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## Foodstuffs – Detection of irradiated food containing bone – Method by ESR spectroscopy

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## Livsmedel – Analys av bestrålade livsmedel innehållande ben – ESR-spektroskopi

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ICS 67.020

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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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Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

The European Standards exist 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 Central Secretariat has the same status as the official versions.

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**CEN**

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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of an identical text or by endorsement, at the latest by June 1997, and conflicting national standards shall be withdrawn at the latest by June 1997.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  $m_s = +\frac{1}{2}$  and  $m_s = -\frac{1}{2}$ , 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 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<sup>®1</sup>) 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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<sup>1</sup>) Suprasil<sup>®</sup> 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 <sup>2)</sup> , power 5 mW to 12,5 mW;
Magnetic field:	342 mT <sup>2)</sup> 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 <sup>3)</sup> sweep rate <sup>3)</sup> 2,5 mT · min <sup>-1</sup> to 10 mT · min <sup>-1</sup> or accumulation of 3 to 5 spectra at greater sweep rate and shorter time constant
Gain:	between about 1,0 × 10 <sup>5</sup> and 1,0 × 10 <sup>6</sup>
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  $g_{\text{symm}} = 2,005$  (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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<sup>2)</sup> 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).

<sup>3)</sup> These values are for the specified magnetic sweep width.