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Identification of Irradiated Foods by Using DNA, Immunochemical, and Biological Methods

– Review –

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Abstract

Ionizing radiation is considered to be an efficient technology to improve food safety and to extend food shelf-life in the food industry, and it has been used in food processing with a number of attributes. Food labeling should be established to enable the consumer to choose food freely, based on label information. A variety of methodologies to determine the physical, chemical, microbiological, and biological changes due to irradiation has been investigated in order to discriminate the irradiated and unirradiated food products for the consumer's free choice in food selection. However, no satisfactory method has been developed so far. In this review, various approaches based on DNA, immunochemical, and biological methods are addressed.

Key words: irradiated food, DNA method, immunochemical method, biological method

INTRODUCTION

Since ionizing radiation is efficient in destroying life forms with relatively low energy input, the number of microorganisms and insects in food could be drastically reduced without any noticeable alteration to the taste or texture of the food. Hence, radiation has been used in food processing with a number of important attributes (1-3). First of all, it can improve the safety of food through the reduction of pathogenic microorganisms and secondly, the shelf-life of food can be extended by reducing decaying organisms. However, like any other food processing technique it must be used properly. The correct dose of radiation (whether gamma rays, electron beams, or X-rays) along with other processing variables such as temperature and atmosphere, must be applied to ensure that the final product is acceptable to the consumer.

One of the key issues related to irradiated foods has been the need to establish detection methods to discriminate the irradiated and unirradiated products in order to give choices to the consumer in food selection. Although many investigations have been performed to detect changes in food exposed to ionizing radiation, no satisfactory method has been developed so far. Currently, a range of tests based on physical, chemical, immunological, biological, or microbiological changes in irradiated food have been researched. In this review, various approaches based on DNA, immunochemical, and biological methods will be discussed.

DNA METHODS

Irradiation causes mutagenesis and transformation as well as reproductive cell death associated with DNA changes. Since most foods are derived from living organisms which all contain DNA, a detection method using DNA could be designed which would have wide applicability. The main radiation-induced lesions are damaged pyrimidine or purine bases, damaged sugar moieties, and strand breaks, either single-strand breaks or double-strand breaks. To analyze these radiation-induced changes, a number of methods based on base damage, DNA denaturation, or strand breaks have been proposed for the purpose of detecting irradiated food. However, one of the difficulties of using various DNA methods is the efficient isolation of DNA from the food in order that subsequent analysis is not interfered with, for instance, due to the gross denaturation or disorganization of DNA structure during isolation or the presence of other food components. Another problem is the handling of food, which also may lead to changes in DNA, e.g. during storage or by other treatments. In the following discussion, only microgel electrophoresis of single cells (comet assay) based on strand breaks and enzyme-linked immunosorbent assay (ELISA) based on base damages will be highlighted.

DNA "comet assay" (3-20)

Principle

Since treatment with ionizing radiation causes DNA fragmentation, migration of DNA in an agarose gel exposed to an electric field offers a simple and rapid tool for identification of irradiated foods. The electric current pulls the charged DNA from the nucleus such that relaxed and broken DNA fragments migrate further and form comets, as broken ends of the negatively charged DNA molecule become free to migrate in the electric field towards the anode. The ability of DNA to migrate is a function of both the size of the DNA and the number of broken 'ends.' Tail length initially increases with damage but reaches a maximum. At low damage levels, stretching of attached strands of DNA, rather than migration of individual pieces is likely to occur. With increasing num-

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bers of breaks, DNA pieces migrate freely to the tail of the comet, and at the extreme, the head and tail are well separated. These two concepts, stretching and migration of separated strands, are generally accepted to explain the DNA migration patterns observed in the comet assay. The distance of DNA migration from the nuclear core is used to evaluate the extent of DNA damage. When DNA damage is minimal and very low doses are used to induce damage, a closer relationship between tail length and dose exists (Fig. 1).

Procedure

Usable parts (i.e., seeds in case of fruits) of samples are isolated, and cells are extracted by using ice-cold phosphate-buffered saline (PBS). The samples should be carefully treated without allowing additional repair or creating additional strand breaks. The extracted cell suspension is mixed with 45°C, 0.8% casting agarose gel solution (1:10). 100 µl is taken from the mixture of cell suspension and spread on an agarose pre-coated slide. Then, the slide is put into a cold lysis buffer (2.5% SDS plus TBE) for 15~60 minutes. Lysis conditions (i.e., salt concentrations, pH, and lysis time in particular) can profoundly affect the ability to detect damage because the extent of cell lysis may depend upon the technique. To remove the impurities obtained from the lysis the slide is placed in a 0.5% TBE buffer (Tris-borate, EDTA) for 5 minutes before electrophoresis. The pre-electrophoresis wash step greatly affects the results of the assay. Residual salt from the lysis solution can be inhibitory to DNA migration and complete removal of the salt requires the use of a much lower voltage. If the slide is not equilibrated with the running buffer before electrophoresis, there will be gradients of salt rising out of the slide and into the buffer during electrophoresis. Salt retards migration of DNA during electrophoresis by particularly neutralizing the charge of the DNA phosphates. Therefore, it necessitates the equilibration of the slide with the running buffer prior to electrophoresis. Electrophoresis is done at 2 volts/cm for 2 minutes in a 0.5% TBE buffer. It

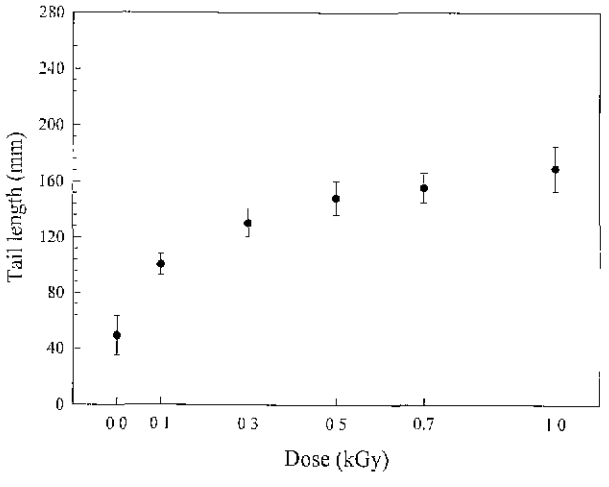

Fig. 1. Dose *vs.* tail length (µm) of the comets from 100 nuclei of wheat kernels irradiated at different doses. Values are the mean (●) and standard deviation (bars) (3).

is more suitable for the determination of relatively large levels of DNA damage to use increased agarose concentration and decreased electrophoresis time. Then, the slide is rinsed with 4°C cold distilled water for 5 minutes and dried for 1 hour at room temperature or 40~50°C oven. Finally, slides are stained with fixing solution and observed through the microscope. The ability to accurately measure DNA damage is dependent upon several technical and biological factors. There are difficulties in comparing the results from different laboratories because of the variability in the application of the technique itself.

Application

The value of this test is that it can be used over a wide range of doses of irradiation and for a variety of products, and that it is simple and rapid. The method was tested on cell suspensions of bone marrow and muscle cells from frozen chicken, turkey, beef, and pork irradiated with doses up to 3 kGy. Experiments with seafood (oysters, mussels), fruits, vegetables, and grains are in progress.

Enzyme-linked immunosorbent assay (ELISA) based on DNA modification (21-25)

Principle

A group of compounds capable of distinguishing small differences between molecules are antibodies. Antibodies bind with a varying degree of specificity to antigens and those which bind with high affinity and extreme specificity can be selected from a heterogeneous population. Although there are many different base modifications, which occur on irradiation of nucleic acids, ELISA is based on two modified bases: thymidine glycol and dihydrothymidine. The two modified bases are conjugated to a number of different molecules such as bovine serum albumin (BSA), or poly-L-alanine to obtain an immune response because compounds of molecular weight less than 1,000 (such as thymidine glycol and dihydrothymidine) do not elicit an immune response. ELISA plates are coated with conjugates of the antigen: i.e., thymidine glycol-BSA or dihydrothymidine-BSA. The test samples are applied to the plate in the presence of the antibody containing cell supernatant. There are two methods: non-competitive (direct) ELISA and competitive ELISA (Fig. 2). In a competitive method, there is competition between the antigen coated onto the plate and the antigen in the sample (Fig. 3). The larger the amount of antigen in the sample is, the lower the amount of antibody which binds to the plate.

Procedure

DNA is modified by using osmium tetroxide or irradiation. Antibodies are prepared corresponding to modified DNA and inhibition assay is followed. Inhibition assay includes the following steps: coat polystyrene microtitre ELISA plates with osmium tetroxide-treated DNA antigen; leave the plates for 3 hours at room temperature; wash the coated plates three times with the coating buffer and leave overnight at 37°C, then seal and store at 4°C; and test the serum and culture medium for antibody.

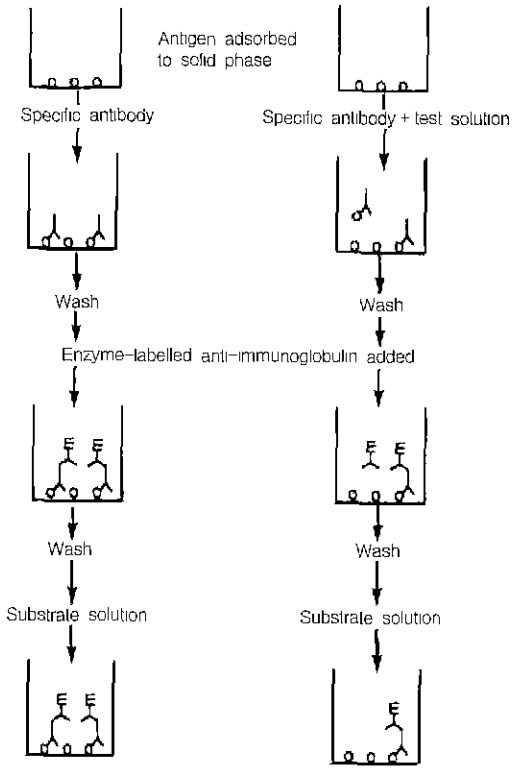

Fig. 2. Non-competitive (left) and competitive (right) ELISA for detection of antibodies (25).

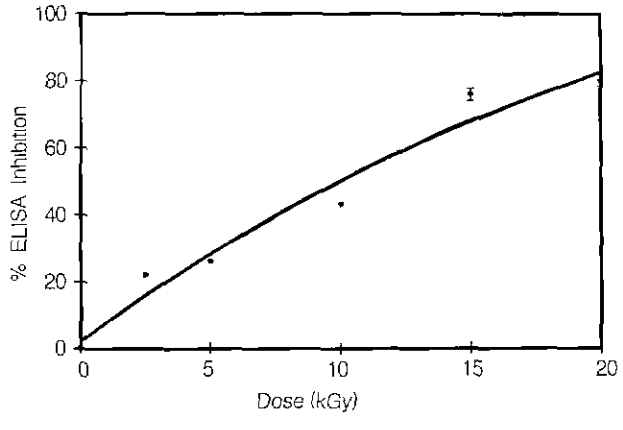

Fig. 3. The effect of irradiation dose on the percentage inhibition in an ELISA assay using dihydrothymine antibody in wheat samples (25).

Application

The ELISA test is rapid and simple to use. And also, it detects specifically the effects of ionizing radiation. This method was successfully practiced with irradiated calf thymus DNA and wheat DNA. By using ELISA, foodstuffs containing DNA can be tested, regardless of the type (fresh, cooked, or frozen).

IMMUNOCHEMICAL METHODS

There are a number of immunochemical methods which merit further research and show promise for the detection of irradiated food. Of those which are currently under examination, the methods based on the radiolytic products formed from proteins and lipids will be discussed.

Immunochemical methods by using changes in egg white proteins (26-29)

Principle

Radiation causes the denaturation of proteins, which results in changes in molecular weight, electrophoretic patterns and inactivation. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDSPAGE) of irradiated egg white protein showed that some components with lower molecular weight were newly produced by irradiation: ovotransferrin (OT), ovomucoid (OM), ovalbumin (OVA). Some bands corresponding to the degraded fragments were visualized more clearly on the membrane by immunoblotting using specific antibodies (Fig. 4).

Procedure

Basically, procedures are composed of three steps: preparation of antisera; polyacrylamide gel electrophoresis; and protein blotting. For preparation of antisera, egg white proteins are dissolved in phosphate buffered saline (PBS) and dissolved proteins are emulsified with an equal volume of Freund's complete adjuvant. Then, emulsified proteins are subcutaneously injected on the back of a rabbit: two booster injections of the same antigen after 14 and 35 days of the first immunization. After 10 days of the last injection bleeding is performed and sera are separated by centrifugation and stored at -80°C before use. SDS-PAGE (10~15% acrylamide) is performed by staining a gel sheet with a mixed solution of 0.2% Coomassie Brilliant Blue R-250 in water, 2-propanol, and acetic acid (5 : 5 : 1 v/v/v), and, then, distaining with 7% acetic acid containing 10% methanol. For protein blotting, electrophoretically separated protein is transferred onto a nitrocellulose (0.45 μm) or polyvinylidene difluoride (PVDF, 0.45 μm) sheet. Nitrocellulose sheet is incubated at 4°C overnight in 3% bovine serum albumin (BSA) in PBS for blocking. After washing the sheet with PBS, it is incubated with rabbit antisera at 37°C for 2 hours with gentle agitation. Then, it

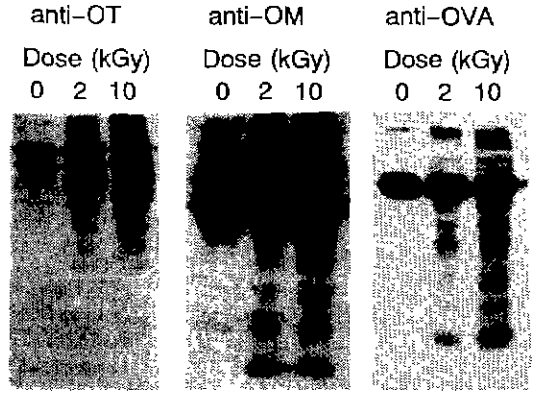

Fig. 4. Immunoblotting analysis of irradiated egg white for anti-OT (left), OM (middle) and OVA (right) (29).

is incubated with peroxidase-coupled anti-rabbit IgG at 37°C for 1 hour following the wash of the sheet with PBS containing 0.02% Tween-20 (PBST). After washing with PBST, the protein bands with reactivity to the specific antibody are visualized by activity staining of peroxidase using 4-chloro-1-naphthol.

Application

The immunochemical method for the identification of irradiated egg is quite effective and would be applicable not only to irradiated egg but also to irradiated egg white proteins added in foods.

ELISA using 2-substituted cyclobutanones (30-32)

Principle

When lipid-containing foods are irradiated, a series of cyclic compounds, 2-substituted cyclobutanones are formed from the corresponding fatty acids. Upon irradiation the four major fatty acids present in most foods, namely palmitic, stearic, oleic, and linoleic acid are converted into 2-dodecyl-, 2-tetradecyl-, 2-tetradecenyl-, and 2-tetradecadienylcyclobutanone. These cyclobutanones can be specifically detected by using antibodies.

Procedure

The basic procedure includes four steps: synthesis of 2-substituted cyclobutanone (2-CB) derivatives; conjugation of 2-substituted cyclobutanones; immunization; and monitoring. 2-substituted cyclobutanone derivatives are synthesized by a multi-step processes. Short chain (C3, C4, C5) 2-CB derivatives are conjugated to large molecular weight carrier proteins to raise antibodies because low molecular weight compounds such as 2-cyclobutanones do not stimulate an immune response. Immunogens are prepared for each of the 2-CB-carrier protein conjugates and antisera are monitored by competitive ELISAs.

Application

It provides a rapid, simple, and sensitive screening method for the detection of radiolytic products in both high and low lipid-containing foods. The ELISA developed using the C10, C12, and C14 cyclobutanone antibodies was validated in a number of food matrices: chicken meat, liquid whole egg, pork and possibly low fat exotic fruits such as papaya and mango.

BIOLOGICAL METHODS

For fresh plant foods, the effects of γ-irradiation are due to changes in biological systems in the food such as the inhibition of sprouting tubers, bulbs and roots, loss of seed viability and delay of maturation and senescence of fruits and vegetables. Moreover, irradiation treatment of fruit and grains is allowed in many countries for purposes such as insect disinfestation and the extension of shelf-life. Some of the detection methods for fruits such as electron spin resonance and thermoluminescence are not sensitive for practical detection, because fruit is usually irradiated with low doses of less than 1 kGy. Changes in the microbial flora and inhibition of root or shoot growth of plant products appear to be reliable indicators of irradiation treatment. However, the weakness of these methods may be the time required to measure the change and the inherent variability of biological systems necessitating the availability of untreated control samples. They could be used in the routine screening of products for irradiation treatment in food control laboratories.

DEFT/APC method (33-43)

Principle

The principle of the method is based on the comparison of results obtained by the direct epifluorescent filter technique (DEFT) and by the conventional aerobic plate counts (APC). DEFT/APC method gives an estimation of microbial numbers pre- and post-irradiation in a sample. The DEFT is used to count the total number of microorganisms, irrespective of viability, in the food sample before any preservation treatment such as irradiation; the microorganisms are captured on a filter and stained with a fluorochrome, acridine orange. The APC reflects the viable microorganisms in the sample at the time of determination capable of growth under the culturing conditions used. Viable organisms, which are fluorescing orange before irradiation, continue to fluoresce orange after irradiation. The difference between the DEFT and APC counts is the number of microorganisms rendered non-viable by the process (Fig. 5). If the log DEFT/APC is at least 3.5 log units, it can be concluded that the sample has been irradiated with a dose level of at least 5 kGy.

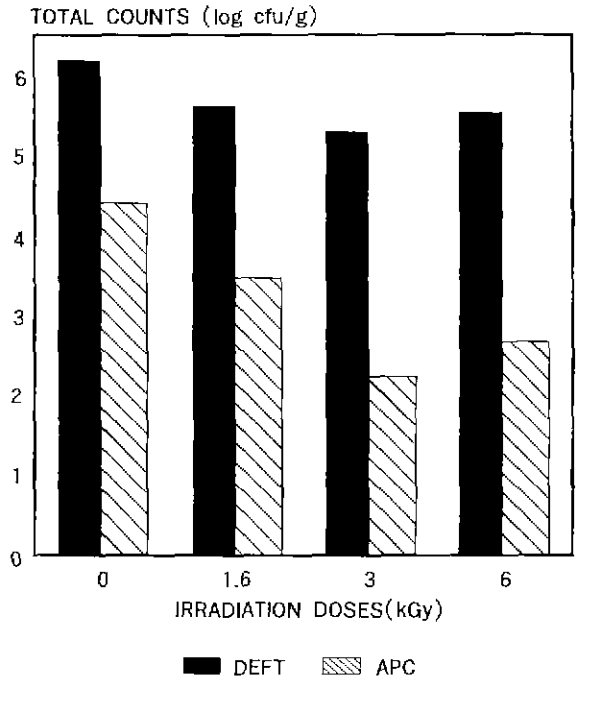

Fig. 5. The logarithmic microbial counts of Norwegian shrimps, both non-irradiated and irradiated, treated with doses of 1.5, 3, and 5 kGy. The results were obtained using the direct epifluorescent filter technique (DEFT) and the conventional aerobic plate count (APC) method (39).

Procedure

The basic procedure includes four steps: pretreatment; filtration; staining; and counting. Samples are pretreated to enable them to be filtered through a 0.6 μm filter prior to the APC and DEFT counts. Usually, different pretreatment procedures are required for different types of sample. For filtration, 0.6 μm Nuclearpore membrane filters are used to trap the microbial cells. Trapped cells are stained with acridine orange and counted with an epifluorescent microscope. For APC, agar plates are incubated at 30°C for 72 hours to allow the individual organisms dispersed through the medium to grow into colonies that can be visually counted.

Application

It has been applied to irradiated samples of minced beef, steak, bacon, pate, gammon, ham, seafood, spices and herbs.

Half-embryo test and germination test (44-48)

Principle

The half-embryo test is based on the inhibition of shoot growth by γ-irradiation. Half-embryo can shoot faster and are significantly more uniform than intact seeds. The half-embryo test is characterized by its easy detection and high sensitivity. The critical dose that inhibits root elongation varies from 0.15 to 0.5 kGy. Root and shoot lengths are more sensitive to γ-irradiation than the germination percentages (Table 1).

Procedure

Seeds are removed from the fruit and washed with distilled water. The outer and inner seed coats are removed with forceps to reveal the embryos. At least 10 half-embryos are placed on distilled water moistened filter paper in a 9 cm covered Petri dish. The dishes are wrapped in aluminum foil and incubated at 25 ~ 35°C. The total number of half-embryos and those producing shoots in each dish are counted after 1, 2, 3, 4, and 7 days incubation.

Application

The half-embryo test was established for the detection of irradiated grapefruit and was applied to other fruits, e.g. apples and cherries, and edible vegetable seeds such as rice, peanut, maize, soybean, red bean, mung bean, and catjang cowpea with a dose above 1.0 kGy. A seedling growth test for the identification of γ-irradiated grape and the other seeds revealed that after treatment with a dose above 1.0 kGy to the seeds, the seedling rate was less than 50% compared with the control. It has been observed that the seed germination of grapefruit is affected by irradiation and shoot growth is totally inhibited by a 0.3 kGy dose. When wheat was irradiated and then germinated in dishes at 25 ~ 28°C for 5 days, germination was inhibited with a dose of 3.3 kGy.

Table 1. Effects of irradiation on wheat germination (45)

Dose (kGy)	Germination rate (%)[1)]	Shoot length (M±SD mm)	Root length (M±SD mm)	Ratio of shoot to root
0	88	67±7	65±9	1.06
0.1	86	67±9	65±18	1.06
0.33	78	54±9[b2)]	38±9[b]	1.42
0.50	78	18±2[b]	10±2[b]	1.80
0.66	78	21±2[b]	12±2[b]	1.75
1.0	84	18±1[b]	11±2[b]	1.64
3.3	60[b]			
6.6	32[b]			
10	2[b]			

[1)]Percentage of shoots longer than 3 mm.
[2)]$p<0.01$

CONCLUSIONS

1. The strength of the comet assay is that it can be used over a wide range of doses of irradiation and for a variety of products, and that it is simple and rapid. The method was tested on cell suspensions of bone marrow and muscle cells from frozen chicken, turkey, beef, and pork irradiated with doses up to 3 kGy. Experiments with seafood (oysters, mussels), fruits and vegetables are underway.

2. The ELISA test based on DNA base damages is specific, rapid and simple to use. This method was successfully practiced with irradiated calf thymus DNA and wheat DNA. ELISA can be used to test foodstuffs containing DNA regardless of the type (fresh, cooked, or frozen).

3. The immunochemical method based on the radiolytic products formed from proteins for the identification of irradiated egg is quite effective and would be applicable not only to irradiated eggs but also to irradiated egg white proteins added to foods.

4. When lipid-containing foods are irradiated, a series of cyclic compounds, 2-substituted cyclobutanones, are formed from the corresponding fatty acids. These cyclobutanones can be specifically detected by using antibodies. It provides a rapid, simple, and sensitive screening method for the detection of radiolytic products in both high and low lipid-containing foods. It has been validated in a number of food matrices: chicken meat, liquid whole egg, pork and possibly low fat exotic fruits such as papaya and mango.

5. DEFT/APC method gives an estimation of microbial numbers pre- and post-irradiation in a sample. The difference between the DEFT and APC counts is the number of microorganisms rendered non-viable by the process. It has been used with irradiated samples of minced beef, steak, bacon, pate, gammon, ham, seafood, spices and herbs.

6. Half-embryo test is based on the inhibition of shoot growth by γ-irradiation. It is characterized by its easy detection and high sensitivity. It was established for the detection of irradiated grapefruit and was applied to other fruits, e.g. apples and cherries, and edible vegetable seeds such as rice, peanut, maize, soybean, red bean, mung bean, and catjang cowpea with doses above 1.0 kGy.

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