a temperature of 103 ± 2 °C. After that place the extraction beakers in the desiccator, allow cooling down to room temperature and weigh them out with an accuracy of 1 mg.

In order to check the weight consistency, dry the samples for another 30 minutes and weigh again after cooling down. Repeat this procedure until the difference between two successive weighings does not exceed 1 mg of the final extract weight. Should the weight increase, take the previous lower value.
Extraction, drying and weighing should be performed consecutively, without interruption.



Sample Soy Drink and extracted fat



Sample Oat Drink and extracted fat

7 Evaluation

The total fat content w in g/100 g (corresponds to %) of the sample is calculated using the following equation:

$$w = \frac{(m2 - m1) * 100}{M0}$$

m1: mass of the empty extraction beaker with boiling stones [g]
m2: mass of the extraction beaker with fat after drying [g]
mo: initial sample weight [g]

The result is rounded to one decimal place.

7.1 Repeatability (milk)

r = 0.01%	for products < 1% fat
r = 0.05%	for products 1-10% fat

8 Sample Details

Sample Name	Lab.No.	Sample Weight g	Fat %	Method	Add. Celite
Organic soy drink	8409	23.0392	1.78	Best Practise Soft	no
Organic soy drink	8409	20.3635	1.77	Best Practise Soft	no
Organic soy drink	8409	19.8704	1.81	Best Practise Soft	yes
Organic soy drink	8409	20.7173	1.81	Best Practise Soft	yes
Organic oat drink	8410	22.8750	1.37	Best Practise Soft	no
Organic oat drink	8410	22.3914	1.36	Best Practise Soft	no
Organic oat drink	8410	22.0695	1.37	Best Practise Soft	yes
Organic oat drink	8410	22.1671	1.36	Best Practise Soft	yes

9 Results Proficiency Test

In December 2022 the lab of C. Gerhardt took part in a Proficiency test of the German MUVA “Chemical Analysis of plant based drinks”.
By means of this test the described method was applied. The lab code was 3.1 + 3.2.
The analyzed samples were Soya Drink (Sample 1) and Oat Drink (Sample 2).
Detailed results are summarized in Table1 and Table2.

Eignungsprüfung zur Qualitätssicherung / Proficiency Testing Study
EPQS Nr. 976 2022 Pflanzendrink / Plant Based Drink
Fett / fat

muva kempten

Tab. 1: / Table 1: Ergebnisse in Probe 1 / Results of sample 1							Zugewiesener Wert ± Unsicherheit / Assigned value ± uncertainty		0,98 (g/100g)	± 0,03 (g/100g)
Lab Code	x_{1lab} Wert 1 Value 1 (g/100g)	x_{2lab} Wert 2 Value 2 (g/100g)	x_{lab} Mittelw. Mean (g/100g)	Methode / Method Beschreibung Description	Norm (Standard) Standard		Kommentar Comment	Bewertung Performance z-Score	z'-Score	Messunsicherheit / measurement uncertainty U_{lab}
1'1	1,00		1,00	Gravimetry			Int. Meth.	0,51		
1'2	keine Ergebisse /no results									
2'1	0,91	0,94	0,93	NMR			Int. Meth.	-1,27		
2'2	0,92	0,95	0,94	NMR			Int. Meth.	-1,02		
3'1	1,03	1,04	1,04	Weibull-Stoldt	VDLUFA VI 15.2.3.			1,53		
3'2	1,01	1,01	1,01	Weibull-Stoldt	VDLUFA VI C 15.2.3.			0,76		
4'1	0,90	0,90	0,90	Butyrom. (Gerber)	VDLUFA VI C 15.3.2			-2,03		
5'1	0,99	0,99	0,99		-		Int. Meth.	0,25		
6'1	0,97	0,97	0,97	Röse-Gottlieb	§ 64 LFGB L 01.00-9			-0,25		
6'2	0,96	0,96	0,96	Röse-Gottlieb	§ 64 LFGB L 01.00-9			-0,51		
7'1	0,97	0,97	0,97	Weibull-Stoldt	§ 64 LFGB L 01.00-9			-0,25		
7'2	0,92	0,89	0,91	NMR			Int. Meth.	-1,78		
8'1	0,91	0,90	0,91	Weibull-Stoldt	§ 64 LFGB L 01.00-20		Person 30	-1,78		
8'2	0,90	0,87	0,89	Weibull-Stoldt	§ 64 LFGB L 01.00-20		Person 32	-2,29		
9'1	keine Ergebnisse / no results									
10'1	1,03	1,00	1,02	Weibull-Stoldt				1,02		
11'1	0,98	0,97	0,98	Weibull-Stoldt	§ 64 LFGB L 01.00-20			0,00		
12'1	1,01	0,99	1,00	Röse-Gottlieb	§ 64 LFGB L 01.00-9			0,51		
12'2	0,97	0,94	0,96	NMR				-0,51		

Table 1: MUVA Proficiency Test – Results of Sample 1

Eignungsprüfung zur Qualitätssicherung / Proficiency Testing Study
EPQS Nr. 976 2022 Pflanzendrink / Plant Based Drink
Fett / fat

muva kempten

Tab. 2: Ergebnisse in Probe 2
Table 2: Results of sample 2

Zugewiesener Wert ± Unsicherheit / Assigned value ± uncertainty: 2,05 ± 0,03 (g/100g)

Lab Code	x_{1lab} Wert 1 Value 1 (g/100g)	x_{2lab} Wert 2 Value 2 (g/100g)	x_{lab} Mittelw. Mean (g/100g)	Methode / Method Beschreibung Description	Norm (Standard) Standard	Kommentar Comment	Bewertung Performance z-Score	z'-Score	Messunsicherheit / measurement uncertainty U_{lab}
1'1	2,10		2,10	Gravimetry		Int. Meth.	0,68		
1'2	keine Ergebisse /no results								
2'1	1,91	1,88	1,90	NMR		Int. Meth.	-2,04		
2'2	1,88	1,91	1,90	NMR		Int. Meth.	-2,04		
3'1	2,10	2,10	2,10	Weibull-Stoldt	VDLUFA VI C 15.2.3		0,68		
3'2	2,09	2,09	2,09	Weibull-Stoldt	VDLUFA VI C 15.2.3.		0,54		
4'1	1,95	2,00	1,98	Butyrom. (Gerber)	VDLUFA VI C 15.3.2		-0,95		
5'1	2,04	2,02	2,03			Int. Meth.	-0,27		
6'1	2,06	2,05	2,06	Röse-Gottlieb	§ 64 LFGB L 01.00-9		0,14		
6'2	keine Ergebisse /no results			Röse-Gottlieb	§ 64 LFGB L 01.00-9				
7'1	2,05	2,05	2,05	Weibull-Stoldt	§ 64 LFGB L 01.00-9		0,00		
7'2	1,85	1,85	1,85	NMR		Int. Meth.	-2,72		
8'1	1,92	2,00	1,96	Weibull-Stoldt	§ 64 LFGB L 01.00-20	Person 30	-1,22		
8'2	2,03	2,03	2,03	Weibull-Stoldt	§ 64 LFGB L 01.00-20	Person 70	-0,27		
9'1	keine Ergebnisse / no results								
10'1	2,05	2,09	2,07	Weibull-Stoldt		Int. Meth.	0,27		
11'1	2,05	2,01	2,03	Weibull-Stoldt	§ 64 LFGB L 01.00-20		-0,27		
12'1	2,01	2,05	2,03	Röse-Gottlieb	§ 64 LFGB L 01.00-9		-0,27		
12'2	1,89	1,92	1,91	NMR			-1,90		

Table 2: MUVA Proficiency Test – Results of Sample 2

10 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 a teaspoon of Silica to the hydrolysis beaker to get it clean 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 Troubleshooting Extraction

9.1 Results 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 Results 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 Varying Results

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

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.

An excerpt from our product portfolio

Fully AUTOMATIC HYDROLYSIS

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.

Fully AUTOMATIC FAT EXTRACTION

SOXTHERM – automatic fast extraction system for fat determination.

Fully AUTOMATIC WATER STEAM DISTILLATION

VAPODEST – fast distillation system for Kjeldahl nitrogen/ protein determination and steam distillation as sample preparation for further analysis.

COMPLETELY AUTOMATIC NITROGEN ANALYSIS

DUMATHERM – nitrogen/protein determination of solid and liquid samples according to the Dumas combustion method. A fast and convenient alternative to the classic Kjeldahl method for almost all sample matrices.

AUTOMATED CRUDE FIBRE DETERMINATION

FIBRETHERM – completely automated processing of the boiling and filtration processes for determining crude fibre, ADF and NDF.

www.gerhardt.de