BRITISH STANDARD

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Foodstuffs —Detection of irradiated food using photostimulated luminescence

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National foreword

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Foodstuffs - Detection of irradiated food using photostimulated luminescence

Produits alimentaires - Détection d'aliments ionisés par photoluminescence

Lebensmittel - Nachweis von bestrahlten Lebensmitteln mit Photostimulierter Lumineszenz

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Management Centre: rue de Stassart, 36 B-1050 Brussels

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Contents

Foreword

This document EN 13751:2002 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of an identical text or by endorsement, at the latest by March 2003, and conflicting national standards shall be withdrawn at the latest by March 2003.

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1 Scope

This European Standard specifies a method for the detection of irradiated foods using photostimulated luminescence (PSL). The technique described here comprises an initial measurement of PSL intensity which may be used for screening purposes, and a calibration method to determine the PSL sensitivity to assist classification. It is necessary to confirm a positive screening result using calibrated PSL or another standardised (e.g. EN 1784 to EN 1788) or validated method.

The method has been successfully tested in interlaboratory trials using shellfish and herbs, spices and seasonings [1]. From other studies it may be concluded that the method is applicable to a large variety of foods [2], [3], [4].

2 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply.

2.1
photostimulated luminescence (PSL)
radiation specific phenomenon resulting from energy stored by trapped charge carriers. Release of this stored energy by optical stimulation can result in a detectable luminescence signal.

2.2
PSL intensity
amount of light detected during photostimulation, in photon count rate

2.3
screening PSL or initial PSL
PSL intensity recorded from the sample as received or following preparation

2.4
calibrated PSL
PSL intensity recorded from the test sample following irradiation to a known dose, after initial PSL measurement

2.5
thresholds
values of PSL intensity used for classification. In screening mode, two thresholds, a lower threshold (T_1) and an upper threshold (T_2) are used to classify the sample

2.6
negative PSL result
PSL intensity below the lower threshold (less than T_1)

2.7
intermediate PSL result
PSL intensity between the upper and the lower threshold (greater than or equal to T_1, less than or equal to T_2)

2.8
positive PSL result
PSL intensity above the upper threshold (greater than T_2)

2.9
dark count
photon count rate from the photomultiplier with an empty chamber in the absence of stimulation

2.10
light count
photon count rate with a reference light source (e.g. ^{14}C loaded scintillant, or equivalent) in the sample chamber

2.11
empty chamber run
PSL intensity measured from an empty sample chamber to ensure absence of contamination of the chamber

3 Principle

3.1 General

Mineral debris, typically silicates or bioinorganic materials such as calcite which originate from shells or exoskeletons, or hydroxyapatite from bones or teeth, can be found on most foods. These materials store energy in charge carriers trapped at structural, interstitial or impurity sites, when exposed to ionising radiation. Excitation spectroscopy has shown that optical stimulation of minerals releases charge carriers [5], [6], [7]. It has subsequently been shown that the same spectra can be obtained from whole herb and spice samples and other foods using photostimulation [2], [8], [9]. PSL measurements do not destroy the sample, therefore whole samples, or other mixtures of organic and inorganic material, can be measured repeatedly. PSL signals, however, decrease if the same sample is measured repeatedly.

The methodology comprises screening (initial) PSL measurements to establish the status of the sample (see 2.3) and an optional second measurement following a calibration radiation dose to determine the PSL sensitivity of the sample (see 2.4).

3.2 Screening PSL

For screening (see 2.3) the signal levels are compared with two thresholds (see 2.5). The majority of irradiated samples produce a strong signal above the upper threshold level. Signals below the lower threshold suggest that the sample has not been irradiated. Signal levels between the two thresholds, intermediate signals, show that further investigations are necessary. The use of thresholds produces an effective screening method which can also be backed up by calibration, by TL as described in EN 1788 or another validated method, e.g. [3], [4], [8].

3.3 Calibrated PSL

For calibration, the sample is exposed to a defined radiation dose after the initial PSL measurement, and then re-measured. Irradiated samples show only a small increase in PSL after this radiation exposure, whereas unirradiated samples usually show a substantial increase in PSL signal after irradiation.

4 Reagents

4.1 Aerosol silicone grease, e.g. Electrolube SC0200H[1)]

4.2 Water, deionized

5 Apparatus

5.1 PSL system, e.g. SURRC PPSL Irradiated food screening system[1)] [10], [11], [12], [13] comprising sample chamber, stimulation source, pulsed stimulation and synchronised photon counting system. For instrumental set-up, see 7.4.

NOTE For the interlaboratory tests, the SURRC PPSL system has been used.

5.2 Disposable Petri-dishes

NOTE For the interlaboratory tests, 5 cm Petri-dishes have been used.

5.3 Radiation source, capable of irradiating samples with a defined radiation dose before measurement of calibrated PSL. In the interlaboratory tests on shellfish and herbs, spices and their mixtures [1], sources delivering ^{60}Co-rays have been employed at a fixed radiation dose of 1 kGy.

Alternative sources may be used providing they have been found satisfactory.

NOTE Other fixed doses can be suitable.

5.4 ^{14}C-Source (optional)

5.5 Laminar flow cabinet (optional)

5.6 Air duster (optional)

6 Sampling technique

Whenever possible, the sample is taken from a light-protected position in the food consignment, since the PSL intensity decreases on exposure to light.

Before analysis, samples should be protected against light exposure. Store them in the dark.

7 Procedure

7.1 General

All dispensing and handling of samples should be carried out under subdued lighting whenever possible. Samples are dispensed into disposable Petri-dishes and introduced to the system.

[1)] Electrolube SC0200H and Scottish Universities Research and Reactor Center Pulsed Photostimulated Luminescence (SURRC PPSL) are examples of products available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement of CEN of these products. Equivalent products may be used if they can be shown to lead to equivalent results.

Samples should be handled with care to avoid cross-contamination during dispensing. It is recommended that samples are dispensed individually, under a laminar flow cabinet (5.5), and fresh tissue is placed on the bench for each sample. The Petri-dish should be covered with a lid to reduce the possibility of contamination.

7.2 Preparation of herb, spice and seasoning samples

Samples are dispensed into clean Petri-dishes, in duplicate. If these test samples lead to inconsistent classifications, a further four aliquots shall be dispensed and classification based on the highest two results. Some samples may require a minimum of preparation; e.g. vanilla pods may need to be cut to fit the dish and wrappings should be removed.

Samples can either be dispensed in a thick layer within the Petri-dish or in a thin layer, applied to a dish already sprayed with silicone grease (4.1) to fix the sample. Thicker layer samples are less likely to be affected by bleaching; subsurface minerals can be exposed by gentle agitation.

NOTE Thin layer samples can also be dispensed into planchets or other shallow containers suitable for irradiation with ^{90}Sr or other sources. If a gamma source is used for calibration either dispensing method is suitable.

7.3 Preparation of shellfish

7.3.1 General

PSL analysis can be conducted using whole samples including shell, shelled whole samples and dissected intestines or minerals extracted by flushing with water (4.2).

If enough sample material is available, it is recommended that samples be divided into at least six portions, i.e. six Petri-dishes.

7.3.2 Whole samples

Whole samples including shell can be placed as received in the Petri-dish. In some cases it may be necessary to cut the shellfish to fit the Petri-dish. If the intestinal tract is visible, it is preferable to place this uppermost.

7.3.3 Shelled whole samples

Shelled whole samples can be placed whole in the Petri-dish, again with the intestinal tract facing upwards, using as many individual shellfish as will fit in the Petri-dish.

7.3.4 Shellfish intestines

Shellfish intestines can be found as a thin dark tube on the convex side of prawns or shrimps, and in the interiors of molluscs. Using a scalpel, slice the flesh open and with tweezers remove the intestinal tract. Repeat this technique on several samples of shellfish (recommended: 6 intestines per Petri-dish).

7.4 Instrumental Set-Up

This section describes the set-up of the SURRC PPSL system, as an example.

The system is used in conjunction with a computer for setting individual measurement parameters (cycle time, thresholds and data recording conditions) for recording quantitative photon counts.

NOTE 1 The system can be used in a stand-alone mode, with simple push button controls, for preliminary measurements. However, the validated procedures which are the subject of this standard apply only to quantitative measurements performed in conjunction with a computer.

The instrumental set-up procedure includes checks on dark count (2.9) and light count (2.10), establishing measurement parameters and checks on irradiated and unirradiated standard materials.

For herbs and spices tested in the interlaboratory trial [1], the threshold settings of T_1 = 700 counts/60 s and T_2 = 5 000 counts/60 s have been shown to be satisfactory. These thresholds refer to the use of 5 cm Petri-dishes. For shellfish tested in the interlaboratory trial [1], the threshold settings of T_1 = 1 000 counts/60 s and T_2 = 4 000 counts/60 s have been shown to be satisfactory.

NOTE 2 The threshold levels are based on results of interlaboratory tests and further experience. They might need to be adjusted in dependence of the PSL sensitivity of the samples, the sensitivity of the instrument and the surface area of the samples (size of petri-dishes). It has been shown that e.g. pepper, nutmeg and clove are less sensitive to PSL.

An empty chamber test (2.11) should be run to ensure that the chamber is free from contamination. This step should be repeated periodically, e.g. at least every 10 samples and also after samples with positive results. An air duster (5.6) can be used to clean the sample chamber.

7.5 Screening Measurements

Run the test samples and record the results over the specified measurement time. The results should be classified according to the pre-set thresholds 2.6 to 2.8.

7.6 Calibrated Measurements

After screening, the sample should be covered to prevent loss of material or contamination, either with the lid of the Petri-dish or, in the case of planchets or shallow containers, some other suitable means. During handling, care should be taken not to shake the sample. The sample should then be exposed to a defined radiation dose (e.g. 1 kGy or a dose comparable to the expected treatment dose). After irradiation, all further handling should take place under subdued lighting whenever possible. After storage overnight at ambient temperature (chilled storage is recommended for shellfish and other perishable materials), perform calibrated measurements according to 7.4 and 7.5.

8 Evaluation

8.1 Negative result

8.1.1 Screening PSL

Negative results (counts less than T_1) indicate that the sample is unlikely to be irradiated. For irradiated samples with insufficient PSL sensitivity, negative results may also occur.

8.1.2 Calibrated PSL

Negative calibrated results (calibrated results reading less than T_1) are indicative of insufficient PSL sensitivity. These are unusual in herbs and spices and should always be associated with negative screening results. With shellfish, negative results after calibration may be more common. Any sample giving negative signals after calibration cannot be classified. In this case, application of TL analysis as described in EN 1788 or another standardized method as described in EN 1784, EN 1785, EN 1786 or EN 1787 or another validated method is recommended.

Negative calibrated results associated with non-negative screening results indicate analytical error and the measurements should be repeated on fresh portions of the sample.

8.2 Intermediate results

8.2.1 Screening PSL

Intermediate screening results (greater than or equal to T_1, less than or equal to T_2) do not allow the irradiation status of the sample to be determined directly: they may be indicative of an irradiation treatment, residual geological signals, or a dilute blend of irradiated material. Application of TL analysis as described in EN 1788 or another standardized method as described in EN 1784, EN 1785, EN 1786 or EN 1787 or another validated method is recommended for all samples giving intermediate screening signals.

8.2.2 Calibrated PSL

Intermediate calibration results might indicate an irradiation treatment if screening results have also been intermediate. Negative screening results and intermediate calibrated results indicate a non-irradiated sample with low sensitivity. Again, assessment using TL analysis as described in EN 1788 or another standardized method as described in EN 1784, EN 1785, EN 1786 or EN 1787 or another validated method is recommended for these cases.

Highly positive screening results (much greater than T_2) in combination with intermediate calibration results indicate analytical error. However, for samples with positive screening results close to T_2, an intermediate calibration result can indicate that the sample had been irradiated at a higher dose than the calibration exposure. Measurements should be repeated, and if necessary followed by assessment of samples by TL analysis or another standardized or validated method.

8.3 Positive results

8.3.1 Screening PSL

Positive screening results (greater than T_2) are strongly indicative of an irradiated sample. For unirradiated samples with high PSL sensitivity (high geological residual signals) positive results may also occur occasionally.

8.3.2 Calibrated PSL

Positive calibrated results (greater than T_2) within the same order of magnitude, as the screening results are indicative of irradiation.

Cases, where positive screening and calibrated PSL results from a series of test samples are close to the threshold T_2 should be analysed using TL analysis as described in EN 1788 or another standardized method as described in EN 1784, EN 1785, EN 1786, and EN 1787, or another validated method.

Pure samples with positive calibrated results, which are much greater than their negative or intermediate screening results are likely to be unirradiated.

In cases where calibrated PSL gives signals which are much greater, e.g. two orders of magnitude or more than screening PSL results, the sample might contain an irradiated component within a blended mixture. TL analysis of these results may prove helpful.

In cases where the calibrated PSL is substantially smaller than the screening results (between 1 and 2 orders of magnitude), this might indicate analytical error and the analyses should be repeated.

9 Limitations

The PSL method may, in principle, be applied to detect irradiation of any food which contains mineral debris. PSL sensitivity of a sample depends on the quantities and types of minerals within the individual sample. Signals below the lower threshold (T_1) are generally associated with unirradiated material, but can derive from low sensitivity irradiated materials. Calibration can help to distinguish these cases. Samples with low sensitivity (negative or intermediate signals after calibration) should be investigated further by TL analysis or another standardized or validated method.

In general, calibrated PSL measurements are recommended for shellfish with low mineral contents and "clean" spices (e.g. nutmeg, ground white and black pepper) to avoid false negative results.

Optimum results are obtained from unblended products. Compound foods e.g. curry powders, and blends may contain debris with a range of PSL sensitivities, in which case calibrated PSL may provide ambiguous results.

The presence of salt in a product may dominate the PSL intensity to an extent which masks signals from any remaining irradiated ingredients. Hydration of the product followed by re-measurement can both identify and rectify this situation.

10 Validation

In the case of shellfish, the method was tested in a small intercomparison organized by SURRC on behalf of the then British Ministry of Agriculture, Fisheries and Food (MAFF) [1] with 5 participating laboratories, each of which analysed 10 irradiated and 5 unirradiated blind samples from 5 warm and cold water species. The 10 irradiated samples consisted of one of each species irradiated to each of 2 doses (0,5 kGy and 2,5 kGy). Participants were asked to measure 6 aliquots of whole samples and 6 of intestines for 60 s, and in each case to use the two highest results to make qualitative screening decisions relative to thresholds of T_1 = 1 000 counts/60 s and T_2 = 4 000 counts/60 s. On this basis all 75 samples were correctly classified (see Table 1). Calibrated PSL measurements were subsequently performed disregarding low sensitivity aliquots. Identical qualitative results were obtained by both screening and calibrated measurements.

Table 1 — PSL screening results from interlaboratory trials of shellfish

	Irradiated		Unirradiated	
	Correctly identified	False negative	Correctly identified	False positive
Shellfish[a]	100 (100 %)	0 (0 %)	50 (100 %)	0 (0 %)

[a] These results refer to a total of 75 blind samples, analysed independently both using whole samples, and as intestinal material. Two results per sample were reported, which were in agreement in all cases.

In another larger interlaboratory test organized by SURRC on behalf of MAFF [1], 8 participants tested 40 varieties of herbs, spices and seasonings, and 4 blends presented blind either in unirradiated form or irradiated with a maximum dose of 10 kGy. Thresholds of T_1 = 700 counts/60 s and T_2 = 5 000 counts/60 s and measurement times of 60 s were used.

662 screening measurements were reported from the samples (345 from irradiated and 317 from unirradiated samples), leading to 577 qualitative classifications based on negative or positive instrumental readings. The irradiation status of 569 (98,6 % of positive or negative outcomes) samples was correctly identified. Eight (1,4 % either false positive or false negative) were incorrect and attributed to operator error. Out of 662 samples examined in the screening study, 85 samples (12,8 %), produced intermediate signals (24 of the 345 irradiated samples, and 61 of the 317 unirradiated samples). These samples required further investigations (see Table 2).

Calibrated measurements were returned from 400 samples (201 irradiated and 199 unirradiated) of which 345 samples were correctly classified. From the 400 samples, 55 determinations (13,8 %) had produced intermediate screening results. After calibration 33 positive results were recorded, confirming the sensitivity to irradiation. This permitted classification of these samples as unirradiated, thus correctly resolving 60 % of the intermediate cases. The remaining 22 intermediate samples (5,5 % of the 400 samples examined here) produced intermediate or negative response to irradiation, and therefore required resolution by another validated or standardized method, such as EN 1788.

The study included four examples of blended mixtures of irradiated spices at 1 %, 5 % and 10 % concentrations in unirradiated spices of matched sensitivity. In this study all blends were correctly identified as containing irradiated material; however, it is recognised that the general problem of detecting minor irradiated components includes variable sensitivity mixtures for which detection performance may be more limited.

Table 2 — PSL screening results from interlaboratory trials of herbs, spices, seasonings and blends

	Irradiated		Unirradiated	
	Correctly identified	False negative	Correctly identified	False positive
Herbs, spices, seasonings and blends[a]	320 (93 %)[b]	1 (0,3 %)[b]	249 (78,5 %)[b]	7 (2,2 %)[b]

[a] A total of 672 samples were distributed to the eight laboratories. PSL screening results were reported for 662 blind samples of herbs, spices, seasonings and blends.

[b] These figures refer to 577 (i.e. 662 minus 85) initial PSL screening results in positive (irradiated) and negative (unirradiated) bands. This does not include intermediate band results.

11 Test report

The test report shall contain at least the following:

a) information necessary for the identification of the sample;

b) a reference to this European Standard;

c) date of sampling and sampling procedure (if known);

d) date of receipt;

e) date of test;

f) the results;

g) length of storage after irradiation and before second PSL measurement;

h) any particular points observed in the course of the test;

i) any operations not specified in the method or regarded as optional which might have affected the results.

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