SLOVENSKI STANDARD
SIST ISO 21527-2:2011

01-junij-2011

Nadomešča:
SIST ISO 7698:1997

Mikrobiologija živil in krme - Horizontalna metoda za ugotavljanje števila kvasovk in plesni - 2. del: Tehnika štetja kolonij v proizvodih z vodno aktivnostjo, nižjo ali enako 0,95

Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 2: Colony count technique in products with water activity less than or equal to 0,95

Microbiologie des aliments -- Méthode horizontale pour le dénombrement des levures et moisissures -- Partie 2: Technique par comptage des colonies dans les produits à activité d'eau inférieure ou égale à 0,95

Ta slovenski standard je istoveten z: **ISO 21527-2:2008**

ICS:

07.100.30 Mikrobiologija živil Food microbiology

SIST ISO 21527-2:2011 **en,fr**

INTERNATIONAL STANDARD

ISO 21527-2

First edition
2008-07-01

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds —

Part 2: Colony count technique in products with water activity less than or equal to 0,95

Microbiologie des aliments — Méthode horizontale pour le dénombrement des levures et moisissures —

Partie 2: Technique par comptage des colonies dans les produits à activité d'eau inférieure ou égale à 0,95

Reference number
ISO 21527-2:2008(E)

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Published in Switzerland

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 21527-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO 21527 consists of the following parts, under the general title *Microbiology of food and animal feedings stuffs — Horizontal method for the enumeration of yeasts and moulds*:

— *Part 1: Colony count technique in products with water activity greater than 0,95*

— *Part 2: Colony count technique in products with water activity less than or equal to 0,95*

This part of ISO 21527, together with ISO 21527-1, cancel and replace ISO 7698:1990, ISO 7954:1987 and ISO 13681:1995.

Introduction

Because of the large variety of food and feed products, the applications of the horizontal method specified in ISO 21527 (all parts) may not be appropriate for certain products. In this case, different methods, which are specific to these products, may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt shall be made to apply the horizontal method as specified in ISO 21527 (all parts) as far as possible.

When ISO 21527 (all parts) is next reviewed, account will be taken of all information then available regarding the extent to which the horizontal method has been followed and the reasons for deviations from this method in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with the horizontal method as specified in ISO 21527 (all parts). It is hoped that when such standards are reviewed they will be changed to comply with ISO 21527 (all parts) so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds —

Part 2: Colony count technique in products with water activity less than or equal to 0,95

WARNING — It is essential that enumeration of moulds is carried out with the greatest care to protect the operator and to prevent contamination of the atmosphere with mould spores.

1 Scope

This part of ISO 21527 specifies a horizontal method for the enumeration of viable osmophilic yeasts and xerophilic moulds in products intended for human consumption or feeding of animals that have a water activity less than or equal to 0,95 (dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereal products, flours, nuts, spices and condiments, etc. [Annex A]), by means of the colony count technique at 25 °C ± 1 °C (Reference [3]).

This part of ISO 21527 does not apply to dehydrated products with water activity less than or equal to 0,60 (dehydrated cereals, oleaginous products, spices, leguminous plants, seeds, powders for instant drinks, dry products for domestic animals, etc.) and does not allow the enumeration of mould spores (Reference [3]). Neither the identification of fungal flora nor the examination of foods for mycotoxins lie within the scope of this part of ISO 21527. The method specified in this part of ISO 21527 is not suitable for enumeration of halophilic xerophilic fungi (i.e. *Polypaecilium pisce, Basipetospora halophila)* such as may be found in dried fish.

2 Normative references

The following referenced documents are indispensable for the application 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.

ISO 6887 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133 (all parts), *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media*

ISO 21527-1, *Microbiology of food and animal feedings stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 1: Colony count technique in products with water activity greater than 0,95*

3 Terms and definitions

For the purposes of this document the terms and definitions given in ISO 21527-1 and the following apply.

3.1
osmophilic yeast
xerophilic mould
fungus which is capable of growth at a water activity less than or equal to 0,95

4 Principle

4.1 Surface-inoculated plates are prepared using a specified selective culture medium. Depending on the expected number of colonies, a specified quantity of the sample (if the product is liquid), or of an initial suspension (in the case of other products), or decimal dilutions of the sample/suspension are used.

Additional plates can be prepared under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

4.2 The plates are then aerobically incubated at 25 °C ± 1 °C for 5 d to 7 d. If necessary, the agar plates are left to stand in diffuse daylight for 1 d to 2 d.

4.3 Colonies/propagules are then counted and, if required (to distinguish yeast colonies from bacterial colonies), the identity of any doubtful colonies is confirmed by examination with a binocular magnifier or microscope.

4.4 The number of yeasts and moulds per gram or per millilitre of sample is calculated from the number of colonies/propagules/germs obtained on plates chosen at dilution levels producing countable colonies. Moulds and yeasts are counted separately, if necessary.

5 Diluent and culture medium

For current laboratory practice, see ISO/TS 11133 (all parts).

5.1 Diluent

5.1.1 General

See ISO 6887 (all parts), ISO 8261 and the specific International Standard dealing with the product concerned.

The use of a diluent containing a sufficient amount of solute [e.g. a 20 % to 35 % (mass concentration) solution of glycerol or D-glucose] is recommended to minimize osmotic shock to xerophilic mould and osmophilic yeast cells when serial dilutions are made prior to plating (References [1], [3]).

NOTE It is possible to add surface-active agents such as sodium poly(oxyethylene)sorbitanmonooleate [1)] [0,05 % (mass concentration)] to diluents to reduce clumping of mould spores and conidia (Reference [3]).

Except for specific preparation of the test sample, the use of 0,1 % (mass concentration) peptone water broth as diluent is recommended .

1) Tween 80 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5.1.2 Composition of 0,1 % (mass concentration) peptone water broth

Enzymatic digest of animal or vegetal tissues	1,0 g
Water	1 000 ml

5.1.3 Preparation of 0,1 % (mass concentration) peptone water broth

Dissolve the components in the water, by heating if necessary.

If necessary, adjust the pH so that, after sterilization, it is 7,0 ± 0,2 at 25 °C.

5.2 Culture medium

5.2.1 Dichloran 18 % (mass concentration) glycerol agar (DG18) (References [4], [5], [6])

5.2.1.1 Composition

Casein enzymatic digest	5,0 g
D-Glucose ($C_6H_{12}O_6$)	10,0 g
Potassium dihydrogenphosphate (KH_2PO_4)	1,0 g
Magnesium sulfate ($MgSO_4 \cdot H_2O$)	0,5 g
Dichloran (2,6-dichloro-4-nitroaniline)	0,002 g
Glycerol anhydrous	220 g
Agar	12 g to 15 g [a]
Chloramphenicol	0,1 g
Water, distilled or deionized	1000 ml

[a] Depending on the gel strength of the agar.

5.2.1.2 Preparation

5.2.1.2.1 General

Suspend all the ingredients except chloramphenicol in the water and bring to the boil to dissolve completely. If necessary, adjust the pH (6.4) so that after sterilization it is 5,6 ± 0,2 at 25 °C.

Add 10 ml of a 1 % (mass concentration) solution of chloramphenicol in ethanol and mix. Dispense the medium in quantities into suitable containers (6.5) of suitable capacity. Sterilize by autoclaving at 121 °C for 15 min.

Immediately cool the medium in a water bath (6.3) maintained at a temperature of 44 °C to 47 °C. Cool to below 50 °C and dispense 15 ml amounts into sterile Petri dishes (6.6).

Allow the medium to solidify, and dry, if necessary, the surface of the plates as described in ISO 7218 and ISO/TS 11133 (all parts).

Use immediately, or store in the dark, according to ISO/TS 11133 (all parts) until required.

CAUTION — Avoid exposure of the medium to light, since cytotoxic breakdown products can result in underestimation of mycoflora in samples.

