Detection methods for irradiated foods

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Introduction

- Irradiation is used in foods to eliminate pathogenic and spoilage microorganisms.
- It causes a cascade of changes in foods.
- Not all the changes are identified nor understood.
- Irradiation is not fully accepted by all consumers.

Introduction (cont.)

- Food irradiation is approved in some countries but only on specific products.
- There is a need for methods to identify irradiated foods to:
  - Check compliance with existing regulations.
  - Give the consumers the opportunity to choose.
  - Facilitate international trade of foods.

Introduction (cont.)

- The irradiation detection methods in foods can be classified in three basic groups:
  - Chemical methods
  - Physical methods
  - Biological methods
- The methods have different degree of development.
- Some are already approved and some are in the research phase.

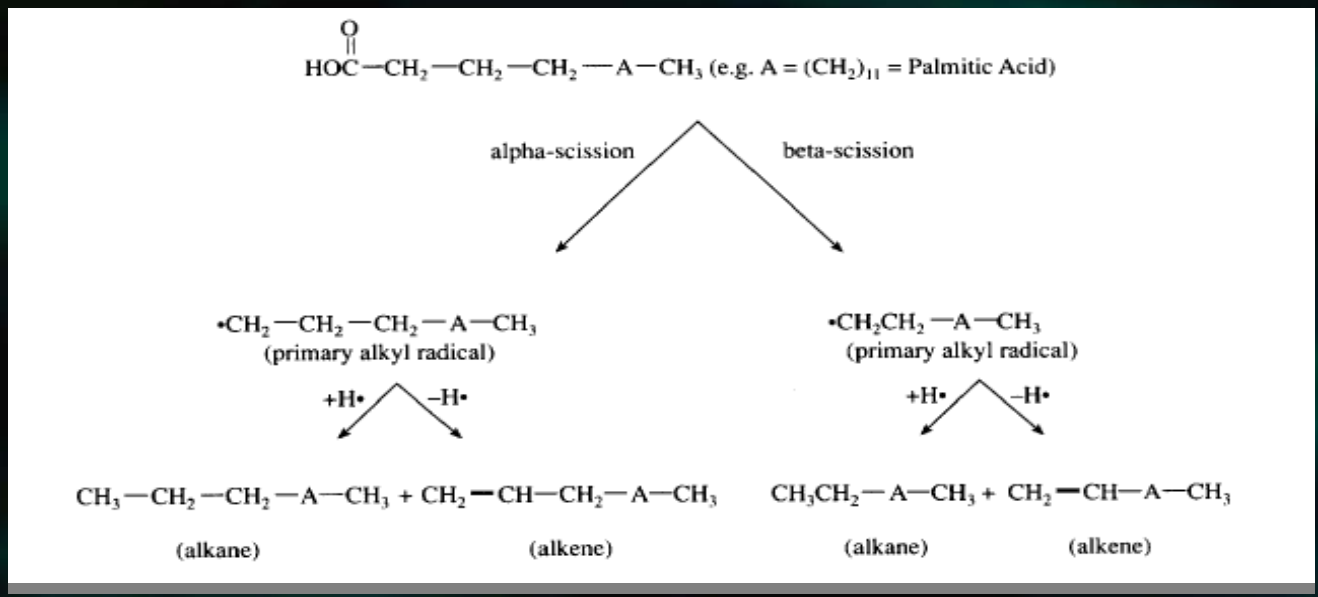
CHEMICAL METHODS

	Status*	Foods studied
Proteins		
o-Tyrosine	B	Chicken, shrimps, shellfish, fish, frog legs, egg white
Formaldehyde	A	Poultry
Crosslinks (electrospray ionization mass spectrometry	A	
Fragmentation (immunochemistry)	B	Egg white
	A	Chicken, shrimps
Lipids		
Hydrocarbons	F	Raw chicken, pork and beef, Camembert, avocado, mango and papaya
	D	Egg powder
	B	Fish, shrimps, shellfish, spices, frogs legs, nuts, beans
2-Alkylcyclobutanones	F	Raw chicken and pork, liquid whole egg
	B	Beef, lamb, shrimps, prawns, mango, papaya, cheese (Camembert, Brie)
Immunochemistry	B	Chicken, prawns
Lipid hydroperoxides	B	Pork, liquor, egg powder, chicken
Cholesterol oxidation products	A	Egg powder, chicken, beef, pork, veal
Carbohydrates		
Optical isomers	?	
Nucleic acids		
Base damage, immunoassay	B	Wheat, prawns
Strand breaks		
Alkaline filtration	B	Crustaceae (interference with storage)
Agarose electrophoresis of mt DNA	B	Beef liver, meat, chicken, fish, prawns
Pulsed field gel electrophoresis	B	Poultry
Microgel electrophoresis "Comet Assay"	D	Frozen chicken, pork
	B (D)	Some seeds
	B	Fish, strawberries, beans, some spices
flow cytometry	B	Onions
Other food constituents		
Evolution of low-molecular gases	B	Frozen chicken, pork, beef, shrimps, spices
Optical isomers	B	Liquor

? = insufficient information
A = concept promising
B = encouraging experiments in one or several laboratories
C = ready for collaborative testing
D = collaborative study with several laboratories already carried out
E = national standard validated by interlaboratory testing
F = international standard validated by interlaboratory testing

Hydrocarbons

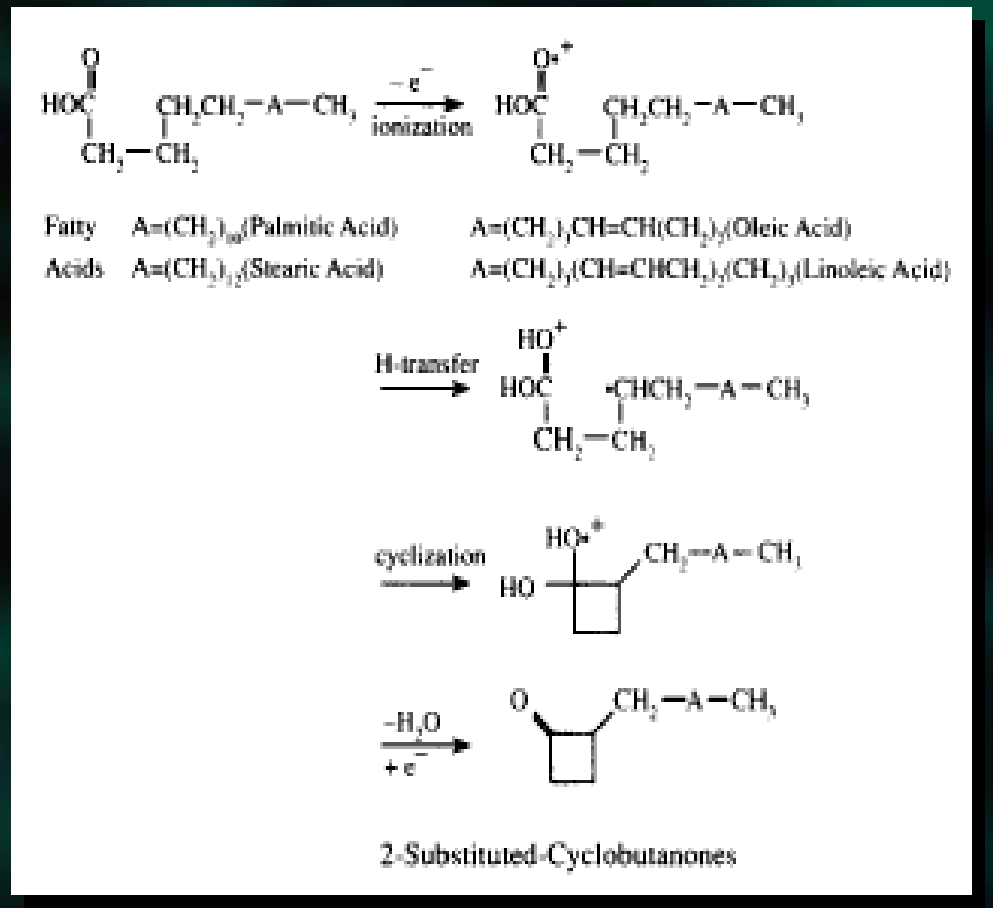
- Irradiated lipids are cleaved in the α or β C-C bond forming Cn-1 or Cn-2 alkanes, alkenes and alka-polyenes.
- Although hydrocarbons are also formed by heat, the radiolytic hydrocarbons have a unique pattern of distribution.



- **The method involves the isolation of fat, separation of the hydrocarbon fraction by adsorption chromatography (florisil) and characterization of hydrocarbons is performed using gas chromatography with a flame ionization detector or a mass spectrometer.**
- **This method is applicable to all foods that contain lipids even though they are present at low concentrations.**
- **This method has been used succesfully on raw chicken, pork and beef, cheese, avocado, papaya and mango, eggs and foods containing eggs.**

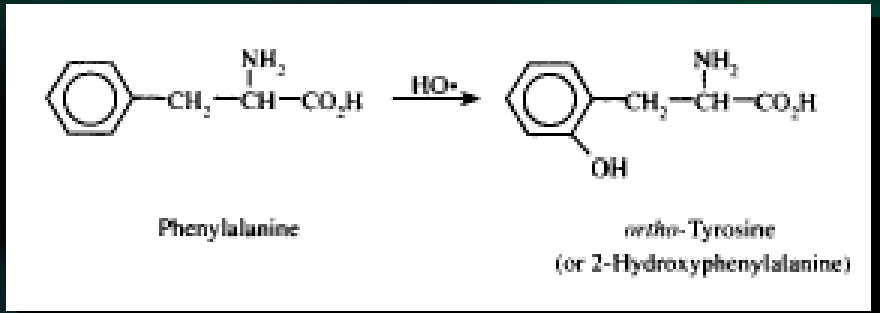
Cyclobutanones

- Cyclobutanones are thought to be radiation specific.
- The detection involves Soxhlet extraction of homogenized sample with hexane, florisil chromatography, followed by gas chromatography/mass spectrometry.



O-Tyrosine

- Formed by the oxidation of phenylalanine residues of protein in food by hydroxyl radicals that are produced by irradiation.
- Hydroxyl radicals produce all three aromatic isomers ortho, meta and para, but ortho isomer is easier to quantitate.
- They are determined by high performance liquid chromatography (HPLC).



DNA

- DNA is a large molecule and easy target for ionizing radiation.
- Upon irradiation DNA molecules suffer denaturation, base modification and fragmentation either by single or double-bond strand breaks.
- So, changes in DNA can be used to identify irradiated foods.

DNA COMET ASSAY

- It detects fragmentation of DNA using the microgel electrophoresis of single cells or nuclei.
- These are embedded in agarose on microscope slides, lysed for disruption of membranes using 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.
- Irradiated cells will show an considerably longer comets (more fragmentation) than unirradiated cells, which will appear nearly circular.

Pros and cons

- The DNA comet assay is a relatively simple and rapid screening test that makes use of low-cost equipments and can be used in all foods containing DNA.
- However this method is not radiation specific because there are other treatments that also induce DNA fragmentation.
- Suspicious samples may need to be confirmed by an officially validated method.

PHYSICAL METHODS

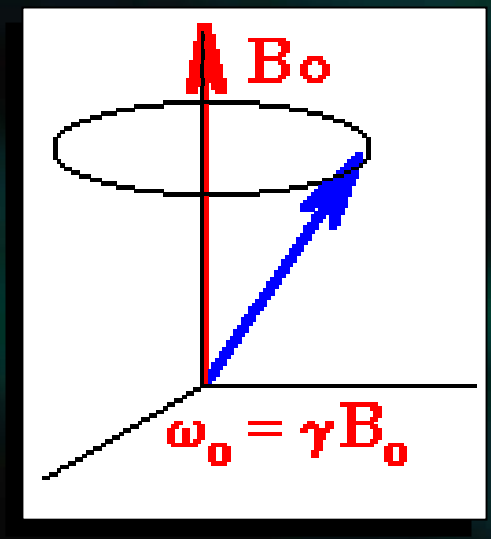
	Status	Foods studied
Changes in physical properties		
Electrical impedance	B	Potatoes
Viscosity of suspensions	D	Pepper
Thermal analysis (e.g. ice nucleation)	A	Fish, prawns, egg white
Near-infrared spectroscopy (NIR)	A	Spices
Nuclear magnetic resonance (NMR)	?	
Detection of free radicals[a]		
Electron spin resonance (ESR)	F	Food containing bone (poultry, meat, fish, frog legs)
	F	Food containing cellulose (pistachio nut shells, paprika powder)
	E	Food containing cellulose (strawberries)
	E	Food containing crystalline sugar (dried mango, dried fig)
	E	Some crustaceae (brown shrimp, Norway lobster)
	D	Food containing cellulose (pepper)
	D	Food containing crystalline sugar (raisins, dried papaya)
	D	Some crustaceae (pink shrimp, crevette, Norway lobster)
	D	Egg shells
	C	Food containing bone fragments (mechanically recovered meat)
	C	Shellfish
	B, C	Food containing cellulose (grapes, various berries -chilled or frozen- french prunes, some spices)
	B	Dehydrated mushrooms, macaroni, snails, gelatin, crustaceae, barley, seeds of fruits (figs, dates)
Luminescence:		
Chemiluminescence	D	Some spices, herbs and dehydrated vegetables
	B	Frozen chicken, wheat flour
	A	Shellfish, crustaceae, poultry bones
Thermoluminescence	F	Food from which silicate minerals can be isolated (herbs, spices, their mixtures, and shrimps)
	E	Fresh fruit and vegetables
	D	Potatoes
	D	Crustaceae and shellfish (measuring silicate minerals)
	D	Crustaceae and shellfish (measuring calcite shells)
	D	Dehydrated fruit and vegetables
Photo-stimulated luminescence	C	Food containing mineral debris or bioinorganic materials (herbs, spices and seasonings, crustaceae and shellfish)

Electron Spin Resonance (ESR) spectroscopy

- It is also called Electron Paramagnetic Resonance (EPR).
- It detects radiation-specific radicals produced upon irradiation of food.
- Free radicals are usually transient species.
- However, in rigid and dry matrices their lifetime extends.

ESR Principle

- Free radicals have unpaired electrons.
- The electron is a charged particle that possesses an intrinsic spin and magnetic moment.



ESR Principle (cont.)

- The energy difference between the two electron spin states is called Zeeman splitting.
- When an external magnetic field is applied to a magnetic particle it attempts to allign with the field and some electrons try to flip.
- However, they need energy to overcome the transition (Zeeman splitting).

ESR Principle (cont.)

- The applied magnetic field changes the frequency of the electron's spin, following the equation:

$$\omega o = Bo\, \gamma$$

where ωo is spin frequency, Bo is the magnetic field strength and γ is the gyromagnetic constant.

- When an electromagnetic wave is applied to the electron and its frequency is equal to the electron spin frequency (induced by the external magnetic field), resonance condition is achieved and flipping occurs.

ESR Principle (cont.)

- The resonance condition is given by the equation:

$$h\nu = g\,\mu_B\,Bo$$

where h is Planck's constant, v is the frequency of the microwave radiation, g is a spectroscopic factor, μ_B is Bohr's magneton and Bo is the resonance magnetic field strength.

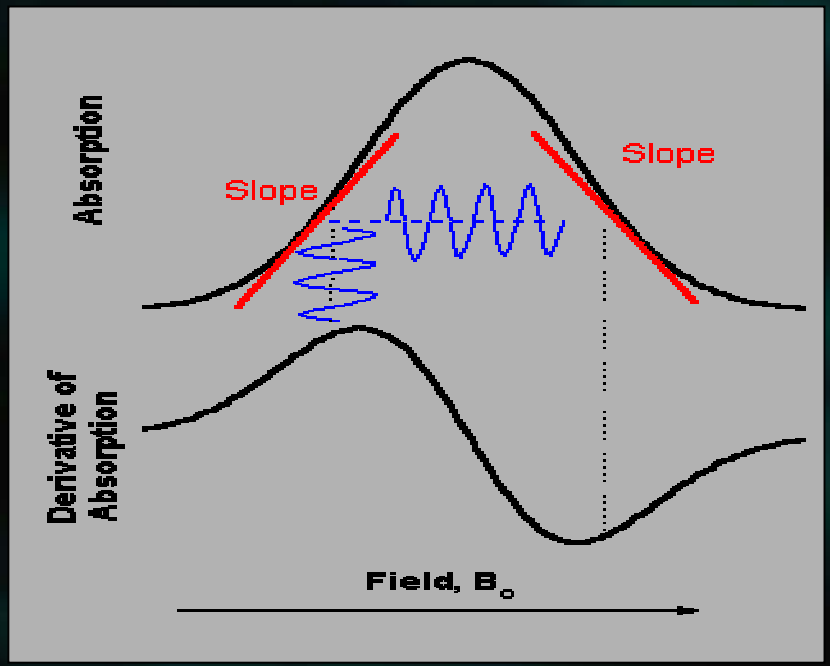
- The Bohr magneton is a unit of magnetic moment, defined by:

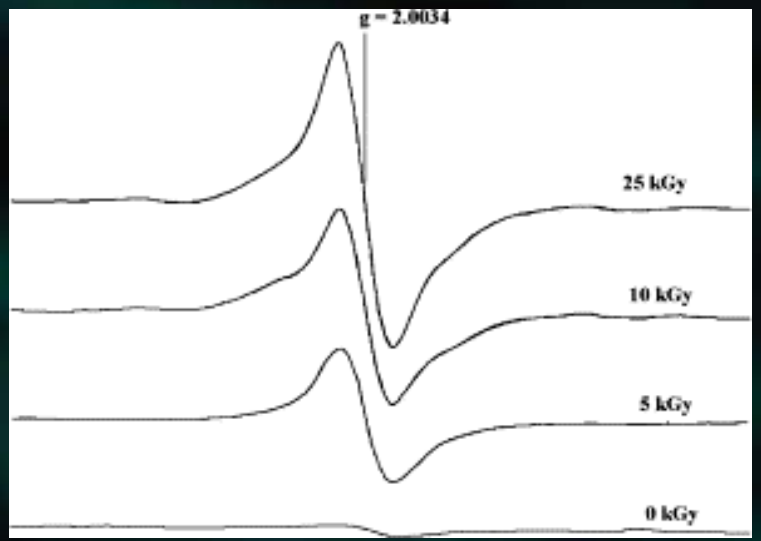
$$\mu_B = (e*h) / (4*\pi*M*c) = 9.2740 \text{ x } 10\text{-}24 \text{ J/T}$$

where e is the charge of the particle, h is Planck's constant, c is the velocity of light and M is the mass of the particle.

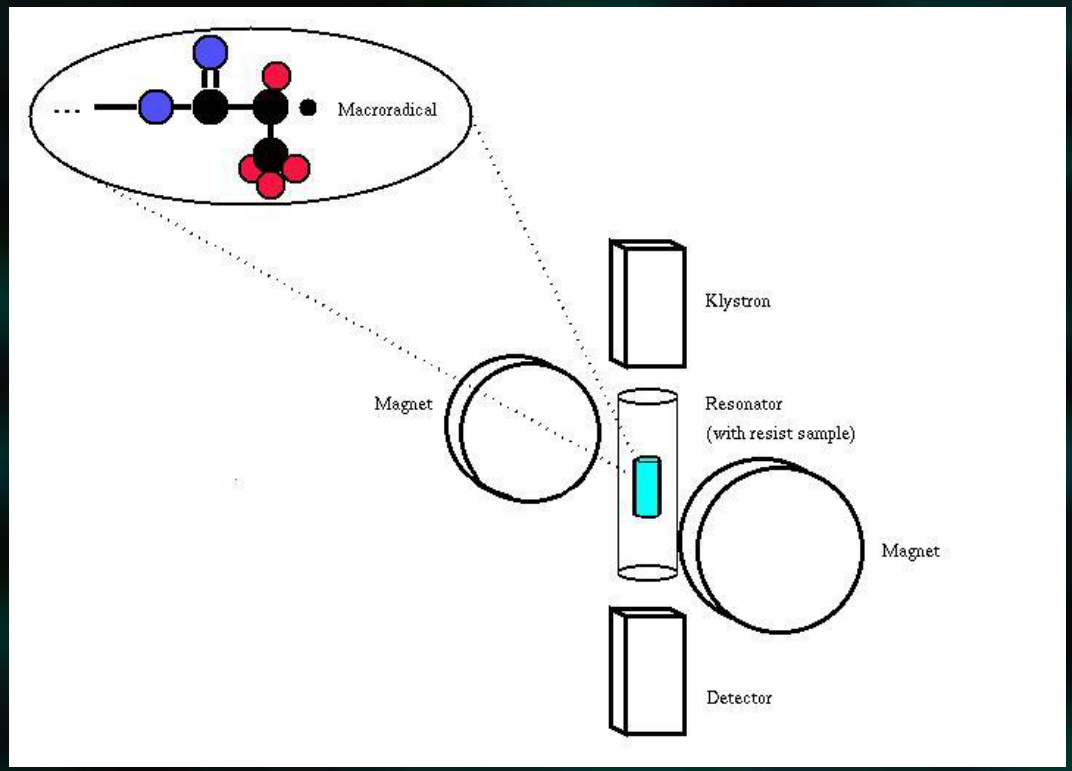
ESR Curve

- The flipping causes energy from the electromagnetic wave to be absorbed.
- The ESR equipment detects this absorption and draws a curve.





ESR Equipment



Pros and Cons

- This technique is non-destructive, very sensitive, specific and combines simplicity with rapid measurement.
- The two most signifficant drawbacks of ESR spectroscopy are the cost of the spectrometer and the special technical skills required to operate it.
- Suitable products: meats (with bone), shellfish, some fruits and seeds.

LUMINESCENCE

- When ionising radiation interacts with an insulating crystal lattice (such as quartz or feldspar), a net redistribution of electronic charge takes place.
- Electrons are stripped from the outer shells of atoms and though most return immediately, a proportion escape and become trapped at ‘meta-stable’ sites within the lattice.

- In order to release these trapped electrons, more energy is required.
- When the necessary energy is applied, these electrons return from the excited state to the electronic ground state by losing part of their excess energy as a photon.
- This phenomenon is called luminescence.

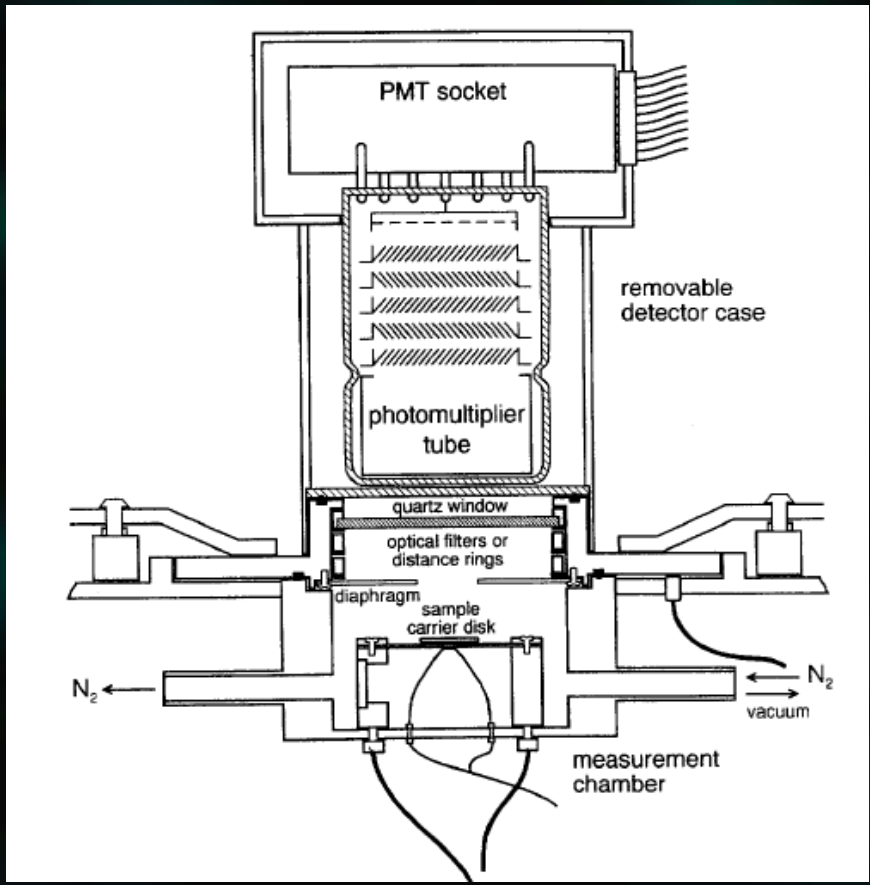
Types of luminescence

- The emission of trapped energy as light may be induced:
  - photochemically (photostimulated luminescence, PSL)
  - thermally (thermoluminescence, TL)
  - chemically (chemiluminescence, CL)
  - solvation (lyo-luminescence, LL).
- In food, crystalline lattices are present in adhering debris of silicate minerals (dust).

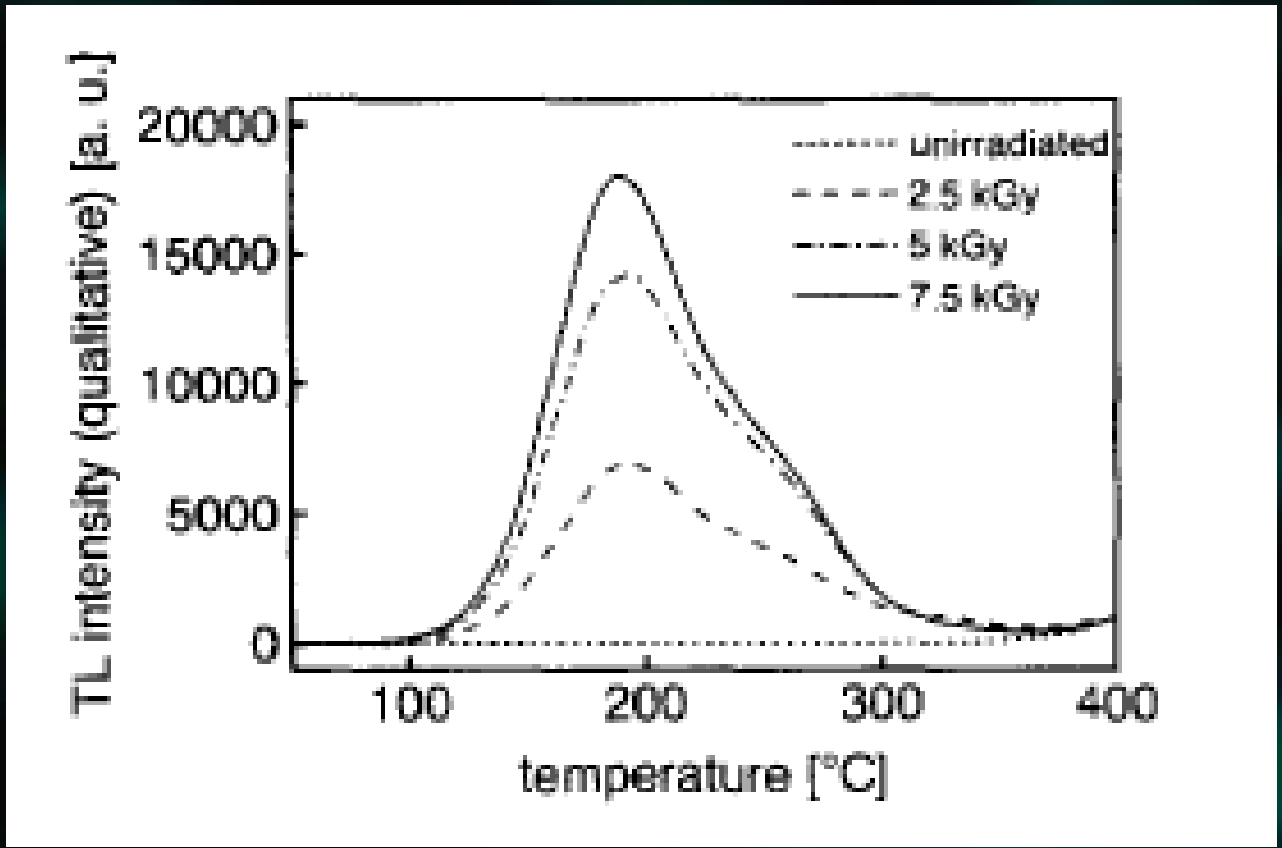
Thermoluminescence

- The TL apparatus consists of a metal planchet on which a disk with sample powder is heated by high frequency current.
- The TL chamber is tightly closed and can be evacuated and flushed with inert gas before measurement.
- The TL glow is measured by a photomultiplier tube.

Thermoluminescence apparatus



Thermoluminescence curve



Thermoluminescence

- A second TL measurement of the same sample after exposure to a known dose of radiation is necessary.
- Thermoluminescence measurement is regularly applied for herbs and spices, also in crustaceae, shellfish, fresh fruits and vegetables.
- Salt may also serve as a TL-sensitive irradiation indicator.

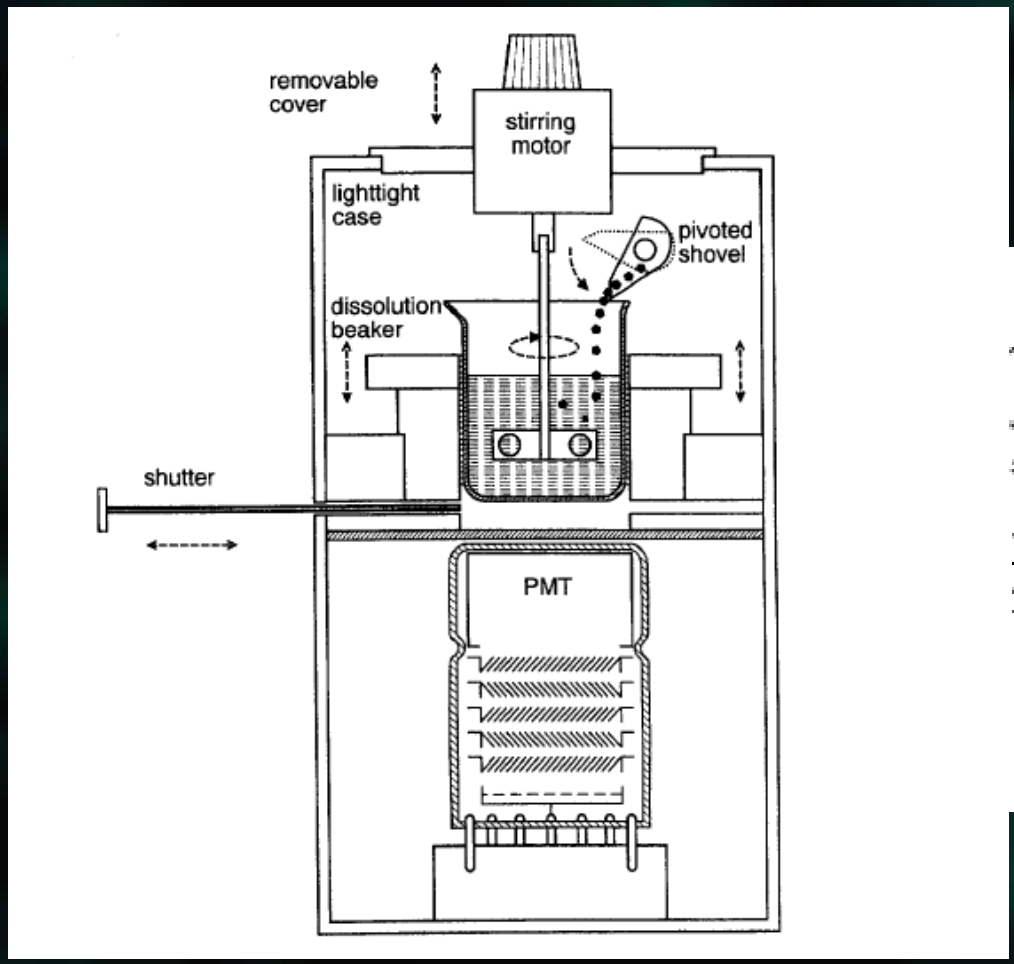
Pros and cons

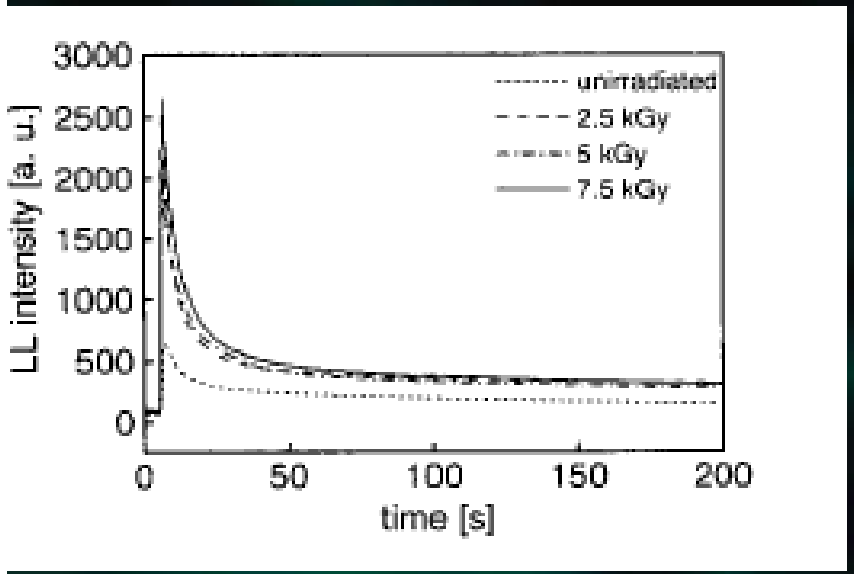
- TL method mostly enables an unequivocal classification of irradiated and unirradiated samples.
- TL technique is laborious, since the minerals have to be prepared free of organic material, there is a small amount of mineral product on food surface, there is natural TL of some materials, stringent quality assurance procedures are necessary, the equipment is costly, and a radiation source is needed.

Lyoluminescence

- The LL is very similar to the TL, except that the sample powder is placed on a shovel and dropped into a glass beaker containing a stirred solvent/sensitiser mixture.
- Upon dissolution of the irradiated solid substance light is emitted.
- The sensitiser is added to give chemiluminescence with the products formed by the reaction of radicals or electrons with the solvent during dissolution.
- Possible use in ground spices and powder milk.

Lyoluminescence





BIOLOGICAL METHODS

	Status*	Foods studied
Histological/morphological characteristics:		
Inhibition of cell division	B	Bulbs and tubers
Lack of wound periderm formation	B	Potatoes
Inhibition of seed germination (shooting, rooting;	D	Citrus fruit
e.g. half-embryo test)	B, C	Apples, cherries
	B	Cereals, legumes
Chromosomal aberrations	A	Cereals, potatoes, strawberries
Electron microscopy	A	Fruit, shrimps
Changes in susceptibility to bacterial spoilage	B	Fish, meat
Changes in microflora:		
Shift in microbial profile	B	Strawberries, fish, shrimps
Radiation resistance	B	Chicken, fish, shrimps
Reduced viability:		
Direct epifluorescent filter technique combined with	D	Spices, chicken
aerobic plate count (DEFT/APC)	B, C	Beef, cod, prawns
Reduced viable Gram-negative bacteria Limulus: Amoebocyte	E	Chicken
Lysate Test combined with count of Gram-negative		
bacteria (LAL/GNB)		
Changes in insects:		
Supra-oesophageal ganglion	B	
Polyphenoloxidase test	B	

LAL and DEFT/APC

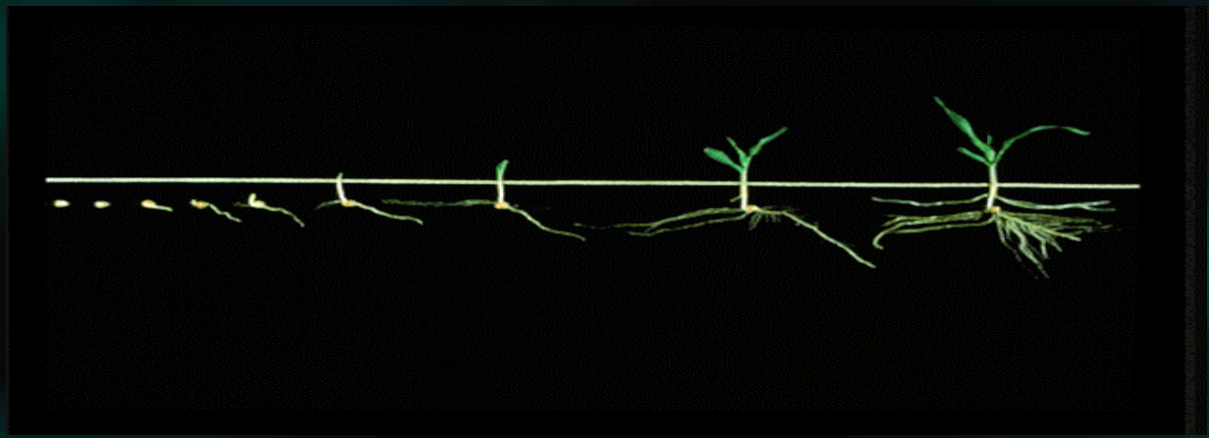
- Both techniques check for the differrence between dead and alive microorganisms.
- Limulus Amoebocyte Lysate (LAL) test checks for total gram-negative organisms and direct epifluorescent filter technique (DEFT) the total number of microorganisms.
- The relationship indicates the number of organisms deactivated by irradiation or other decontaminating treatments.

Pros and cons

- Economical option for screening large numbers of samples.
- Determination not possible when there are few microbes.
- Tests an effect that is not radiation-specific.
- Some spices have antimicrobial compounds that may decrease APC.

Germination

- This assay is limited to vegetable seeds.
- It relies on the fact that irradiated seeds germinate signifficantly slower than control seeds.
- This test is very simple and cheap, but slow since it takes a few days to get the results.



CONCLUSIONS

- Detection methods are being continously developed.
- Today, there is no single method that can be used in all products.
- The selection of a method to test for irradiated foods depends mainly on the product to be analyzed. the degree of precision required, and the costs.