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English version

Foodstuffs - Microbiological screening for irradiated food using LAL/GNB procedures

Produits alimentaires - Dépistages microbiologique des aliments ionisés en utilisant la technique LAL/GNB

Lebensmittel - Mikrobiologisches LAL/GNB-Screeningverfahren zum Nachweis von bestrahlten Lebensmitteln

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Contents

Foreword

This document (EN 14569:2004) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by April 2005, and conflicting national standards shall be withdrawn at the latest by April 2005.

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

1 Scope

This document specifies a microbiological screening method comprising two procedures, which are carried out in parallel. It permits the identification of an unusual microbiological profile in poultry meat. The presence of a large excess population of dead micro-organisms can under certain circumstances be presumptive of irradiation treatment, which means, that the results of the procedure of the determination of endotoxin concentration in the test sample using the *Limulus* amoebocyte lysate (LAL) test and of the procedure of the enumeration of total Gram negative bacteria (GNB) in the test sample are not radiation specific. Therefore, it is recommended that a positive result be confirmed using a standardized reference method for the detection of irradiated food, e.g. EN 1784, EN 1785 or EN 1786 [1] to [3].

This screening method has been successfully tested by inter-laboratory trials [4], [5], [6] and the procedure is generally applicable to whole or parts of poultry, e.g. breast, legs, wings of fresh, chilled or frozen carcasses with or without skin.

The method can also provide information about the microbiological quality of a product prior to irradiation.

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.

ENV ISO 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory (ISO/TS 11133-1:2000).*

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

3 Principle

The method determines the number of viable Gram negative bacteria present in the test sample and the concentration of bacterial endotoxin present on the surfaces of Gram negative bacteria as lipopolysaccharides (LPS) serving as a measure for the estimation of the amount of total Gram negative bacteria, both viable and dead (Figure C.1). If the difference is high, it is assumed that the sample has been treated by a method of preservation, possibly by treatment with ionising radiation.

4 Procedures

The two procedures that are required to be carried out are:

Procedure 1: Enumeration of total Gram negative bacteria (GNB) in the test sample (according to Annex A)

Procedure 2: Determination of endotoxin concentration in the test sample using the *Limulus* amoebocyte lysate (LAL) test (according to Annex B).

Samples should be analysed immediately upon receipt (by both GNB and LAL) to reduce any further microbial proliferation. If this is not possible, samples should be stored at – 20 °C until examination, which should be carried out as soon as possible.

5 Evaluation

In accordance with the methods specified in Annexes A and B the number of GNB forming units shall be expressed as $\log_{10}$ colony forming unit (cfu) GNB per gram and the concentration of endotoxin expressed as $\log_{10}$ Endotoxin Units (EU) per gram.

The difference between the GNB and the endotoxin results shall be calculated as ($\log_{10}$ EU/g - $\log_{10}$ cfu GNB/g).

Samples that are identified as having high endotoxin levels but low levels or no detectable GNB show an unusual microbiological profile. Thus, when $\log_{10}$ EU/g - $\log_{10}$ cfu GNB/g is greater than 0 the sample is suspected of having been irradiated and should be subjected to further radiation specific tests.

Samples that are identified as having high endotoxin levels and similarly high Gram negative bacteria counts show a normal microbiological profile. Thus, when $\log_{10}$ EU/g - $\log_{10}$ cfu GNB/g is lower or equal 0, the sample is not suspected of having been irradiated.

Samples that are identified as having endotoxin titres $\log_{10}$ EU/g of lower than 2,0 and low levels of GNB, i.e. $\log_{10}$ cfu GNB/g of lower than 2,0 should be considered inconclusive and should be subjected to further radiation specific tests.

6 Limitations

This method can give only an indication of a possible treatment by ionising radiation. A high amount of dead microorganisms in comparison to the viable fraction can be due to several other reasons. It is therefore necessary to confirm a possible treatment by ionising radiation by a standardized reference method for the detection of irradiated foods. The method is of particular use to routine microbiological laboratories, which may be involved in the examination of foods.

It should be noted that freezing after irradiation can influence the ratio of GNB to EU due to the loss of viability of microorganisms. Conversely, re-growth of bacterial flora can occur in irradiated samples which are stored unfrozen.

7 Validation

The method was developed by the UK Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Norwich (FSL) and validated by collaborative trial [4], [5], [6]. Twenty UK laboratories participated in the trial (16 Public Analyst Laboratories, 3 Public Health Laboratories and FSL). A commercial poultry processor supplied a single batch of boneless chicken breasts with skin and a single batch of boneless chicken breast fillets. Within 24 h of slaughter, both batches of chicken were transported from the processing plant, under refrigeration conditions (<5 °C), to a commercial irradiation facility. The batch of chicken breasts were subdivided; one third of the batch were retained as control (unirradiated) samples; one third were irradiated at an overall average dose of 2,5 kGy and the remainder irradiated at 5 kGy. The skinless chicken breast fillets were sub-divided into two. Half were retained as controls and the remaining half were irradiated at 2,5 kGy. Samples were irradiated using a Cobalt 60 source. Amber perspex dosimeters[1)] were used to estimate the dose received by measuring spectrometrically a change in absorbance at 530 nm. All chicken pieces were randomly coded and packaged in insulated containers under chill conditions. Samples were then delivered by overnight carrier to arrive at participating laboratories the following morning. Participants were instructed to commence the analysis immediately upon arrival of the samples. Each participant received 10 samples for analysis comprising control samples of chicken breasts with and without skin; chicken breasts with skin irradiated at 2,5 kGy and 5 kGy and skinless breasts irradiated at 2,5 kGy. All samples were dispatched randomly as blind ‘pairs’. Participants were asked to examine each sample only once and report the results obtained from the LAL test and enumeration of GNB.

Using the evaluation criteria detailed in Clause 5, 100 % of all unirradiated samples, 97 % of chicken with skin irradiated at 2,5 kGy, 82 % of those irradiated at 5 kGy and 88 % of the skinless fillets irradiated at 2,5 kGy were correctly identified. All other samples were reported as ‘inconclusive’ due to the very low EU titres obtained.

1) Amber perspex dosimeter is an example of a suitable product commercially available from Harwell, UK which was used for the interlaboratory test. This information is given for the convenience of users and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

8 Test report

The test report shall contain at least the following:

a) information necessary for identification of the sample;

b) a reference to this document;

c) date of sampling and sampling procedure (if known);

d) date of receipt and thermal status of the sample when received;

e) date of test;

f) storage conditions of the sample in the laboratory after receipt;

g) the result;

h) any particular points observed in the course of the test;

i) any operations not specified in the method or regarded as optional which might have affected the results.