ANNEX III

METHODS OF ANALYSIS TO CONTROL THE COMPOSITION OF FEED MATERIALS AND COMPOUND FEED

H.DETERMINATION OF CRUDE OILS AND FATS

1. Purpose and scope

This method is for the determination of crude oils and fats in feed. It does not cover the analysis of oil seeds and oleaginous fruit.

The use of the two procedures described below depends on the nature and composition of the feed and the reason for carrying out the analysis.

1.1. Procedure A — Directly extractable crude oils and fats

This method is applicable to feed materials of plant origin, except those included within the scope of Procedure B.

1.2. Procedure B — Total crude oils and fats

This method is applicable to feed materials of animal origin and to all compound feeds. It is to be used for all materials from which the oils and fats cannot be completely extracted without prior hydrolysis (e.g. glutens, yeast, potato proteins and products subjected to processes such as extrusion, flaking and heating).

1.3. Interpretation of results

In all cases where a higher result is obtained by using Procedure B than by Procedure A, the result obtained by Procedure B shall be accepted as the true value.

2. Principle

2.1. Procedure A

The sample is extracted with light petroleum. The solvent is distilled off and the residue dried and weighed.

2.2. Procedure B

The sample is treated under heating with hydrochloric acid. The mixture is cooled and filtered. The residue is washed and dried and submitted to the determination according to Procedure A.

3. Reagents

3.1. Light petroleum, boiling range: 40 to 60 ^{o}C. The bromine value must be less than 1 and the residue on evaporation less than 2 mg/100 ml.

3.2. Sodium sulfate, anhydrous.

3.3. Hydrochloric acid, c = 3 mol/l

3.4. Filtration aid, e.g. Kieselguhr, Hyflo-supercel.

4. Apparatus

4.1. Extraction apparatus. If fitted with a siphon (Soxhlet apparatus), the reflux rate shall be such as to produce about 10 cycles per hour; if of the non-siphoning type, the reflux rate shall be about 10 ml per minute.

4.2. Extraction thimbles, free of matter soluble in light petroleum and having a porosity consistent with the requirements of point 4.1.

4.3. Drying oven, either a vacuum oven set at 75 ± 3 °C or an air-oven set at 100 ± 3 °C.

5. Procedure

5.1. Procedure A (see point 8.1)

Weigh 5 g of the sample to the nearest 1 mg, transfer it to an extraction thimble (4.2) and cover with a fat-free wad of cotton wool.

Place the thimble in an extractor (4.1) and extract for six hours with light petroleum (3.1). Collect the light petroleum extract in a dry, weighed flask containing fragments of pumice stone[1].

Distil off the solvent. Dry the residue maintaining the flask for one and a half hours in the drying oven (4.3). Leave to cool in a desiccator and weigh. Dry again for 30 minutes to ensure that the weight of the oils and fats remains constant (loss in weight between two successive weighings must be less than or equal to 1 mg).

5.2. Procedure B

Weigh 2,5 g of the sample to the nearest 1 mg (see point 8.2), place in a 400 ml beaker or a 300 ml conical flask and add 100 ml of hydrochloric acid (3.3) and fragments of pumice stone. Cover the beaker with a watch glass or fit the conical flask with a reflux condenser. Bring the mixture to a gentle boil over a low flame or a hot-plate and keep it there for one hour. Do not allow the product to stick to the sides of the container.

Cool and add a quantity of filtration aid (3.4) sufficient to prevent any loss of oil and fat during filtration. Filter through a moistened, fat-free, double filter paper. Wash the residue in cold water until a neutral filtrate is obtained. Check that the filtrate does not contain any oil or fats. Their presence indicates that the sample must be extracted with light petroleum, using Procedure A, before hydrolysis.

Place the double filter paper containing the residue on a watch glass and dry for one and a half hours in the air oven (4.3) at 100 ± 3 °C.

Place the double filter paper containing the dry residue in an extraction thimble (4.2) and cover with a fat-free wad of cotton wool. Place the thimble in an extractor (4.1) and proceed as indicated in the second and third paragraphs of point 5.1.

6. Expression of result

Express the weight of the residue as a percentage of the sample.

7. Repeatability

The difference between the results of two parallel determinations carried out on the same sample by the same analyst shall not exceed:

— 0,2 %, in absolute value, for contents of crude oils and fats lower than 5 %,

— 4,0 % relative to the highest result for contents of 5 % to 10 %,

— 0,4 %, in absolute value, for contents above 10 %.

8. Observations

8.1. For products with a high content of oils and fats, which are difficult to crush or unsuitable for drawing a homogeneous reduced test sample, proceed as follows.

Weigh 20 g of the sample to the nearest 1 mg and mix with 10 g or more of anhydrous sodium sulfate (3.2). Extract with light petroleum (3.1) as indicated in point 5.1. Make up the extract obtained to 500 ml with light petroleum (3.1) and mix. Take 50 ml of the solution and place in a small, dry, weighed flask containing fragments of pumice stone. Distil off the solvent, dry and proceed as indicated in the last paragraph of point 5.1.

Eliminate the solvent from the extraction residue left in the thimble, crush the residue to a fineness of 1 mm, return it to the extraction thimble (do not add sodium sulfate) and proceed as indicated in the second and third paragraphs of point 5.1.

Calculate the content of oils and fats as a percentage of the sample by using the following formula:

$(10m_1 + m_2) \times 5$

where:

m_1 = weight in grams of the residue after the first extraction (aliquot part of the extract),

m_2 = weight in grams of the residue after the second extraction.

8.2. For products low in oils and fats the test sample may be increased to 5 g.

8.3. Pet foods containing a high content of water may need to be mixed with anhydrous sodium sulfate prior to hydrolysis and extraction as per Procedure B.

8.4. In paragraph 5.2 it may be more effective to use hot water in place of cold water to wash the residue after filtration.

8.5. The drying time of 1,5 h may need to be extended for some feed. Excessive drying shall be avoided as this can lead to low results. A microwave oven can also be used.

8.6. Pre-extraction by Procedure A prior to hydrolysis and re-extraction by Procedure B is recommended if the crude oil/fat content is greater than 15 %. To some extent this depends on the nature of the feed and the nature of the oil/fat in the feed.

(**1**) Where the oil or fat has to undergo subsequent quality tests, replace the fragments of pumice stone by glass beads.

Changes to legislation:
There are currently no known outstanding effects for the Commission Regulation (EC) No 152/2009, Division H..