EURL-MP-method_006 (version 1)
Determination of ergot sclerotia (*Claviceps purpurea* Tul.) in whole kernel cereals by visual screening

Analyte group:	**Undesirable substances (Directive 2002/32/EC)**
Analyte(s):	Ergot sclerotia
Commodity group:	Whole kernel cereals
Commodities validated:	Whole kernel cereals
Technique:	Visual screening

Modifications compared to previous version:
Not applicable

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1 Introduction

Infestation of cereals, primarily rye, by ergot sclerotia (spore bodies of the mould *Claviceps purpurea*) has a long history. Symptoms were indicated as Saint Anthony's fire from the Middle Ages. Major outbreaks were reported in north Norway early 17th Century and in New England in the late 17th Century (the Salem witchcraft trials). Increased attention was given to monitoring after an outbreak in France (Pont St. Esprit) in 1951. (data taken from van Raamsdonk et al., 2016 [1]).

The intoxications are caused by ergot alkaloids present in the ergot sclerotia. There is a certain relationship between the level of ergot sclerotia and ergot alkaloids [2]. The quantity of ergot sclerotia is used as measure in legislation. Directive 2002/32/EC includes a level 1000 mg/kg of ergot sclerotia for feed materials [3]. The current limit in unprocessed cereals for food use with the exception of maize and rice intended for use as food ingredient is 500 mg/kg (Regulation (EC) 1881/2006 [4], amended by Regulation (EU) 2015/1940 [5]). A new legal limit of 200 mg/kg for food is currently being prepared by the Commission.

2 Scope

This document describes the visual / macroscopic and gravimetric method for the detection and quantification of ergot sclerotia in whole kernel materials. The application concerns food and feed raw materials that are granular, such as cereals, seeds or grains.

3 Principle

A sample consisting of 2 kg of material is divided in equal portions of 500 gram. Two portions are screened for ergot sclerotia. If the combined level of contamination exceeds the threshold value (mg/kg) of 60% of the legal limit, the two remaining portions are screened additionally. The combined results of either two or four portions is reported in mg/kg.
This procedure is developed in the years 2016 to 2019 in order to handle unavoidable inhomogeneity when dividing a sample with large units into test portions. A revised version of Regulation (EC) 152/2009 [6], currently under preparation by the Commission, will include this procedure. Standard CEN/EN 15587:2018 [7] provides a protocol based on the examination of a representative test portion of 250 grams. In case the contamination level exceeds 500 mg/kg the entire sample of 1 kg has to be analysed. Target material include a range of extraneous material, damaged kernels, impurities (Besatz) and ergot sclerotia. This CEN protocol will be superseded by the larger amounts of the sample and the test portions as included in the amended version of Regulation (EC) 152/2009 currently under preparation.

The method complies with the framework for visual research as presented in the draft Guidelines for visual methods, under preparation by an international expert group coordinated by WFSR [8].

4 Reagents

Not applicable.

5 Equipment

Any reference to type and/or product is only to inform the user and to identify the equipment and does not imply exclusion of similar equipment.

Usual laboratory equipment. In particular, the following, can be used:

5.1 **Analytical balance**, accuracy: 0.001 g

5.2 **Laboratory balance**, accuracy: 0.01 g

5.3 **Sieve** with a mesh seize of 0.5 mg (500 µm)

5.4 **Sample divider**, capable of dividing a sample of a weight of 2 kg in four portions of 500 g each

5.5 **Plate or tray**, with edges or borders and a sufficient area for screening of the matrix material by hand and selection of the target material (ergot sclerotia)

5.6 **Magnifying glass**, aid for supporting the screening process

5.7 **Stereo microscope (binocular)**, with a magnification range of 8x to 64x for verification of the identity of the selected ergot sclerotia

6 Procedure

The technician must be trained in the field of specific anatomical, histological and morphological characteristics (structures) of feed and food materials and should have access to relevant literature and reference materials. The steps are described in paragraph 7 and Annex A.

6.1 Samples

The sample of 2 kg is to be sieved using a sieve with a mesh seize of 0.5 mm (500 µm) in order to remove powdery components.

6.2 Test portion

The cleaned sample is divided in four subsamples of equal weight, approximately 500 g each, by using the sample divider. The weight of each portion is recorded. The portions will be properly labelled and stored.

7 Determination of ergot sclerotia

Select the ergot sclerotia or fractions thereof from the sample material of a portion. Spread the material proportionally in the tray. The grains or kernels should preferably form a layer of not more than one grain or kernel thick. Inspect every grain based on shape and colour and move it to a pile of cereal grains in the corner of the tray. Separate every particle which fits the description of an ergot sclerotium or part of it. Special attention should be given to grains infested by smut or otherwise damaged that might mimic ergot sclerotia. Definitions of common types of damaged grains or inclusions are given in CEN/EN

15587:2018. Documentation for proper identification is given in the IAG method A4 [9] for determination of ergot sclerotia. The minimum size of the fragments to be selected is 0.5 mm. Confirm the identity of the selected ergot sclerotia by using the stereo microscope (binocular), with support of information from handbooks and reference material.

Analyse two of the portions and store the results per portion in terms of sample weight (g), number of sclerotia (n) and weight of selected sclerotia (mg). Calculate the contamination from the pooled results of both portions as mg ergot sclerotia per kg (mg/kg) (Annex A).

If the contamination level exceeds the threshold value (**8.1**), analyse the second set of two portions. Store the results per portion in terms of sample weight (g), number of sclerotia (n) and weight of selected sclerotia (mg). In this case, calculate the total contamination found in the entire sample of approx. 2 kg sample material by pooling the weights of the four fraction of sclerotia and report as mg ergot sclerotia per kg (mg/kg).

A flow chart of the procedure is presented in Figure 1.

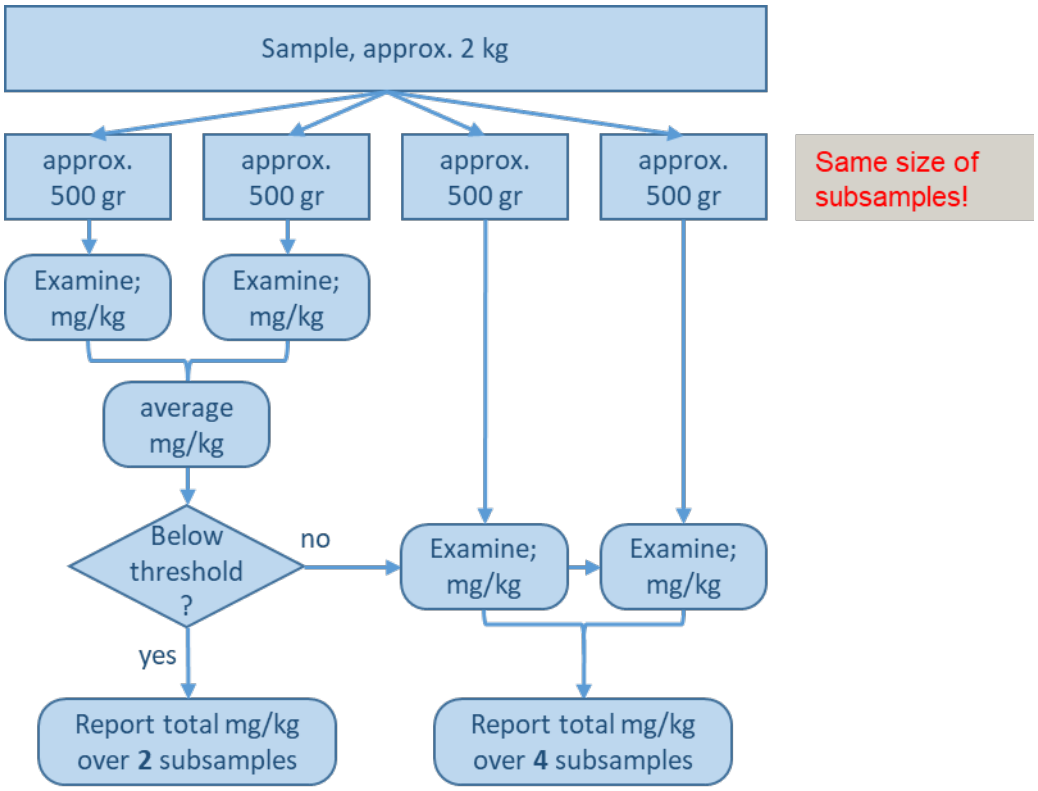

Figure 1. Flow chart for the detection of undesirable substances in whole kernel sample material (ergot sclerotia).

The results are stored in a table, according to Table 1 in Annex A.

8 Evaluation and calculations

8.1 Threshold value

The provisional threshold value will be set at 60% of the legal limit, as follows:

- Feed, legal limit 1000 mg/kg (Directive 2002/32/EC) [3]: threshold value 600 mg/kg
- Food, proposed legal limit 200 mg/kg (future amendment of Regulation (EC) 1881/2006) [4]: threshold value 120 mg/kg

8.2 Final result

The combined results in terms of mg/kg, based on either two portions or on four portions, will be reported. The other (intermediate) results will be stored for background documentation and for quality control according to the table in Annex A.

9 References

1. van Raamsdonk LWD *et al*. (2016) IAG ring test visual detection of ergot sclerotia in rye 2015. RIKILT report 2016.013, pp. 28.
2. Mulder PPJ *et al*. (2012) Dutch survey ergot alkaloids and sclerotia in animal feeds. RIKILT Report 2012.005, pp. 1-54.
3. EU (2002) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Official Journal of the European Communities, L140: p. 10-21.
4. EU (2006) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L364: p. 5-24.
5. EU (2015) Commission Regulation (EU) 2015/1940 of 28 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia in certain unprocessed cereals and the provisions on monitoring and reporting. Official Journal of the European Communities, L283: p. 1-4.
6. EU (2009) Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union, L54: p. 1-130.
7. CEN (2018) EN 15587:2018 - Cereals and cereal products - Determination of Besatz in wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), rye (*Secale cereale* L.) and feed barley (*Hordeum vulgare* L.), pp. 28.
8. IAG (2008) Method for the determination of ergot (*Claviceps purpurea* Tul.) in animal feedingstuff. IAG-Method A4, pp. 6.
9. WFSR (2021) Quality assurance and control of visual methods. Part 1 - Theory and principles. Under preparation.

Annex A Checklist for analysis

Technician:

Date:

Lab. journal / page:

A.1 Preparation of test portion

- ☐ Divide the test sample in four subsamples of equal weight, approximately 500 grams **(6.2)**
- ☐ Follow the steps described in the determination of ergot sclerotia **(7)**.

Table 1. Results of the analysis of ergot sclerotia

Portion	Sample weight (g) (**S**)	Count sclerotia (number)	Weight sclerotia (mg) (**x**)	Combined result (mg/kg)	Comment
1 (a)				$x = \frac{x_a + x_b}{S_a + S_b}$	Combined result of **2** portions
2 (b)					
If content in terms of mg/kg does exceed the threshold value, also analyse portion 3 and 4 and take the results of all four portions into consideration:					
3 (c)				$x = \frac{x_a + x_b + x_c + x_d}{S_a + S_b + S_c + S_d}$	Combined result of all **4** portions
4 (d)					