SLOVENSKI STANDARD
SIST EN 1786:1998

01-november-1998

Živila - Detekcija obsevane hrane, ki vsebuje kosti - Metoda z ESR spektroskopijo

Foodstuffs - Detection of irradiated food containing bone - Method by ESR spectroscopy

Lebensmittel - Nachweis von bestrahlten knochen- bzw. grätenhaltigen Lebensmitteln - Verfahren mittels ESR-Spektroskopie

Produits alimentaires - Détection d'aliments ionisés contenant des os ou des aretes - Méthode par spectroscopie RPE

Ta slovenski standard je istoveten z: **EN 1786:1996**

ICS:

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

SIST EN 1786:1998 **en**

EUROPEAN STANDARD **EN 1786**

NORME EUROPÉENNE

EUROPÄISCHE NORM December 1996

ICS 67.020

Descriptors: foodstuffs, irradiated foodstuffs, ionizing radiation, food analysis, detection of irradiation treatment, meat bones, fish bones, ESR-spectroscopy

English version

Foodstuffs - Detection of irradiated food containing bone - Method by ESR spectroscopy

Produits alimentaires - Détection d'aliments ionisés contenant des os ou des arêtes - Méthode par spectroscopie RPE

Lebensmittel - Nachweis von bestrahlten knochen- bzw. grätenhaltigen Lebensmitteln - Verfahren mittels ESR-Spektroskopie

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Foreword

This European Standard has been prepared by CEN/TC 275 "Food analysis - Horizontal methods" the secretariat of which is held by DIN.

This European Standard was elaborated on the basis of a protocol developed following a concerted action of 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.

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of an identical text or by endorsement, at the latest by June 1997, and conflicting national standards shall be withdrawn at the latest by June 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, 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 method for the detection of meat containing bone and fish containing bone which have been treated with ionizing radiation, by analysing the electron spin resonance (ESR) spectrum, also called electron paramagnetic resonance (EPR) spectrum, of the bones, see [1] to [11].

Interlaboratory studies have been successfully carried out with beef bones, trout bones and chicken bones, see [12] to [18]. Since the radiation induced ESR signal is attributed to hydroxyapatite (see 7.1), which is the principal component of bones, it is expected that the application of the method can be extended to all meat and fish species containing bones. These expectations are consistent with laboratory experience (see Annex B).

The detection limit depends on the state of mineralization of the bones which is usually lower for small species (see clause 8).

2 Definition

For the purposes of this standard, the following definition applies:

ESR spectrum: The signals obtained by the method described in this European Standard. They are either due to paramagnetic compounds formed by irradiation or to compounds originally present.

3 Principle

ESR spectroscopy detects paramagnetic centres (e.g. radicals). 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 an identification of irradiated samples it may be helpful to measure the g values of the ESR signals. For further information, see [1] to [12].

Radiation treatment produces radicals which can be quite stable in solid and dry components (e.g. bones) of the food, and can be detected. The intensity of the signal obtained increases with the concentration of the paramagnetic compounds and thus with the applied dose.

4 Apparatus

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 **g value measurement unit** including frequency counter, magnetic field probe (nuclear magnetic resonance (NMR) Gaussmeter), or any other built-in g value measurement unit.

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

4.4 **Balance**, accurate to the nearest 1 mg (optional).

4.5 **Laboratory vacuum oven**, or **freeze dryer**.

5 Sampling plan

(No specification yet)

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

6 Procedure

6.1 Sample preparation

Remove flesh as completely as possible from a bone sample, e. g. using a scalpel, to get the bone as clean as possible. If necessary, split the bone and remove the marrow. Then dry the bone in a freeze dryer for about 18 h or for about 3 h at approximately 40 °C in a laboratory vacuum oven (4.5). Remove a suitable sample piece (about 100 mg, 3,0 mm to 3,5 mm thick and 5,0 mm to 10,0 mm long) for analysis from the dried bone. Place the sample in a standard ESR tube (4.3).

NOTE: The samples may be measured in powdered form or as bone fragments. 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

Use a time constant and sweep rate appropriate for an ESR signal with a peak to peak linewidth of approximately 0,4 mT. For example, the following ESR spectrometer settings have been found to be satisfactory:

Microwave radiation:	9,5 GHz[2]), power 5 mW to 12,5 mW;
Magnetic field:	342 mT[2]) centre field,
	sweep width 5 mT to 20 mT;
Signal channel:	50 kHz or 100 kHz modulation frequency,
	0,2 mT up to 0,4 mT modulation amplitude;
	50 ms to 200 ms time constant [3])
	sweep rate [3]) 2,5 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 about 1,0 x 10^5 and 1,0 x 10^6
Temperature:	room temperature

6.2.2 Analysis of sample

Analyse the sample prepared as described in 6.1 in an ESR tube (4.3).

Although the g value of the individual signals of the ESR spectrum does not need to be determined in most cases, it can be used for positive identification of irradiated samples (see 7.2).

7 Evaluation

7.1 Assessment of the ESR spectra

Irradiated samples are recognized by the appearance of a typical asymmetric signal having g values of 2,002 and 1,998 (see 7.2). This signal is attributed to trapped radicals in hydroxyapatite produced by the action of ionizing radiation on the bone.

A low-intensity symmetrical signal having a g value of g_{symm} = 2,005 (see figure A.1) is sometimes present in the ESR spectra (e.g. in the case of bones containing marrow).

Typical examples of ESR spectra of unirradiated and irradiated bones (chicken thigh in this case) are shown in the figures A.1, A.2 and A.3. Note that the ESR spectra of bones irradiated at relatively low doses (less than 2 kGy to 3 kGy) reveal a combination of the radiation-specific and the non-specific signal frequently (see figure A.2). At doses less than 0,5 kGy, the non-specific signal may be stronger than the specific one. In contrast, the radiation-specific signal is frequently the only one observed (see figure A.3) in the case of high doses (more than 3 kGy).

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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]) These values are for the specified magnetic sweep width.

7.2 Calculation of the g value

Using the measured values obtained as described in 6.2.2, calculate the g value (g_{signal}) using equation 1:

$$g_{signal} = \frac{71{,}448 \cdot \nu_{ESR}}{B} \quad \text{... (1)}$$

where:

ν_{ESR} is the microwave frequency, in gigahertz (GHz);
B is the magnetic field (magnetic flux density), in millitesla (mT) (10 Gauss = 10 Gs = 1 mT).

The procedure for calculating the g value of the signals from bone is to measure the frequency (e.g. frequency counter) and the field B (e.g. gaussmeter) at the positions of the arrows in figures A.1 to A.3.

The following values are found in bones:

g_{symm} = 2,005 ± 0,001 (no proof of irradiation);
g_1 = 2,002 ± 0,001 (irradiated);
g_2 = 1,998 ± 0,001 (irradiated).

8 Limitations

Detection of irradiated bone samples is typically possible above a dose of approximately 0,5 kGy, covering the majority of commercial applications.

Detection limits and stability are influenced by the degrees of mineralization and crystallinity of hydroxyapatite in the sample.

In general, the bones of larger animals and species are highly mineralized with low minimum detectable doses. However, variations within individual animals and species have been noted, see [19] to [21].

In case of meat bones the results of this detection method are not significantly influenced by heating of the sample (e.g. boiling in water). Detection of irradiation treatment is not significantly influenced by storage times of up to 12 months. For poorly mineralized fish bones it has been noted that non radiation induced signals are strongly enhanced if the temperatures recommended (6.1) for drying are exceeded and may interfere with the radiation specific signals.

9 Validation

This European Standard is based on interlaboratory tests with meat bones and fish bones [12] to [18].

In an interlaboratory test carried out by the Community Bureau of Reference (BCR) [13], [16], 21 laboratories identified coded samples of beef bones and trout bones 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 negative[1])	No of false positive[2])
Beef bone	84	0	0
Trout bone	84	5[3])	0

[1]) False negatives are irradiated samples identified as unirradiated.
[2]) False positives are unirradiated samples identified as irradiated.
[3]) The five false negatives were due to misinterpretation of the spectra.

In an interlaboratory test carried out by the German Federal Health Office (Bundesgesundheitsamt, BGA) [17], 18 laboratories identified coded samples of chicken and trout bones which were either unirradiated or irradiated to about 2 kGy, 4 kGy or 6 kGy (see table 2).

Table 2: Interlaboratory data

Product	No of samples	No of false negative[1])	No of false positive[2])
Chicken bone	108	0	0
Trout bones	108	0	2[3])

[1]) False negatives are irradiated samples identified as unirradiated.
[2]) False positives are unirradiated samples identified as irradiated.
[3]) The two false positives were due to misinterpretation of the spectra.

10 Test report

The test report shall contain at least the following:

a) information 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

Field: 350 mT ± 10 mT (3500 Gs ± 100 Gs).

Gain $3,2 \cdot 10^5$

Figure A.1: Typical ESR spectrum of an unirradiated chicken thigh sample

Field: 350 mT ± 10 mT (3500 Gs ± 100 Gs)

Gain $3,2 \cdot 10^5$

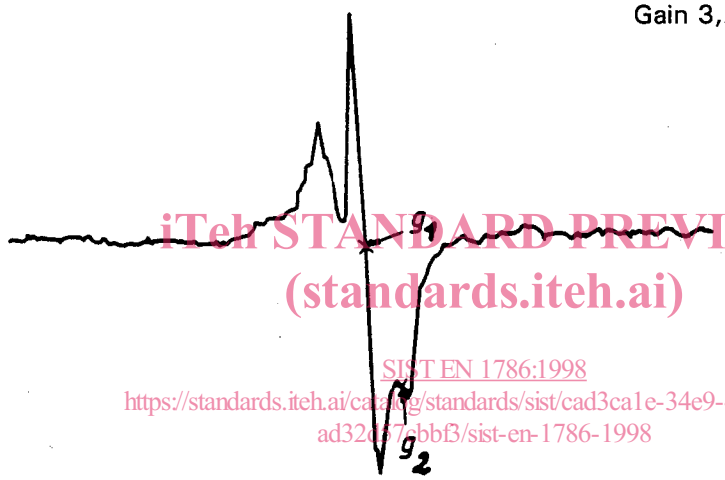

Figure A.2: Typical ESR spectrum of a chicken thigh sample irradiated with a dose of approximately 1 kGy

Field: 350 mT ± 10 mT (3500 Gs ± 100 Gs)

Gain $1,0 \cdot 10^5$

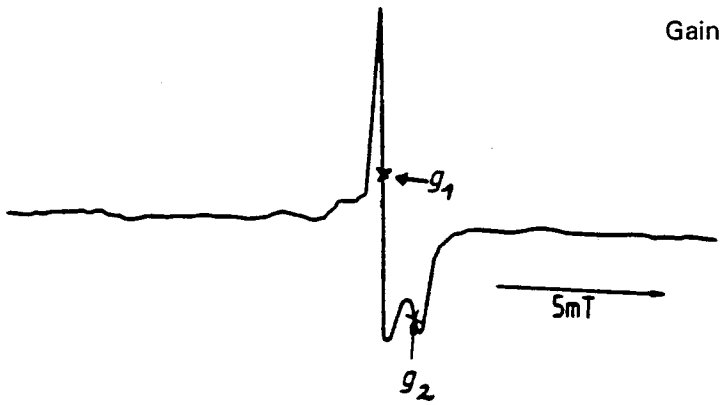

Figure A.3: Typical ESR spectrum of a chicken thigh sample irradiated with a dose of approximately 3 kGy