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Rapid detection of irradiated frozen hamburgers

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Abstract

DNA comet assay can be employed as a rapid and inexpensive screening test to check whether frozen ground beef patties (hamburgers) have been irradiated as a means to increase their safety by eliminating pathogenic bacteria, e.g. *E. coli* O157:H7. Such a detection procedure will provide an additional check on compliance with existing regulations, e.g. enforcement of labelling and rules in international trade. Frozen ready prepared hamburgers from the market place were 'electron irradiated' with doses of 0, 1.3, 2.7, 4.5 and 7.2 kGy covering the range of potential commercial irradiation. DNA fragmentation in the hamburgers was made visible within a few hours using the comet assay, and non-irradiated hamburgers could be easily discerned from the irradiated ones. Even after 9 months of frozen storage, irradiated hamburgers could be identified. Since DNA fragmentation may also occur with other food processes (e.g. temperature abuse), positive screening tests shall be confirmed using a validated method to specifically prove an irradiation treatment, e.g. EN 1784 or EN 1785.

Keywords: Food irradiation detection; Meat; Hamburger; DNA comet assay

1. Introduction

Treating food with ionising radiation is one of the many antimicrobial interventions available to producers, which can be included as a critical control point (CCP) within the frame of a HACCP system to produce microbiologically safe food products. The safety and nutritional adequacy of irradiated food are recognised by the scientific community and international organisations (Diehl, 1995; WHO, 1999). In view of the increasing incidence of foodborne diseases in the past few decades—up to 30% of the population in industrialised countries suffers from infections or intoxication caused by food each year—attention is concentrating on remedies to arrest this development (Press Release WHO/4 of 25 January 2000; WHO, 2000; CEC, 2000). The use of ionising radiation for treating refrigerated or frozen, uncooked meat, meat by-products, and certain other meat products (e.g. ground beef and hamburger) to reduce levels of foodborne pathogens (and to extend shelf-life) is now authorised in the USA (FSIS, 1999), and commercial applications are growing.

Irradiation of meat food products will substantially reduce the level of harmful microbial pathogens such as *E. coli* O157:H7, *Salmonella*, *Clostridium perfringens*, and the protozoan parasite *Toxoplasma gondii*. The Food Safety and Inspection Service (FSIS) in the United States believes that ground beef in future is likely to be irradiated in relatively large quantities initially because *E. coli* O157:H7 can be effectively eliminated from the meat without cooking it. FSIS reported that if 25% of all ground beef is irradiated, the health and economic benefits from the reduction of diseases could range from USD 56.5 million to 138 million. This is a conservative estimate, as even higher benefits could be anticipated according to FSIS (FSIS, 1999).

Since irradiated beef patties (as other irradiated food) do not look or taste different, and have the same nutritional value as their unirradiated counterparts, information about their being radiation processed for additional safety seems imperative. Certification of

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irradiated hamburgers would tell the consumer that these products have implemented additional safety. In order to have an independent check on such paperwork, analytical detection methods for the irradiation treatment of food have been developed. Particularly in Europe, emphasis has been laid on development of such detection methods directly on the food product, and several of these methods are now established as European Standards, e.g. EN 1784 to EN 1788 (Delincée, 1998). Two of these CEN Standards, namely gas chromatographic analysis of hydrocarbons (EN 1784) and of 2-alkylcyclobutanones (EN 1785) could be used to detect irradiated hamburgers, since these methods rely on radiation-induced changes in the fatty part of the food, and hamburgers contain enough fat for these analyses. Both methods, however, require rather expensive equipment, and sample preparation and analysis may be quite time consuming.

A rapid and inexpensive screening test employing DNA Comet Assay to identify radiation treatment of food has been described by Cerda et al. (1997). Ionising radiation—like various chemical or physical treatments—causes fragmentation of DNA. When food containing DNA is irradiated, modification of these large molecules occurs including fragmentation. This fragmentation can be studied by microgel electrophoresis of single cells or nuclei. DNA fragments will migrate out of the nucleus forming a tail in the direction of the anode, giving the damaged cells the appearance of a comet. This test is, therefore, also called DNA Comet Assay. Irradiated cells will show an increased extension of DNA thus considerably longer comets (more fragmentation) than unirradiated cells. Non-irradiated cells appear nearly circular or with only slight tails.

The DNA Comet Assay has yielded good results with whole cuts of various meats e.g. beef, pork, poultry, or exotic meats (Cerda et al., 1997; Cerda, 1998a, b; Cerda and Koppen, 1998; Kruszewski et al., 1998; Khan and Delincée, 1999; Villavicencio et al., 2000). The method has already been employed in different frozen meats as an import control in Sweden (Merino and Cerda, 2000). It is also proposed as a European Standard (prEN 13784). Since processing of beef to hamburgers may influence DNA appearance, additional testing with ready-prepared hamburgers was required. In addition, the effect of storage has been studied.

2. Experimental

Ready-prepared frozen hamburgers were purchased in a local supermarket and irradiated—while still frozen—with an electron beam (2.2 MeV accelerated electrons with an average dose rate of 260 Gy/s, generated by a Van de Graaff accelerator, Vivirad-High Voltage Co., Handschuheim, France, by courtesy of the research centre AÉRIAL in Strasbourg). The intended radiation dose levels were 0, 1.5, 3.0, 4.5 and 7.0 kGy, covering the range of potential commercial irradiation (FSIS, 1999). To obtain good dose uniformity, hamburgers were cut into 2–3 mm thick slices for sufficient penetration, which was controlled by GAFchromic film dosimeters (McLaughlin et al., 1991) positioned both above and below the slices. In addition, a 100 μm-thick copper scattering foil (Strasser et al., 1991) was employed. Dose uniformity was $\pm 10\%$. Applied doses turned out to be 0, 1.3, 2.7, 4.5 and 7.2 kGy. Samples were stored frozen ($-18^{\circ}C$) for at least 9 months.

DNA Comet Assay was carried out in principle as previously described (Cerda et al., 1997). For each dose of radiation, at least triplicate slides were prepared. Briefly, about 2.5 g of very thin slices of meat were cut with a scalpel from the frozen hamburgers, transferred to a small beaker with 5 ml of ice-cold phosphate buffered saline (PBS) and stirred for 5 min at about $500\,min^{-1}$. The suspension was filtered sequentially through 500 and 200 μm sieve cloth, and left to settle on ice for about 2 min. The supernatant was used as cell extract. The cells were embedded in agarose on microscope slides, lysed for about 10 min for disruption of membranes using a detergent (2.5% SDS), and electrophoresed at a potential of 2 V/cm for 2.0 min using a 45 mM Tris-borate, 1 mM EDTA buffer, pH 8.4. Silver staining was employed to visualise DNA. Slides were evaluated with a standard transmission microscope.

3. Results and discussion

Fig. 1 illustrates the results obtained by Comet Assay on irradiated or non-irradiated frozen hamburgers. At a single glance the irradiated samples can be discerned from the non-irradiated ones, the latter showing a few but easily recognisable nearly intact cells with only slight tails. These images could not be found in irradiated samples, in which longer comets were observed. With increasing dose the tails of the comets became more pronounced, illustrating the higher degree of DNA fragmentation. Although even in the non-irradiated sample a variety of differently shaped comets could be observed, the presence of nearly intact cells indicates that the sample has not been irradiated.

Temperature abuse (or just storage) may also lead to a degradation of these nearly intact cells as DNA fragmentation of abused samples has been observed (Cerda et al. 1997; Cerda, 1998a, b; Cerda and Koppen, 1998; Kruszewski et al., 1998; Marchioni, personal communication). However, in properly frozen and stored samples, non-irradiated items could be discriminated from irradiated hamburgers, even after 9 months of storage.

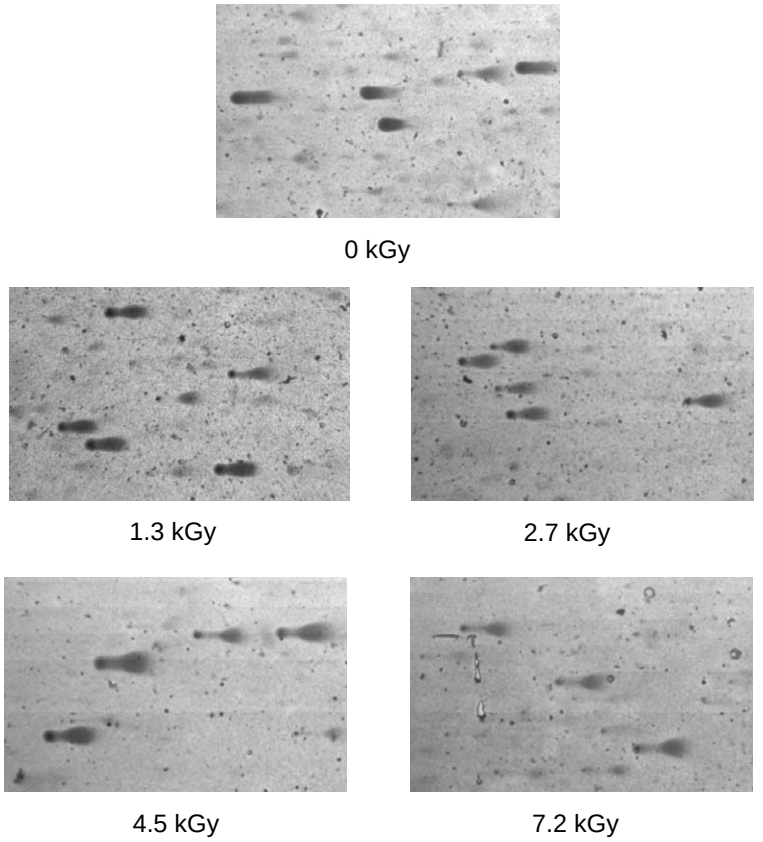

Fig. 1. DNA Comet Assay on frozen hamburgers (Anode to the right; silver staining).

4. Conclusions

DNA Comet Assay is a rapid and inexpensive screening test for detection of irradiated frozen hamburgers. Positive results need to be confirmed by a validated method to specifically prove an irradiation treatment, e.g. EN 1784 or EN 1785.

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