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Utgåva 1

Livsmedel – Analys av bestrålade livsmedel med hjälp av DEFT/APC – Screeningmetod

Foodstuffs – Detection of irradiated food using Direct Epiflourescent Filter Technique/Aerobic Plate Count (DEFT/APC) – Screening method

ICS 07.100.30; 67.040.00

Språk: engelska

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The European Standard EN 13783:2001 has the status of a Swedish Standard. This document contains the official English version of EN 13783:2001.

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English version

Foodstuffs - Detection of irradiated food using Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) - Screening method

Produits alimentaires - Détection d'aliments ionisés en utilisant la technique d'épifluorescence après filtration et dénombrement de la flore aérobie sur milieu gélosé (DEFT/APC) - Méthode par criblage

Lebensmittel - Nachweis der Bestrahlung von Lebensmitteln mit Epifluoreszenz-Filtertechnik/aerober mesophiler Keimzahl (DEFT/APC) - Screeningverfahren

This European Standard was approved by CEN on 29 September 2001.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

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Foreword

This European Standard 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of an identical text or by endorsement, at the latest by May 2002, and conflicting national standards shall be withdrawn at the latest by May 2002.

This European Standard was elaborated on the basis of a protocol developed following a concerted action supported by the Commission of European Union (XII C.) (BCR), and a screening investigation carried out by the Danish National Food Agency and the local Environment and food Agency, MLK FYN, DK Odense. Experts and laboratories from E.U. and EFTA countries, contributed jointly to the development of the concerted action protocol.

WARNING: The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

The annexes A, B and C are informative.

This standard includes a Bibliography.

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

1 Scope

This European Standard specifies a microbiological screening method for the detection of irradiation treatment of herbs and spices, using the combined direct epifluorescent filter technique (DEFT) and aerobic plate count (APC). The DEFT/APC technique is not radiation specific, therefore, it is recommended to confirm positive results using a standardised method (e.g. EN 1788, prEN 13751) to specifically prove an irradiation treatment of the suspected food.

The method has been successfully tested in interlaboratory tests with herbs and spices [1] to [5].

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

ISO 4833, *Microbiology – General guidance for the enumeration of microorganisms - Colony count technique at 30 °C.*

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

3 Principle

The method is based on the comparison of the APC with the count obtained using DEFT. The APC gives the number of viable microorganisms in the sample after a possible irradiation and the DEFT count indicates the total number of microorganisms, including non-viable cells, present in the sample. The difference between the DEFT count and the APC count in spices treated with doses of 5 kGy to 10 kGy is generally about or above 3 to 4 log units. Similar differences between DEFT and APC counts can be induced by other treatments of the foods leading to death of microorganisms, e. g. heat, thus positive results shall be confirmed.

A known volume of sample is filtered through a membrane filter at reduced pressure in order to concentrate the microorganisms on the filter. The microorganisms are stained with a fluorochrome, acridine orange (AO), resulting in an orange and orange-yellow fluorescence under illumination with blue light at 450 nm to 490 nm. These microorganisms are counted using an epifluorescence microscope to give the DEFT count. However, microorganisms which were non-viable before irradiation show green fluorescence and are not counted. In parallel, the APC is determined from a second portion of the same test sample [6] to [10].

4 Reagents

4.1 General

During the analysis use only reagents of recognized analytical grade. All the reagents used in the DEFT and APC determinations should be sterilized by membrane filtration through 0,2 µm pore size membrane filters or by autoclaving.

4.2 Peptone saline diluent

4.2.1 Composition

Sodium chloride	8,5 g
Peptone	1,0 g
Distilled or demineralized water	1000 ml

4.2.2 Preparation

Dissolve the components in the water. Adjust the pH, if necessary, so that after sterilization the final pH is 7,2 ± 0,2 at 20 °C to 25 °C. Sterilize in the autoclave (5.13) at 121 °C ± 1°C for 15 min. The diluent may be stored in a glass bottle at 4 °C to 6°C for not more than two weeks.

4.3 Buffer, pH 3,0

4.3.1 Citric acid solution, substance concentration $c(C_6H_8O_7 \cdot H_2O)$ = 0,1 mol/l

4.3.1.1 Composition

Citric acid monohydrate	21 g
Distilled or demineralized water	1000 ml

4.3.1.2 Preparation

Dissolve the citric acid monohydrate in the water. The solution may be stored in a sterilized glass bottle at 4 °C to 6 °C for not more than three months.

4.3.2 Sodium hydroxide solution, c(NaOH) = 0,1 mol/l

4.3.2.1 Composition

Sodium hydroxide	4,0 g or
Sodium hydroxide solution, 1 mol/l	100 ml
Distilled or demineralized water	900 ml

4.3.2.2 Preparation

Dissolve the sodium hydroxide or dilute the sodium hydroxide solution in the water. The solution may be stored in a glass bottle at 4 °C to 6 °C for not more than three months.

4.3.3 Complete buffer, pH 3,0

4.3.3.1 Composition

Citric acid solution (4.3.1)	100 ml
Sodium hydroxide solution (4.3.2)	54 ml

4.3.3.2 Preparation

Mix the citric acid solution and the sodium hydroxide solution. Adjust pH to 3,0 ± 0,2 with citric acid solution or sodium hydroxide solution. Sterilize the buffer through a membrane filter of pore size 0,2 µm (5.3) before use. The solution may be stored in a glass bottle at 4 °C to 6 °C for not more than three weeks.

4.4 Acridine orange solution

4.4.1 Buffer solution, pH 6,6

4.4.1.1 Composition

Citric acid solution (4.3.1)	35,5 ml
Sodium hydroxide solution (4.3.2)	100 ml

4.4.1.2 Preparation

Mix the citric acid solution and the sodium hydroxide solution. Adjust pH to 6,6 ± 0,2 with citric acid solution or sodium hydroxide solution. Sterilize the buffer through a membrane filter of pore size 0,2 µm (5.3) before use. The buffer solution may be stored in a glass bottle at 4 °C to 6 °C for not more than three weeks.

4.4.2 Complete acridine orange solution

4.4.2.1 Composition

Acridine orange	0,025 g
Buffer, pH 6,6 (4.4.1)	100 ml

4.4.2.2 Preparation

Dissolve the acridine orange in the buffer solution (4.4.1). Sterilize the acridine orange solution through a 0,2 µm pore size membrane filter (5.3) before use. The solution may be stored in a brown glass bottle at 4 °C to 6 °C for not more than one week.

NOTE 1 Concentrated acridine orange solution is commercially available and is recommended for safety reasons.

NOTE 2 As acridine orange is regarded as a mutagenic substance, disposable gloves and face masks should be used when weighing the stain.

4.5 2-Propanol

4.6 Triton® X-100[1)], 1 % cleaning solution

4.6.1 Composition

Triton® X – 100	10 ml
Distilled or demineralized water	1000 ml

4.6.2 Preparation

Mix Triton X-100 with warm (80°C) water. Sterilize the solution through a 0,2 µm pore size membrane filter (5.3). The solution may be stored in a glass bottle at 4 °C to 6 °C for not more than three weeks.

[1)] Triton® X-100 is an example of a suitable product available commercially. This information is only given for the convenience of users of this International Standard and does not constitute an endorsement by CEN of this product.

4.7 Tryptone-Yeast Extract-Glucose-Agar (Plate count agar)

4.7.1 Composition

Tryptone	5,0 g
Yeast Extract	2,5 g
Dextrose (Glucose)	1,0 g
Agar (according to the gel strength of the agar used)	12 g to 18 g
Distilled or demineralized water	1000 ml

4.7.2 Preparation

Dissolve the components or dehydrated complete medium in the water while heating until boiling. Adjust the pH, if necessary, so that after sterilization the final pH is 7,2 ± 0,2 at 20 °C to 25 °C. Sterilize in the autoclave (5.13) at 121 °C ± 1 °C for 15 min.

5 Apparatus

Usual laboratory apparatus in accordance with ISO 7218 and, in particular, the following:

5.1 Apparatus for membrane filtration of sample suspensions

Filtration equipment made of stainless steel or glass should be used. The bottom filter should be of sintered glass or stainless steel intended for filters with a diameter of 25 mm (5.5). The filter tower volume should be at least 10 ml. The filtration equipment is placed vertically on a suction flask or a manifold connected to a water pump or vacuum pump with a pressure regulator. The vacuum during filtration should usually be approximately 70 kPa.

NOTE The use of a water pump is not very suitable if the water pressure cannot be regulated.

5.2 Filter funnel

and suitable suction flasks made of glass for sterile filtration of reagents and diluent.

5.3 Membrane filters,

cellulose ester or similar, pore size 0,2 µm, e.g. diameter 30 mm and/or 47 mm, for sterile filtration of reagents.

5.4 Membrane filters,

polypropylene, diameter 25 mm, pore size 10 µm, for prefiltration of samples.

5.5 Membrane filters,

white polycarbonate, diameter 25 mm, pore size 0,6 µm for membrane filtration of sample test solution.

5.6 Sterile fast filter paper,

for filtration of spice samples.

5.7 Epifluorescence microscope,

with suitable light and filter combination (450 nm to 490 nm)

5.8 Optics,

100 x immersion objective, ocular with magnification of 10 x. (Using a tube magnification of 1,25 a total magnification of 1250 x is achieved.)

5.9 Microscope slide,

e. g. 76 mm x 26 mm.