

Foodstuffs — DNA Comet Assay for the detection of irradiated foodstuffs — Screening method

The European Standard EN 13784:2001 has the status of a
British Standard

ICS 67.050

National foreword

This British Standard is the official English language version of EN 13784:2001.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Food analysis — Horizontal method, which has the responsibility to:

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Lebensmittel — DNA-Kometentest zum Nachweis von bestrahlten Lebensmitteln — Screeningverfahren

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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of an identical text or by endorsement, at the latest by May 2002, and conflicting national standards shall be withdrawn at the latest by May 2002.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.). Experts and laboratories from E.U. and EFTA countries, contributed jointly to the development of this 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.

Annex A is 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 screening method for foods that contain DNA. It is based on micro-gel electrophoresis of single cells or nuclei to detect DNA fragmentation presumptive to irradiation treatment [1] to [8]. The DNA Comet Assay is not radiation specific, therefore, it is recommended to confirm positive results using a standardized method to specifically prove an irradiation treatment of the respective food, e.g. EN 1784, EN 1785, EN 1786, EN 1787, EN 1788, EN 13708, and prEN 13751.

Interlaboratory studies have been successfully carried out with a number of food products, both of animal and plant origin such as various meats [9] to [11], seeds, dried fruits and spices [6], [12]. Other studies [13] to [32] demonstrate that the method is applicable to a large variety of foodstuffs, but also that limitations exist (see clause 8).

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).

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*.

3 Principle

DNA fragmentation can be caused by various chemical or physical treatments including ionizing radiation. When food containing DNA is treated by ionizing radiation, modification of these large molecules occurs including fragmentation either by single- or double-strand breaks. This fragmentation can be studied by microgel electrophoresis of single cells or nuclei. These are embedded in agarose on microscope slides, lysed for disruption of membranes using a detergent and electrophoresed at a set voltage. DNA fragments will stretch or migrate out of the cells forming a tail in the direction of the anode giving the damaged cells the appearance of a comet. This comet assay to measure DNA damage can be carried out under various conditions. Both alkaline and neutral protocols exist. In general, under alkaline conditions both DNA single- and double-strand breaks and alkali-labile sites are measured, whereas under neutral conditions only DNA double-strand breaks are observed. However, using neutral conditions [1] single-strand breaks also exert an influence on the comet appearance, due to relaxation of supercoiled DNA in the nucleus [7], [8]. Irradiated cells will show an increased extension of the DNA from the nucleus towards the anode thus considerably longer comets (more fragmentation) than unirradiated cells. Unirradiated cells will appear nearly circular or with only slight tails (see Figure A.1).

This European Standard describes the use of a simple agarose single-layer set-up employing neutral pH combined with a low voltage and short electrophoresis time.

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 1 according to EN ISO 3696.

4.2 Hydrochloric acid, substance concentration $c(\text{HCl}) = 1 \text{ mol/l}$