SLOVENSKI STANDARD
SIST EN 1787:2001

01-februar-2001

Nadomeġļa:
SIST EN 1787:1998

Živila - Ugotavljanje obsevane hrane, ki vsebuje celulozo z ESR spektroskopijo

Foodstuffs - Detection of irradiated food containing cellulose by ESR spectroscopy

Lebensmittel - ESR-spektroskopischer Nachweis von bestrahlten cellulosehaltigen Lebensmitteln

Produits alimentaires - Détection par spectroscopie RPE d'aliments ionisés contenant de la cellulose

Ta slovenski standard je istoveten z: EN 1787:2000

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

SIST EN 1787:2001 **en**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 1787

March 2000

ICS 67.050

Supersedes EN 1787:1996

English version

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This European Standard was approved by CEN on 21 February 2000.

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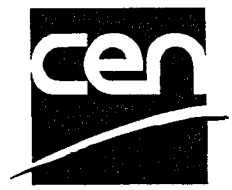

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 1787:2000 E

Contents

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 supersedes EN 1787:1996.

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of an identical text or by endorsement, at the latest by September 2000, and conflicting national standards shall be withdrawn at the latest by September 2000.

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.

This draft European Standard was elaborated on the basis of a protocol developed following a concerted action supported by the Commission of European Union (XII C.5). Experts and laboratories from E.U. and EFTA countries, contributed jointly to the development of this protocol.

1 Scope

This draft European Standard specifies a method for the detection of foods containing cellulose which have been treated with ionizing radiation, by analysing the electron spin resonance (ESR) spectrum, also called electron paramagnetic resonance (EPR) spectrum, of the food, see [1] to [13].

Interlaboratory studies have been successfully carried out with pistachio nut shells, [14] to [18], paprika powder, [19] and [20] and fresh strawberries [21].

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.

EN ISO 3696 Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)

3 Principle

ESR spectroscopy detects paramagnetic centres (e.g. radicals). They are either due to irradiation or to other compounds present. An intense external magnetic field produces a difference between the energy levels of the electron spins $m_s = + ½$ and $m_s = -½$, leading to resonance absorption of an applied microwave beam in the spectrometer. ESR spectra are conventionally displayed as the first derivative of the absorption with respect to the applied magnetic field.

The field and frequency values depend on the experimental arrangements (sample size and sample holder), while their ratio (i.e. g value) is an intrinsic characteristic of the paramagnetic centre and its local coordination. For further information, see [1] to [13].

Radiation treatment produces radicals which can be detected in solid and dry parts of the food. The intensity of the signal obtained increases with the concentration of the paramagnetic compounds and thus with the applied dose.

4 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following:

4.1 Commercially available X-Band ESR spectrometer including magnet, microwave bridge, console with field-controller and signal-channel, rectangular or cylindrical cavity

4.2 ESR tubes, of internal diameter about 4,0 mm (e.g. Suprasil®[1]) quartz tubes)

4.3 Balance, accurate to the nearest 1 mg (optional)

4.4 Laboratory vacuum oven, or **freeze dryer**

4.5 Electric blender

4.6 Filter paper

4.7 Scalpel

4.8 Water of at least grade 3 according to EN ISO 3696

5 Procedure

5.1 Sample preparation

5.1.1 Shells and stones

Remove pieces of suitable size (about 50 mg to 100 mg, 3,0 mm to 3,5 mm in diameter) from the shells or stones of the food, e.g. using a scalpel. Drying (e. g. in a freeze-dryer or at approximately 40 °C in a laboratory vacuum oven (4.4)) is usually not necessary in the case of nutshells but recommended for pips and kernels of fruits.

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[1]) Suprasil® is an example of a product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement of CEN of this product.

5.1.2 Spices

Use about 150 mg to 200 mg of the spice sample. Drying (e. g. in a freeze-dryer or at approximately 40 °C in a laboratory vacuum oven (4.4)) is usually not necessary.

5.1.3 Strawberries

Strawberry samples should be measured immediately after receipt. Otherwise store the samples at approximately - 18 °C until analysis.

For ESR measurement about 200 mg of seeds (achenes) of strawberries are needed. These can be gained usually from about 80 g of strawberries.

For separation of the small seeds from the main fruit body either peel off the skin (recommendation: in frozen state) or use the whole fruit (without stalks and leaves). Homogenize the strawberries in an electric blender (4.5). Add 500 ml of water to the fruit pulp and stir thoroughly. Allow the seeds to settle and decant most of the water together with the floating fruit pulp. Repeat this procedure once or twice to remove any remaining fruit pulp.

Place the seeds on filter paper to remove adhering water. Dry the seeds in a freeze dryer or at approximately 40 °C in a laboratory vacuum oven (4.4) e.g. for 2 h.

Do not grind the seeds since grinding will diminish the signal to noise ratio and can cause a change of the shape of the ESR spectrum.

Storing samples in the frozen state will not adversely affect the detection of treatment with radiation.

5.2 ESR Spectroscopy

5.2.1 Spectrometer settings

Use a time constant and sweep rate appropriate for an ESR signal with a peak to peak linewidth of approximately 0,8 mT. For example, the following ESR spectrometer settings have been found to be satisfactory:

Microwave radiation:	9,78 GHz[2]), power 0,4 mW (for pistachio nuts), to 0,8 mW (for paprika powder and strawberries)[3])
Magnetic field:	348 mT centre field[2]), sweep width 20,0 mT;
Signal channel:	50 kHz or 100 kHz modulation frequency, 0,4 mT to 1,0 mT modulation amplitude; 100 ms to 200 ms time constant[4]) sweep rate 5 mT min^{-1} to 10 mT min^{-1} or accumulation of 3 to 5 spectra at greater sweep rate and shorter time constant;
Gain:	between approximately 10^4 and 10^6;
Temperature:	ambient temperature.

5.2.2 Analysis of sample

Analyse the sample prepared as described in 5.1 in an ESR tube (4.2).

6 Evaluation

A single signal C (see figure A.1 and A.3) is observed in the ESR spectra of all food containing cellulose, including unirradiated samples. In the case of irradiated samples, the intensity of this signal is usually much greater and, in addition, a pair of lines occurs to the left (at lower field) and right (at higher field) of the central signal.

This pair of lines is due to cellulose radicals formed by the ionizing radiation. The spacing of this radiation-induced signal pair is about 6,0 mT and is symptomatic of radiation treatment having taken place (see figure A.2 and A.4).

In some types of food, broad lines of low intensity due to paramagnetic Mn^{2+} ions are observed in addition to the signals mentioned. However their position in the magnetic field is different, and the spacing between two manganese lines being about 9,0 mT (coupling constant) differs from the spacing of the irradiation specific signals.

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[2]) These values are for the specified microwave frequency and magnetic field; if the frequency is higher (lower) the magnetic field strength will be higher (lower).

[3]) If saturation is suspected, the microwave power should be reduced, see [10].

[4]) These values are for the specified sweep rate.

7 Limitations

Detection limits and stability are influenced by the crystalline cellulose content and the moisture content of the samples. Positive identification of the cellulose radicals is evidence of irradiation but the absence of this signal does not constitute evidence that the sample is unirradiated.

Detection of irradiated pistachio nuts has been validated for doses of 2 kGy and above and stability is not expected to present limitations for detection of irradiation for at least one year after treatment.

Detection of irradiated paprika powder has been validated for doses of 5 kGy and above. Stability of cellulose radicals in paprika powder is largely dependent on storage conditions, (especially humidity), and may be shorter than the shelf-life of the products.

Detection of irradiated fresh strawberries has been validated for doses of 1,5 kGy and above. Detection of irradiated berries has been analysed for doses of 0,5 kGy and above. Detection is typically limited to about the first 3 weeks after treatment. Stability of cellulose radicals in berries depends on storage conditions and may be shorter than the shelf-life of the products.

8 Validation

This draft European Standard is based on two interlaboratory tests with pistachio nut shells [14] to [18], one interlaboratory test with paprika powder [19], [20] and one with fresh strawberries [21].

In an interlaboratory test carried out by the Community Bureau of Reference (BCR) [17], [18], 21 laboratories identified coded samples of pistachio shells which were either unirradiated or irradiated to about 2 kGy, 4 kGy or 7 kGy (see table 1).

Table 1 - Interlaboratory data

Product	No of samples	No of false negatives[1])	No of false positives[2])
Pistachio shells	84	15	2

[1]) False negatives are irradiated samples identified as unirradiated.
[2]) False positives are unirradiated samples identified as irradiated.

After improvement of the first protocol, in an interlaboratory test carried out by the German Federal Health Office (Bundesgesundheitsamt, BGA) [16], 17 laboratories identified coded samples of pistachio shells which were either unirradiated or irradiated to about 4 kGy or 6 kGy (see table 2).

Table 2 - Interlaboratory data

Product	No of samples	No of false negatives[1])	No of false positives[2])
Pistachio shells	68	0	1

[1]) False negatives are irradiated samples identified as unirradiated.
[2]) False positives are unirradiated samples identified as irradiated.

In an interlaboratory test carried out by the BGA [19], [20], 20 laboratories identified coded samples of paprika powder which were either unirradiated or irradiated to about 5 kGy or 10 kGy (see table 3).

Table 3 - Interlaboratory data

Product	No of samples	No of false negatives[1])	No of false positives[2])
Paprika powder	160	0	1

[1]) False negatives are irradiated samples identified as unirradiated.
[2]) False positives are unirradiated samples identified as irradiated.

In an interlaboratory test carried out by the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) [21], 23 laboratories identified coded samples of fresh strawberries which were either unirradiated or irradiated to about 1,5 kGy or 3 kGy (see table 4).

Table 4 - Interlaboratory data

Product	No of samples	No of false negatives [1])	No of false positives [2])	No of inconclusive results
Strawberries	184	7	0	2

[1]) False negatives are irradiated samples identified as unirradiated.
[2]) False positives are unirradiated samples identified as irradiated.

9 Test report

The test report shall contain at least the following:

a) information necessary for identification of the sample;
b) a reference to this European Standard;
c) the result;
d) date of sampling and sampling procedure (if known);
e) date of receipt;
f) date of test;
g) any particular points observed in the course of the test;
h) any operations not specified in the method or regarded as optional which might have affected the results.

Annex A (normative)

Figures

Figure A.1- ESR spectrum of an unirradiated pistachio nut shell
(C) = unspecific central ESR-signal

Figure A.2- ESR spectrum of a pistachio nut shell, irradiated with 4,0 kGy, with irradiation specific line pair of the cellulose radical, spacing 6,05 mT ± 0,05 mT
(C) = unspecific central ESR-signal

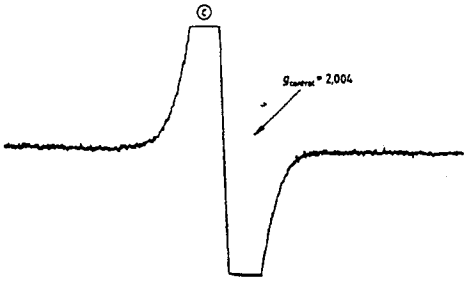

Figure A.3- ESR spectrum of seeds from unirradiated strawberries (C) = unspecific central ESR-signal

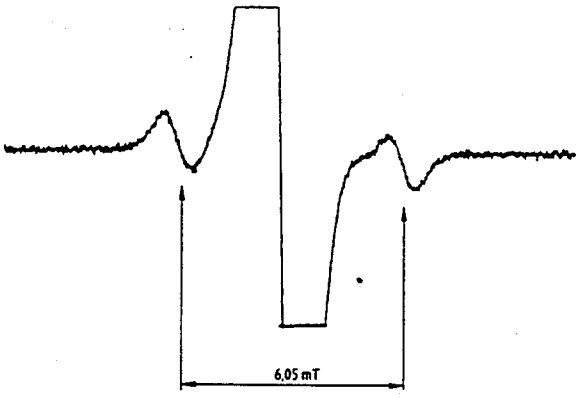

Figure A.4- ESR spectrum of seeds from strawberries, irradiated with 3,5 kGy, with irradiation specific line pair of the cellulose radical, spacing 6,05 mT ± 0,05 mT, (C) = unspecific central ESR-signal

Annex B (informative)

Further information on the applicability

Laboratory experience has been gained to support the application of this method to the following sample types:

Raspberries, blueberries (chilled or frozen), french prunes, coconuts, almonds and walnuts.

A list of tested food has been published [22].

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