Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of Saccharomyces cerevisiae used as feed additive

EESTI STANDARDI EESSÕNA

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Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
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Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of Saccharomyces cerevisiae used as feed additive

Aliments des animaux: Méthodes d'échantillonnage et d'analyse - Détection et dénombrement des souches de Saccharomyces cerevisiae utilisées comme additifs pour l'alimentation animale

Futtermittel: Probenahme- und Untersuchungsverfahren - Nachweis und Zählung von Saccharomyces cerevisiae als Futtermittelzusatzstoff

This European Standard was approved by CEN on 2 August 2021.

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European foreword

This document (EN 15789:2021) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2022, and conflicting national standards shall be withdrawn at the latest by May 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 15789:2009.

The main changes compared to the previous edition are as follows:

— Amendment of the title;

— Extension of the scope of application to all *Saccharomyces cerevisiae* strains used as feed additive;

— Updating of normative cross references;

— Supplement of phosphate buffered saline with Tween® 80;

— Addition of the option to use Tween® 80 supplemented phosphate buffered saline for the preparation of the initial suspension as well as diluent for serial dilutions;

— Removal of the chromogenic culture medium for the enumeration of *Saccharomyces cerevisiae*;

— Addition of the option to use spread plates as well as a spiral plater for enumeration;

— Preparation of initial suspensions generally conducted with tempered tPBS;

— Unification of the homogenization time for the preparation of initial suspensions to one minute for all feed matrices;

— Adjustment of the cultivation time and temperature to 48 h to 72 h at (30 ± 1) °C;

— Addition of a procedure for the investigation of feeding stuffs containing high amounts of copper in the informative Annex A;

— Adjustment of the range of accepted colony numbers for counting from '≥ 30 to ≤ 350' to '≥ 10 to ≤ 200' colonies per plate.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This methodology has been developed to enumerate yeasts (*Saccharomyces cerevisiae*) used as feed additives to enable the European Commission to control proper labelling of animal feeding products. It was compiled first during the EU project SMT4-CT98-2235 "Methods for the official control of probiotics used as feed additives" [1].

The procedure has been validated for one commercially used *Saccharomyces cerevisiae* strain [2]. As the method is not selective for this particular *Saccharomyces cerevisiae* strain, it can be assumed, that it can also be applied to enumerate other *Saccharomyces cerevisiae* strains in their respective dosage form in feed provided that the added yeast is present in far higher numbers than any other yeast.

The method has not been validated for other yeast species (e.g. *Kluyveromyces marxianus*).

This method is not applicable for the detection of any ubiquitous or pathogenic yeasts in food and animal feeding stuffs.

1 Scope

This document specifies general rules for the enumeration of *Saccharomyces cerevisiae* in feeding stuffs (additives, premixtures and compound feeds excluding mineral feeds) that contain *Saccharomyces cerevisiae* as a single microorganism component or in a mixture with other microorganisms. Applying the method to premixtures and compound feeds with critical amounts of copper demands a special procedure (see Annex A). The document is not applicable to mineral feeds, which are defined as complementary feeding stuffs composed mainly of minerals and containing at least 40 % crude ash (Regulation (EC) 767/2009) [3].

There are different categories of feed samples:

a) Additives containing about 10^{10} colony forming units (CFU)/g;

b) Premixtures containing about 10^{11} CFU/kg;

c) Compound feeds, meal or pellets which contain about 10^{9} CFU/kg.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
Saccharomyces cerevisiae
unicellular fungus which mostly reproduces vegetatively by budding

Note 1 to entry: This description is based on their characteristics as used for this document.

Note 2 to entry: Budding cells are broadly ellipsoidal with multilateral bud formation. They show no or simple pseudohyphae.

Note 3 to entry: *S. cerevisiae* forms colonies on the specified selective medium after incubation for 48 h to 72 h at 30 °C under aerobic conditions fitting the description in 9.6.