Method for Determination of Castor (*Ricinus communis* L.) seed husks in Animal Feedingstuff, IAG-Method A6

International Association of Feedingstuff Analysis
Section Feedingstuff Microscopy

1. Objective and field of application

The method is used for both qualitative and quantitative determination of castor seed husks in animal feedingstuff.

2. Principle

Castor seed husks are determined by macroscopic and microscopic identification of the seed husk fragments. Quantification is done by weighing the amount of identified seed husk fragments in sieved fractions with particle size > 0.5 mm.

3. Reagents

3.1 Embedding agents

3.1.1 Chloral hydrate (ß = 60 %)

3.1.2 Water

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

4. Equipment and accessories

4.1 Optical equipment

4.1.1 Stereo microscope (up to 70x magnification); recommended additional equipment: image support system

4.1.2 Compound microscope (up to 400x magnification); recommended additional equipment: polarization, phase contrast, image support system

4.1.3 Magnifier (up to 10x magnification)

4.2 Mortar and pestle

4.3 Sieves (supporting document (9)

4.4 Analytical balance (accuracy 0.001 g)

4.5 Additional laboratory equipment is listed in supporting document (9)

4.6 Reference material

5. Procedure

Pelleted feedingstuff has to be depelleted roughly before analysing (4.2). Qualitative determination is performed macroscopically and microscopically considering the castor seed husks in all sieve fractions. Quantitative determination is performed by selecting and weighing the seed husks > 0.5 mm found in the laboratory sample or an aliquot of it.

5.1 Preparation of the laboratory sample

Non-pelleted or coarsely crushed laboratory sample (approximately 100 g) is weighed (4.4) and fractionated by density according to supporting document (9) using water for separation (8.1). Castor seed husk particles will be found in the sediment. After drying the sediment it is fractionated by particle size (8.2) according to supporting document (9). For quantification the weight of each fraction is recorded (4.4)

5.2 Identification

Castor seed husks are identified based on characteristic features of the fragments of the seed husks. The identification of seed husk fragments may be facilitated through comparison with reference material (4.6) and existing descriptions (10). The endosperm tissue and the germ of castor seeds do not possess diagnostic features. After previous treatment for oil production the cells are crushed down beyond recognition.

Morphology: Castor seeds are 10 - 20 mm long, elongate oval and slightly flattened. The husks are brown, reddish-brown to black, brindled whitish or mottled. The surface of the relatively thick husk is smooth, shining and of brittle character. At one edge of the seed there is a pale white excrescence, the so called caruncula.

Anatomy of seed husk: The epidermis cells are diagnostically important due to their polygonal, mostly pentagonal or heptagonal shape and their reticulate and ledged swellings of the cell walls. The attached collapsed tissue layer without diagnostic features is adjoined by a cell layer of cubical cells with incorporated calcium carbonate crystals.

Also diagnostically important is the cell layer adjoining inwardly to this layer. It is a layer of brown, about 200 µm high palisade cells lying tightly together showing a characteristic bending which can be seen also by stereo microscope at higher magnifications.

5.3 Quantification

The quantification of castor seed husk fragments is performed using the sieve fractions > 0.5 mm, deriving from the dried sediment. Material identified as castor seed husk fragments is separated from the sample and weighed (4.4). An aliquot of the sieved fractions may be used if necessary.

The weight of the castor seed husk fragments determined in the different sieve fractions is summarized and recorded as milligram castor seed husks per kilogram feedingstuff (mg/kg) (6.1).

6. Calculation and report

6.1 Calculation

The amount of castor seed husk fragments in mg/kg (ppm) feedingstuff (original sample) is calculated using the following formula:

$$\mathbf{C} = \frac{\mathbf{BC \times 1000}}{\mathbf{E}} \text{ [mg/kg]}$$

C = amount of component in mg/kg (ppm) feedingstuff

BC = selected fragments of component in the laboratory sample or an aliquot of it [mg]

E = total weight of the laboratory sample or an examined aliquot of it [g]

6.2 Report

6.2.1 Negative result:

As far as was discernible using a microscope, seeds or seed husk fragments of castor (*Ricinus communis*) were not found in the submitted sample.

6.2.2 Positive result:

As far as was discernible using a microscope x mg castor (*Ricinus communis*) seed husk fragments/kg feedingstuff were found in the submitted sample. For quantification castor seed husk fragments > 0.5 mm are considered.

6.2.3 Possible supplement to the report:

The sieve fraction > 0.5 mm amounts to xx % of the laboratory sample.

7. Validation

not applicable

8. Remarks

8.1 Separation by water as separation fluid is done according to supporting document (9, 5.3.3.2 - separation by slurrying or separation in a weak water flow).

8.2 Fractionating by particle size is done according to document (9, 5.3.1).

8.3 This method has been developed by the International Association of Feedingstuff Analysis (IAG) – Section Feedingstuff Microscopy.

9. Supporting document

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

10. Literature

- FREUND, H. (ed.), 1976: Handbuch der Mikroskopie in der Technik. Band VIII: Mikroskopie der Nahrungs- und Futtermittel, der Drogen und Genußmittel. Umschau-Verlag, Frankfurt/ Main
- GASSNER, G.; Hohmann, B. und Deutschmann, F., 1989: Mikroskopische Untersuchung pflanzlicher Lebensmittel. 5. Auflage, Gustav Fischer-Verlag, Stuttgart, New York, 66-70
- HAHN, H. und Michaelsen, L., 1996: Mikroskopische Diagnostik pflanzlicher Nahrungs-, Genuß- und Futtermittel, einschließlich Gewürze. Springer-Verlag, Berlin, Heidelberg, New York
- HOHMANN, B., 1973: Über Ricinus-Besatz in Ölsaatrückständen. Kraftfutter 56, 562
- HOHMANN, B., 1973: Über Ricinus-Rückstände in Futtermitteln. In: Tagungsprotokoll der Internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Sektion Futtermittelmikroskopie, Luxemburg
- HUSS, W., 1968: Mikroskopischer Nachweis und Bestimmung des Anteils an Rizinussamenschalen. EWG-Dokument Nr. 7781/3/VI/68-D
- ISO-Methode Nr. 5061-1983 E: International Standard: Animal feeding stuffs - Determination of castor oil seed husks - Microscopical method
- KNAUR, W. 1940: Anatomie und Entwicklungsgeschichte der rauhschaligen Rizinussamen und der Nachweis von Rizinus in Futtermitteln. Zeitschr. Tierernährg. Futterm.-Kunde 4, 125-182
- 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
- MEZAROS, L. und Deutschmann, F., 1975: Atlas für die Mikroskopie von Nahrungsgrundstoffen und Futtermitteln. Teil I: Ölsaaten und deren Verarbeitungsrückstände. In: Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch, Bd. XI, Hrsg. L. Schmitt), Verlag Neumann-Neudamm, Melsungen
- RÖZSE, E., 2005: Atlas of Feed Microscopy. Part II: The Examination of Non-desired Materials in Feed. National Institute for Agricultural Quality Control, Budapest
- VAN VUCHELEN, 1966: Mikroskopische Analysenmethoden: Bestimmung des Anteils an Rizinussamenschalen. EWG Vorschlag Nr. 13.506/VI/66-D

- VÖHRINGER, H., 1997: Leitfaden für die Futtermittel-Qualitätskontrolle durch Mikroskopie. Hoffmann-La Roche, 3. Fassung, Wien