Total Fat Determination in Cheese
according to the Randall method

Reference: **AOAC 933.05** Fat in Cheese
Tested with **VELP Scientifica SER 158/6 Solvent AutoExtractor** (Code S303A0380) and **HU6 Hydrolysis Unit** (Code F30300110)

Introduction

Cheese is a great source of calcium and protein. It also contains high amounts of vitamins A and B12, along with zinc, phosphorus, and riboflavin. Cheese is also a high-fat and high-calorie food. Indeed types of cheese are often classified according to the fat content, and the dough of the cheese changes accordingly from soft to hard.

Anyway, there is nothing so bad that it is not good for something: fat in cheese is indeed what carries the flavors, and makes the lipid-soluble vitamins A, D, E and K accessible to the body.

High-fat cheeses like blue cheese, Brie, and sharp cheddar contain also small amounts of conjugated linoleic acid (CLA). This is a fatty acid that naturally occurs in foods, and it has been shown to be anti-carcinogenic, besides preventing heart disease and obesity.

Fat % d.m.*	Classification	Dough
> 45	High fat	Hard
25 – 45	Medium fat	Semi-hard
10 – 25	Low fat	Semi-soft
< 10	Skim	Soft

*d.m.= on dry matter

Fat Determination in Cheese

Hot solvent extraction process with SER 158 Series can be summed up in 5 steps, for a fully automatic process completely unattended:

| STEP 1 IMMERSION	→	STEP 2 REMOVING	→	STEP 3 WASHING	→	STEP 4 RECOVERY	→	STEP 5 COOLING

During IMMERSION the sample is immersed in boiling solvent. Then the REMOVING step automatically lowers the level of the solvent to below the extraction thimble. During WASHING the condensed solvent flows over the sample and through the thimble to complete the extraction process. The fourth step involves solvent RECOVERY. Approximately 90% of the solvent used is collected in the internal recovery tank. The final step is the COOLING of the extraction cups containing the extracted matter. The cups are raised to prevent burning. The extraction cups containing the extract are placed in a drying oven, cooled in a desiccator and weighed for the extract percentage calculation.

Sample

Semisoft cheese   Fat labeled content: 22 %

In order to determine the total fat in food and feed samples a direct extraction with solvents is not efficient because a part of lipids is chemically bonded with other components. The determination of the total fat content of these samples, requires a *preliminary hydrolysis* using hydrochloric acid 4 N, followed by filtration and washing, in order to free completely the fat molecules. Then the hydrolyzed sample is ready for the extraction and it can be easily transferred to SER 158 Solvent AutoExtractor avoiding any possible sample loss and ensuring improved accuracy of results (see the following picture).

Step 1	Step 2
Hidrolysis with HU6	Extraction with SER 158
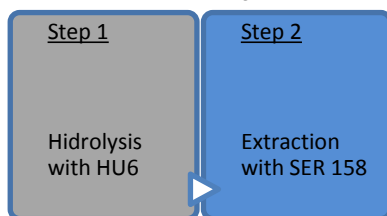
Chemicals and Equipments Required

- Analytical balance, min. 3 decimals
- Glass sand, 0.4-0.8 mm (Code A00000089)
- Celite 545 (Code A00000097)
- Glass crucibles P1 (Code A00000086)
- Hydrochloric acid 4N

- Test tube 250 ml (Code A00000144)
- Glass bottle for waste collector (Code A00000088)
- Glass extraction cups (Code A00000290)
- Viton seals
- Petroleum Ether 40 – 60 °C as solvent

All the accessories required for the hydrolysis are included in the kit code A00000085.

Sample Preparation

- **Hydrolysis Crucibles Preparation**

Put 25 g of glass sand in glass crucibles P1 and 3 g of Celite 545: the two layers resulting have not to be mixed together otherwise the phase in Celite may bypass the crucible negatively affecting the end result.

- **Sample Preparation in Test Tube**

Mix the sample with a spoon and weigh 3 g of homogenized sample (M_{sample}) and 2 g of Celite 545 in the test tube. In each test tube add 50 ml of 4N hydrochloric acid, shake gently and carefully, and finally add a further 50 ml hydrochloric acid in order to rinse the sample residue that may remain on the walls of the test tube.

- **Glass Extraction Cups Preparation**

Keep the empty glass extraction cups in oven at 105 °C for 1 hour. Cool them in a desiccator and record the accurate weight of the tare (M_{tare}).

Hydrolysis Procedure with HU 6

Place 6 crucibles P1 on the HU6 and connect the aspirating tubes: one side needs to be placed on the sealing ring of the crucible and the other one has to be in contact with the corresponding test tube.
Set 170 °C for 30 minutes.
Place the 6 test tubes in the heating block, lower the glassware and activate the vacuum pump.
  ➤ *In case of foam, add 4 N hydrochloric acid drop by drop inside the test tubes.*
At the end of the procedure, switch off and allow aspiration of the content of the test tubes in the crucible.
Raise the glassware to its maximum point and secure it, by tightening the knob.
Then, add hot water (40-50 °C, about 250 ml) slowly, in order to aspirate all the residues of hydrolysis in the test tubes.
Remove the aspirating tubes and the crucibles containing the sample after the washing: mix the layer of hydrolyzed sample with the layer of Celite by using a spatula in order to break any clot.
  ➤ *Be careful not to damage the layer of glass sand.*
This operation helps the sample drying, which must take place in an oven at 105 °C.

If dried test portions will not be immediately extracted, put them in a desiccator, or let the crucibles cool to room temperature and then, mix the layer of hydrolyzed sample and Celite with a spatula to obtain a powdery hydrolyzed.
  ➤ *Be careful not to damage the layer of glass sand.*

Extraction Procedure with SER 158

Fix the crucibles with the crucible holders (Code A00000293). The extraction cups containing the crucibles can now be placed on the ultra-fast heating plate of SER 158.

On the ControlPad™ select "Analysis", and then method "Total fat in cheese" including the following parameters:

- Immersion Time: 60 minutes
- Removing Time: 10 minutes
- Washing Time: 50 minutes
- Recovery Time 30 minutes
- Cooling Time: 3 minutes
- Petroleum Ether 40-60 °C, 100 ml

Close the safety guard and add the solvent using the automatic solvent dispensing system **SolventXpress™** to minimize exposure to the solvent ensuring operator safety.
Press START to begin the extraction process. At the end of analysis position the extraction cups containing the extract in a drying oven (1 hour at 105 °C), cool them in a desiccator to room temperature and record the accurate weight (M_{tot}).

Typical Results on Cheese

Analysis results are calculated automatically and stored in the ControlPad™ when entering the weights into the software (manually or automatically through a balance). The extract percentage calculation is performed by using the following formulas:

$$Extract\ (g) = (Total - Tare)$$
$$Extract\ (\%) = Extract \times 100\ /\ (Sample)$$

Where:

Sample = sample weight (g)
Tare= weight of the empty extraction cup (g)
Total = weight of the extraction cup + extract (g)

Tare (g)	Sample (g)	Total (g)	Extract (g)	Extract (%)
133.5103	5.0782	134.6117	1.10	21.7
134.4137	5.0895	135.5335	1.12	22.0
133.3980	5.0115	134.4984	1.10	22.0
135.0785	5.0655	136.2112	1.13	22.4
133.4737	5.0421	134.5857	1.11	22.1
132.4648	5.0483	133.5793	1.11	22.1
			Average ± SD%	**22.0 ± 0.2**
			RSD% **	**0.98**
Fat Labeled content: 22 %				

** RSD% = (Standard Deviation x 100) / Average

Conclusion

The results obtained are reliable and reproducible in accordance with the expected values, with a low relative standard deviation (RSD < 1%), that means high repeatability of the results.
Therefore, SER 158 Solvent AutoExtractor and HU6 hydrolyses unit are ideal for the fat content determination in cheese.

Benefits of HU 6 Hydrolysis Unit are:
- safety with performance, reducing manual handling to the minimum
- no sample transfer required when passing from HU 6 to SER 158
- suitable for both acid and basic hydrolysis

Benefits of hot solvent extraction (Randall) by using SER 158 Solvent AutoExtractor:
- up to 5 times faster than Soxhlet (hot solvent vs.cold solvent)
- low solvent consumption (high solvent recovery, approximately 90%) - limited cost per analysis
- no exposure to solvent
- worldwide official method
- full traceability with automatic result calculation and on-board archive