SLOVENSKI STANDARD
SIST EN 1788:1998

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Živila - Detekcija obsevane hrane, iz katere lahko izoliramo silikatne minerale - Metoda s termoluminescenco

Foodstuffs - Detection of irradiated food from which silicate minerals can be isolated - Method by thermoluminescence

Lebensmittel -Nachweis von bestrahlten Lebensmitteln, von denen Silikatmineralien isoliert werden können - Verfahren mittels Thermolumineßenz

Produits alimentaires - Détection des aliments ionisés dont les minéraux silicatés peuvent etre isolés - méthode par thermoluminescence

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English version

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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 supported by 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of an identical text or by endorsement, at the latest by June 1997, and conflicting national standards shall be withdrawn at the latest by June 1997.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 irradiation treatment of food 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] and shrimps [4], [5]. From other studies, including an interlaboratory test on fresh fruits and vegetables, it may be concluded that the method, after suitable modification, is applicable to a large variety of foods including sea-food [6] to [38]. At present, this standard pertains only to the detection of the irradiation treatment of herbs, spices, their mixtures and shrimps.

2 Normative references

This European Standard incorporates by dated and undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this draft European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

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

3 Definitions

For the purposes of this standard, the following definitions apply:

3.1 Thermoluminescence (TL): The light emission which occurs on heating a solid material in addition to black body radiation, due to the thermally stimulated release of trapped charge carriers.

3.2 TL intensity: The amount of light detected per unit temperature interval at a given heating rate. The integrated TL intensity over a stated temperature interval is measured in photon counts or coulombs.

3.3 Glow curve: The variation of TL intensity with temperature. The integral of the glow curve is expressed in counts or coulombs depending on the apparatus used.

3.4 Glow 1: The glow curve recorded from the minerals of the prepared sample as received.

3.5 Glow 2: The glow curve recorded from the minerals of the prepared sample after measurement of Glow 1 and a subsequent exposure to a fixed known dose of radiation for the purpose of normalization.

3.6 TL glow ratio: The ratio of integrated TL intensities of Glow 1 to Glow 2, evaluated over a stated temperature interval.

3.7 Minimum Detectable integrated TL- intensity Level (MDL): The full process blank level (Glow 1) plus three standard deviations (full process blank levels should be measured in parallel with sample extractions using portions of the same stock solutions and following the procedure in all stages) defines the MDL, which should be consistent with freedom from contamination of discs, glassware and reagents.

3.8 Background of the TL reader: Integrated TL intensity measured without sample disc over the whole temperature range studied.

4 Principle

Silicate minerals contaminating food products 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 food products, mostly by a density separation step. In order not to obscure the TL, the isolated silicate minerals should be as free as possible of organic constituents of the food. A first glow of the separated mineral extracts is recorded (Glow 1). Since various amounts 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 the irradiation 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 only on the silicate minerals which can be separated from various foods and is principally not influenced by the kind of food product.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade. Water shall be of at least grade 3 in accordance with EN ISO 3696. All reagents shall be kept free from particulate contamination throughout the procedure.

5.2 Sodium polytungstate $Na_6[H_2W_{12}O_{40}] \times H_2O$ solution with a density of 2 g/ml. The solution may be recovered and purified for re-use [2].

5.3 Hydrochloric acid, c[1]) (HCl) = 1 mol/l

5.4 Ammonium hydroxide solution, c (NH_4OH) = 1 mol/l

5.5 Acetone

5.6 Nitrogen gas, oxygen free, for flushing the TL heating chamber

5.7 Silicone spray (optional)

5.8 Ethanol

6 Apparatus

6.1 General

All laboratory surfaces and glassware should be carefully cleaned.

Use usual laboratory apparatus and, in particular, the following:

6.2 TL reader with glow curve recording facility and data evaluation; heating rate: about 6 °C/s; maximum temperature required: at least 350 °C; equipped with a suitable photomultiplier tube, e. g. a bi-alkali photocathode photomultiplier tube, in combination with filters to reject black body radiation. Acceptable filter combinations are Corning 7/59® + Schott KG 1® filters or Schott BG 39®[2]), or equivalent.

6.3 Stainless steel discs or shallow cups having a diameter to suit the TL reader (usually about 9 mm up to 10 mm) and a thickness of 0,25 mm up to 0,5 mm.

6.4 Radiation source, capable of irradiating samples with a defined radiation dose before measurement of Glow 2. In the interlaboratory tests on herbs, spices, their mixtures and shrimps [1] to [5], various sources delivering ^{60}Co-γ-rays have been employed at a fixed radiation dose of 1 kGy.

NOTE 1: Other fixed doses may be suitable.

NOTE 2: Alternatives to ^{60}Co-γ-rays may be used, provided they have been found satisfactory.

6.5 Ultrasonic bath, capable of fitting several beakers of 150 ml.

6.6 Nylon disposable sieves, comprised of e.g.:

6.6.1 Mini-sieve set (51 mm in diameter), consisting of 2 rings between which the nylon sieve cloth is clamped.

6.6.2 Nylon sieve cloth with pore sizes of 125 μm and 250 μm.

6.7 Centrifuge, supplied with a swing-out rotor and suitable glass tubes, e.g. of 10 ml to 15 ml capacity with pointed bottom; providing a centrifugal acceleration of about 1000 g at the outer end of the tubes.

6.8 Vortex for centrifuge tubes (optional)

6.9 Vacuum pump (optional)

6.10 Laboratory oven, set to (50 ± 5) °C.

7 Sampling technique

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

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[1]) c is the substance concentration

[2]) Corning 7/59®, Schott KG 1® and Schott BG 39® are examples of suitable products available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of these products.

Before analysis, samples should be protected against light exposure. Store them in the dark. Avoid exposure of samples to temperatures in excess of 100 °C, since heating reduces TL intensity.

8 Procedure

8.1 General

Several procedures for mineral separation may be used, e.g. handpicking, rinsing by water, density separation and/or acid hydrolysis. It has to be ascertained that the mineral isolation procedure does not affect the qualitative classification as to whether the food has been irradiated or not, when compared to the procedures described below which have been found satisfactory.

The isolated silicate minerals should be free of organic material. The presence of organic matter could induce spurious (non-radiation induced) luminescence, or in extreme cases could obscure TL. Samples with organic residues are blackened by the TL measurement process.

Furthermore, during isolation the minerals should be protected against light exposure, i.e. not exposed to strong light or unnecessarily kept exposed to light, to prevent optical bleaching. It may be favourable to use subdued light conditions. Some authors prefer to work under safelight conditions. However, the interlaboratory tests with herbs, spices, their mixtures and shrimps [1] to [5] demonstrate that protection from strong light under usual laboratory conditions may prove satisfactory.

The amount of silicate minerals required for TL analysis, is in the range of 0,1 mg to 5 mg.

The lowest amount of minerals depends on the outcome of Glow 2. The lowest acceptable integrated TL intensity for Glow 2, shall be at least 10 times the MDL, see also 8.4.6.

8.2 Isolation of silicate minerals from food

8.2.1 Preconcentration step of minerals

8.2.1.1 Herbs, spices and their mixtures

Preconcentration of minerals by wet sieving is recommended for most samples using the following procedure. If enough minerals can be isolated just by collection or washing, proceed immediately to the density separation step (as described in 8.2.2).

Suspend 3 g to 20 g of the sample (depending on the degree of mineral contamination) in a 100 ml to 150 ml glass beaker with 50 ml to 100 ml water added.

Treat the sample in the beaker with ultrasound for about 5 min (to shake loose the adhering minerals).

Sieve the sample in portions through a 250 μm nylon mesh (for coarse samples like aromatic herbs) or through a 125 μm nylon mesh (6.6) (for fine samples like ground spices) into a large beaker (e.g. 500 ml to 1000 ml), rinsing the minerals through with water each time e.g. using a strong jet of water from a wash bottle. Discard the constituents retained by the sieve cloth. Use a fresh nylon sieve cloth for each sample. Allow to settle for about 5 min.

Decant most of the water from the large beaker together with as much organic material as possible, leaving the minerals in only a few millilitres of water. If there are still fairly large amounts of organic material left, add water to a depth of 1 cm to 2 cm, swirl, wait for about 5 s to 10 s to let the minerals settle again and then decant again. Repeat this step until only small amounts of organic material are left together with the minerals.

Transfer the mineral fraction to a centrifuge tube (6.7), e.g. using a Pasteur pipette.

Centrifuge for 1 min at 1000 *g*. Alternatively allow sedimentation for 5 min. Decant off or remove the water by suction, leaving the mineral fraction behind.

8.2.1.2 Shrimps

NOTE: Minerals can be associated with various parts of shrimps including the intestines. Intestinally entrained minerals are by preference selected for analysis. The intestine shines through the skin on the convex side of the animals as an 1 mm to 2 mm broad dark tube.

Cut the skin carefully using a scalpel and remove the intestine by means of forceps. Transfer intestines of several animals to a Petri dish and cut with a scalpel. After adding some drops of water, separate minerals from intestinal membranes. Transfer minerals by means of a Pasteur pipette to a suitable, e. g. 10 ml to 15 ml centrifuge glass tube.

Centrifuge for about 1 min at 1000 *g*. Alternatively allow sedimentation for 5 min.

Decant off or remove the water by suction, leaving the mineral fraction behind.

8.2.2 Density separation step to free the minerals from organic material.

To the mineral fraction in the centrifuge tube (6.7) add 5 ml of sodium polytungstate solution (5.2). Shake vigorously (Vortex) and agitate in an ultrasonic bath for about 3 min. (In some cases the pre-concentration step for herbs, spices and their mixtures (8.2.1.1) can be omitted. In this case place 0,5 g to 1 g of the sample in a suitable centrifuge tube, e. g. 15 ml, and add about 5 ml of polytungstate solution. Shake vigorously for a short time (Vortex), and agitate with ultrasound for about 5 min to 15 min.)

Centrifuge for 2 min at 1000 *g*. Silicate minerals (density 2,5 g/ml to 2,7 g/ml) will sediment whereas organic components will float.

Fill up the tube with water to facilitate removal of the organic material. Extract the upper water layer and the organic material either by decantation or vacuum suction, leaving the minerals behind in the lower polytungstate layer. If necessary, clean the tube side by wiping with a small moist tissue. If not all organic material is removed, fill the tube with water again and repeat extraction.

Extract the sodium polytungstate layer, being careful to leave the mineral fraction behind. If too much organic material is still present, again add sodium polytungstate solution and repeat the procedure.

The sodium polytungstate solution may be collected and purified for re-use [2].

Wash the minerals twice to remove the tungstate residues by filling the tube with water, allow the minerals to settle or centrifuge shortly at 1000 *g* and remove the water.

To dissolve carbonates adhering to the silicate minerals, add 1 ml to 2 ml of hydrochloric acid (5.3), agitate, and leave for 10 min in the dark. If necessary, increase the amount or concentration of hydrochloric acid.

Neutralize the acid using the ammonium hydroxide solution (5.4), fill up the tube with water, allow the minerals to settle or centrifuge shortly. Remove the water and wash the mineral residue twice with water.

To displace the residual water, add about 3 ml of acetone (5.5) and agitate. If the acetone becomes turbid, remove it and add fresh acetone.

8.3 Fixing the minerals on discs for TL measurement

Carefully clean stainless steel discs (6.3), e. g. by rinsing in water, ultrasonic agitation, several washings in acetone, a second ultrasonic treatment, drying in an oven, and storage under dust free conditions. (The cleaning procedure may be checked as described in annex A.)

Transfer the isolated minerals in acetone to a disc using a Pasteur pipette. After suction of the mineral suspension into the pipette, the minerals will immediately sediment to the outlet of the pipette and can then be easily transferred dropwise (allow the acetone to evaporate in between) in an adequate amount to the disc. Store the discs overnight at 50 °C in a laboratory oven (6.10).

As an alternative to dropping minerals on the disc, transfer the mineral suspension in acetone to one or a set of clean flat bottomed tubes each containing a clean stainless steel disc. Place these tubes upright in a laboratory oven at 50°C overnight. The acetone will dry off, leaving a deposit of minerals adhering to the discs.

The deposit of minerals may be fixed on the disc by using silicone spray (5.7).

8.4 Thermoluminescence (TL) measurements

8.4.1 General

For comparison of different analyses, identical measuring conditions should be assured. Measure the background of the TL reader regularly and ensure that it remains at the same level. Clean the infra-red barrier filter and the heating plate (planchet) regularly with ethanol (5.8).

To reduce spurious TL, flush the TL heating chamber with nitrogen (5.6), at a constant flow rate during each measurement.

8.4.2 Measurement conditions

The following instrument settings have been found satisfactory.

Initial temperature:	70 °C
Heating rate:	6 °C/s
Final temperature:	350 °C to 500 °C

8.4.3 Measurement of Glow 1

Place the disc with the mineral deposit (as prepared in 8.3) on the heating plate of the TL reader (6.2), and glow it under the specified conditions.

8.4.4 Irradiation for the purpose of normalization

After measurement of Glow 1, irradiate the discs with the mineral deposit with a defined radiation dose using the radiation source (6.4).

With herbs, spices, their mixtures and shrimps which are irradiated in commercial practice for decontamination purposes with radiation doses at or above 1 kGy, the interlaboratory tests have shown that a fixed radiation dose of about 1 kGy with a ^{60}Co-γ-source proves satisfactory [1] to [5]. It shall be noted that the classification criteria and TL limits (8.4.7) depend on the radiation dose used for normalization.

NOTE: Some studies indicate that suitable alternatives to ^{60}Co-γ-rays may be used, see e.g. [2], [15], and [19].

The applied radiation dose for normalization should be controlled by adequate dosimetry.

The discs should be packed in a manner which protects them from loss of material, exposure to light or cross contamination. It is essential that the minerals on the discs to be irradiated, and subsequently measured for Glow 2, are identical with the minerals measured during Glow 1. If significant loss of minerals occurs, the discs should be rejected. This may be checked by visual inspection or by weighing of the discs.

After irradiation of the discs, store them overnight at 50 °C in the laboratory oven (6.10) before recording Glow 2.

8.4.5 Measurement of Glow 2

Measure Glow 2 under the same conditions as Glow 1 (8.4.2).

8.4.6 Estimation of MDL

Measure full process blank levels in parallel with sample extractions using portions of the same stock solutions and following the procedure in all stages. Calculate the MDL in accordance with 3.7. Contamination will be indicated by higher blank levels.

8.4.7 TL limits for Glow 2

Reject any sample with a Glow 2 lower than 10 times the MDL, evaluated over a stated temperature interval. In this case no assessment can be made of whether irradiation treatment of the food product has occurred.

If the TL of Glow 2 approximates the counting saturation limit, reject the sample and repeat the analysis using a smaller amount of minerals. Alternatively, a restrictive aperture or a neutral density filter to reduce count rate may be effective (both for Glow 1 and Glow 2).

9 Evaluation

Base the identification of irradiated foods by TL analysis on the value of the TL glow ratio (3.6), evaluated over a recommended temperature interval. In addition, shapes of glow curves offer support for identification.

The recommended temperature interval for evaluation of the TL glow ratio is in the range of 150 °C to 250 °C. Practically, temperature intervals may be defined by evaluating the glow curve of a well characterized phosphor like feldspar or lithium fluoride (see annex B).

A temperature interval comprising ± 10 °C up to ± 40 °C around the optimal temperature may be chosen. The absolute temperature scale can be determined in the TL reader using calibrated thermocouples. The optimal temperature is a function of post-irradiation delay and storage temperature.

Calculate the integral of Glow 1 and Glow 2 over the recommended temperature interval (see 8.4.7), and the TL glow ratio (3.6).

TL glow ratios from irradiated samples are typically greater than 0,5, whereas those from unirradiated samples are generally below 0,1.