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<Review>

General Survey of Detection Methods for Irradiated Foods

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Abstract

The development of detection techniques is needed, in order for regulating authorities to determine whether or not a particular food sample has been irradiated, and label it accordingly so that a consumer's free choice can be exercised. The chemical and physical changes brought about in foods by practical doses of irradiation are very small, and therefore very sensitive methods are required. A number of promising approaches have been developed and evaluated. These include chemical, physical and biological methods ranging from the very simple to highly sophisticated techniques.

1. Introduction

Food irradiation can improve the safety of food through the reduction of pathogenic microorganisms which can be present in untreated foods. However, consumers must have the free choice to purchase irradiated or non-irradiated foods. Moreover, to promote international trade in irradiated foods and to prevent abuse of the technology, identification methods are needed. The need for tests on the food itself became important as consumers began to demand the clear labelling of irradiated food. Ideally, a rapid and sensitive method applicable to wide range of foods should be developed. However, despite the many investigations performed to detet changes in food subjected to ionizing radiation, no satisfactory method has been developed so far. Currently, a range of tests based on physical, chemical and microbiological changes in irradiated food have been researched. The various approaches which have been investigated and the foods studied are summarized in Table 1[1-4].

2. Physical Methods

The physical properties of foods are sometimes altered upon exposure to ionizing radiation. The stability of organic radicals produced by irradiation are increased if the unpaired electron is incorporated into the complex polymeric system, as in peptides and polysaccharides, and is structurally isolated from the water.

2.1. Electron Spin Resonance Spectroscopy (ESR)

Electron spin resonance (ESR) is one of the most

promising physical techniques for the Detection of irradiated foods. It relies on the detection of trapped free radicals and, therefore is satisfactory only for foods in which radicals are essentially immobilized, such as dried foods, bone containing foods and seafoods with hard cuticles [5-8]. There are three factors affecting the intensity of ESR signals on bone containing foods produced by radiation :·(i) origin (animal species), (ii) age of animals, (iii) kind of bone fragments taken for ESR measurement. The ESR dose estimation could indicate whether or not radiation treatment was applied in accordance with GIP(Good Irradiation Practice). The cellulose-derived radical is detectable several months after irradiation, but its special intensity decreases to about 10% of its intensity. Thus this ESR method is adequate for the detection of irradiated fruits and vegetables, whose shelf-life is expected to be limited to a few weeks at the maximum. Although ESR is a well established, non-destructive method of free radical analysis, the equipment is expensive and highly skilled operators are required.

2.2. Thermoluminescence(TL)

In solid dielectric material, energy is stored during irradiation as trapped charge carriers. By thermal stimulation, excess energy can be released as luminescence emission. In TL, isolated silicate minerals are heated under controlled conditions which give rise to measurable glow curves. Depending on the 'depth of the trap', excess energy is released as light emission at a certain temperature. The amount of light emitted can then be correlated with the irradiation dose originally absorbed. For a clear identification of irradiation, TL intensities have to be normalized and this is achieved by re-irradiation of the samples with a dose of at least 1 kGy. The TL ratio of the first glow curve and second glow curve is approximately the same if the sample has been irradiated prior to examination [9-11]. The TL method provies a useful means to ensure that irradiated herbs and spices are properly labelled. Instead of thermal stimulation for release of trapped energy, light can be used as a stimulus. Emissions from irradiated foods show Anti-Stokes behaviour, since their wavelengths are shorter than the wavelengths used for excitation. The photostimulated luminescence(PSL) can be rapidly performed without the need for sample preparation or re-irradiation and can be applied to a similar broad range of foods as TL. A low-cost PSL instrument has been designed for fast screening applications [12]. A burst of light emission ('chemiluminescence') may occur when water is added to irradiated solids. This can be detected by a suitably sensitive photomultiplier and correlated with a radiation dose.

2.3. Viscometry

A significant reduction in gel-forming capability after gamma irradiation could be observed in several spices containing large amounts of starch reduced by irradiation. The viscosity changes are quite stable on storage, so that the irradiation effects can be detected even after years. A normalized parameter of black and white peppers is better for detecting irradiation treatment than a viscosity value itself. The method offers a means for rapid detection of irradiated black pepper in a cost-effective way [13-16].

2.4 Impedance and Conductivity

Irradiation may cause changes in the electrical properties of foods. Ions and charged molecules oscillate in response to the applied field. Most instruments operate within the frequency range of about 2~10 kHz. At the lower end of this range

capacitance predominates, while at the higher end of the range, conductance predominates. Therefore overall readings from instruments reflect a combination of these effects. Irrardiated potatoes could be identified by the measurement of impedance [17]. The ratio of the impedance magnitude at 5kHz, measured at 22~25℃ at the apical region of a potato tuber with 1 mA of alternating current, results in the best detection of irradiated potatoes.

2.5. Hydrogen and Carbon-Monoxide

Measurement of gas evolution is a promising screening test to detect irradiated food. Hydrogen is produced by irradiation but diffuses out unless the gas is trapped. Irradiated pepper has been evaluated by hydrogen detectors [18]. The probe consists of a palladium-coated field-effect transistor and is adequate to monitor the head-space of irradiated food, especially offering a test for irradiated frozen food in which positive detection gives conclusive evidence of irradiation. Irradiated meat products are detected by the formation of radiolytically derived carbon monoxide (CO). The method has the advantage in that it can be used for boneless food samples. The use of multiple gas sensors increase the reliability of the test, which is cheap and rapid and easy to perform.

3. Chemical Methods

Foods are complex mixtures of many substances competing with one another to absorb the ionizing radiation. The formation of free radicals leads to a wide variety of radiolytic products on which analytical detection methods could be based. Pattern recognition and analysis of variance have been applied to different foodstuffs in attempts to detect the effects produced by irradiation[19].

3.1. Lipids : Volatiles

Irradiation can induce a number of chemical changes in the fatty acids found in foods. Attention has been focused on the volatile products, as these may be responsible for the off-flavours developed in irradiated meats. The nature of the volatiles depends on the composition of the fat in irradiated food, while their quantity depends on the radiation dose. The relatively simple measurement of lipid-derived volatile products is applicable to any food containing lipids, especially high-fat foods such as meat, fish, shellfish, and eggs. Cleavage of lipids occurs primarily near the ester carbonyl, producing alkanes and alkenes with 1 or 2 fewer carbon atoms which are most suitable as possible indicators of irradiation [20-23]. The four major fatty acids in most foods, namely, palmitic, stearic, oleic and linoleic acid, give 2-dodecyl-, 2-tetradecyl-, 2-tetradecenyl- and 2-tetradecadienyl cyclobutanone, respectively, following irradiation. These radiation-induced volatiles are isolated from the lipids by Florisil column chromatography and subsequently identified by GC/MS (gas chromatography/mass spectrometry). To obtain more clean chromatograms, sample preparation by a two-step HPLC (LC) coupled on-line to the gas chromatograph is the method of choice. Although equipment costs are high, and this approach requires the use of relatively sophisticated analytical equipment and a few hours to complete, the technique has postential to estimate the irradiation dose by a dose-response relationship. Lipid oxidation products are another possible form, while they are affected by a wide variety of conditions and factors [24].

3.2. Proteins : o-Tyrosine

Changes in the chemical composition of proteins

have been examined by a number of investigators using electrophoresis or gel permeation chromatography, but with only limited success. Products derived from aromatic amino acids are highly radiation-sensitive in meat, poultry and fish. At one time o-tyrosine was thought to be a unique radiolytic product [25, 26]. The simplicity of the methodology suggests that the technique may be

Table 1. Analytical Detection Methods for Irradiation Treatment of Foods

Detection methods and foods under evaluation
Physical methods
1. ESR : To detect the free radicals in irradiated dry foods and bone Meat(Bone), Fish (Bone), Seeds & Herbs, Egg (Shell : Research), Vegetables (Research), Fruit (Fresh, Dried : Research), Mechanically Deboned Meat (Research)
2. TL : Emission of light on heating an irradiated food Shellfish (Meat), Seeds & Herbs, Meat (Bone : S), Fish (Bone : S), Vegetables(Research), Fruit (Fresh, Dried : Research)
2*. PSL : Seeds & Herbs(Research)
3. Viscosity : Accompanied the chain scission of polymers in some foods Seeds & Herbs
4. Conductance/Impedance Vegetables
5. Gas evolution
Chemical methods : The detection of low levels of radiolysis products using conventional methods of analysis, such as gas-liquid chromatography
1. Lipids : Analyses for low molecular weight volatiles generated from fatty acids during the irradiation of lipids
1*. Hydrocarbons Meat (Flesh), Egg (Contents), Cheese, Fruit (Fresh)
1**. Cyclobutanones Meat(Flesh), Egg(Contents)
1***. Peroxides
2. Proteins
2*. o-Tyrosine : Detection of o-tyrosine generated during the irradiation of proteins
3. DNA : Detection of changes in DNA, in particular single and double strand breaks and chemical modification of bases
3*. DNA-Comet Meat (Bone, Flesh)
3**. Mitochondrial DNA Meat(Flesh : Research)
4. Immunological methods : Cyclobutanones and others
Biological methods : Based on the direct physiological effect of irradiation on the target foodstuff, e.g. the ability of bulbs and tubers to germinate and initiate growth, and on the indirect ones, e.g. assessment of the micro-organisms' viability
1. DEFT/APC Seeds & Herbs
2. Germination

* S= Screening method only

At present, no single method is universally applicable to all foods, but several specific methods are sufficiently reproducible for individual food types.

valuable, along with others, for protein-rich foods.

3.3. DNA Damage

Since ionizing radiation affects DNA by causing lesions in specific bases, single strands and double strands, DNA damage should therefore be a means of detecting whether or not a food has been irradiated and quantifying the dose received. However, radiation on strand breaks cannot be isolated in meat because of the strong enzymatic degradation of cellular DNA during storage. It appears necessary to isolate the irradiated DNA from cell enzymes in order to make the DNA strand rupture more specific to radiation. Mitochondrial NDA(mt DNA) is protected against enzymatic reactions by the mitochondrial walls, but not against irradiation. The reduced average size of DNA strands and their increased mobility could be analysed by a electrophoretic gel. Though the detection method has the advantage of being of a very general application, at least in principle, the main difficulty is to purity the mitochondrial genetic material. A further promising technique for detecting DNA fragments in irradiated food is the microgel electrophoresis of single cells. It utilizes change in mobility by exposing the nuclei of lysed cells embedded in agarose on a microscope slide to an electric current. The proportion of DNA in the 'tail' of the comet compared with the amount of DNA remaining in the 'head' is a measure of DNA damage. This rapid and simple screening method has been shown to be applicable in chicken, onions and potatoes [27-29].

3.4. Immunological Methods

An immunoassay can be simple, robust and very rapid, and can be carried out in non-specialized laboratories. Coupled to an enzyme-linked immunosorbent assay (ELISA) test, using antibodies cyclobutanone haptens may further enhance its sensitivity to both high and low fat-containing foods as well as the specificity of this technique. For the more specific DNA probe method, ELISA, antibodies are raised against thymidine glycol and dihydrothymidine, whose presence indicate radiation exposure. The material was detectable for at least 20days after irradiation, and was not found in non-irradiated control samples [20-32]

4. Biological Methods

Because the cell structure of plant and animal tissue may be affected by ionizing radiation, the changes, which may be observed macroscopically, could serve as detectors of irradiation. The embryo test holds promise for foods containing viable seeds [33-36]. Many foods are known to be contaminated with microorganisms. The direct epifluorescent filter technique (DEFT) and aerobic plate count (APC) can be used together to measure the total microorganisms present before and after irradiation.

4.1. DEFT/APC

The difference between the results of DEET and APC is a measure of microbial inactivation, which is related to the radiation dose received. Originally a method developed for the rapid enumeration of microorganisms in raw milk samples, it is now used for forzen meat and vegetables, alcoholic beverages, confectionery and dried foods, as well as hygiene testing. The DEFT could be used to aid the detection of irradiated foods by direct comparison with a conventional APC. Spices may be judged to have been irradiated if the DEFT count is larger than the APC count by a factor of more than 10^4. The simple, easy method is used for screening with a range of different foods such

as meat, fish, seafood and herbs and spices. However, this microbiological test is not specific and the results need to be confirmed using another technique [37-39].

4.2. Supercooling

Modification of the number of nucleation centres in water-containing biological tissue could happen through sorption of radiolysis products (e.g. free radicals). Frozen, irradiated cod, mushroom and chicken flesh showed significantly greater supercooling when monitored with a differential scanning calorimeter. Ther method can be applied to water-containing foods and it is cheap [40, 41].

5. Conclusions

Sound, properly validated analytical methods with international recommendations will help promote the acceptance of irradiated foods. Currently, the available methods can be divided into screening methods and detection methods. The screening methods include DEFT/APC, as well as different methods based on electrophoresis showing DNA and protein damage. Detection methods used to confirm the screening results include TL, ESR and the identification of lipid volatiles. Some detection tests, such as TL and PSL, depend on changes in adhering minerals, while other tests depend on changes in intrinsic components. Presently, the leading methods of identification are ESR spectroscopy to detect and quantify radiation-treated meat containing bone, and gas chromatography to determine lipid volatile hydrocarbons in irradiated fatty foods.

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