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⟨2250⟩ DETECTION OF IRRADIATED DIETARY SUPPLEMENTS

INTRODUCTION

Federal regulations do not permit irradiation of dietary ingredients or dietary supplements for sanitation purposes. Under section 201(s) of the Federal Food, Drug, and Cosmetic Act [21 USC 321(s)], irradiation is considered an additive, and as such it requires FDA approval. Foods that are irradiated should be adequately labeled according to international and national guidelines with statements such as “Treated with radiation” or “Treated by irradiation” in addition to information required by other regulations, including the irradiation logo, the Radura [21 USC 321(s)]. Overexposure to irradiation may have negative effects on product quality. Currently, several independent methods are used to identify irradiated foodstuffs, including dietary supplements. These methods have been validated and are recognized worldwide.

Procedures based on luminescence are widely applied and include screening by photostimulated luminescence (PSL), which is a rapid, simple preliminary screening method to detect irradiation, and a subsequent thermoluminescence (TL) procedure to confirm that the sample has been irradiated. PSL is less time consuming than TL because the inorganic/silicate mineral source of luminescence does not need to be isolated from any organic components present. Both PSL and TL signal intensities are affected by irradiation dose as well as by the nature and amount of inorganic material. TL analysis is one of the detection methods used for confirming the presence of irradiated foods, herbs, spices, vegetables, and fruits, although it has certain limitations: for example, samples must contain sufficient amounts of silicates that can be successfully separated from the samples. The lengthy preparation and need for irradiation in all cases limits TL to a small number of laboratories.

The procedures described in this chapter can be used both by regulatory authorities and by producers and suppliers of foods, including dietary supplements, to detect undeclared irradiated products in the market for purposes of determining compliance with regulations.

PRINCIPLES OF PHOTOSTIMULATED LUMINESCENCE AND THERMOLUMINESCENCE

Most of the natural dietary ingredients that are either cultivated or wild contain silicate minerals, calcite, or hydroxyapatite. Exposure to ionizing radiation from gamma rays (^{60}Co or ^{137}Cs), electron beams (up to 10 MeV), or x-rays (up to 5 MeV) stimulates electrons in those types of crystals, resulting in energized electrons being stored in the crystal lattice. Trapped high-energy electrons can be released by stimulation with light or controlled heat, leaving electron holes in the crystal lattice. The energy thus released is detected as luminescence. PSL occurs if light is applied to the system, and TL occurs when heat is applied to the system. The recorded luminescence intensity is proportional to the initial radiation dose absorbed.

Samples for both PSL and TL measurements must be taken from a light-protected position of the bulk samples, and exposure to high temperature and light must be avoided after the samples are taken from the bulk. Sample preparation and subsequent PSL or TL measurement must be conducted under subdued lighting conditions when possible.

TL has been widely used for detection of irradiation in food and dietary ingredients from which silicate minerals can be isolated. In this chapter, PSL and TL are described as the most appropriate detection methods for the materials typically used as dietary ingredients. The two methods are both radiation-specific phenomena, based on the observation that photon counts of irradiated samples usually are higher than those of nonirradiated samples. However, all available methods have some limitations in terms of their specific application range, product-to-product variation, complexity of or interference from the food matrix, and low concentration of radiation-induced markers.

GENERAL PROCEDURE FOR DETECTION OF IRRADIATION IN DIETARY INGREDIENTS AND DIETARY SUPPLEMENTS

Screening Using Photostimulated Luminescence

PSL, also known as optically stimulated luminescence, is based on the emission of light in the 300–600 nm range from irradiated samples when illuminated at longer wavelengths in the near-IR region. PSL analysis allows multiple measurements to be performed without sample pretreatment in a short period of time. PSL is also a nondestructive method that does not require separation of inorganic minerals and organic compounds. PSL is based on optical stimulation of mineral debris, typically silicates, and bioinorganic materials such as calcite, feldspar, or hydroxyapatite, and its sensitivity depends on the quantities and types of minerals present in the sample. Before the measurement of PSL, two thresholds are set: the lower typically is set at 700 counts/min (T1), and the upper typically is set at 5000 counts/min (T2). These two thresholds serve to classify the samples. After initial screening of PSL intensity from the samples is performed, the results are classified into negative (counts/ min less than T1), intermediate (counts/min NLT T1 and NMT T2), or positive (counts/min more than T2). A second measurement after a known irradiation dose, known as calibrated PSL (CalPSL), is applied in cases of poorly defined sample matrices whose luminescence sensitivities are not well established. By comparing the screening PSL measurement against its CalPSL response, analysts can take into account variation in detection sensitivity due to variable amounts and different types of minerals present in a given sample. CalPSL measurements are recommended to rule out false negative results due to low mineral content.

INSTRUMENTATION

The PSL system consists of pulsed IR sources for photostimulation, a single-photon counting system for highly sensitive detection of luminescence, a sample chamber, and a computer for data analysis.

The instrumental setup procedure includes checks of irradiated and nonirradiated materials, as well as establishing measurement parameters (cycle time, thresholds, and data-recording conditions). Measurement of initial background counts and periodic measurement of counts in the empty chamber should be conducted in subdued lighting to confirm lack of instrument contamination. The PSL signals (photon counts) emitted from the sample/second are automatically accumulated in the computer and are reported as counts/min. [Note—All experiments should be conducted under subdued lighting. In order to minimize the risk of cross-contamination, all preparations are performed in a laminar-flow cabinet.]

PHOTOSTIMULATED LUMINESCENCE MEASUREMENT

Procedure

PSL screening—Dispense and weigh two portions of the sample (5–10 g, depending on the density of the product) into two separate 50-mm disposable Petri dishes to cover the Petri base in a thin layer. Dispense samples in subdued lighting to minimize bleaching and under a laminar-flow cabinet to minimize cross-contamination. Measure the PSL of the sample for 1 min on the duplicate aliquots, and calculate the mean.

Calibrated PSL (CalPSL)—[Note—CalPSL testing may be done only once as part of the validation of the procedure for each material.]

Dispense and weigh two portions of the sample (5–10 g, depending on the density of the product) into 50-mm disposable Petri dishes to cover the Petri base in a thin layer. Measure the PSL of the sample for 1 min on duplicate aliquots, and for each sample calculate the mean. Irradiate the sample to a known dose of 1 kGy after the initial screening. Repeat the PSL measurement (CalPSL).

Evaluation—Classify results in the following manner:

<T1: negative, no evidence of PSL
>T1 and <T2: intermediate, weak PSL signal
>T2: positive, stronger signal.

Acceptance criteria—Nonirradiated samples produce negative results in the PSL screening and are known to produce positive results in the CalPSL. [Note—Negative signals in the PSL screening are generally associated with nonirradiated material but can result from low-sensitivity irradiated materials. To evaluate whether a sample is a low-sensitivity material, assessment of the signals using the CalPSL option is necessary. Positive signals in the PSL screening are associated with irradiated material. Samples that are classified as intermediate require further investigation by the TL method to determine their irradiation status.]

Confirmatory Analysis Using Thermoluminescence

The TL analysis is based on physical changes in silicate minerals that are present in many food samples and dietary ingredients. The silicate minerals are able to store the absorbed radiation energy. A TL reader measures the amount of light that is emitted during controlled heating. The TL of the sample (TL_1) is compared to that of the same sample following irradiation at 1 kGy (TL_2). If the TL ratio (TL_1/TL_2) is greater than 0.1, the sample is considered to be irradiated. In order to use the TL method for the detection of irradiated foods or dietary ingredients, silicate minerals must be isolated from the samples.

INSTRUMENTATION

TL measurements can be performed with a TL detector that meets the specifications in Table 1.

Table 1. Thermoluminesence Detector Specifications[a]

Radiation[b]	Photon: energies >5 keV Neutron: thermal to 100 MeV Electron/beta: energies >70 keV
Measurement ranges	10 µGy to 1 Gy (1 mrad to 100 rad) linear 1 Gy to 20 Gy (100 rad to 2000 rad) supralinear
Repeatability	For 1 mGy (100 mrad) ^{137}Cs doses, <2% standard deviation of 10 sequential measurements
Heating plate	50° to approximately 500°, approximately 6°/s

[a] Harshaw TLD 3500 (Thermo Fisher Scientific, Waltham, MA) and Nanogray TL2000 (Nanogray, Osaka, Japan) are suitable.

[b] Gamma rays from ^{60}Co or 10-MeV electron beams are suitable radiation sources.

PROCEDURE FOR SAMPLE PREPARATION

Sodium polytungstate solution: Prepare a solution of sodium polytungstate in water with a final density of 2 g/mL.

Mineral extraction: Suspend 3–20 g of sample with 50–100 mL of pure water, and sonicate for about 5 min. Sieve through a 250-µm nylon mesh, rinsing the mesh with water each time by using a strong jet from a wash bottle into a larger beaker (500–1000 mL). Allow the minerals to settle for 5 min and decant most of the water, leaving the minerals in <50 mL of water. Transfer the mineral fraction to a centrifuge tube, and centrifuge for 1 min at 1000 × g. Discard the water layer.

Preconcentration density separation: Add 5 mL of the *Sodium polytungstate solution* to the minerals in the centrifuge tube. Shake or mix on a vortex mixer, then sonicate for 5–15 min. Centrifuge for 2 min at 1000 × g. Silicate minerals (density 2.5–2.7 g/mL) precipitate, whereas organic components float. Carefully overlay a layer of water on the *Sodium polytungstate solution*, and discard the water layer and the organic materials by decantation or vacuum suction, leaving the minerals behind in the polytungstate layer. Carefully remove the *Sodium polytungstate solution* layer, and wash the minerals twice with water (centrifuge at 1000 × g briefly). Add 1–2 mL of 1 M hydrochloric acid, shake, and leave for 10 min in the dark to dissolve carbonates adhering to the silicate materials. Neutralize the acid with 1 M ammonium hydroxide. Fill the tube with water. Allow the minerals to settle, and centrifuge. Discard the water, and wash the minerals twice with water.

Fixing the minerals on disc for TL measurement: Add 3 mL of acetone to the preconcentrated minerals, and shake to displace residual water. Use a stainless steel disc suitable for the TL reader in use. [Note—Discs typically are 9–10 mm in diameter and 0.25–0.50 mm in thickness]. Carefully clean the disc by rinsing in water, wash two to three times with acetone, and dry in an oven. Store under dust-free conditions. Record the weight of the clean disc immediately before use. Transfer the minerals (in acetone), and dry the disc at 50° overnight (lab oven). Weigh the disc, and calculate the mass of minerals. Repeat the extraction, if necessary, to meet the system suitability requirements. [Note—The mineral sample amount is typically between 0.1 mg and 5 mg]. The deposited minerals can be fixed on the disc by using silicone spray (or by layering with 0.2% carboxymethylcellulose) and drying at 50° overnight.

Blank discs: Use clean discs.

TL MEASUREMENT

TL measurements are performed using a TL reader. Register the TL emission as a function of temperature (glow curves).

Minimum detectable integrated TL intensity level (MDL): Integrate the TL intensity of the first glow of the blank discs, and calculate the standard deviation. The MDL is three times the standard deviation of the integrated TL intensity of the blank discs.

System suitability: The integrated TL intensity of the irradiated sample (TL_2) should be at least 10 times the MDL.

Measurement of TL_1

First-glow TL_1 measurement— Set the instrument to an initial temperature of 70°, a heating rate of 6°/s, and a final temperature of 350°. Flush the chamber with nitrogen. Place the sample disc on the heating plate of the TL reader, and measure the glow curve. Determine TL_1 as the integrated TL signal between 150° and 250°. Measure the background glow for the sample (BG_1) after cooling the sample to 50°. After measuring TL_1 and BG_1, measure the weight of the sample (BW_1).

Irradiation—Irradiate the disc with the minerals, with a defined radiation dose of about 1 kGy. [Note—A source of ^{60}Co gamma rays is suitable.] Store at 50° overnight.

Measurement of TL_2

Second-glow TL_2 measurement— Measure the TL for the irradiated sample (TL_2) as described for TL_1. Measure the background glow for the irradiated sample (BG_2) after cooling the sample to 50°, and record the weight of the sample plate with the sample (BW_2). Measure the blank levels for process control (without the sample), following the procedures at each stage. Calculate the MDL as the integrated TL intensity of the blank plus three standard deviations.

TL glow ratio: The TL glow ratio per sample weight is calculated as described below:

$$\text{TL glow ratio} = [(TL_1 - BG_1)/BW_1]/[(TL_2 - BG_2)/BW_2]$$

TL_1 = irradiated sample for the first-glow measurement

BG_1 = background glow for the irradiated first-glow sample

BW_1 = weight of the irradiated first-glow sample

TL_2 = irradiated sample for the second-glow measurement

BG_2 = background flow for the irradiated second-glow sample

BW_2 = weight of the irradiated second-glow sample

Evaluation: TL glow ratios from irradiated samples are typically greater than 0.1. The ratios from nonirradiated samples are below 0.1.

Acceptance criteria: The temperature that gives the maximum glow from TL_1 measurement must be equal to or higher than the one from a nonirradiated sample. The TL glow ratio must be NMT 0.1 for nonirradiated materials.

Topic/Question	Contact	Expert Committee
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