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Use of the DNA Comet Assay to detect beef meat treated by ionizing radiation

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Abstract

The DNA Comet Assay has been described as a rapid and inexpensive screening test to identify radiation treatment of food. In this work, this method was applied to detect the treatment of beef meat pieces either by gamma rays or electron beam. The dose levels were 2.5, 4.5, and 7.0 kGy for chilled samples, and 2.5, 4.5, 7.0 and 8.5 kGy for frozen samples. The analyses were made over periods of 15 and 30 days after irradiation for the chilled and frozen samples, respectively. The effects of gamma rays and electron beam on DNA migration in the test were similar. The DNA Comet Assay, under neutral conditions, made it easy to discriminate between irradiated and non-irradiated beef.

Keywords: DNA Comet Assay; Irradiation detection; Beef meat; Gamma rays; Electron beam

1. Introduction

At present, over 40 countries, including Brazil, have approved the radiation processing of foods (ICGFI, 2004). Food irradiation is a physical treatment of food with high-energy ionizing radiation. It can be used to prolong the shelf life of food products and/or to reduce health hazards associated with certain products due to the presence of pathogenic micro-organisms (Diehl, 1995; Molins, 2001; WHO, 1999). In Brazil, food irradiation has been permitted since 1973 (Brazil, 1973). According to Resolution-RDC No. 21 from 26/01/2001, any food can be treated by ionizing radiation considering that the minimum absorbed dose must be sufficient in order to achieve the intended objective, and the maximum dose must be lower than that dose which may compromise the functional properties and/or the sensorial attributes of food (Brazil, 2001). This legislation is based upon World Health Organization recommendations (WHO, 1999).

At the international conference on "The Acceptance, Control of, and Trade in Irradiated Food", it was recommended that governments should encourage research into detection methods (Anon, 1989). A number of physical, chemical, and biological techniques to detect irradiated foods have been discussed in the literature (Delincée, 1998, 2002a; Haire, Chen, Janzen, Fraser, & Lynch, 1997; Raffi, 1998). Ten international standards regarding different detection procedures for irradiated food have been adopted by the European Committee for Standardization (CEN) and are now available to food control agencies. These Standards have also been

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endorsed as general Codex Methods. One of these methods is the DNA Comet Assay EN 13784 (CEN, 2001) which has been described as a rapid and inexpensive screening test to identify radiation treatment of food (Cerda, Delinceé, Haine, & Rupp, 1997). If the test is carried out under neutral conditions, mainly DNA double-strand breaks are observed, and on electrophoresis of single cells the DNA fragments migrate out of the cells forming a tail in the direction of the anode giving the damaged cells the appearance of a comet. The head of the comet is formed by the remaining nucleus, whereas the tail is dominated by the fragments. The extension of the tail is closely related to the damage intensity (Fairbairn, Olive, & O'Neil, 1995; McKelvey-Martin et al., 1993). Östling and Johanson (1984) observed that fragment migration was a function of radiation dose. With increasing radiation dose more DNA fragmentation occurs, and these fragments migrate further during the electrophoresis. Thus, irradiated cells will show an increased extension of the DNA from the nucleus towards the anode, whereas unirradiated cells will appear nearly circular or with only slight tails.

This method is restricted to foods that have not been subjected to heat or other treatments, which induce DNA fragmentation. Advantages are its simplicity, low cost and speed of measurement (Cerda et al., 1993; Cerda, Delinceé, 1997; Delincée, 1998).

The purpose of this study is to evaluate the practical use of the DNA Comet Assay as a technique for identification of irradiated beef meat, both chilled and frozen. The use of ionizing radiation for treating refrigerated or frozen, uncooked meat, meat by-products, and certain other meat products to reduce levels of foodborne pathogens (and to extend shelf life) has recently been authorised in the USA. Also, other countries including Brazil, have authorised irradiation of meat (ICGFI, 2004). In this paper, the effects of different radiation sources, such as gamma rays from ^{60}Co and electron beam on the DNA damage has been investigated. In addition, the effect of storage has been studied.

2. Materials and methods

2.1. Samples

Refrigerated and frozen meat samples, rump of beef, were purchased in local butcher's shop in São Paulo, Brazil.

2.2. Irradiation

The samples, 5.0 g for each dose level, were packed in plastic bags and labelled. Irradiation was performed using an electron accelerator (Radiation Dynamics Inc., USA; $E = 1.5$ MeV, $I = 25$ mA) or ^{60}Co gamma source (Gammacell 220, AECL; dose rate 5.31 kGy/h). Harwell Amber 3042 Dosimeters were used for the measurement of radiation dose. The radiation dose levels were 2.5, 4.5, and 7.0 kGy for refrigerated samples (4 °C ± 2), and 2.5, 4.5, 7.0, and 8.5 kGy for frozen samples (−18 °C ± 1). Samples were stored post-irradiation for fresh beef and frozen beef up to 15 days and 1 month, respectively.

2.3. Methodology

The DNA Comet Assay was performed essentially as described in the European Standard EN 13784 (CEN, 2001). Briefly, 0.2 g of crushed meat samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. Cell suspension (100 μl) was mixed with 500 μl of low-melting agarose (0.8% in PBS). Hundred microliters of this mixture was spread on precoated slides. The coated slides were immersed in lysis buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 min. The slides were placed in a horizontal electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. Silver staining was carried out for 20 min following fixing. The DNA fragment migration patterns of 100 cells for each dose level were evaluated with a standard transmission microscope. The comet tail length was measured from the middle of the nucleus to the end of the tail. Chilled and frozen samples were tested on days 1, 4, 7 and 15, and 1, 4, 7, 15 and 30 after irradiation, respectively. Results were evaluated statistically using Tukey's test (Montgomery, 1984).

3. Results and discussion

The gradual increase of radiation-induced DNA damage in the beef samples was characteristic for the cells, showing extended migration patterns of DNA fragments, as already described (Cerda & Koppen, 1998) in fresh chicken samples. The comet cells were classified on a morphological basis as illustrated in Fig. 1. Short tail cells with relatively little DNA degradation were classified as type 1. Others types are: type 2, long tail; type 3, long tail wider at the end; type 4, long tail separated from the head of the comet; type 5, almost no DNA is left in the head of the comet and the tail appears as a cloud, far from the head.

It was verified that DNA migration increased concomitantly in relation to applied radiation dose in both frozen and chilled beef meat samples. Irradiated samples clearly showed a more pronounced DNA migration than the control, permitting the control to be distinguished from irradiated samples until one month after treatment (Fig. 2). Some differences between gamma radiation and electron beam treatment in the extent of DNA migration were observed, but these differences were not statistically

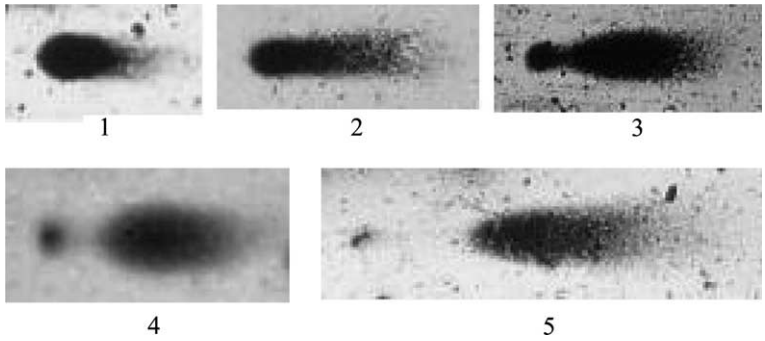

Fig. 1. Photomicrographs of comet types (1–5) in irradiated beef samples.

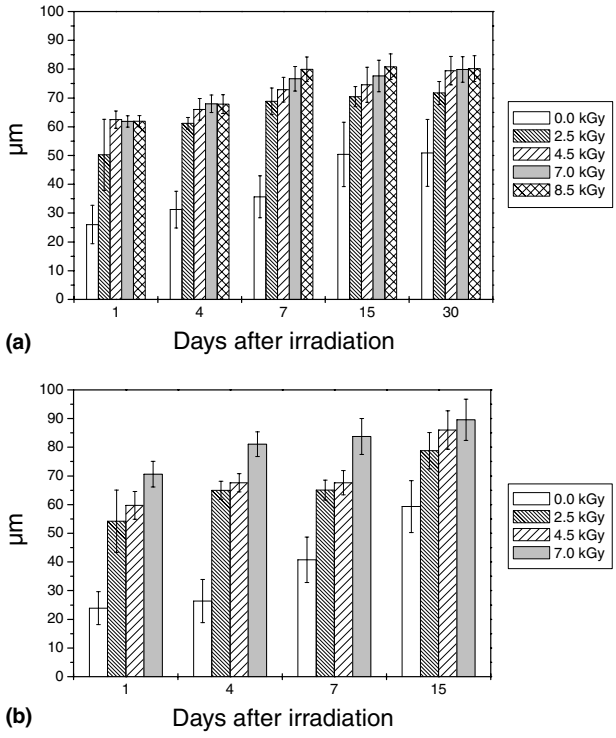

Fig. 2. DNA migration in beef samples treated with gamma rays at: (a) frozen and (b) chilled conditions.

significant. However, significant differences ($p < 0.01$), with regard to the extent of DNA migration, were mostly found for different storage times. At short storage times (up to 4 days) in frozen samples no increase in DNA migration was noted.

Evaluation of comet types in frozen beef samples permitted rapid discrimination between non-irradiated samples and samples irradiated with doses of at least 2.5 kGy due to the presence of type 1 comets, in untreated samples on days 1, 4 and 7 after irradiation. This discrimination was possible on days 15 and 30 due to prevalence of type 2 comets in untreated samples. Similarly, in chilled samples, the prevalence of type 1 comets in the control on days 1 and 4 after treatment by ionizing radiation, and the predominance of type 2 comets on days 7 and 15 in untreated samples allowed a clear discrimination between irradiated and non-irradiated samples.

In Fig. 3 some histograms of the extent of DNA migration are displayed as a function of gamma radiation dose level over the storage period in frozen beef meat samples. On days 1, 4 and 7, the untreated samples showed a predominance of comets with DNA migration ranging from 20 to 40 μm, and on days 15 and 30 these values ranged from 40 to 60 μm. On the other hand, the gamma-irradiated samples on day 1 following irradiation, showed values of DNA migration mainly in the range of 60–80 μm, whereas the values of the electron-irradiated ones were between 40 and 60 μm. It can also be observed that there is a tendency for displacement of histogram bars to the right on the x-axis (i.e., increased extent of migration) with higher radiation doses and longer storage times. In this way, there was substitution of less for more damaged cells when increasing radiation dose level. Furthermore, it was verified that storage time influenced DNA degradation. Chilled gamma-irradiated samples showed values of DNA migration ranging from 20 to 40 μm on days 1 and 4, whereas these values were between 40 and 60 μm on days 7 and 15. Similar results were obtained in chilled samples exposed to electron beam.

The DNA Comet Assay has already been applied for detection of radiation processing in different meat products. In the work of Cerda (1998a), fresh chicken legs, pork chops and salmon, all irradiated with 10 MeV electrons and stored at 2 °C, could be detected by comet pattern observations on days 1, 7 and 14 after irradiation. In the interlaboratory trial carried out by Cerda (1998b), with irradiated frozen chicken and pork samples, even dose estimates were made on the basis of the comet shapes. In that study, from a total of 148 analysed samples, 138 were identified correctly. In Sweden, the DNA Comet Assay was used to control imported possibly irradiated meat and poultry (Merino & Cerda, 2000). Khan and Delincée (1998) could distinguish irradiated rainbow trouts (*Salmo gairdneri*) at doses of 1.0 and 2.0 kGy from untreated ones after 11 days frozen storage. Cerda and Koppen (1998) studied DNA degradation in chilled chicken by the DNA Comet Assay, and observed that

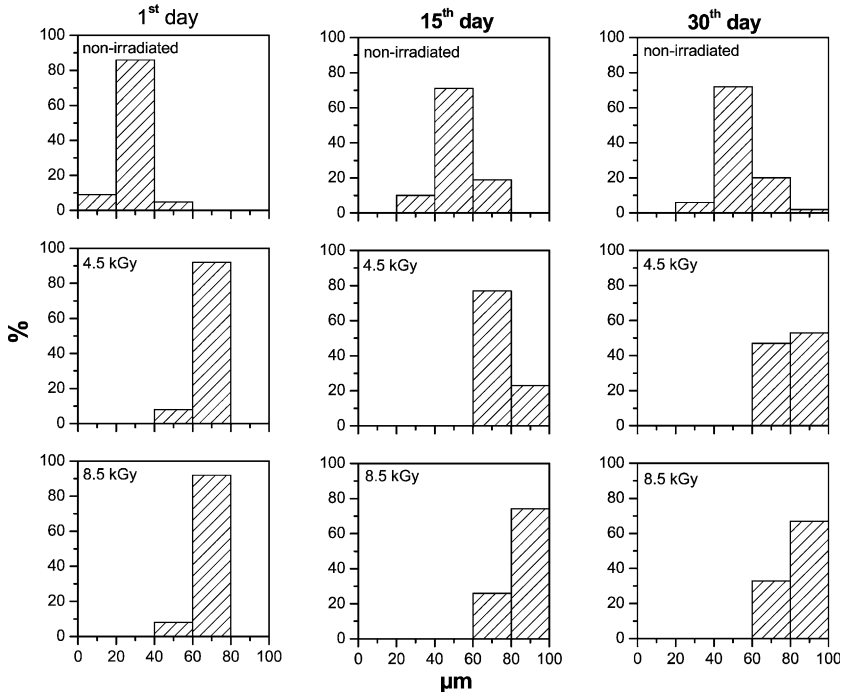

Fig. 3. Distribution histograms of DNA migration extents as a function of radiation dose and storage time in frozen beef meat samples treated with gamma rays.

DNA natural degradation in unirradiated samples increased with regard to storage time, which was reflected in the length and shapes of comets. On day 10, DNA fragmentation increased significantly as compared to the 1st day. They concluded that the DNA Comet Assay also can be used as a rapid method in order to verify the hygienic status of fresh chicken. An increase in DNA migration with storage time was also observed in refrigerated and frozen beef samples applying the Comet Assay (Park et al., 2002). These authors also reported serious DNA damage on repetitive freezing–thawing cycles. However, only a low detergent concentration of 0.2% SDS for lysis was used in their experiments. Furthermore, studies in refrigerated exotic meats such as boar, jacare and capybara (Villavicencio, Mancini-Filho, & Delincée, 2000), and in refrigerated pork (Araújo, Marín-Huachaca, Mancini-Filho, Delincée, & Villavicencio, 2004) and poultry (Villavicencio, Araújo, Marín-Huachaca, Mancini-Filho, & Delincée, 2004) have offered good results. Unirradiated hamburgers could be discriminated easily from those irradiated, even after 9 months frozen storage (Delincée, 2002b). Khan, Khan, and Delincée (2003) applied the Comet Assay on some fresh and frozen samples of meats (lamb, beef, turkey) and fresh seafood to detect an irradiation treatment. The test was successful for meats, but not for fish as only salmon could be identified, whereas irradiated halibut, herring, plaice, saithe and squid could not be detected. These authors applied only storage times up to 6 days post-irradiation and for most cases with meat samples the test was successful. However, in fresh lamb autolytic degradation of DNA on storage precluded adequate detection. The Comet Assay has in the meantime also been used under alkaline conditions for detection of irradiated pork, beef and chicken. However, increased tail formation under these conditions leads to higher background levels for the unirradiated samples (Miyahara, Saito, Ito, & Toyoda, 2002).

It should finally be recognized that the DNA Comet Assay is not radiation specific, therefore, it is recommended to confirm positive results using a validated reference method to prove the irradiation treatment. In this work, the DNA Comet Assay was shown to be a reliable screening method for the detection of processing by ionizing radiation, either gamma rays or electron beam, of both chilled and frozen beef meat until 1 month after irradiation.

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