DETECTION OF IRRADIATED FOOD

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Food irradiation uses electromagnetic radiation of energy levels sufficient to cause disinfestation or decontamination of treated product without causing induced radioactivity. Approval of facilities to be use for food irradiation is given by national competent authorities.

Since 2001 Brazil has one of the most comprehensive legislation (following Codex recommendations) within the scope of the Ministry of Health with no restricted list of products nor doses to be applied.

Different countries legislations concerning foods and food ingredients treated with ionizing radiation generally establish:

- a list of foods and food ingredients authorized,
- technical aspects for carrying out the process,
- labeling of irradiated foods and
- conditions for authorizing food irradiation.

That is the case for **EUA**.

The list of products authorized for irradiation within the whole **European Union** contains only a single food category: *"dried aromatic herbs, spices and vegetable seasonings"*, in spite of the fact that each European country hold and apply his own legislation inside their borders.

As other food processes, irradiation produces physical and chemical changes, but the extent of these changes differs significantly. In comparison to thermally sterilized foods, for instance, the extent of chemical changes in radiation-sterilized foods is relatively small and uniform.

An ideal detection method of irradiated food should:

- measure a specific radiation effect, proportional to the dose
- and should not be affected by processing parameters and storage conditions or
- length of time between irradiation processing and analysis.

- In 1993, the European Commission gave a mandate to the European Committee for Standardization (CEN) to standardize irradiated food detection methods.

- These European methods (European Community, 2009) have been adopted by the Codex Alimentarius Commission as General Methods and are referred to in the Codex General Standard for Irradiated Foods in section 6.4 on "Post-irradiation verification" (Codex, 2002).

Ten current methods used to identify irradiated foods (ENs)

- Detection of Irradiated food containing fat - GC analysis of hydrocarbons [EN 1784:2003]
- Detection of Irradiated food containing fat – GC/MS analysis of 2-Alkylcyclobutanones [EN 1785:2003]
- Detection of irradiated food containing bone - Method by ESR spectroscopy [EN 1786:1996]
- Detection of irradiated food containing cellulose. Method by ESR spectroscopy [EN 1787:2000]
- Thermoluminescence detection of irradiated food from which silicate minerals can be isolated [EN 1788:2001]
- (Cont.)

Ten current methods used to identify irradiated foods (ENs)

- Detection of irradiated food containing crystalline sugar by ESR spectroscopy [EN13708:2001]
- Detection of irradiated food using photostimulated luminescence [EN 13751:2002]
- Detection of irradiated food using Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) — Screening method [EN 13783:2001]
- DNA Comet Assay for the detection of irradiated foodstuffs — Screening method [EN 13784:2001]
- Microbiological screening for irradiated food using LAL/GNB procedures [EN 14569:2004]

1. Detection of Irradiated food containing fat - GC analysis of hydrocarbons

- Upon irradiation, in the fatty acid moieties of triglycerides breaks occur mainly in the α and ß positions with respect to the carbonyl groups **resulting in the respective Cn-1 and the Cn-2 hydrocarbons (HC).**
- To predict these chief radiolytic products, **the fatty acid composition of samples has to be known.** For detection of HC the fat is isolated from the sample by melting it out or by solvent extraction. The HC fraction is obtained by adsorption chromatography prior to separation using gas chromatography (GC) and detection with a flame ionization detector or a mass spectrometer.
- Saturated HC are frequently present both as contaminants and as naturally occurring compounds in food.
- Detection of irradiated **raw meat** and **Camembert** has been validated for doses of about 0.5 kGy
- Detection of irradiated **fresh avocado, papaya and mango** has been validated for doses of approximately 0.3 kGy and above.

2. Detection of Irradiated food containing fat – GC/MS analysis of 2-Alkylcyclobutanones

- During irradiation, the acyl-oxygen bond in triglycerides is cleaved and this reaction results in the formation of **minute 2-alkylcyclobutanones** containing the **same number of carbon atoms** as the parent fatty acid and the alkyl group is located in ring position 2. Thus, **if the fatty acid composition is known, the 2-alkylcyclobutanones formed can be predicted**.

- The 2-dodecylcyclobutanone (DCB), 2-tetradecylcyclobutanone (TCB) and 2 (tetradec-5'-enyl) cyclobutanone formed from **palmitic**, **stearic and oleic** acid, respectively, during irradiation.

- The 2-alkylcyclobutanones are extracted using n-hexane or n-pentane along with the fat and then fractionated using adsorption chromatography prior to separation using **GC/MS.**

- Detection of **irradiated raw chicken** has been validated for doses of approximately 0.5 kGy; **irradiated liquid whole egg, raw pork, salmon and Camembert** has been validated for doses of approximately 1 kGy.
- Results with mangoes and papayas, using their seeds as the source of lipid, were not considered satisfactory and hence validation is not extended to these products.

3. Detection of irradiated food containing bone - Method by ESR spectroscopy

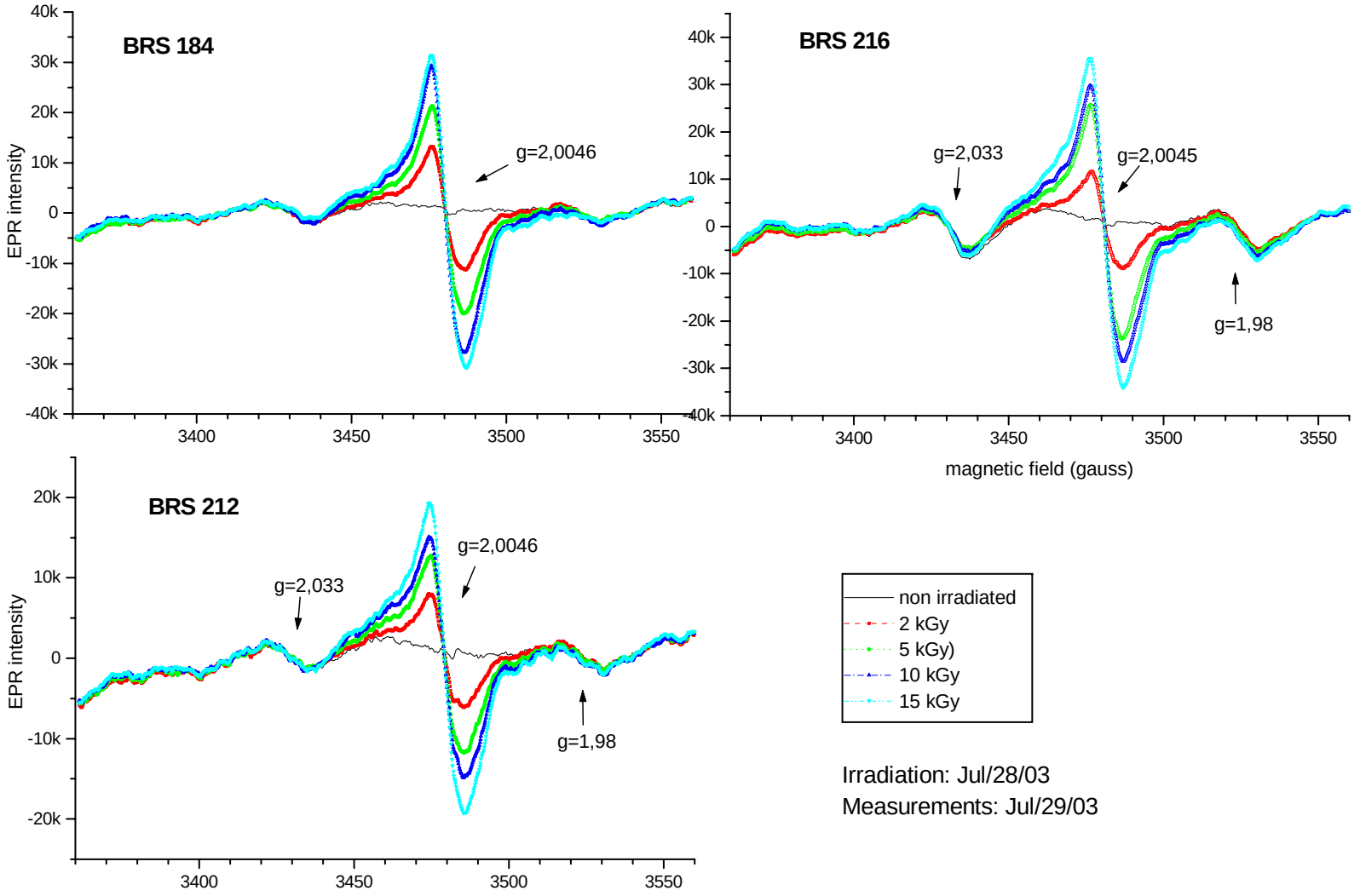
- Electron Spin Resonance (ESR) spectroscopy detects paramagnetic centers (e.g. radicals). Radiation treatment produces radicals which can be quite stable in solid and dry components (e.g. bones) of the food, and can be detected. The intensity of the signal obtained increases with the concentration of the paramagnetic compounds and thus with the applied dose.

- Detection of irradiated bone samples is typically possible above a dose of approximately 0.5 kGy, covering the majority of commercial applications. Detection limits and stability are influenced by the degrees of mineralization and crystallinity of hydroxyapatite in the sample.

4. Detection of irradiated food containing cellulose Method by ESR spectroscopy

- ESR spectroscopy detects paramagnetic centres (e.g. radicals).
- Detection limits and stability are influenced by the **crystalline cellulose** and **moisture** content.
- Positive identification of the cellulose radicals is evidence of irradiation but the absence of this signal does not constitute evidence that the sample is unirradiated.
- Detection of **irradiated pistachio nuts** has been validated for doses of 2 kGy
- Detection of **irradiated paprika powder** has been validated for doses of 5 kGy.
- Detection of **irradiated fresh strawberries** has been validated for doses of 1.5 kGy
- Detection is typically limited to about the first 3 weeks after treatment.

EPR spectra of the three soybean samples (obtained 16h after irradiation)



Irradiation: Jul/28/03
Measurements: Jul/29/03

5. Thermoluminescence detection of irradiated food from which silicate minerals can be isolated

- 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 thermoluminescene (TL) glow curves. Silicate minerals are isolated from the foodstuffs, mostly by a density separation step. 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 irradiated samples yield higher TL glow ratios than that of unirradiated samples.

- Detection of irradiated herbs, spices (6 kGy).

- irradiated shellfish (0.5 kGy to 2.5 kGy)

- irradiated fresh and dehydrated fruits and vegetables (1kGy).

6. Detection of irradiated food containing crystalline sugar by ESR spectroscopy

- Different mono- or disaccharides may dominate in the sample producing different ESR spectra after irradiation.
- 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.
- 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.

7. Detection of irradiated food using photostimulated luminescence

- 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 ionizing radiation.

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

- The PSL method may, in principle, be applied to detect irradiation of any food which contains mineral debris. Signals below the lower threshold (T1) are generally associated with unirradiated material, but can derive from low sensitivity irradiated materials. Calibration can help to distinguish these cases.

- 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.

8. Detection of irradiated food using Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) — Screening method

- 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.

- A limitation of the method is encountered when there are too few microbes in the sample (APC <103 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).

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

- DNA fragmentation can be caused by various chemical or physical treatments including ionizing radiation. This fragmentation can be studied by microgel electrophoresis of single cells or nuclei.

- 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, which will appear nearly circular or with only slight tails.

- The DNA Comet Assay is working as a screening test and may detect irradiation of any food containing DNA: 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.

10. Microbiological screening for irradiated food using LAL/GNB procedures

- This is a microbiological screening method comprising two procedures, which are carried out in parallel. It permits the identification of an unusual microbiological profile in poultry meat, for example. The presence of a large excess population of dead micro-organisms can under certain circumstances be presumptive of irradiation treatment, which means, that the results of the procedure of the determination of endotoxin concentration in the test sample using the Limulus amoebocyte lysate (LAL) test and of the procedure of the enumeration of total Gram negative bacteria (GNB) in the test sample are not radiation specific.

- This method can give only an indication of a possible treatment by ionising radiation. The method is of particular use to routine microbiological laboratories, which may be involved in the examination of foods.

Conclusions

- There are no a perfect and universal method to identify food products submitted to radiation processing.

- Nevertheless, it is important to have some sensitive analytical methods to detect irradiation processing independently if the food products have been labeled as such.

- Proper control of irradiation processing of food is very critical to facilitate trade of irradiated foods and to enhance consumer confidence, consumer choice and safety.

Thank you!

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