



Sample Preparation for the Macroscopic and Microscopic Analysis, IAG-Method A1



International Association of Feedingstuff Analysis
Section Feedingstuff Microscopy



1. Objective and field of application

The method is used for sample preparation for the macroscopical and microscopical analysis of animal feedingstuffs. It describes the production of a representative sample and the preparation of the sample for analysis by crushing and fractioning.

2. Definitions

Sample for examination: representative part of the sample material provided for examination that is used for the production of the sample ready for analysis.

Sample for analysis: representative portion of the sample for examination prepared for analysis.

Depelletise: sample material provided in pressed form (e.g. pellets) is transformed into a not pressed, coarse-grained or powdery state eventually e.g. by influence of moisture.

Fractionate: to separate the sample material by particle size, density or solubility.

Separation fluid: fluid of known/given density for separation of the sample material into a fraction of specifically heavier and specifically lighter particles compared to the density of the separation fluid.

Sediment: fraction of specifically heavy particles in a sample that sink to the bottom in a certain separation fluid and can therefore be separated from specifically lighter particles.

Flotate: fraction of specifically light particles in a sample that float on the surface of a certain separation fluid and can therefore be separated from the sediment.

3. Reagents

3.1 Ethanol, $\beta = 96 \%$

3.2 Acetone

3.3 Tetrachloroethylene, $\rho = 1.63 \text{ g/cm}^3$

3.4 Nitric acid, $w = 10 \%$

3.5 Hydrochloric acid, $w = 25 \%$

3.6 Sodium hydroxide solution, $w = 2.5 \%$

3.7 Potassium hydroxide, $w = 3\%$

The reagents listed may be replaced by others which produce comparable results.



4. Equipment and accessories

- 4.1 Analytical balance (accuracy = 0,001 g)
- 4.2 Sample divider
- 4.3 Mortar and pestle
- 4.4 Grinding mill
- 4.5 Sieves fitted with wire nettings or perforations with different mesh sizes (e.g. 2.0 mm, 1.0 mm, 0.5 mm, 0.25 mm) and collecting tray; recommended additional equipment: sieve towers, sieve shaker
- 4.6 Oven (up to 130 °C)
- 4.7 Laboratory glassware
- 4.8 Filters (e.g. paper, gaze)
- 4.9 Freeze dryer
- 4.10 Hot plate or Bunsen burner

5. Procedure

5.1 Preparation of a sample for examination

The sample for examination is supposed to represent the sample material provided for examination and consists of at least 100 g. The material is divided by hand or with a sample divider.

5.1.1 Division by hand

5.1.1.1 Procedure 1:

Small portions of the homogeneously mixed sample material are picked from a number of spots by a spoon or a spatula, given into a vessel and mixed again. If necessary, several samples for examination can be produced in this way. An aliquot portion of the sample material provided for examination is taken for retain sample.

5.1.1.2 Procedure 2:

The well-mixed sample material is spread plainly on an even pad. The sample is divided into four sectors of similar size. The respective portions lying diagonally opposite are joined. This way the sample is divided into two portions. If necessary, several samples for examination can be produced in this way. An aliquot portion of the sample material provided for examination is taken for retain sample.



5.1.2 Division by a sample divider:

The sample material is given on a sample divider (4.2). If necessary, several samples for examination can be produced in this way. An aliquot portion of the sample material provided for examination is taken for retain sample.

5.2 Reduction of the sample for examination to small pieces

Depending on the quality of the sample for examination it must be reduced to small pieces or depelleted. Very fatty material can be defatted (5.4.1) by acetone (3.2).

5.2.1 Not pressed samples for examination

Finely milled material is not reduced to smaller pieces. Coarse material (at least 10 g) is crushed to smaller pieces mechanically by mortar and pestle (4.3) or milled (4.4).

5.2.2 Pressed samples for examination

Depending on the demands for analysis, pressed samples for examination are crushed or reduced to smaller pieces mechanically or are depelleted.

5.2.2.1 Pressed material (at least 10 g) is mechanically crushed by mortar and pestle (4.3) or milled (4.4)

5.2.2.2 At least 10 g of the pressed material is mixed with at least three times as much water. The suspension is stirred up several times and left standing until the pellets disintegrate. Then the depelleted material is filtered (4.8) and dried at room temperature or freeze-dried (4.9).

5.2.2.3 For depelleting at high humidity pressed material (at least 10 g) is left standing in humid atmosphere at 70 °C (4.6) until the pellets disintegrate. The material is crushed, sieved (4.5) and dried at room temperature immediately to prevent the particles from sticking together again.

5.3 Fractionation of the sample

In order to fractionate the sample material is separated by particle size, solubility or density.

5.3.1 Fractionation by particle size

In order to fractionate by particle size coarse fractions (particle size > 0.5 mm) and fine fractions (particle size < 0.5 mm) are obtained by sieving (4.5). For quantitative analysis the sieve fractions are weighed (4.1).



5.3.2 Fractionation by solubility of the particles

Depending on their solubility in different solvents certain particles can be separated:

Water-soluble substances (e.g. molasses) can be separated from the remaining material by dissolving in water. Fatty substances can be dissolved in or separated by ethanol (3.1) or acetone (3.2). For quantitative analysis the fractions are weighed (4.1).

5.3.3 Fractionation by density

The sediment is separated from the flotage in a separation fluid by different procedures.

5.3.3.1 Separation by particular separation fluids

Not pressed sample material (at least 10 g) is weighed (4.1) and suspended in the five- to tenfold volume of a particular separation fluid (e.g. tetrachloroethylene, 3.3) in an adequate vessel (4.7). The suspension is shaken several times or stirred by a glass bar to support the separation. After sufficient settling time flotage and sediment are separated and dried. For quantitative analysis the fractions are weighed (4.1).

5.3.3.2 Separation by water as separation fluid

5.3.3.2.1 Separation by slurring

Not pressed sample material (at least 10 g) is weighed (4.1) and stirred in water in a beaker (4.7). The specifically heavier particles sink to the bottom, the suspended particles are decanted carefully. This procedure is repeated until the sediment is separated from the suspending particles. The sediment remaining after decanting the fluid is transferred into a flat glass dish and dried in an oven (4.6) at approx. 100 °C until the weight is constant. Particles sticking together are carefully crushed or cracked in a mortar (4.3). For quantitative analysis the fractions are weighed (4.1).

5.3.3.2.2 Separation in a weak water flow

The fine-grained sample (at least 10 g) is weighed and transferred into a cylinder which is filled with water afterwards. A weak water flow is conducted into the suspension by a glass tube dipped into the water down to the lower third of the cylinder so that light particles are floated. This is done until no suspended particles are left. The fluid above the sediment is decanted. The sediment is transferred into a flat glass dish and dried in an oven (4.6) at approx. 100°C until the weight is constant. Particles sticking together are carefully crushed or cracked in a mortar (4.3). For quantitative analysis the fractions are weighed (4.1).



5.3.4 Fractionation of pieces of tissue (acid-base-decomposition)

Characteristic pieces of tissue of feedingstuffs that are very sugary, paste-like, pulpy samples or samples rich in starch can be concentrated by acid-base-decomposition.

The sample is boiled with the tenfold volume of nitric acid (3.4) or hydrochloric acid (3.5) for about 1 minute (4.10). After adding approx. 100 ml of water the sample is filtered (4.8) and rinsed with warm water. The residue is then boiled for about 1 minute with the twofold volume of sodium hydroxide solution (3.6) or potassium hydroxide solution (3.7). After neutralisation with hydrochloric acid (3.5) the sample is filtered (4.8) and washed. In the supernatant of the filter the specifically heavy particles are in the cone end of the filter cone and the specifically light particles are on the upper margin of the filter.

5.4 Removal of interfering constituents from the sample

Constituents interfering with the sample preparation or analysis can be removed from the sample.

5.4.1 Interfering constituents (e.g. molasses or fat) can be removed from the sample according to the procedure of fractioning by solubility of the particles (5.3.2).

5.4.2 Starch can be removed from sugary, paste-like, pulpy feedingstuffs or feedingstuffs rich in starch according to the procedure of fractionating of pieces of tissue (5.3.4) or in a weak water flow (5.3.3.2).

5.4.3 Expected mucilage production (e.g. linseed) in the separation by water (5.3.3.2) can be avoided by soaking the sample in water for approx. 1 hour. After decanting the water the sample is suspended in the threefold volume of nitric acid (3.4) or hydrochloric acid (3.5) for about 3 to 5 minutes (4.10). After that the sample is washed with water, neutralized with sodium hydroxide solution (3.6) and washed again with water.

6. Literature

- BIHLER, E., 1972: Eine Möglichkeit der Vorbereitung von pelletierten Mischfutterproben für die mikroskopische Untersuchung. In: Tagungsprotokoll der Internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Sektion Futtermittelmikroskopie, Oldenburg
- DEUTSCHMANN, F. und Mäckel, H. G., 1976: Grundlagen der mikroskopischen Diagnostik pflanzlicher Rohstoffe. In: Handbuch der Mikroskopie in der Technik, Band VIII. Umschau-Verlag, Frankfurt/Main



- FREUND, H. (ed.), 1976: Mikroskopie der Nahrungs- und Futtermittel, der Drogen und Genussmittel. In: Handbuch der Mikroskopie in der Technik, Band VIII. Umschau-Verlag, Frankfurt/Main
- GASSNER, G.; Hohmann, B. und Deutschmann, F., 1989: Mikroskopische Untersuchung pflanzlicher Lebensmittel. 5. Auflage, Gustav Fischer-Verlag, Stuttgart, New York
- HAHN, H. und Michaelsen, L., 1996: Mikroskopische Diagnostik pflanzlicher Nahrungs-, Genuss- und Futtermittel, einschließlich Gewürze. Springer-Verlag, Berlin, Heidelberg, New York
- HUß, W., 1976: Die mikroskopische Untersuchung von Futtermitteln. In: Handbuch der Mikroskopie in der Technik, Band VIII. Umschau-Verlag, Frankfurt/Main
- HUß, W., 1959: Vorbereitung von Pressfuttermitteln für die Untersuchung mit dem Stereomikroskop. In: Tagungsprotokoll der Internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Sektion Futtermittelmikroskopie, Stuttgart-Hohenheim
- HUß, W., 1968: Mikroskopischer Nachweis und Bestimmung des Anteils an Ricinussamenschalen. EWG-Dokument Nr. 7881/3/VI/68-D
- KLEIN, H., Marquard, R., 2003: Futtermittel-Mikroskopie. Atlas zur mikroskopischen Untersuchung pflanzlicher und tierischer Futtermittel. AgriMedia-Verlag, Bergen/Dumme
- MALKOMESIUS, E.; Nehring, K.; Claus, G. und Kummer, H., 1951: Die Untersuchung von Futtermitteln. In: Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (Methodenbuch, Band III, 2. Auflage, Hrsg. R. Herrmann), Neumann-Verlag, Radebeul und Berlin
- MÜLLER, M., 1995: Die Gefriertrocknung als Möglichkeit der Vorbereitung von pelletierten Mischfutterproben. In: Tagungsprotokoll der Internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Sektion Futtermittelmikroskopie, Hamburg
- Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), 1993: Versandmuster und Analysenprobe. Kap. 2 in: Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (Methodenbuch) Bd. III. Die chemische Untersuchung von Futtermitteln. 4. Erg., VDLUFA-Verlag, Darmstadt
- Verordnung über Probenahmeverfahren und Analysemethoden für die amtliche Futtermittelüberwachung (Futtermittel-Probenahme- und Analyse-Verordnung) vom 27. April 2004 – BGBl. Teil I Nr. 21, 856
- VÖHRINGER, H., 1997: Leitfaden für die Futtermittelqualitätskontrolle durch Mikroskopie. Hoffmann-La Roche, 3. Fassung, Wien