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Identification of irradiated refrigerated pork with the DNA comet assay

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Abstract

Food irradiation can contribute to a safer and more plentiful food supply by inactivating pathogens, eradicating pests and by extending shelf-life. Particularly in the case of pork meat, this process could be a useful way to inactivate harmful parasites such as *Trichinella* and *Taenia solium*. Ionizing radiation causes damage to the DNA of the cells (e.g. strand breaks), which can be used to detect irradiated food. Microelectrophoresis of single cells ("Comet Assay") is a simple and rapid test for DNA damage and can be used over a wide dose range and for a variety of products. Refrigerated pork meat was irradiated with a ^{60}Co source, Gammacell 220 (A.E.C.L.) installed in IPEN (São Paulo, Brazil). The doses given were 0, 1.5, 3.0 and 4.5 kGy for refrigerated samples. Immediately after irradiation the samples were returned to the refrigerator (6°C). Samples were kept in the refrigerator after irradiation. Pork meat was analyzed 1, 8 and 10 days after irradiation using the DNA "Comet Assay". This method showed to be an inexpensive and rapid technique for qualitative detection of irradiation treatment.

Keywords: Gamma radiation; Food irradiation detection; DNA comet assay; Pork meat

1. Introduction

In order to give the consumer the assurance that meat processed by irradiation is a safe product, a great deal of research has been developed in the world. The effect of irradiation on the hygienic quality of meat and meat products is considerated as related to the control of meat-borne parasites of humans; elimination of pathogens from fresh meat and poultry and elimination of pathogens from processed meat (Brito et al., 2002).

Correct and comprehensive information about food irradiation and irradiated food must reach consumers in order to enable them to reach decisions based on well-founded reasons (Bruhn, 1995). A food that has been irradiated must be labeled to control the process, and to ensure a free consumer choice (Morehouse, 2002) and methods to identify irradiated foods are highly desirable (Marin-Huachaca et al., 2002). Methods based on DNA alterations could be applied to a large amount of foods (Cerda et al., 1997; Delincée, 1998, 2002a,b).

2. Experimental

2.1. Samples

Refrigerated pork meat samples were obtained from the local market in São Paulo, Brazil. Samples were

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packed in polyethylene bags, labeled and identified with its respective irradiation doses.

2.2. Irradiation

Samples were irradiated in Instituto de Pesquisas Energéticas e Nucleares (IPEN—CNEN/SP) at doses 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.

2.3. Methodology

Samples were analyzed 1, 8 and 10 days after irradiation. The DNA Comet Assay was carried out for the detection as described by Cerda et al. (1997). European Standard—"DNA Comet Assay" EN 13784 (Delincée, 2002a,b).

3. Results and discussion

The results show that with the increase of irradiation dose, a increase of migration distance of DNA fragments also occurs. Intact nuclei of round shape can be observed in non-irradiated samples (comets type 10), until big fragments represented in typical comets of variable tail length (comets type 20–50), as shown in Fig. 1.

Depending on the structure of DNA fragments formed (Fig. 2), an approximate estimation of dose applied could be done. Intact nuclei could be observed in non-irradiated samples and also in minor quantity in those with lower irradiation doses (Fig. 3). Non-irradiated samples show different types of comets probably due to natural DNA degradation in dead cells (Fig. 3). A large storage time and others factors such as frozen/heat cycles also induces DNA degradation.

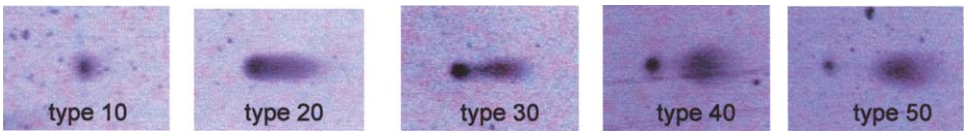

Fig. 1. Types of comets photomicrographies.

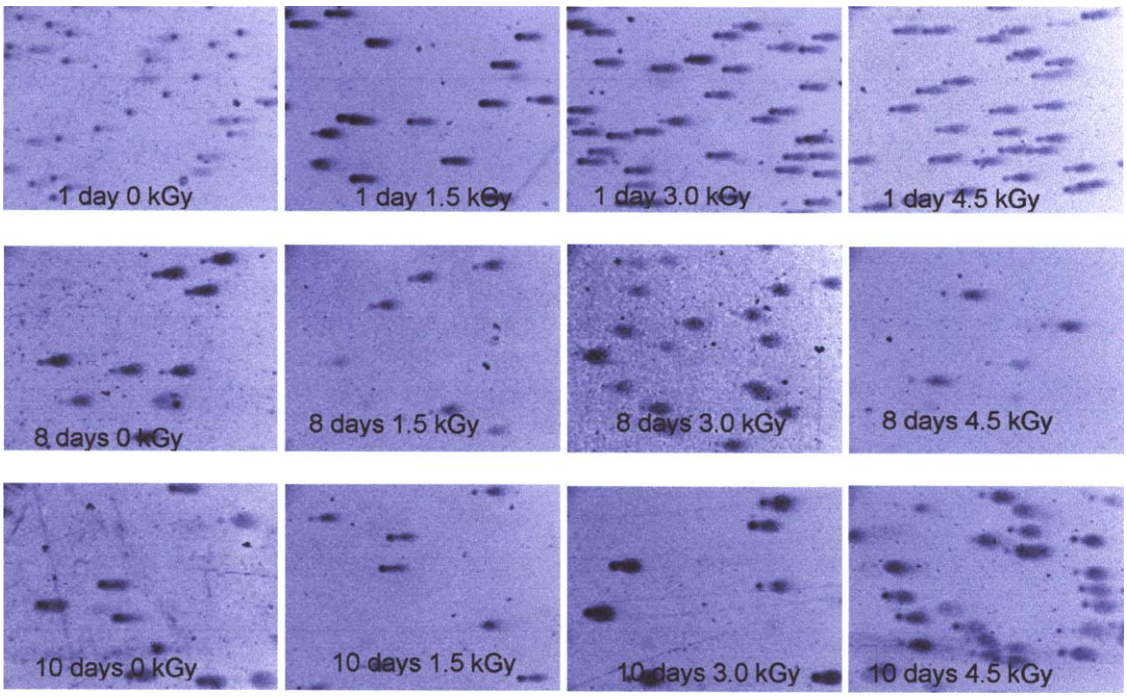

Fig. 2. Refrigerated pork meat: different irradiation doses and storage time.

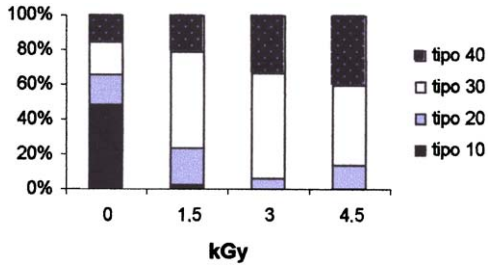

Fig. 3. Percentage of comets types 1 day after irradiation.

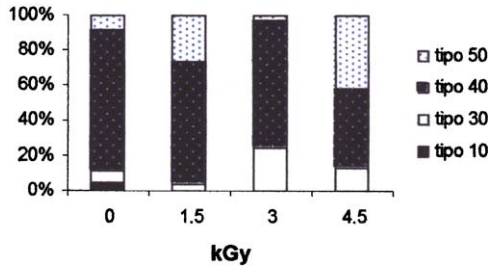

Fig. 4. Percentage of comets types 8 day after irradiation.

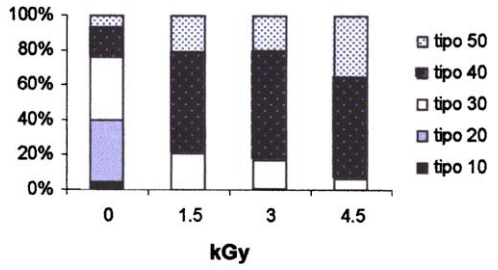

Fig. 5. Percentage of comets types 10 days after irradiation.

It was observed in Figs. 4 and 5 that with higher doses, a great DNA quantity would be fragmented, forming the typical tail in comet structure.

At doses above 1.5 kGy, different types of comets were observed when compared to non-irradiated samples (Fig. 2).

Our results are in accordance with similar studies shown in literature. Delincée (2002a,b), has got similar results with irradiated frozen hamburger. Several authors, Villavicencio et al. (1998, 2000), Delincée (1993, 1998, 2002a,b), Cerda et al. (1997), using DNA Comet Assay to detect irradiated food, showed that due to radiation effect on food DNA alterations were found similar to our study. It is worth to say that as most studies realized, we also did not use an image analyzer to quantify comets types, due to the facility and simplicity of this method. Using this technique, an effective screening of DNA fragmentation induced by radiation is obtained.

4. Conclusion

In Compliance with the results found, it was observed that DNA Comet Assay could be used satisfactorily with the studied samples. Further studies with others samples must be realized to verify this technique in a large variety of foods. Microgel electrophoresis of cells or nuclei is a fast technique, simple and inexpensive for a qualitative detection of irradiation treatment. In case of suspected samples or positive results, it is necessary to confirm by a validated method to prove an irradiation treatment.

Acknowledgements

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