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Meat and meat products — Enumeration of lactic acid bacteria — Colony-count technique at 30 °C

Viande et produits à base de viande — Dénombrement des bactéries lactiques — Technique par comptage des colonies à 30 °C

Reference number
ISO 13721:1995(E)

Foreword

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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.

International Standard ISO 13721 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 6, *Meat and meat products*.

Annex A of this International Standard is for information only.

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Meat and meat products — Enumeration of lactic acid bacteria — Colony-count technique at 30 °C

1 Scope

This International Standard specifies a method for the enumeration of viable lactic acid bacteria in meat and meat products, including poultry, by counting the colonies growing in a solid medium after aerobic incubation at 30 °C for 3 days.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3100-2:1988, *Meat and meat products — Sampling and preparation of test samples — Part 2: Preparation of test samples for microbiological examination.*

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

ISO 7218:—[1], *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

3 Definition

For the purposes of this International Standard, the following definition applies.

3.1 lactic acid bacteria: Bacteria which form colonies at 30 °C in a solid selective medium (MRS at pH 5,7) under the test conditions specified in this International Standard.

4 Principle

4.1 Preparation of two poured plates, using MRS agar at pH 5,7 contained in Petri dishes. Inoculation of the plates with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

4.2 Inoculation of other pairs of poured plates, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

Incubation of the plates at 30 °C for 72 h.

4.3 Calculation of the number of lactic acid bacteria per gram or millilitre of test sample from the number of colonies obtained in the dishes selected.

5 Dilution fluid and culture medium

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Dilution fluid

See ISO 6887.

5.3 Culture medium (MRS medium) at pH 5,7 (see reference [1])

1) To be published. (Revision of ISO 7218:1985)

NOTE 1 The use of commercially available media is acceptable, however attention is drawn to the fact that variations in composition may occur between products from different manufacturers.

5.3.1 Composition

Peptone	10,0 g
Meat extract	8,0 g
Yeast extract	5,0 g
Triammonium citrate [$(NH_4)_3C_6H_5O_7$]	2,0 g
Sodium acetate (CH_3COONa)	5,0 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,2 g
Manganese sulfate tetrahydrate ($MnSO_4 \cdot 4H_2O$)	0,05 g
Dipotassium hydrogen phosphate (K_2HPO_4)	2,0 g
Glucose ($C_6H_{12}O_6$)	20,0 g
Sorbitan monooleate (Tween 80)	1,0 g
Agar	12 g to 18 g [1]
Water	1 000 ml

1) Depending on the gel strength of the agar.

5.3.2 Preparation

5.3.2.1 Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH (6.7) using hydrochloric acid (approx. 1 mol/l solution) so that after sterilization it is 5,7 at 25 °C.

Transfer the medium to bottles of capacity not more than 300 ml.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C. If the medium is to be used immediately, cool it to 47 °C in the water bath (6.5) before use.

If not, before beginning the microbiological examination, in order to avoid any delay when pouring the medium, completely melt the medium in a boiling water bath (6.6), then cool it to 47 °C in the water bath (6.5).

5.3.2.2 If there is a risk of extensive yeast contamination (e.g. in dried sausage), add sorbic acid to the MRS medium as follows.

Dissolve 1,4 g of sorbic acid in about 10 ml of a 1 mol/l solution of sodium hydroxide. Adjust the pH to 5,8 and sterilize by filtration. Add this solution to 1 000 ml of sterilized MRS agar. The final pH of the medium should be 5,7.

6 Apparatus and glassware

NOTE 2 Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory apparatus and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or **wet sterilization (autoclave)**

See ISO 7218.

6.2 Incubator, capable of operating at 25 °C ± 1 °C.

6.3 Petri dishes, made of glass or plastic, of diameter 90 mm to 100 mm.

6.4 Total-delivery (blow-out) pipettes, having wide openings and having a nominal capacity of 1 ml, graduated in 0,1 ml divisions.

6.5 Water bath, or similar apparatus, capable of operating at 47 °C ± 2 °C.

6.6 Boiling water bath

6.7 pH-meter, accurate to within ± 0,1 pH unit at 25 °C.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 3100-1[2].

Store the sample, if necessary, in such a way that deterioration and change in composition are prevented.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 3100-2.

9 Procedure

9.1 Test portion, initial suspension and dilutions

Prepare the intial suspension and dilutions in accordance with ISO 6887.

9.2 Inoculation and incubation

NOTE 3 Surface plating in combination with incubation under anaerobic or microaerobic conditions can be applied instead of the pour-plating procedure described. Candle jars may be used to obtain appropriate conditions.

9.2.1 Take two sterile Petri dishes (6.3). Using a sterile pipette (6.4), transfer to each dish 1 ml of the test sample if the product is liquid, or 1 ml of the initial suspension in the case of other products.

Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample if the product is liquid, or 1 ml of the first decimal dilution of the initial suspension (10^{-2}) in the case of other products.

Repeat the procedure described with the further dilutions, using a fresh sterile pipette for each decimal dilution.

NOTE 4 If high numbers of lactic acid bacteria are expected, plate out only those dilutions necessary to achieve the correct colony range (9.3).

9.2.2 Pour into each Petri dish approximately 15 ml of the MRS medium (5.3) which has been prepared then cooled to approximately 47 °C in the water bath (6.5). The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium (5.3) is poured into the dishes shall not exceed 15 min.

Carefully mix the inoculum with the medium by horizontal movements and allow the mixture to solidify, with the Petri dishes standing on a cool horizontal surface.

9.2.3 Invert the prepared dishes and incubate them in the incubator (6.2) set at 25 °C for 72 h ± 3 h.

9.3 Counting and selection of colonies

After the specified period of time (see 9.2.3), count the colonies in each dish. Retain dishes containing fewer than 300 colonies at two successive dilutions.

10 Expression of results and calculation

10.1 Calculate the number N of lactic acid bacteria present in the test sample, as the weighted mean from two successive dilutions, using the equation:

$$N = \frac{\sum a}{V(n_1 + 0{,}1n_2)d}$$

where

$\sum a$ is the sum of the colonies counted on all the dishes from the two successive dilutions which contain at least 15 colonies;

V is the volume of inoculum applied to each dish, in millilitres;

n_1 is the number of dishes retained at the first dilution;

n_2 is the number of dishes retained at the second dilution;

d is the dilution factor corresponding to the first dilution retained.

Round off the results calculated to two significant figures. For this, if the last figure is below 5, the preceding figure is not modified; if the last figure is 5 or more, the preceding figure is increased by one unit. Proceed stepwise until two significant figures are obtained.

Take as the result the number of lactic acid bacteria per millilitre (liquid product) or per gram (other product), expressed as a number between 1,0 and 9,9 multiplied by the appropriate power of 10.

EXAMPLE

$$N = \frac{\sum a}{V(n_1 + 0{,}1n_2)d} = \frac{168 + 215 + 14 + 25}{1(2 + 0{,}1 \times 2)10^{-2}} =$$

$$= \frac{422}{0{,}022} = 19\ 182$$

Round off to two significant figures, namely 19 000 or $1{,}9 \times 10^4$ lactic acid bacteria per gram of product.

10.2 If the two dishes, at the level of the test sample (liquid product) or of the initial suspension (other product), contain less than 15 colonies, calculate the arithmetical mean y of the colonies counted on two dishes.

Express the result as follows:

— for liquid products: estimated number of lactic acid bacteria per millilitre $N_E = y$

— for the other products: estimated number of lactic acid bacteria per gram $N_E = y \times \frac{1}{d}$

where d is the dilution factor of the initial suspension.

10.3 If the two dishes at the level of the test sample (liquid product) or of the initial suspension (other product) do not contain any colonies, express the result as follows:

— less than 1 lactic acid bacterium per millilitre (liquid product)

— less than $1 \times \frac{1}{d}$ lactic acid bacterium per gram (other product)

where d is the dilution factor of the initial suspension.

11 Confidence limits

For calculation of the confidence intervals, see ISO 7218.

12 Test report

The test report shall specify:

— the method in accordance with which sampling was carried out, if known;

— the method used;

— the test result(s) obtained; and

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

The test report shall include all information necessary for the complete identification of the sample.

Annex A
(informative)

Bibliography

[1] Pharmacopoeia of Culture Media for Food Microbiology: De Man, Rogosa and Sharpe agar with sorbic acid (MRS-S agar). *Int. J. Food Microbiol.*, **5**, 1987, pp. 230-232.

[2] ISO 3100-1:1991, *Meat and meat products — Sampling and preparation of test samples — Part 1: Sampling.*

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