Method for the Determination of Ergot *(Claviceps purpurea* Tul.*)*

IMPORTANT PRELIMINARY REMARK

This document provides guidance for the control of ergot sclerotia in cereals to verify the compliance with the maximum level established in Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
This document does not replace the application of international standards for the determination of ergot in cereals such as EN 15587:2008 "Cereal and cereal products – Determinaiton of Besatz in wheat, durum wheat, rye, triticale and feed barley".

1. Objective and field of application

The method is used for both qualitative and quantitative determination of ergot in cereals

2. Principle

Ergot in cereals is determined by the macroscopic and microscopic identification of the ergot sclerotia and fragments. Quantification is done by weighing the amount of identified ergot sclerotia and fragments with a particle size > 0,5 mm.

3. Reagents

3.1. Chloralhydrate, ß = 60%
3.2. Sodium hydroxide (pelleted)
3.3. Potassium hydroxide (pelleted)
3.4. Ethanol, σ = 50%
3.5. Acetone
The reagents listed can be replaced by others which yield comparable results.

4. Equipment and accessories

4.1. Optical equipment
4.1.1. Stereo microscope (up to 70x magnification)
4.1.2. Magnifier (up to 10x magnification)
4.2. Sieve fitted with square meshes of width of 0.5mm
4.3. Analytical balance (accuracy 0,001 g)
4.4. Reference material

5. Procedure

The examination is performed in cereals.
Qualitative determination of the ergot sclerotia is performed macroscopically and microscopically considering ergot and its fragments in all sieve fractions (sieve fraction > 0.5 mm and < 0.5mm). However this qualitative determination is not necessary for checking compliance.
Quantification is performed by selecting and weighing of ergot and its fragments with a particle size > 0,5 mm out of the test sample or an aliquot of it.

5.1. Preparation of the test sample

Cereals (at least 1 kg) are weighed (4.3) and used directly for the investigation (5.2 and 5.3).

5.2. Identification of ergot

Ergot sclerotia are identified based on their characteristic features. The identification may be facilitated by comparison to reference material (4.4).

Morphology: *Claviceps purpurea* Tul. sclerotia are elongated with a length up to several centimetres, coloured dark violet to black. The shape is similar to cereal kernels. They only consist of fungal hyphae.

Anatomy: Cross sections through the random parts of ergot sclerotia show very small, narrow interconnected hyphae which yield a dense pseudoparenchymatic tissue. The cells contain lots of fat oil. The outer layers of the hyphae are coloured dark violet to black, whereas the inner parts are coloured light pink to violet.

For the identification of ergot fragments the following colour reaction can, if necessary, be used. The staining procedure is only applicable to fresh sclerotia material
Therefore a filter paper is soaked with a solution of 3 ml ethanol (3.4) and 2 sodium hydroxide pellets (3.2) or 2 potassium hydroxide pellets (3.3).
The sample is distributed on the filter paper.
After app. 5 min. a red-violet halo around the ergot fragments is observed. The dark violet colouring of the outer hyphae layers is dissolved also in chloralhydrate (3.1) and colours it violet.

5.3. Quantification

The quantification of ergot is performed using the sieve fractions > 0,5 mm.
Material identified as ergot in each fraction is selected and weighed. An aliquot of the sieved fractions may be used if necessary. The ergot content of the fraction > 0.5 mm is summarized and expressed in mg/kg cereals (6.1).

6. Calculation and report

6.1. Calculation

The amount of ergot fragments in mg/kg cereals (original sample) is calculated using the following formula:

$C = BC \times 1000/E$ [mg/kg]

C = amount of ergot in mg/kg cereals
BC = selected ergot fragments in the test sample (or an aliquot of it) [mg]
E = total weight of the test sample (or an examined aliquot of the test sample) [g]

6.2. Report

6.2.1. Negative result:
As far as was discernible using a microscope, ergot (with particle size > 0,5 mm) was not found in the submitted sample.

6.2.2. Positive result
As far as was discernible using a microscope x mg ergot/kg cereals were found in the submitted sample. For quantification of ergot, particles > 0,5 mm are considered