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Identification of irradiated refrigerated poultry with the DNA comet assay

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Abstract

Food irradiation could make a significant contribution to the reduction of food-borne diseases caused by harmful bacteria such as *Salmonella* and parasites. In fact these organisms cause an increasing number of diseases and eventually deaths all over the world, also in industrialized countries. Radiation processing has the advantage that in addition to eliminating pathogens, thereby enhancing food safety, it also extends shelf life through destruction of spoilage organisms. The DNA molecule because of its big size is an easy target for ionizing radiation, therefore, changes in DNA offer potential to be used as a detection method for the irradiation treatment. In our study, poultry has been irradiated and changes in DNA analyzed by the Comet Assay. Samples were packed in plastic bags and irradiated. Doses were 0, 1.5, 3.0 and 4.5 kGy. Immediately after irradiation the samples were returned to the refrigerator (4°C). Samples were analyzed 1 and 10 days after irradiation. This method proved to be an inexpensive and rapid screening technique for qualitative detection of irradiation treatment.

Keywords: Gamma radiation; Food irradiation detection; DNA comet assay; Poultry

1. Introduction

Food irradiation is recognized as an effective measure to decrease microbiological contamination and to extend shelf life both for fresh and industrially processed foods (Molins, 2001). This treatment when used together, good manufacturing practices and food preparation decreases the probability that pathogenic microorganisms and parasites (in meats and other foods) reaching consumers (Diehl, 2002).

Since the DNA molecule is a susceptible target to ionizing radiation (as well as physical and chemical treatments), DNA damage in cells can be analyzed using the DNA "Comet Assay".

In order to check compliance with existing regulations, detection of radiation treatment by analyzing the food itself is highly desirable (Delincée, 1998). At present, in Europe, a number of standard detection methods have been approved (Delincée, 2002). Meanwhile these nine detection methods are also adopted as general Codex Methods. The DNA Comet Assay is one of these as it detects DNA damage induced by ionizing radiation. This method has been studied in many food items such as meat, fish, grains, and fruits (CEN.EN 13784, 2001). In this paper, its suitability was tested for irradiated refrigerated poultry from Brazil.

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2. Experimental

2.1. Samples

Refrigerated (4°C) poultry meat (chicken) samples were obtained from the local market in São Paulo, Brazil. Samples were packed in polyethylene bags, labeled and identified with its respective irradiation doses.

2.2. Irradiation

Three samples of each dose were irradiated in Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP) at dose levels of 0; 1.5; 3.0 and 4.5 kGy using a ^{60}Co gamma ray facility (Gammacell 220, A.E.C.L., dose rate: 5.41 kGy/h). Immediately after irradiation, samples were replaced in refrigerator (4°C). Harwell Amber 3042 Dosimeters were used for the measurement of radiation dose.

2.3. Methodology

Samples were analyzed 1 and 10 days after irradiation employing the DNA Comet Assay as described by Cerda et al. (1997) and in the European Standard (CEN.EN 13784, 2001).

3. Results and discussion

By employing the DNA comet assay it is possible to detect the irradiation treatment of commercial poultry samples using the various radiation doses applied in this study. With increasing irradiation doses the samples showed an increasing migration distance of DNA fragments. Similar results previously published by different authors with different foodstuffs (CEN.EN 13784, 2001) have shown that non-irradiated DNA is mostly with an intact structure (comet type 10). As an indicator of the radiation process, following the different doses some expressive and typical comets of variable tail length (comets type 20–50) appeared.

Fig. 1 illustrates the scale of typical types of comets which are found in most of irradiated meat samples.

Using this scale type as a reference and depending on the structure of DNA fragments formed, an approximate estimation of the dose applied could be done. Intact nuclei could be mainly observed in non-irradiated samples immediately after irradiation (Figs. 2 and 3). Some different types of comets probably due to natural DNA degradation in dead cells could also be observed in non-irradiated samples, particularly after storage. Storage time and other factors such as frozen/heat cycles also induces DNA degradation and can mislead the result of the analysis (Cerda and Koppen, 1998; Cerda, 1998; Park et al., 2000; Khan et al., 2003).

With increasing radiation doses, a larger quantity of DNA is fragmented, forming the typical tail in comet structure (Figs. 2 and 4).

The results are in accordance with previous studies (Cerda et al., 1997; Villavicencio et al., 1998, 2000; CEN.EN 13784, 2001; Khan et al., 2003) using DNA comet assay to detect irradiated food. It is worth saying that in this study no image analyzer to quantify comets types was required, due to the ease and simplicity of the method. Using this screening technique, an effective

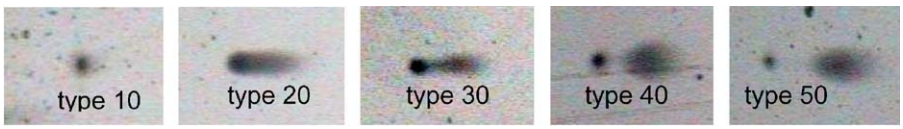

Fig. 1. Scale of Comet types.

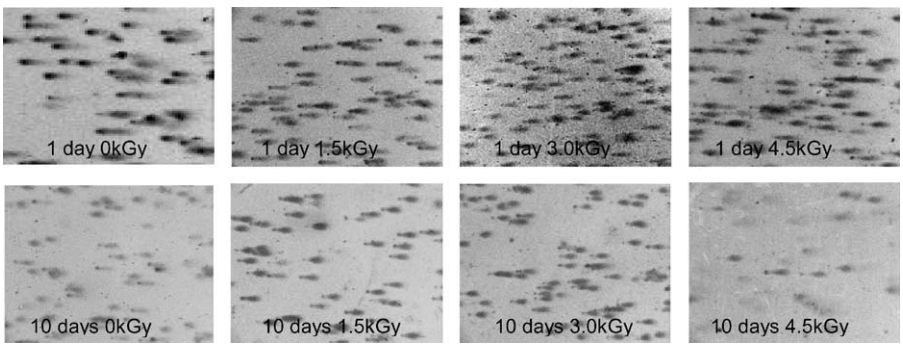

Fig. 2. Refrigerated poultry meat: different irradiation doses and storage time.

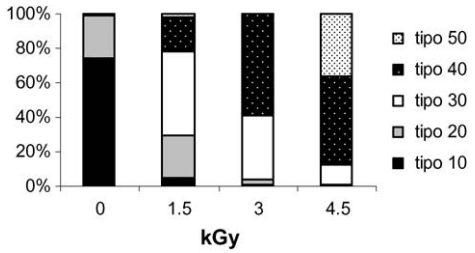

Fig. 3. Percentage of comets types 1 day after irradiation.

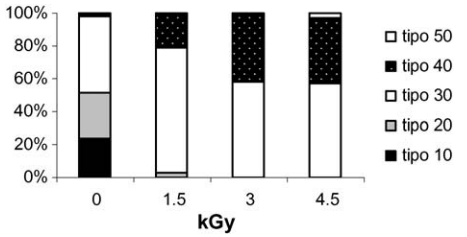

Fig. 4. Percentage of comets types 10 day after irradiation.

measure of DNA fragmentation induced by radiation is achieved in order to control and enforce the labeling of irradiated foods.

4. Conclusion

For control of labelling, e.g. for export trials of foods treated by irradiation for sanitary reasons, the DNA comet assay can be utilized as a screening technique. Satisfactory results were obtained on irradiated refrigerated poultry samples from Brazil. Further studies with other samples and techniques need to be realized. Microgel electrophoresis of cells or nuclei is a fast technique, simple and inexpensive for a qualitative detection of the irradiation treatment. However, in case of suspected samples or positive results, it is necessary to confirm by another validated method to prove an irradiation treatment.

Acknowledgements

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