SLOVENSKI STANDARD
oSIST prEN 1787:2019

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Živila - Detekcija obsevane hrane, ki vsebuje celulozo, s spektroskopijo ESR

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: prEN 1787

ICS:

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

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EUROPEAN STANDARD

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

Lebensmittel - ESR-spektroskopischer Nachweis von bestrahlten cellulosehaltigen Lebensmitteln

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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European foreword

This document (prEN 1787:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document will supersede EN 1787:2000.

The predecessor of this document 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 EU and EFTA countries, contributed jointly to the development of this protocol.

The changes between this document and the previous edition are as follows:

— The scope contains the information that it has been shown that false-positive results can appear when analysing bleached nuts.

— The clause on limitations was amended by the information that, having identified, that the method yields false positives with certain matrices (e.g. bleached nuts), it is in the responsibility of the analyst to ensure that the method is fit for purpose on the matrix on which it is applied.

— The clause on limitations was furthermore amended by the information that it has been shown that with nuts in shells (hazelnuts, walnuts, pistachio), a bleaching can lead to comparable positive signals which can be mixed up with radiation induced signals.

— The Figures A.5 to A.8 were included to show how figures from non-irradiated but bleached nutshells can be differentiated from irradiated shells from hazelnuts and walnuts.

— This new version was editorially updated according to current rules.

1 Scope

This document 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]. However, it has been shown that false-positive results can appear when analysing bleached nuts. For further information, see Clause 7 on limitations.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

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

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

4 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 ms = + 1/2 and ms = −1/2, 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, sample holder and spectrometer specifications), 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 specific radicals which can be mostly 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.

5 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following:

5.1 Commercially available X-Band ESR spectrometer including magnet (electro or permanent), microwave bridge, console with field- controller and signal-channel, rectangular or cylindrical cavity.

5.2 ESR tubes, suitable for the ESR spectrometer used (e.g. Suprasil®[1] quartz tubes,)

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

5.4 Laboratory vacuum oven or **freeze dryer**

5.5 Electric blender

5.6 Filter paper

5.7 Scalpel, pincers

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

6 Procedure

6.1 Sample preparation

6.1.1 General

Do not grind the samples since grinding could either diminish the signal to noise ratio and can also cause a change of the shape of the ESR spectrum or induce other ESR signals [25].

6.1.2 Shells and stones

Remove pieces of suitable size (about 50 mg to 100 mg, e.g. 3,0 mm to 3,5 mm in diameter) from the shells or stones of the food, e.g. using a scalpel or pincers. Drying (e.g. in a freeze-dryer or at approximately 40 °C in a laboratory vacuum oven (5.4)) is usually not necessary in the case of nutshells but recommended for pips and kernels of fruits.

6.1.3 Spices

For example 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 (5.4)) is usually not necessary.

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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.

6.1.4 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 (5.5). 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 (5.4) e.g. for 2 h.

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

6.2 ESR Spectroscopy

6.2.1 Spectrometer settings

These following parameters have been proven to be successful in the interlaboratory test (see Clause 8). They should be optimized as per sample and ESR spectrometer specifications.

Use a time constant and sweep rate (or sweep time) 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:	Frequency 9,78 GHz[2], power 0,4 mW (for e.g. pistachio nuts), to 0,8 mW (for e.g. paprika powder or strawberries)[3];
Magnetic field:	348 mT centre field[2]), sweep width 20 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} (sweep time 2,4 min to 1,2 min) 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.

[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.

6.2.2 Analysis of sample

Analyse the sample prepared as described in 6.1 in an ESR tube (5.2).

7 Evaluation

A single central signal c, with a g value of g approximately 2,004 (Figure A.1, A.2, A.3, A.4, A.6 and A.8) 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, a pair of lines occurs to the left (at lower magnetic field) and right (at higher magnetic 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 6,05 mT ± 0,05 mT and is symptomatic of radiation treatment having taken place (see Figures A.2, A.4, A.6 and A.8).

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.

Confirmation of sensitivity to radiation can be achieved, where necessary, by irradiating the sample and re-testing.

8 Limitations

Having identified, that the method yields false positives with certain matrices (e.g. bleached nuts), it is in the responsibility of the analyst to ensure that the method is fit for purpose on the matrix on which it is applied.

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 for validated matrices 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.

It has been shown that with nuts in shells (hazelnuts, walnuts, pistachio), a bleaching can lead to comparable positive signals which can be mixed up with radiation induced signals (see [23], [24] and Figures A.5 to A.8).

9 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).