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Effects of the ionizing radiations, freezing and thawing duration on chicken liver cells quality

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ABSTRACT

All food storage processes modify the food. Irradiation reduces and could stop cell division, avoid infestation, reduce contamination and delay food decomposition. The cold chain is a succession of steps which maintains the food at low temperature. Defrosted food shall never be frozen again, the best way being to consume it quickly then avoiding multiplication and acceleration of microbial growth, which causes decay and nutrients damage. The Comet Assay indicates DNA damage and can then be used to control the overall quality of the food and in a certain extent to evaluate the damage caused by irradiation and storage on liver chicken cells. In this work, different thawing temperatures and radiation doses were checked to establish a "DNA damage index" by using the Comet Assay. Samples were irradiated in a ^{60}Co irradiator with 1.5, 3.0 and 4.5 kGy radiation doses. Our results showed that no intact cells were detected in frozen samples: however, irradiated liver samples *in natura* showed some intact cells depending on the applied radiation doses.

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1. Introduction

Quite all the conservation process change both the chemical composition and the external aspect of foods (Édira et al., 2004). For example, heat process can promote changes in concentration of volatiles or trace elements (Ben, 1999). Such losses of various macro or micro-nutrients and trace elements may be detrimental for the human health (Belitz and Grosch, 1988). The effect of irradiation on the hygienic quality of meat and meat products is considerable as related to the control of meat-borne parasites of humans elimination of pathogens from fresh meat and elimination of pathogens from processed meat (Brito et al., 2002). But this treatment may induce some off flavors and some nutritional losses. Commercial irradiation of foods has been allowed in Brazil since 1973, and now more than 20 different food products are approved (Villavicencio et al., 2002). Freezing or food storage at sub-freezing temperatures may induce the formation and growth of ice crystals that can damage the cell walls, reducing the water content on the cells involving protein denaturation and subsequent losses of nutrients. On the other hand, freezing does not kill microorganisms, but only stop their proliferation and inhibits their metabolism by removing the bio-available water through the formation of extra and intracellular ice crystals. During the cold chain process, chilled meat should be kept at temperatures between −1.5 and +7 °C, frozen meat is kept at temperatures lower than −12 °C and super frozen meat products have to be maintained at temperatures lower than −18 °C (Luchiare, 2006). A temperature from −5 to −7 °C inhibits the bacterial growth by the reduction of water availability and by the reduction of the enzymatic activity of living cells. A temperature below −20 °C suppresses the water availability and inhibits all the enzymatic activities. Thawing frozen food results in proliferation microorganisms and acceleration of their growth, because of the presence of nutrients, lost by the damage cells (Brito et al., 2002), providing the food deterioration and therefore, nutrients losses (Silva Jr., 2002). The Comet Assay is a biological test, originally used to identify irradiated foods by visual microscope observation of the DNA damage degree. This method is cheap and allows a quick identification of lysed cellular material. The degraded cells look like comets. The gradual increase of DNA damage increases the length of comet tail. For the highest DNA fragmentation, the comet does not increase any more although DNA fragments continue migration from head to tail (Faullimel et al., 2005). As freeze–thaw cycles induces DNA fragmentation because of the reactivation of cytosolic enzymes, due to the increase of temperature, the Comet Assay seems to be a good candidate for the detection of such temperature abuses during conservation of frozen meat products. On the other hand, irradiation also induces DNA damages (Araújo et al., 2004). The food that has been irradiated must be labeled to control the process and to ensure a free consumer choice (Morehouse, 2002).

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Methods to identify irradiated foods are highly desirable (Marin-Huachaca et al., 2002). It becomes interesting to investigate them, if these two processes give occurrence to different single cell electrophoretic patterns allowing then the Comet Assay to differentiate between an irradiated sample from a non-irradiated one, but which was submitted to bad storage conditions.

2. Experimental

2.1. Samples

The samples of liver chicken *in natura* were obtained in local market in São Paulo, Brazil. During transportation, samples were stored in thermal bags, packed with indication of their respective irradiation doses, storage time and temperature. They were frozen at −13 °C and defrost during 1 and 3 h.

2.2. Irradiation

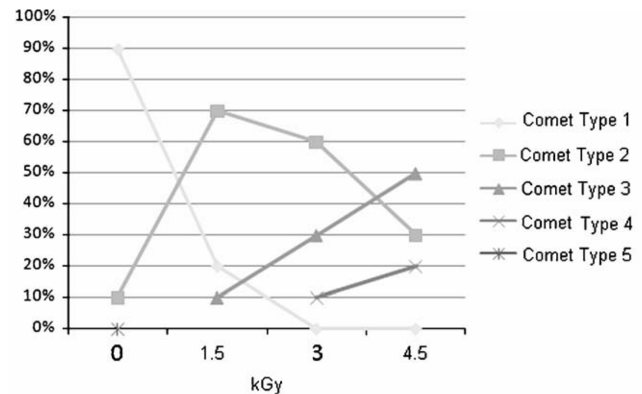
The samples of liver chicken were irradiated at room temperature at the Institute for Nuclear and Energy Research, (IPEN-CNEN/SP), Center of Radiation Technology at doses of 1.5, 3.0 and 4.5 kGy, using a ^{60}Co gamma ray facility (Gammacell 220, A.E.C.L) with a dose rate 2.64 kGy/h. Absorbed doses were controlled with Harwell Amber 3042 Dosimeters.

2.3. Methodology

The non-irradiated *in natura* samples were analyzed immediately on arrival at the laboratory. The irradiated samples were analyzed immediately after irradiation and the frozen samples were analyzed seven days after being irradiated and frozen. The samples which have been defrosted were analyzed after 1 and 3 h after thawing. All the samples were analyzed using the Comet Assay as described by Cerda et al. (1997) and in the European Standard (CEN.EN 13784, 2001). A total of 5 g of crushed (thin pieces of liver chicken) 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 600 µl of low-melting agarose (0.8% m:v in PBS). A total of 100 µl of this mixture was spread on pre-coated 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 without SDS. The electrophoresis conditions were 2 V/cm for 2 min (100 mA). SyBr Gold staining solution (15 µL SG+150 mL PBS) during 10 min at 4 °C. The DNA electrophoretic pattern was recorded on 100 cells for each treatment applied on the food. The morphological evaluations were performed with a fluorescence microscope (420/510 nm). The comets tail lengths were measured from the middle of the nucleus to the end. The samples with 5.0 g were packed in plastic bags and labelled.

3. Results and discussion

The DNA Comet Assay was applied to detect an irradiation treatment on samples of chicken liver (Villavicencio et al., 1997). With increasing irradiation doses, the samples showed an increasing migration distance of DNA fragments. Similar results already published by different authors with different foodstuffs (CEN.EN 13784, 2001) have shown that non-irradiated DNA is



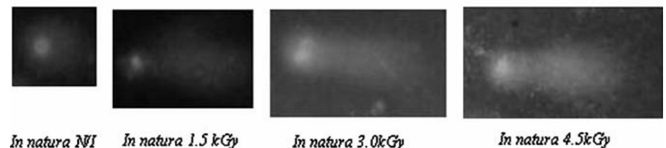
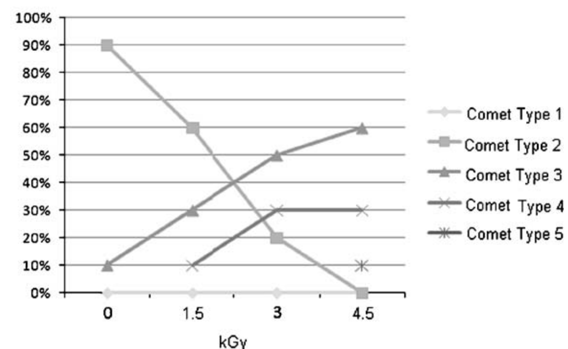


Fig. 2. Type and percent of comets in liver chicken, all *in natura*, not irradiated and irradiated (1.5, 3 and 4.5 kGy). Photos of comets stained with SyBr Gold and visualized in a fluorescence microscope.



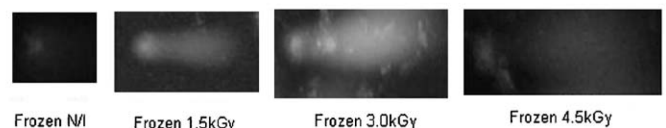


Fig. 3. Type and percent of comets in liver chicken, not irradiated and irradiated (1.5, 3 and 4.5 kGy), frozen during seven days. Photos of comets stained with SyBr Gold and visualized in a fluorescence microscope.

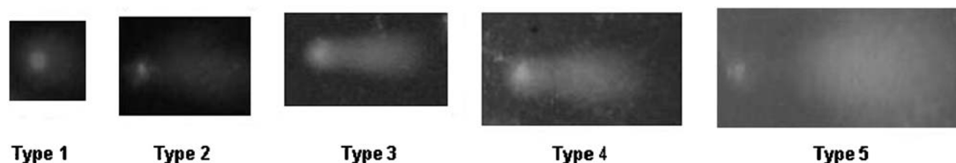


Fig. 1. Scale of typical types of comets which were found in most irradiated, frozen and defrosted chicken liver samples. Photos of comets stained with SyBr Gold and visualized in a fluorescence microscope.

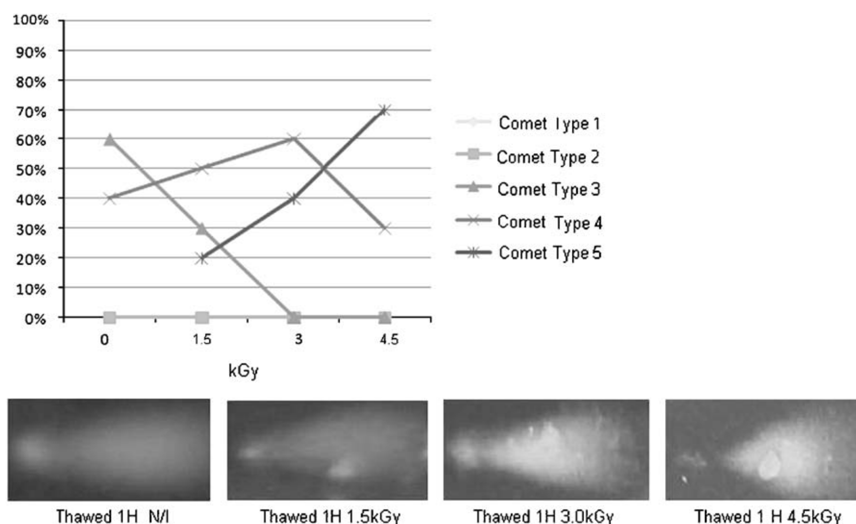


Fig. 4. Type and percent of comets in liver chicken, not irradiated and irradiated (1.5, 3 and 4.5 kGy), frozen during seven days and defrosted during 1 h. Photos of comets stained with SyBr Gold and visualized in a fluorescence microscope.

mostly with an intact structure and present comet of type 1 (Fig. 1). 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, as already shown by other authors (Cerda and Koppen, 1998; Cerda, 1998; Park et al., 2000; Khan et al., 2005). We propose to classify the comet as a function of their length which gives an indication of the dose absorbed by the analyzed sample (Fig. 1).

Using this as reference, an estimation of the DNA degradation can be made. Storage time and other factors such as freeze–thaw cycles can be quantified. It is clear that the irradiation, freezing, thawing and refreezing were responsible factors of the DNA degradation. The lowest degradation could be observed in liver chicken *in natura* not irradiated (Fig. 2).

On the other hand, in samples which were frozen, it was not possible to find any comet of type 1 (Fig. 3). Freezing process seems to degrade with the same intensity all cells present in the samples. This overall degradation does not occur in the case of food irradiation. Indeed radiation processing do not degrade all the cells, but seems to leave some intact cells.

During thawing, the cell walls are broken and food micro-nutrients are dragged along with the intracellular water. Moreover, during thawing, the temperature rise above 0 °C and the enzymatic activity is boosted again, giving rise to a DNA fragmentation, due to the activity of cytosolic enzymes. Thawing duration contributes then to the DNA degradation amplitude as presented in Figs. 4 and 5.

The higher degradation was fonud on cells that were irradiated, frozen and defrosted during 3 h (Fig. 5). The same way that the increase of irradiation dose, occur an increase of the cellular degradation (Fig. 2), is possible to say that higher storage time of the product, higher is the cells degradation (Fig. 3) and the same way occur with the defrost time (Figs. 4 and 5).

Our results are in accordance with similar studies shown in literature. Delincée (2002a) and Delincée (2002b) have got similar results with irradiated frozen hamburger. Several authors, Villavicencio (1998), Villavicencio et al. (2000), Delincée (1993, 1998, 2002a, 2002b), and Cerda et al. (1997), using DNA Comet Assay to detect irradiated food, showed that due to the radiation effect on food DNA alterations were found similar to our study. Time–temperature abuse, Faullimel et al. (2005). It is worth to say

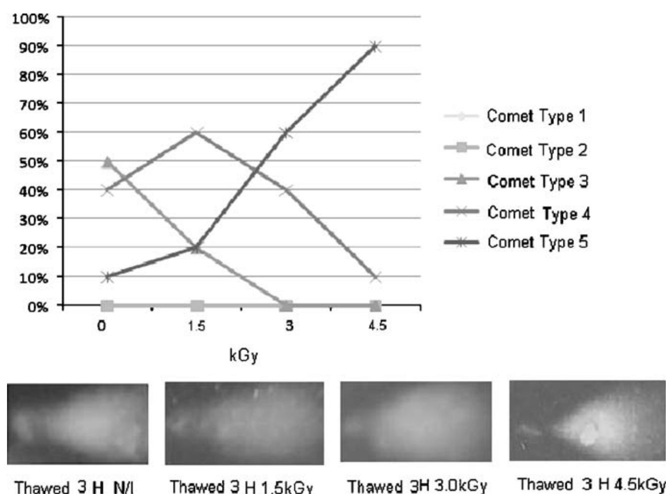


Fig. 5. Type and percent of comets in liver chicken, not irradiated and irradiated (1.5, 3 and 4.5 kGy), frozen during seven days and defrosted during 3 h. Photos of comets stained with SyBr Gold and visualized in a fluorescence microscope.

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 showed, it was observed that Comet Assay could be used to detect the overall quality of liver chicken by using the proposed screening method. It was possible to avoid the use of a costly image analyser as proposed by other authors (Faullimel et al., 2005). This technique is fast and simple for a qualitative detection of irradiation treatment or for non-irradiated food having been submitted to temperature abuses.

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