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DETECTION OF IRRADIATION TREATMENT OF FOODS USING DNA 'COMET ASSAY'

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ABSTRACT

Microgel electrophoresis of single cells (DNA comet assay) has been investigated to detect irradiation treatment of some food samples. These samples of fresh and frozen rainbow trout, red lentil, gram and sliced almonds were irradiated to 1 or 2 kGy using 10 MeV electron beam from a linear accelerator. Rainbow trout samples yielded good results with samples irradiated to 1 or 2 kGy showing fragmentation of DNA and, therefore, longer comets with no intact cells. Unirradiated samples showed shorter comets with a significant number of intact cells. For rainbow trout stored in a freezer for 11 days the irradiated samples can still be discerned by electrophoresis from unirradiated samples, however, the unirradiated trouts also showed some longer comets besides some intact cells. Radiation treatment of red lentils can also be detected by this method, *i.e.* no intact cells in 1 or 2 kGy irradiated samples and shorter comets and some intact cells in unirradiated samples. However, the results for gram and sliced almond samples were not satisfactory since some intact DNA cells were observed in irradiated samples as well. Probably, incomplete lysis has led to these deviating results.

KEYWORDS

Irradiated food; detection methods; DNA; microgel electrophoresis

INTRODUCTION

During the last few years, several analytical methods have been investigated for the detection of irradiation treatment of foods. Some of these methods have shown very promising results and have been successfully tested in interlaboratory blind trials. For some of these methods, international standards, such as European Standards by the European Committee for Standardization (CEN) have been formulated (Delincée, 1996). These include methods based on ESR spectroscopy of foods containing bones or cellulose, thermoluminescence of foods contaminated with silicate minerals or gas chromatographic analysis of foods containing lipid-derived radiolytic products, such as long chain hydrocarbons or 2-alkylcyclobutanones. However, all these methods require sophisticated equipments and the procedure for analysis is time consuming. Therefore, there is need to find out rapid and simple tests which do not require expensive equipments for screening foods items while suspected samples can then be further investigated by more reliable methods given above.

For foods containing DNA, a sensitive technique to detect fragmentation of DNA as a result of radiation treatment is microgel electrophoresis, also called comet assay (McKelvey-Martin *et al.* 1993, Cerda *et al.* 1997). Cerda *et al.* (1993) reported a simple method based on electrophoresis

of fragmented DNA in irradiated meat samples for detection of irradiation treatment. In this rapid and simple method, after silver staining (Delincée, 1995) the migration patterns of DNA indicates a possible radiation treatment. In the present report, application of this method for detection of irradiation treatment of fresh and frozen rainbow trout (*Salmo gairdneri)*, red lentil (*Lens esculenta*), gram (*Cicer arietinum*) and sliced almond *(Prunus amygdalus)* has been investigated.

EXPERIMENTAL

Fresh trouts were purchased from local market in Karlsruhe. After irradiation, the samples were stored in a freezer. The samples of pulses and sliced almond were also purchased from local market. Irradiation (1 and 2 kGy) was carried out using 10 Mev electrons from a linear accelerator and GAF films were used for dosimetry.

Assay for animal cells was carried out by a procedure similar to one described previously (Cerda *et al.* 1993, Delincée, 1995b): Small samples (*ca.* 1 g) from irradiated and unirradiated rainbow trout were mixed thoroughly in 5 ml phosphate buffered saline (PBS) and after filtration, a 100 µl aliquot of this cell suspension was mixed with 1000 µl of low-melting agarose (0.8 % in PBS). 100 µl of this mixture was spread on pre-coated slides. Lysis of the cells was carried out for 10 minutes with 2.5 % SDS in 45 mM Tris-borate, 1 mM EDTA buffer, pH 8.4. Using the same buffer but devoid of SDS, electrophoresis was performed at 2 V/cm for 2.5 minutes. Silver staining was carried out for 10 minutes (Delincée, 1995a) following fixing and the slides were examined under a microscope for comets resulting from migration of fragmented DNA. For plant cells (red lentils, gram and sliced almonds), the samples (*ca.* 1 g) were grounded in a mortar and mixed with 3 ml PBS. A procedure similar to that described above was followed except that electrophoresis time was 2 minutes and staining was carried out for 20 minutes. Duplicate measurements for each sample were carried out and at least 200 cells were counted for each dose level. Length of the comet migration out of the cell or nuclei after electrophoresis was measured by a transmission microscope.

RESULTS AND DISCUSSION

Using microgel electrophoresis of food items, it is often possible to detect irradiation treatment by just a glance at a slide with nice comets. However, a more thorough analysis by measuring migration length of the comets (in μm) was carried out. Frequency histograms were drawn by plotting number of comets versus length of comets. Although a number of cells with different shapes and lengths were observed in control sample of rainbow trout also, some intact cells with no or only slight comets (ca. 10 µm migration) were always present in non-irradiated samples, as shown in Figures 1 and 2 for fresh rainbow trout. However, for irradiated samples, no intact cells were observed. The length of comets for irradiated samples was much longer because of fragmentation of DNA, as shown in Figures 1 and 2 for radiation dose of 1 and 2 kGy, respectively. It was further observed that comets for 2 kGy were relatively longer than comets for 1 kGy. This relative length of comets can give some indication about the amount of radiation dose delivered to the sample.

The trout samples after irradiation were stored in a freezer (ca. -18 °C) for 11 days and then analyzed by microgel electrophoresis. The results were similar to those shown in Figures 1 and 2 for fresh trout samples. However, some longer comets were present in control sample as well besides intact cells. Nevertheless a clear distinction between irradiated and unirradiated trout samples was possible even after 11 days frozen storage.

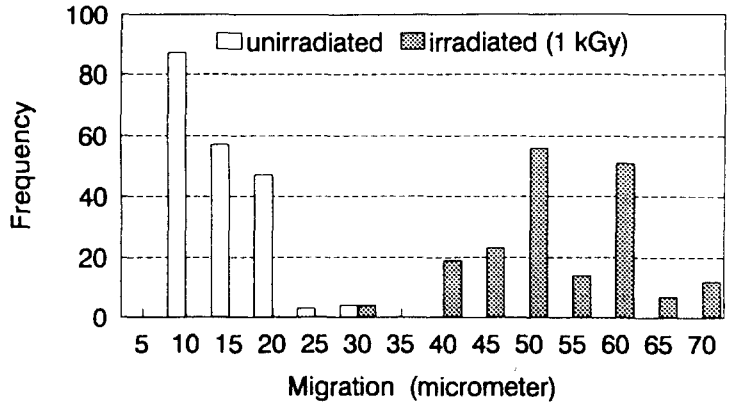

Figure 1. Frequency histogram of DNA comets from unirradiated and irradiated (1 kGy) rainbow trout.

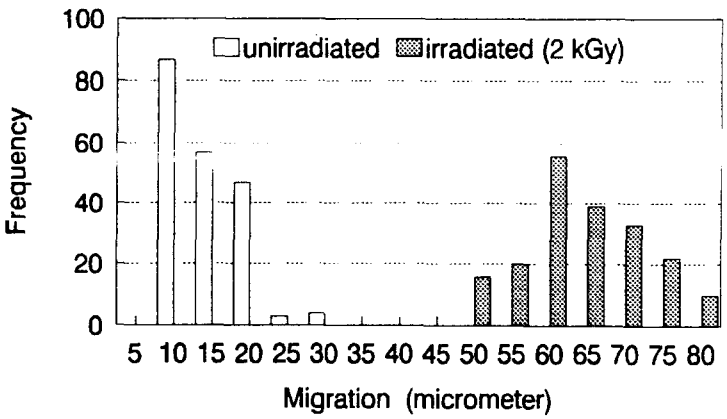

Figure 2. Frequency histogram of DNA comets from unirradiated and irradiated (2 kGy) rainbow trout.

Microgel electrophoresis for fragmented DNA was also applied to other food samples as well, such as lentils, gram and sliced almond. The results for unirradiated and irradiated (1 kGy) samples of red lentils are shown in Figure 3.

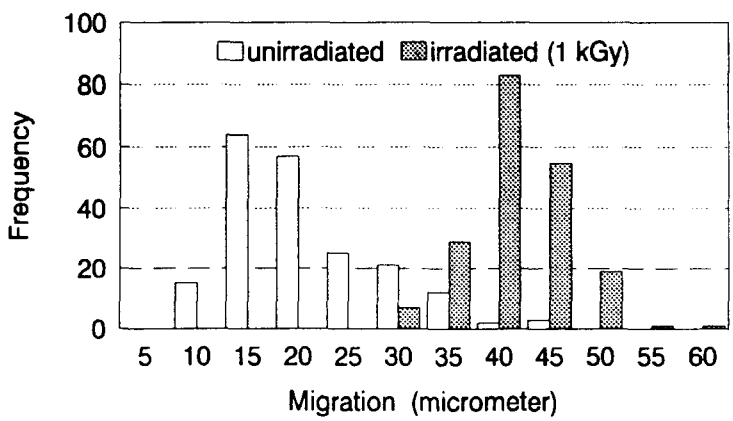

Figure 3. Frequency histogram of DNA comets from unirradiated and irradiated (1kGy) lentils.

In control sample, some intact cells with slight comets were visible together with longer comets. In samples irradiated to 1 kGy, no intact cells were observed and DNA comets exhibited

relatively longer migration distances. The number of longer comets were also higher than in the control samples. A similar histogram was obtained for lentil samples irradiated to 2 kGy. Therefor, a clear distinction between irradiated and unirradiated samples of lentils irradiated to at least 1 kGy is possible using comet assay.

Comet assay tests were also performed for gram and sliced almond. In gram, samples irradiated to 1 kGy also showed some intact cells like unirradiated samples. Gram samples irradiated to 2 kGy, however, showed no intact cells and somewhat larger migration of DNA comets. These results suggest that using the present procedure, gram samples irradiated to at least 2 kGy can be identified using comet assay. For sliced almonds, the samples irradiated to 1 or 2 kGy showed significant numbers of intact cells similar to the control samples. Frequency histograms for irradiated and unirradiated samples were similar, therefore, identification was not possible. In these cases probably a stronger lysing agent, longer lysing time or longer electrophoresis time may lead to better identification of irradiated samples of gram and sliced almond. [NOTE: In the mean time, experiments with plant tissues have shown that in general lysis times of at least 15 minutes are required to make cell membranes permeable. By using these stronger lysis conditions, successful identification of irradiated almonds has been achieved (Cerda *et al.* 1997)]. Several plants give different size and shapes of comets and their susceptibility to radiation is not the same. Therefore, experience with individual samples is needed to clearly differentiate between irradiated and unirradiated samples. Unequivocal identification of suspected samples can be achieved by using other detection methods.

It is concluded that the comet assay procedure can be used as a routine screening test for rainbow trout and red lentils. However, for gram and almond an improved procedure would be needed. Microgel electrophoresis appears to be a very simple and rapid screening test for detection of irradiated foods and does not require costly equipments.

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