comisión del codex alimentarius

ORGANIZACIÓN DE LAS NACIONES UNIDAS PARA LA AGRICULTURA Y LA ALIMENTACIÓN

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Tema 6 del programa **CX/MAS 02/7-Add.1**

PROGRAMA CONJUNTO FAO/OMS SOBRE NORMAS ALIMENTARIAS
COMITÉ DEL CODEX SOBRE MÉTODOS DE ANÁLISIS Y TOMA DE MUESTRAS
24ª reunión
Budapest (Hungría), 18 - 22 de noviembre de 2002

RATIFICACIÓN DE LAS DISPOSICIONES SOBRE MÉTODOS DE ANÁLISIS EN LAS NORMAS DEL CODEX
MÉTODOS DE ANÁLISIS PARA LA DETECCIÓN DE ALIMENTOS IRRADIADOS
(Propuestas por la Comunidad Europea)

Métodos generales del Codex para la detección de alimentos irradiados

A petición de la UE, el CCMAS aprobó en su 23ª reunión cinco métodos para la detección de alimentos irradiados que fueron adoptados posteriormente por la Comisión del Codex Alimentarius en su 24ª reunión como métodos generales del Codex. En el curso del procedimiento de aprobación, la Comisión del Codex Alimentarius animó al CCMAS a conceder una mayor importancia a los métodos validados cuya utilización sería conveniente en países en vías de desarrollo.

Normalización de otros métodos

Desde la 23ª reunión del CCMAS, el Comité europeo para la normalización (CEN) homologó 4 métodos adicionales para la detección de alimentos irradiados. Tres de ellos (EN 17351, EN 13783, EN 13784) deberían satisfacer la solicitud antes mencionada de la Comisión del Codex Alimentarius ya que estos métodos pueden aplicarse con material de bajo coste. Además, el CEN actualizó uno de los métodos generales del Codex (EN 1788).

Propuesta

a) La Comunidad Europea desea solicitar al CCMAS que apruebe las siguientes normas como métodos generales del Codex:

- EN 13708:2001 Detección mediante espectroscopia ESR de alimentos irradiados que contienen azúcar cristalino
- EN13751:2002 Detección mediante luminiscencia fotoestimulada de alimentos irradiados
- EN 13783:2001 Detección de alimentos irradiados mediante la utilización de la técnica directa de filtro epifluorescente/recuento en placa aeróbica (DEFT/APC) - método de cribado
- EN 13784:2001 Test cometa del ADN para la detección de productos alimenticios irradiados - método de cribado

b) La Comunidad Europea desea solicitar al CCMAS que actualice el método general del Codex EN 1788:1996 sustituyéndolo por

- EN 1788:2001 Detección de alimentos irradiados a partir de los cuales pueden aislarse minerales de silicato - método por termoluminiscencia.

Anexo: resumen de las 5 normas del CEN (ámbito de aplicación, principios, limitaciones, validación y bibliografía)

ANNEX 1

EN 13708

Foodstuffs — Detection of irradiated food containing crystalline sugar by ESR spectroscopy

Scope

This European Standard specifies a method for the detection of foods containing crystalline sugars 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 [7].

Interlaboratory studies have been successfully carried out on dried figs, dried mangoes, dried papayas and raisins [1] to [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 magnetic field and microwave 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 co-ordination. For further information, see [1] to [7].

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.

Limitations

While the general formation processes of radiation-induced radicals are known, identification of the specific radicals responsible for individual signals has not yet been achieved. Nevertheless, the association between radiation treatment and the signals illustrated in clause 5 and figures A.2 and A.4 has been demonstrated in a number of studies [1] to [7].

Multicomponent ESR spectra prove prior irradiation but the absence of the specific spectrum does not constitute evidence that the sample is unirradiated. Different mono- or disaccharides may dominate in the sample producing different ESR spectra after irradiation. Moreover, if no sugar crystals are present in the sample, irradiation will not produce specific ESR signals.

Detection of irradiated dried figs, dried mangoes, dried papayas and raisins has been validated. The lower limit of detection mainly depends on the crystallinity of the sugar in the sample. Detection of irradiation treatment is not significantly influenced by storage of at least several months.

The applicability of this method depends on the presence of sufficient quantities of crystalline sugar in the sample at all stages of handling between irradiation and testing. Confirmation of sensitivity to radiation can be achieved, where necessary, by irradiating a portion of the sample and re-testing. It is important that dried fruits have not been re-hydrated prior to testing.

Validation

This European Standard is based on two interlaboratory tests, one with dried papayas and raisins, [1], [2], and one with dried mangoes and dried figs [3].

In an interlaboratory test carried out by the Community Bureau of Reference (BCR) [1], [2], 21 laboratories identified coded samples of dried papayas and raisins which were either unirradiated or irradiated to about 0,5 kGy, 1 kGy, 2 kGy, 4 kGy or 7 kGy (see Table 1).

Table 1 – Interlaboratory data

Product	No of samples	No of false negatives[a]	No of false positives[b]
Raisins	126	7[c]	1
Dried papayas	126	2[d]	0

[a] False negatives are irradiated samples identified as unirradiated.

[b] False positives are unirradiated samples identified as irradiated.

[c] Obtained from the 19 samples irradiated at 0,5 kGy.

[d] Obtained from the 21 samples irradiated at 0,5 kGy

In another interlaboratory test carried out by the German Federal Institute for Health Protection and Veterinary Medicine (BgVV) [3], 17 laboratories identified coded samples of dried mangoes and dried figs which were either unirradiated or irradiated to about 1 kGy, 3 kGy or 5 kGy (see Table 2)

Table 2 – Interlaboratory data

Product	No of samples	No of false negatives[a]	No of false positives[b]
Dried mangoes	184	0	0
Dried figs	184	2	0

[a] False negatives are irradiated samples identified as unirradiated.

[b] False positives are unirradiated samples identified as irradiated.

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ANNEX 2

EN 13751

Foodstuffs — Detection of irradiated food using photostimulated luminsecence

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].

Principle

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).

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].

CALIBRATED PSL

For calibration, the sample is exposed to a defined radiation dose after the initial PSL measurement, and then remeasured. 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.

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.

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.

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ANNEX 3

EN 13783 - Foodstuffs — Detection of irradiated food using Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) — Screening method

Scope

This European Standard specifies a microbiological screening method for the detection of irradiation treatment of herbs and spices, using the combined direct epifluorescent filter technique (DEFT) and aerobic plate count (APC). The DEFT/APC technique is not radiation specific, therefore, it is recommended to confirm positive results using a standardised method (e.g. EN 1788, prEN 13751) to specifically prove an irradiation treatment of the suspected food.

The method has been successfully tested in interlaboratory tests with herbs and spices [1] to [5].

Principle

The method is based on the comparison of the APC with the count obtained using DEFT. The APC gives the number of viable microorganisms in the sample after a possible irradiation and the DEFT count indicates the total number of microorganisms, including non-viable cells, present in the sample. The difference between the DEFT count and the APC count in spices treated with doses of 5 kGy to 10 kGy is generally about or above 3 to 4 log units. Similar differences between DEFT and APC counts can be induced by other treatments of the foods leading to death of microorganisms, e. g. heat, thus positive results shall be confirmed.

A known volume of sample is filtered through a membrane filter at reduced pressure in order to concentrate the microorganisms on the filter. The microorganisms are stained with a fluorochrome, acridine orange (AO), resulting in an orange and orange-yellow fluorescence under illumination with blue light at 450 nm to 490 nm. These microorganisms are counted using an epifluorescence microscope to give the DEFT count. However, microorganisms which were non-viable before irradiation show green fluorescence and are not counted. In parallel, the APC is determined from a second portion of the same test sample [6] to [10].

Limitations

A limitation of the method is encountered when there are too few microbes in the sample (APC < 10^3 cfu/g). If fumigation or heat treatment has been used for decontamination, the DEFT/APC-difference of counts can be similar to the difference of counts obtained after irradiation. However, the use of fumigation can be detected.

Some spices such as cloves, cinnamon, garlic and mustards contain inhibitory components with an anti-microbial activity which may lead to decreasing APC (false positive result).

Validation

The combined DEFT/APC method has been applied to herbs and spices, [2], [3]. When samples of allspice, peppers and cardamom were irradiated with a dose of at least 10 kGy, the differences between DEFT and APC were greater than 6 log units for irradiated allspice and white pepper, greater than 4,5 log units for black pepper, and greater than 7 log units for cardamom [2]. When spices such as peppers, paprika, cardamom, cinnamon and ginger and herbs such as thyme, marjoram, basil and oregano were analyzed after irradiation with doses of 5 kGy and 10 kGy, differences between DEFT and APC varied between 3,9 and 6,8 log units and between 5,7 and 7,5 log units, respectively [3].

A BCR collaborative study [1] was conducted including 192 samples of whole allspice, whole and powdered black peppers, whole white pepper, paprika powder, cut basil, cut marjoram, and crushed cardamom unirradiated or irradiated with doses of 5 kGy and 10 kGy and analyzed by eight laboratories. The average values of the differences between DEFT and APC in samples irradiated with doses of 5 kGy and 10 kGy were 5,1 and 6,1 log units, respectively. For all irradiated samples analyzed, the differences between DEFT and APC count generally increased to at least 3,5 log units, whereas the difference in the case of unirradiated spices was insignificant. The reproducibility relative standard deviations for the differences were 12,3 %, 19,9 % and 20,7 % with the doses of 10 kGy and 5 kGy and for unirradiated samples, respectively, indicating acceptable variabilities among laboratories.

The probability of judging an unirradiated sample as irradiated (false positive result) using the limit of 4,0 log units is low [1]. On the other hand, the limit of 4,0 log units will sometimes give false negative result. This has been demonstrated for samples of basil [1] and [11].

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ANNEX 4

EN 13784

Foodstuffs — DNA Comet Assay for the detection of irradiated foodstuffs — Screening method

Scope

This European Standard specifies a screening method for foods which contain DNA. It is based on micro-gel electrophoresis of single cells or nuclei to detect DNA fragmentation presumptive to irradiation treatment [1] to [8]. The DNA Comet Assay is not radiation specific, therefore, it is recommended to confirm positive results using a standardized method to specifically prove an irradiation treatment of the respective food, e.g. EN 1784, EN 1785, EN 1786, EN 1787, EN 1788, EN 13708, and prEN 13751.

Interlaboratory studies have been successfully carried out with a number of food products, both of animal and plant origin such as various meats [9] to [11], seeds, dried fruits and spices [6], [12]. Other studies [13] to

[32] demonstrate that the method is applicable to a large variety of foodstuffs, but also that limitations exist (see clause 8).

Principle

DNA fragmentation can be caused by various chemical or physical treatments including ionizing radiation. When food containing DNA is treated by ionizing radiation, modification of these large molecules occurs including fragmentation either by single- or double-strand breaks. This fragmentation can be studied by microgel electrophoresis of single cells or nuclei. These are embedded in agarose on microscope slides, lysed for disruption of membranes using a detergent and electrophoresed at a set voltage. DNA fragments will stretch or migrate out of the cells forming a tail in the direction of the anode giving the damaged cells the appearance of a comet. This comet assay to measure DNA damage can be carried out under various conditions. Both alkaline and neutral protocols exist. In general, under alkaline conditions both DNA single- and double-strand breaks and alkali-labile sites are measured, whereas under neutral conditions only DNA double-strand breaks are observed. However, using neutral conditions [1] single-strand breaks also exert an influence on the comet appearance, due to relaxation of supercoiled DNA in the nucleus [7], [8]. Irradiated cells will show an increased extension of the DNA from the nucleus towards the anode thus considerably longer comets (more fragmentation) than unirradiated cells. Unirradiated cells will appear nearly circular or with only slight tails (see Figure A.1).

This European Standard describes the use of a simple agarose single-layer set-up employing neutral pH combined with a low voltage and short electrophoresis time.

Limitations

The DNA Comet Assay may, in principle, be applied to detect irradiation of any food containing DNA. The comet assay has already been applied successfully to a number of foodstuffs, both animal foods, e.g. chicken, duck, quail, pheasant, pork, boar, beef, veal, lamb, deer, fish (trout, salmon), and plant foods, e.g. almonds, figs, lentils, soybeans, carioca and macaçar beans, strawberries, grapefruit, linseed, sesame seeds, sunflower seeds, rosé pepper.

It is emphasized that the comet assay is working as a screening test, and the results need to be confirmed by another technique specific for irradiation, since DNA fragmentation may be obtained by other means (see for example mustard seeds as described below in paragraph 8).

Each new type of foodstuff shall be tested before unknown samples are analyzed: lysis solution and treatment time, as well as electrophoresis time and field strength can be changed in order to obtain an adequate migration of DNA in the gel.

Since at present, knowledge of DNA comet patterns for various foodstuffs is still limited, it would be of advantage to use products from different sources to gain experience with the variability of comet patterns. Also the effect of irradiation and storage parameters should be further studied. In addition, the preparation of cell suspensions is an important step since this is a necessary prerequisite for applying the comet assay. For some foodstuffs, e.g. papayas, tamarinds [32], a lot of crude cell debris interferes with the pattern of comets, and some additional steps in the preparation of the cell suspension to arrive at a suitable background are necessary. For some other products, it might be difficult to obtain appropriate cells, e.g. for some nuts, some spices, some fish [31] and [32]. For heated foods, no cells will be available due to extensive heat damage. Thus, no appropriate cells can usually be found for blanched shrimps or for cooked (microwaved) chicken [6].
In the case of meat, it should be recognized that depending on storage conditions (temperature and time between slaughter and freezing) of the fresh meat, a natural (enzymatic) degradation of DNA takes place, producing DNA fragmentation [33]. The comets in this case are of varying shapes. The presence of many cells showing advanced DNA degradation might be only a sign of poor storage conditions. Also, repeated freeze-thawing of meat produces extensive DNA damage (fragmentation). The patterns are similar to those obtained from natural DNA degradation and can usually be distinguished from irradiated samples which give a homogenous pattern [6]. Only if all the cells show advanced DNA degradation one can not determine whether the sample was irradiated or not. This is a clear limitation of the comet assay.

Another limitation may be insufficient lysis of the cells to make membranes permeable, this permeability enabling the DNA fragments to migrate out of the nuclei. In earlier experiments [4], [5], [15], [17], [19], [20] insufficient lysis was experienced using an SDS concentration of only 0,1%. By increasing the SDS concentration to 2,5% this problem was overcome at least for animal cells [6]. For some plant cells the duration of lysis time had to be increased, e.g. up to 30 min to 60 min for soybeans or grapefruits [6], [26],

[30]. For some other plant products, e.g. mushroom spores from *Agaricus bisporus*, lysis of the cell wall was not achieved [6], [19], and therefore, the comet assay could not be applied. Probably, insufficient lysis is also the reason for intact cells being observed in irradiated samples, such as dried gram, sliced almonds [28], red kidney beans and tamarinds [32]. Stronger lysis solutions or longer duration of incubation might alleviate this problem.

For some food items cell preparations could be obtained but difficulties arose to differentiate between unirradiated and irradiated samples, e.g. for some nuts, some seeds or some spices [31]. Difficulties were also observed for food items to be irradiated with low radiation doses, such as onions and potatoes [6] and [32]. Possibly, the use of sophisticated image analysis may help with identification, but just by visual inspection the judgement whether the sample was irradiated or not was doubtful.

A special case was observed for mustard seeds, which showed a comet pattern corresponding to an irradiated sample with clear comets and no intact cells. This sample was tested by thermoluminescence (EN 1788), but shown to be unirradiated. Also the germination capacity of the mustard seeds indicated no irradiation treatment [6]. Similar problems were encountered with a millet sample. Although only comets could be observed in the sample, other detection methods like thermoluminescence measurements (EN 1788) or hydrocarbon formation from the lipid part of the food (EN 1784) identified the sample as unirradiated [34]. Probably the state of the seeds, whether in dormancy or not, may play a role.

Validation

The procedure as described in this European Standard is based on interlaboratory studies with animal (chicken, pork) [9] to [11] and plant foods [6], [12] as well as on studies with a number of food items [13] to [32].

In an interlaboratory test organized on behalf of the Swedish National Food Administration, nine participating laboratories analyzed three kinds of coded cell suspensions made of irradiated and unirradiated chicken bone marrow, chicken and pork muscle tissue. The radiation doses varied between 0 kGy, 1 kGy, 2,5 kGy, 3 kGy, and 5 kGy. From a total of 162 samples dispatched, valid results were reported from 148 samples. Of these, 138 were correctly identified. From 106 irradiated samples, 99 were correctly detected, while 39 out of 42 unirradiated were correctly classified, see Table 1 [11].

Table 3 — Interlaboratory data for chicken and pork

Product	Total No. of samples	No. of samples (valid results)[a]	No. of correct identifications	No. of false negatives[b]	No. of false positives[c]
Chicken bone marrow	54	54	52	1	1
Chicken muscle	54	46	42	3	1
Pork muscle	54	48	44	3	1
All	162	148	138	7	3

[a] no results reported for lacking samples.

[b] false negatives are irradiated samples identified as unirradiated.

[c] false positives are unirradiated samples identified as irradiated.

It should be recognized that some laboratories did not have much experience with the comet assay at the time of this collaborative trial. Although each laboratory received a set of reference samples made from chicken bone marrow irradiated with 0 kGy, 1 kGy, 3 kGy or 5 kGy and labelled with the given doses, difficulties with this new method were experienced in some laboratories. However, six laboratories successfully identified all the samples correctly.

A further collaborative trial [6] was conducted with a variety of plant items, namely almonds, figs, lentils, linseed, rosé pepper, sesame seeds, soybeans and sunflower seeds. The coded samples were either unirradiated or irradiated with doses of 0,2 kGy, 1 kGy or 5 kGy. In addition to the 20 coded samples, the participants

received a reference set of 12 samples with known radiation dose. Four laboratories participated in this intercomparison. Of the total 78 answers received, 74 were correct (95%). The results are shown in Table 2.

Table 4 — Interlaboratory data for plant cells tested (for 10 months of storage after irradiation)

Sample	Total No. of samples	No. of samples (valid results)[a]	No. of correct identification	No. of false negatives[b]	No. of false positives[c]
Unirradiated	32	31	29	0	2
Irradiated	48	47	45	2	0
All	80	78	74	2	2

[a] One laboratory did not provide results with soybeans, since it had problems with the lysing conditions.

[b] False negatives are irradiated samples identified as unirradiated.

[c] False positives are unirradiated samples identified as irradiated.

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EN 1788, *Foodstuffs - Thermoluminescence detection of irradiated food from which silicate minerals can be isolated.*

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ANNEX 5

EN 1788

Foodstuffs — Thermoluminescence detection of irradiated food from which silicate minerals can be isolated

Scope

This European Standard specifies a method for the detection of irradiation treatment of food and/or food ingredients by thermoluminescence analysis of contaminating silicate minerals. This method is applicable to those foodstuffs from which a sufficient amount of silicate minerals can be isolated.

The method has been successfully tested in interlaboratory tests with herbs and spices as well as their mixtures [1] to [3], shellfish including shrimps and prawns [4] to [6], both fresh and dehydrated fruits and vegetables [7] to [9], and potatoes [10]. Other studies [11] to [46] demonstrate that the method is applicable to a large variety of foodstuffs.

1 Principle

Silicate minerals contaminating foodstuffs store energy by charge trapping processes as a result of exposure to ionizing radiation. Releasing such energy, by controlled heating of isolated silicate minerals, gives rise to measurable TL glow curves.

Silicate minerals are therefore isolated from the foodstuffs, mostly by a density separation step. In order not to obscure the TL, the isolated silicate minerals should be as free of organic constituents as possible. A first glow of the separated mineral extracts is recorded (Glow 1). Since various amounts and/or types of minerals (quartz, feldspar etc.) exhibit very variable integrated TL intensities after irradiation, a second TL glow (Glow 2) of the same sample after exposure to a fixed dose of radiation is necessary to normalize the TL response.

The TL glow ratio, thus obtained, is used to indicate radiation treatment of the food, since the population of irradiated samples on principle yields higher TL glow ratios than that of unirradiated samples. Glow shape parameters offer additional evidence for identifying irradiated foods. This method of TL analysis relies solely on the silicate minerals which can be separated from various foods and is not principally influenced by the kind of food product.

Limitations

This method of TL analysis can, in principle, be applied to detect irradiation of any food from which silicate minerals can be isolated. Detection limits and stability of the method depend on the quantities and types of minerals recovered from individual samples, and the glow temperature intervals selected for analysis. Minerals from unirradiated samples show a residual geologically derived TL signal with maximum intensity at glow curve temperatures above 300 °C, and minor components in the 200 °C to 300 °C region which can influence

detection limits. The stability of TL signals is strongly influenced by glow curve temperature and is greater for higher temperatures. For temperatures from 200 °C to 250 °C TL signals are stable for many years.

The method has been validated with samples which have either been wholly irradiated or unirradiated. In cases where irradiated and unirradiated products are mixed or blended, the outcome of the analysis depends on the relative sensitivities of the irradiated and unirradiated components.

Detection of irradiated herbs, spices and their mixtures has been validated for doses of approximately 6 kGy, and above, and timescales up to nine months covering the range of commercial applications [1] to [3]. Other studies [11] to [13], [15] to [17], [19], [20], [21], [23] to [27], [29] to [34], [36], [37], [40] to [43] have shown that the method may be applied to doses above 1 kGy, and timescales up to several years.

Detection of irradiated shellfish, has been validated for the range of 0,5 kGy to 2,5 kGy and for time scales covering the shelf life of commercial applications [4] to [6]. Other studies [19] to [23], [27], [31], [35], [39], [42] have shown the applicability of TL analysis to shellfish.

Detection of irradiated fresh and dehydrated fruits and vegetables has been validated for doses of about 1 kGy for fresh fruits and vegetables [7], [8] and a radiation dose of about 8 kGy for dehydrated fruits and vegetables [9]. Other studies [11] to [14], [17] to [21], [23], [25], [27], [28], [31], [38], [42] have shown the applicability of TL analysis to fruits and vegetables.

In some cases problems may arise due to a limited amount of silicate minerals present on the samples. In one interlaboratory test [8], participating laboratories were only able to obtain valid results on 97% of strawberries, 82% of avocados, 48% of mushrooms, 83% of papayas and 95% of mangoes, due to a restricted amount of sample and consequently minerals. In practice, larger sample volumes will mostly overcome these problems.

A similar problem occurred in another interlaboratory test on fresh fruits and vegetables [7] and in one on dehydrated fruits and vegetables [9]. In the latter study, particularly, the apple samples showed very low mineral contents. Sufficiently materials could only be obtained from 75% of the samples.

It should be recognized that irradiation of fresh fruits and vegetables for disinfestation purposes occur at lower dose levels than those used in the present interlaboratory tests [7], [8]. In this special case a similar procedure as for potatoes [10] can be adapted (see 8.4.4).

Detection of irradiated potatoes has been validated for radiation doses as low as about 50 Gy about four months after irradiation [10]. Other studies [7], [11], [13], [15], [18], [23], have shown the applicability of TL analysis to potatoes. One of these studies [15] has shown that detection of irradiated potatoes is possible during the whole shelf life.

As mineral debris occur ubiquitously on all foodstuffs which have been exposed to wind and soil, all kinds of agricultural products may be evaluated by TL. In addition to the above mentioned produce, also bulbs such as onion and garlic [18], [23], cereals [44] and pulses [45], [46] have been tested by TL.

Validation

The procedure as described in this European Standard is based on interlaboratory studies with herbs, spices, their mixtures [1] to [3], shellfish [4] to [6], fresh and dehydrated fruits and vegetables [7] to [9], and potatoes [10] as well as on studies with other food items [11] to [46].

In the case of herbs and spices, the method was tested in a small preliminary intercomparison organized by the Community Bureau of Reference (BCR) with six participating laboratories, each of which analysed 12 irradiated and unirradiated herbs and spices [1].

In another, larger interlaboratory test organized by the former German Federal Health Office (Bundesgesundheitsamt, BGA, successor institute: Federal Institute for Health Protection of Consumers and Veterinary Medicine, BgVV), 14 participants tested 18 different herbs and spices or mixtures three and/or nine months after irradiation with a dose of about 6 kGy or 11 kGy respectively. Of a total of 317 samples examined, 99,1% were correctly identified. Only three irradiated samples were classified as unirradiated. None of the unirradiated samples were classified as irradiated [2], [3].

In an interlaboratory test organized by the German BgVV, 23 participating laboratories analyzed coded shrimp samples, namely Vietnam Cat Tiger and China Reds, which were either unirradiated or irradiated with doses of 1 kGy or 2 kGy. Of a total of 125 samples examined, 123 samples were identified correctly. Two

samples irradiated with 1 kGy were identified as unirradiated using a fixed threshold value of 0,50 for the TL glow ratio. If the glow curve shape had been considered additionally to the TL ratio, all samples would have been identified correctly [4], [5].

In an interlaboratory test with shellfish organized on behalf of the British Ministry of Agriculture Fisheries and Food (MAFF), seven participating laboratories analyzed five species, namely prawns (Norway lobsters), black tiger prawns, brown shrimps, mussels and king scallops. The coded samples were either unirradiated or irradiated with doses of 0,5 kGy or 2,5 kGy. From a total of 105 samples, adequate amounts of silicate minerals could be isolated from 103 samples, and these 103 samples were all identified correctly [6].

In an interlaboratory test with fruits and vegetables organized on behalf of the British MAFF, nine participating laboratories analyzed five types of fruits and vegetables, namely strawberries, avocados, mushrooms, papayas and mangoes. These were presented for blind analysis in three conditions: unirradiated, irradiated to 1 kGy, and irradiated to 1 kGy and optically bleached. From a total of 405 samples, valid results were obtained from 327 samples, all of which were identified correctly. The remaining 78 samples did not yield adequate amounts of silicate minerals [8].

In an interlaboratory test with dehydrated fruits and vegetables organized by the French Centre Technique de la Conservation des Produits Agricoles (CTCPA), eight participating laboratories analyzed five kinds of dehydrated fruits and vegetables, namely apple cubes, sliced carrots, leeks and onions, and powdered asparagus. The coded samples were either unirradiated or irradiated with a dose of about 8 kGy and analyzed six months after irradiation. From a total of 240 samples, adequate amounts of silicate minerals could be isolated from 220 samples. Participants were asked to apply fixed thresholds to the TL glow ratio; considering the sample as irradiated at a value higher than 0,5 and unirradiated at a value lower than 0,1, whereas samples with TL glow ratios between 0,1 and 0,5 were considered as in doubt. 202 samples of the 220 samples were identified correctly, two unirradiated samples were deemed irradiated, (probably due to miscoding), and 16 samples were classified as in doubt or yielded inconsistent results [9].

In an interlaboratory test organized by the German BgVV, 22 participating laboratories analyzed coded potato samples which were either unirradiated or irradiated with doses of about 50 Gy, 160 Gy or 310 Gy. Applying the identification criteria according to clause 9, 216 samples out of a total number of 220 samples were identified correctly. Two samples had to be excluded due to inconsistent results, whereas one unirradiated sample was identified as irradiated and one irradiated sample was identified as unirradiated [10].

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