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Application of a rapid screening method to detect irradiated meat in Brazil

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Abstract

Based on the enormous potential for food irradiation in Brazil, and to ensure free consumer choice, there is a need to find a convenient and rapid method for detection of irradiated food. Since treatment with ionising radiation causes DNA fragmentation, the analysis of DNA damage might be promising. In this paper, the DNA Comet Assay was used to identify exotic meat (boar, jacaré and capybara), irradiated with ^{60}Co gamma rays. The applied radiation doses were 0, 1.5, 3.0 and 4.5 kGy. Analysis of the DNA migration enabled a rapid identification of the radiation treatment.

Keywords: Food irradiation detection; Meat; DNA; Comet assay

1. Introduction

The microbiological safety of meat and meat products has received increased attention from regulators, consumers, researchers, industry, and the media (Nutsch et al., 1997). Irradiation processing can be used as a valuable method of food preservation. The process has the purpose of achieving partial or complete inactivation of cells of specific pathogens or of potential spoilage microorganisms that may be naturally present on unprocessed foods. Radiation pasteurization when used in conjunction with proper food processing and preparation techniques, greatly decreases the probability that food-borne pathogens associated with meat, poultry, and other foods will reach consumers (Thayer et al., 1996). Since the large molecule of DNA is an easy target for ionising radiation, changes in DNA offer potential as a detection method (McMurray et al., 1996). A rapid and sensitive technique to detect DNA fragmentation is the microgel electrophoresis of single cells or nuclei, also called "comet assay". The test is restricted to foods that have not been subjected to heat or other treatments, which also cause DNA fragmentation. Advantages are its simplicity, low cost and speed of measurement. It has already been used for the control of imported meat in Sweden. Although irradiation has been confirmed to be a very efficient method for the production of healthy, wholesome and microbiologically safe food, consumers should be able to make their own free choice between irradiated and non-irradiated food. For this purpose labelling is indispensable. In order to

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check compliance with existing regulations, detection of radiation treatment by analysing the food is highly desirable (Delincée, 1998). This paper intends to report the application of the DNA comet assay to detect the irradiation treatment in special kind of meats.

2. Experimental

2.1. Samples

Refrigerated meat samples, boar (*Sus scrofa*), jacaré (*Caiman yacaré*) and capybara (*Hydrochoerus hydrochoeris* (L.)), were received from a local butcher's shop in São Paulo. The samples were packed and labelled in plastic bags with the packaging date and the irradiation dose for each product sample.

2.2. Irradiation

This was performed with a ^{60}Co source, Gammacell 220 (A.E.C.L.) in São Paulo, Brazil. The dose rate was 8.36 kGy/h. The applied doses were 0, 1.5, 3.0 and 4.5 kGy. The temperature during the irradiation was $20 \pm 2^{\circ}$C and immediately after irradiation the samples were returned to the refrigerator (6°C).

2.3. Analysis

Three samples of each product were used for each dose and samples were analysed 1 day after irradiation. The DNA "comet assay" was performed as described by Cerda et al. (1997). The slides were observed in a photomicroscope and documented photographically.

3. Results and discussion

The results show that the distance of DNA migration, "comet length", increases with radiation dose, for all samples. Also, the size of the dose may be indicated by the shape of the comet. This is not, however, an obligatory requirement of methods for detection of irradiated food. It is of most importance to be able to distinguish between non-irradiated and irradiated samples.

In analysing unirradiated samples, intact cells could be observed. However, in some cases, a small quantity of different "comet" cells in unirradiated samples was visible. Fig. 1a shows very nice intact cells for jacaré meat in the unirradiated sample. At the dose of 1.5 kGy, a "comet" structure becomes visible for the sample, as a "tail" starts to develop in addition to the "head" (Fig. 1b). At 3.0 kGy, (Fig. 1c) more "comets" with lengths greater than at 1.5 kGy can be seen and, in particular, the amount of DNA in the tail increases.

At the highest radiation dose permitted in the USA to process refrigerated meat, 4.5 kGy, special types of "comets" were observed. The amount of

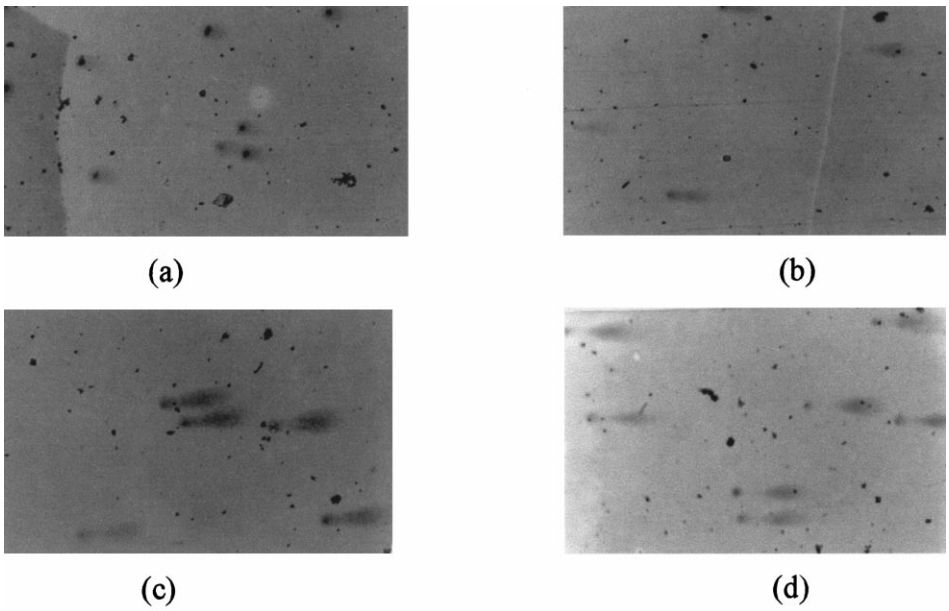

Fig. 1. DNA "comet assay" on refrigerated jacaré meat samples. Anode to the right; silver staining; microscope objective ×10. (a) Non irradiated; (b) irradiated with 1.5 kGy; (c) irradiated with 3.0 kGy; and (d) irradiated with 4.5 kGy.

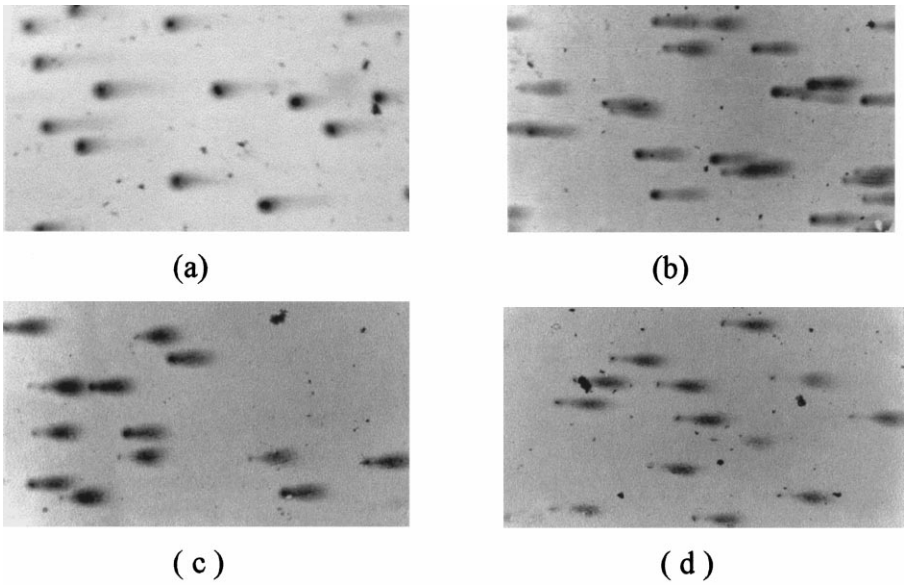

Fig. 2. DNA "comet assay" on refrigerated boar meat samples. Anode to the right; silver staining; microscope objective ×10. (a) Non irradiated; (b) irradiated with 1.5 kGy; (c) irradiated with 3.0 kGy and; (d) irradiated with 4.5 kGy.

DNA in the tail has further increased, and the tail starts to separate from the head. The length and shape of the "comets" reflects the amount of DNA fragmentation, indicating differences in radiation dose. Unirradiated samples are recognised by the presence of cells or nuclei with practically no tail. The majority of the images are of this type (around 90% of the total). At the same time, "comets" of varying shapes appear, representing natural DNA degradation in dead cells (apopthosis). Using the microscope at a magnification of 100 ×, hundreds of cells can be screened on a slide in a short time. In boar (Fig. 2) and capybara (Fig. 3) samples, similar results were observed. These experiments are the first ones

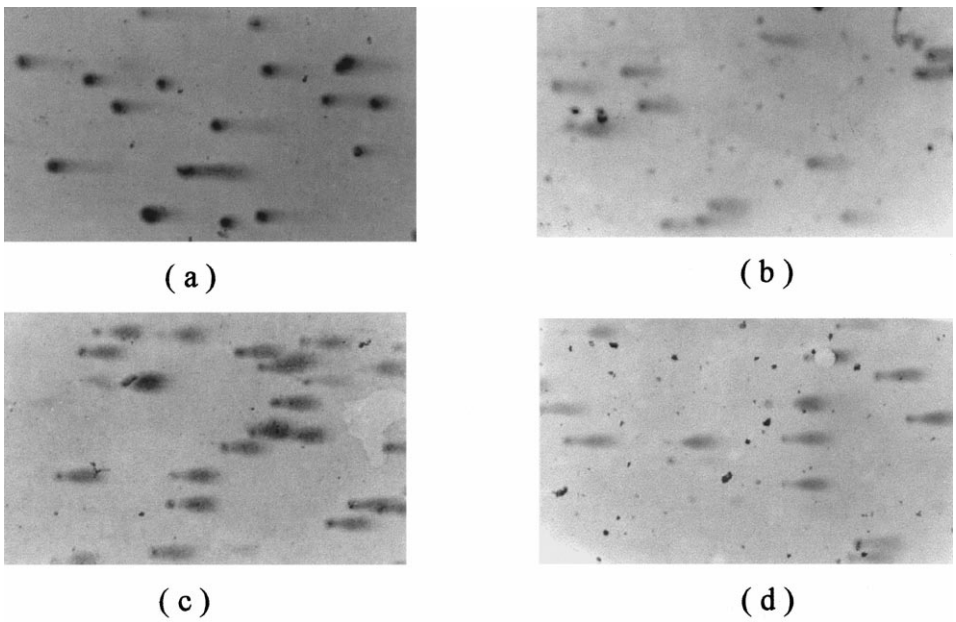

Fig. 3. DNA "comet assay" on refrigerated capybara meat samples. Anode to the right; silver staining; microscope objective ×10. (a) Non irradiated; (b) irradiated with 1.5 kGy; (c) irradiated with 3.0 kGy and; (d) irradiated with 4.5 kGy.

reporting the use of the comet assay with these exotic meats.

Cerda (1998) reported that the "comet assay" can be used for the detection of fresh irradiated chicken, pork and fish. Khan and Delincée (1998), studying irradiated rainbow trout, concluded that the comet assay procedure can be used as a routine screening test. The DNA comet assay offers considerable promise as a simple low-cost and rapid screening test for qualitative detection of irradiation treatment of a wide variety of foods (Cerda et al., 1997). However, the test is restricted to foods not subjected to heat or other treatments, which also induce DNA fragmentation. Recent studies from Cerda and Koppen (1998) reported that using the comet assay the DNA of fresh chicken can be checked, and a general picture of the bacterial contamination may be obtained, thereby using the assay as a freshness indicator.

4. Conclusion

The DNA "comet assay" offers high potential as a rapid screening test for qualitative detection of irradiation treatment of foods. Micro electrophoresis of single cells can be used over a wide dose range and for a large variety of products. It is a simple and rapid test for DNA damage. Suspected samples may subsequently be analyzed by official methods.

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