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ORIGINAL ARTICLE

Evaluation of irradiation in foods using DNA Comet assay

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Abstract Comet assay is a rapid, inexpensive and sensitive biological technique to detect DNA damage in food stuffs by irradiation. In this study the Comet assay is applied on foods of plant and animal origins. Samples were irradiated by using Co-60 gamma-radiation source. The applied doses were 2, 6 and 10 kGy for food of plant origin and 0.5, 1 and 2 kGy for meat items. The un-irradiated and irradiated samples were clearly differentiated on the basis of DNA fragmentation. During the electrophoresis study, it was found that in un-irradiated cells DNA remained intact and appeared as Comets without tail whereas in irradiated cells Comets with tails were visible due to stretching of fragmented DNA. Moreover, it was also revealed that the DNA tail length was dose dependent. Dry food stuffs (seeds) showed good results as compared to moist foods (meat, fruits and vegetables) due to the absence of background damage.

Keywords Comet assay · DNA fragmentation · Irradiation · Electrophoresis · Absorbed dose

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Introduction

Since 1990, the radiation processing of more than 250 food commodities have been commercialized in various parts of the world. There is an extending list of food commodities which are being irradiated including meat, poultry, vegetables, fruits, cereal, grains and spices (Huachaca et al. 2002). This technique is used to extend the shelf life of foods by reducing or nullifying the microbial load that contaminate the food stuffs. This decontamination reduces health hazards due to the absence of food borne microorganism (Huachaca et al. 2005). Many studies support the use of ionizing radiation for the effective control of pests, reduction of weight loss during post-harvest storage and enhancement of international trade (Bhatti and Kwon 2007; Bhatti et al. 2009). Food irradiation is prohibited in some countries but some do permit it with proper labeling (Chaudhuri 2002). Hence in both the cases the availability of reliable and sensitive detection method in the hands of food control and custom clearance agencies is indispensible (Huachaca et al. 2002). DNA Comet assay provides an inexpensive, rapid, convenient, simple, qualitative and to some extent quantitative method for the irradiation detection of varieties of foods, which are exposed to even low radiation absorbed doses. This assay has increasingly been recognized as a valuable tool for regulatory studies (Koppen and Cerda 1996; Delincee 1998; Park et al. 2000; Villavicencio et al. 2000, 2004; Khan et al. 2002a; Huachaca et al. 2005; Kumaravel and Jha Awadhesh 2006; Cutrubinis et al. 2007).

The DNA molecule because of its large size is a susceptible target for ionizing radiation, therefore changes in DNA offer potential to be used as a biological marker for the detection of radiation treatment. This susceptibility of DNA is a cause to kill microorganisms, insects or parasites effectively in food (Villavicencio et al. 2004). The DNA

Table 1 Optimized conditions to evaluate DNA Comet assay for various food commodities

Food item	Sedimentation time, min	Lysis time, min
Lamb	5-Mar	20
Beef	5-Mar	30
Chicken	5-Mar	15
Fish	5-Mar	15
Onion	15-Oct	15
Potato	15-Oct	45
Ginger	15-Oct	45
Apple	15-Oct	45
Mango	15-Oct	45
Peach	15-Oct	45
Linseed	15-20	60
Chicken	15-20	60
Maize	15-20	60
Wheat	15-20	60

Comet assay technique was applied for the first time on food stuff to measure qualitative as well as quantitative DNA damage in single cell (Cerda et al. 1997). It is now widely used in a variety of research areas such as radiation biology, genetic toxicology and cancer research (Fairbairn et al. 1995; Cerda et al. 1997; Park et al. 2000).

DNA Comet assay is a single cell gel electrophoresis in which DNA migrates out of the cell in the direction of anode, appearing like "Comet". The size and shape of a Comet as well as the distribution of DNA within the Comet have been co-related with the extent of DNA damage (Khan et al. 2002a; Kumaravel and Jha Awadhesh 2006). After radiation treatment, electrophoresis of irradiated cells caused stretching of fragmented DNA. They are stained as Comets with tails and can be clearly distinguished from un-irradiated cells, which remain intact and appear as Comets without tail (Khan et al. 2005).

In the present study, foods of animal and plant origins were used and DNA Comet assay was employed to check its validation for the identification of irradiated foods of both animal and plant sources.

Materials and methods

About 250 g of meat (beef, lamb, chicken and fish), vegetables (onion, potato and ginger), fruit (apple, mango and peach) and seeds (linseed, wheat, maize and chick pea) were purchased from local market. Samples were packed in polyethylene pouches and were labeled for the respective absorbed radiation doses. Meat samples were kept refrigerated before and after irradiation till analysis, vegetables and fruits were frozen after irradiation while the seeds were kept at room temperature (30 °C). The samples were irradiated at Nuclear Institute for Food and Agriculture, Peshawar, Pakistan, using Co-60 gamma ray facility. The fruit, vegetables and seeds were irradiated to the absorbed doses of 2, 6 and 10 kGy and the meat samples were exposed to 0.1, 0.5 and 2 kGy dose levels. Samples were analyzed a week after irradiation employing the DNA Comet assay as described in the European standard with slight modifications (Cerda et al. 1997; ECS 2001).

Different parameters optimized for DNA Comet assay for different food items are listed in Table 1. The slides of un-irradiated and irradiated samples were stained with ethidium bromide (ICN, USA) and evaluated using fluorescence microscope (Labomad, USA).

It is possible to analyze Comets without image analysis software because human eye can readily distinguish Comets representing different levels of DNA fragmentation. In this study Comet data is based on visual scoring as recommended by various authors working on DNA Comet assay (Collins et al. 1995; Cerda et al. 1997; Miyahara et al. 2002; Khan et al. 2002b; Wong et al. 2005). Moreover, many studies revealed that there is an excellent co-relation between visual scoring and other DNA damage measuring parameters (tail length,% of DNA in tail, tail moment) calculated by using computer image analysis (Wong et al. 2005). Two slides for each sample were prepared, 25 Comets on each slides (Total 50 Comets per sample) were classified into several categories based on distortion of DNA. Each Comet was assigned a type ranging from type 1 (undamaged) to type 6 (maximum damage). Then an overall score on arbitrary scale was derived for each sample.

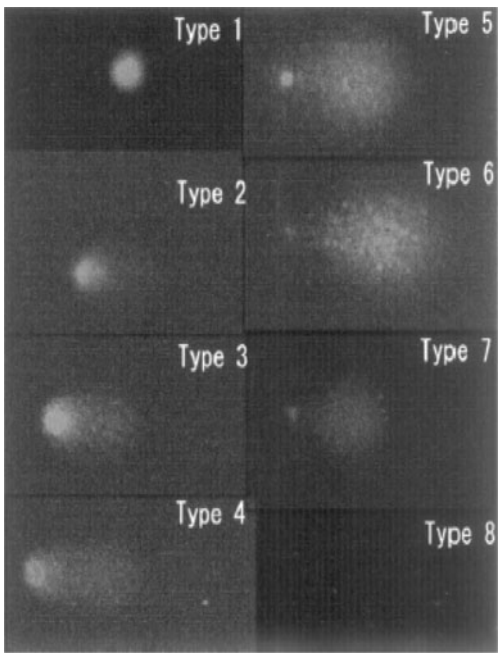

Fig. 1 Typical Comets for types 1–8

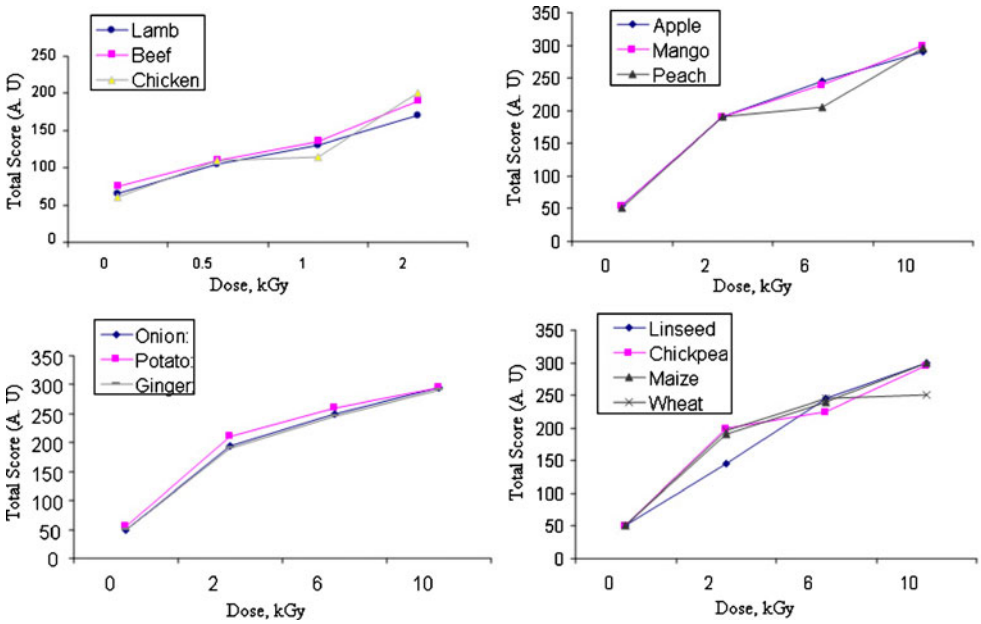

Fig. 2 Absorbed dose response curves for food items (n=3)

Results and discussion

By employing DNA Comet assay the radiation treatments to different food commodities were evaluated. Comet types are classified according to Fig. 1 and the score for each sample was tabulated by using following formula (Miyahara et al. 2002):

$$\text{Total scores} = (\text{No. of Comets of type } 1 \times 1) + (\text{No. of Comets of type } 2 \times 2) + \ldots\ldots + (\text{No. of Comets of type } 6 \times 6)$$

This calculated score is the index of DNA damage. In case of un-irradiated meat samples the total scores were found to be 60, 65 and 75 for lamb, chicken and beef samples, respectively which are shown in Fig. 2. The Comets of type 2 and 3 which represent slightly distorted DNA were also present in these samples along with Comet type 1 representing intact DNA. The appearance of Comet types 2 and 3 in the un-irradiated meat samples indicate background damage.

For un-irradiated vegetables the total scores obtained were 50, 55 and 50 for onion, potato, ginger, respectively. In case of un-irradiated fruit samples the total scores were 55, 55 and 50 for apple, peach and mango, respectively. In the un-irradiated seed (linseed, chickpea, maize and wheat) samples no Comet type other than type 1 was visible, Fig. 2 showing that background damage was negligible. The total score of un-irradiated vegetables and fruits is less as compared to the total score of un-irradiated meat samples indicating less background damage.

The lamb, chicken and beef samples irradiated to an absorbed dose of 2 kGy showed the total scores of 170, 200 and 190, respectively. There was a higher DNA damage found in chicken samples as compared to lamb and beef. The vegetables, fruits and seed samples irradiated to the same absorbed doses showed almost the same trend of DNA damage, which is illustrated in Fig. 2.

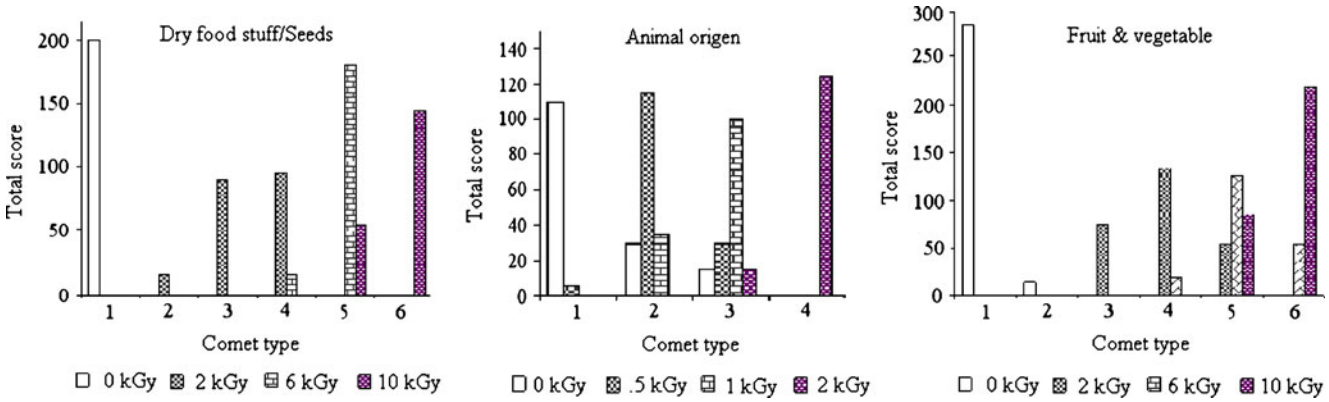

Fig. 3 Distribution of Comet type in different type of foods

In all, the un-irradiated food samples (meat, vegetables, fruits and seeds) Comets of type 1 were predominant, whereas in the irradiated samples the number of Comet types increased with the radiation absorbed dose as shown in Fig. 3. These differences in appearance of Comets were due to the relaxation of super coiled DNA in the nucleus to different extent depending on irradiation doses, which has already been mentioned (Fairbairn et al. 1995; Cerda et al. 1997). In fact, no measurement is required to decide whether the sample is irradiated or not. The irradiation identification can be done only by visualizing the appearance of Comets at a glance. Blind trials have been successfully carried out for variety of food stuffs by this technique. More clear results were obtained for dry food stuffs (seeds) as compared to fresh foods (meat, vegetables and fruits) most likely because DNA damage by other factors is eliminated in dry food stuff. In fresh food stuff cells slightly diffused DNA were also observed especially in non-irradiated meat samples that may be due to natural DNA degradation (Miyahara et al. 2002). Moreover, storage time and environmental toxicologies particularly for fresh food stuff may also cause DNA degradation and mislead the result of analysis. Hence, positive results should be confirmed by other radiation specific identification methods (Koppen and Cerda 1996; Cerda et al. 1997; Huachaca et al. 2002; Khan et al. 2002b, 2005; Villavicencio et al. 2004).

Conclusion

Evaluation of different food items showed that DNA of un-irradiated food samples mostly have intact structure, whereas irradiated samples showed different degrees of DNA damage depending upon irradiation dose. Though this technique is not radiation specific but still it can be valuable for preliminary screening as it is rapid, inexpensive, simple and can be employed for on the spot applications in custom clearance sites for the compliance of labeling regulations.

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