Identification of Radiation Treatment of Frozen Chicken and Fresh Turkey using DNA Comet Assay

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Summary: DNA comet assay was applied to check the detection of radiation treatment of frozen chicken and fresh turkey. The cells from unirradiated and irradiated samples of chicken and turkey were extracted in cold PBS, embedded in agarose on microscope slides, lysed for 15 minutes in 2.5 % SDS and subjected to electrophoresis at a rate of 2V/cm for 2 minutes. After silver staining these slides were evaluated through an ordinary transmission microscope. In irradiated samples, fragmented DNA (due to radiation treatment) stretched towards the anode and cells appear as comets. The density of DNA material in the tails increased with increasing radiation dose. However in case of unirradiated samples, the large molecule of DNA remained relatively intact and there was only minor or no migration of DNA; thus cells were round or had very short tails. Therefore, clear discrimination between unirradiated and irradiated food samples is possible. Hence, DNA comet assay provides an inexpensive and quick screening method for several kinds of foods containing DNA including frozen chicken and fresh turkey.

Introduction

The radiation processing of foods have gained significant importance throughout the world. The process is useful to decrease or eliminate food-borne pathogens from meat and meat products [1-3]. The treatment can be used for reducing food-borne illnesses as well as associated medical and productivity costs [4].

On the other hand, proper control of radiation treatment of food is very important in order to facilitate international trade of irradiated food, to enhance the consumer confidence in the process and to ensure free choice of consumers. During the last two decades, considerable research efforts have been directed for finding identification methods for foods that have been irradiated. European Committee of Standardization (Comite Europeen de Normalisation, CEN) has adopted some methods for the detection of irradiated foods, such as Electron Spin Resonance (ESR) spectroscopy, thermoluminescence (TL) and photostimulated luminescence (PSL) [5]. However, these validated methods require expensive instrumentation and are time consuming. DNA comet assay, on the other hand, provides a versatile, rapid and simple test involving relatively inexpensive instrumentation, which can be used for the screening of irradiated food [6].

Ionizing radiation causes DNA damage and radiation-induced changes in DNA could serve as marker for detection method for irradiated foods. Microgel electrophoresis of single cells/ nuclei, also called "DNA comet assay", can be used to detect DNA damage [7, 8]. The cells are embedded on microscope slides; cell membranes are disrupted to make permeable using a detergent and subjected to electrophoresis at set voltage. As a result, DNA fragments stretch or migrate in the direction of the anode giving the damaged cell the appearance of a comet. The comets appear with regular tails of definite shapes and sizes depending upon the applied radiation dose. The cells from unirradiated samples stained as nuclei without tails or with very short dispersed tails.

The application of this technique to detect irradiation treatment of several foods has been described [6, 9-15]. In the present investigations, irradiated and unirradiated samples of frozen chicken and fresh turkey have been analyzed for identification of radiation treatment using DNA comet assay.

Results and Discussion

Samples of Frozen Chicken

The irradiated and unirradiated samples of frozen chicken were analysed using lysis time of 15 minutes. These samples could be discriminated from

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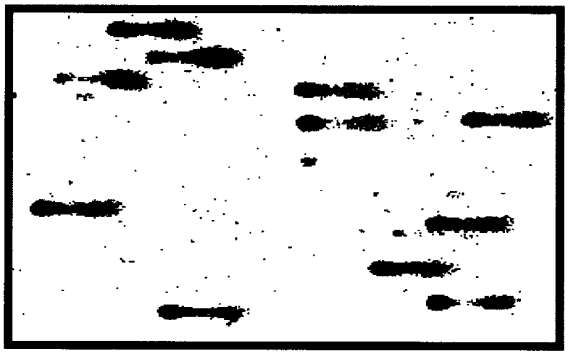
each other just at a glance under the microscope. In principle, the unirradiated samples should consist of more or few numbers of round cells indicating that no radiation treatment of the food samples has undergone. On the other hand, irradiated samples should show only the comets and there should be no intact cells, which can be seen as a round stain. The present study showed a nice discrimination between unirradiated and irradiated samples of chicken thus making the assay very successful for analysis. The size and shape of comets for different employed doses of radiation were different and were consistent with the applied dose. Because of this reason a rough dose estimate on the basis of shapes and sizes of comets was also possible. For instance, the comets for samples irradiated to 3 kGy show wide and dense tails due to the presence of more fragmented DNA material as compared to samples irradiated to 1 kGy. The facts have been shown in Fig. 1, which shows the photographs of DNA comet assay of unirradiated and irradiated samples of muscle tissues from chicken legs.

In order to determine the duration of post-irradiation applicability of assay, the controlled and treated samples of frozen chicken were analysed after 1, 2 and 4 days of irradiation. The analyses showed that numbers of intact cells in form of round stains along with some comets were present in controlled samples even on 4th day of storage in deep freezer (-20 $^{\circ}$C). Therefore, it was concluded that the unirradiated and irradiated samples of frozen chicken could be distinguished after a storage period of 4 days.

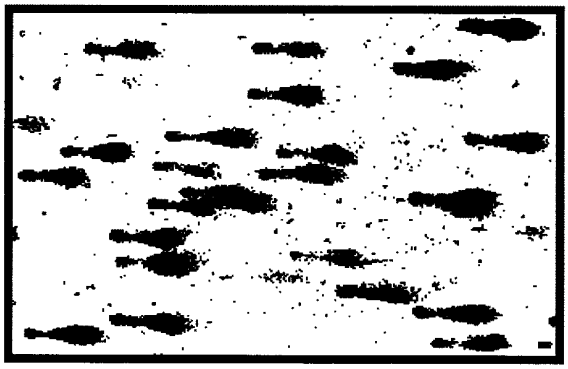
Samples of Fresh Turkey

The irradiated and unirradiated samples from the fresh meat of turkey were taken and analysed for the discrimination. The controlled samples contained several intact cells in form of big wide and denser stains along with a minor migration of some DNA material. No typical comet was found in this case thus giving a strong evidence for the absence of radiation treatment. The samples irradiated to 1 and 5 kGy contained only the comets and no intact cells (like that observed in unirradiated samples) were found in both these cases. Thus this type of pattern of DNA migration was associated with the evidence of radiation treatment in both the samples. Both the irradiated samples could be distinguished for the difference in radiation dose. The

Un-irradiated (0 kGy)



Irradiated to 1 kGy



Irradiated to 3 kGy

Fig. 1: Typical DNA Comet Assay for Samples of unirradiated and irradiated frozen Chicken; Silver staining; anode to the right; microscope object x 20.

cells irradiated to 1 kGy showed thin cylindrical comets but those irradiated to 5 kGy showed comets with wide and denser tails. This difference in shape and size was dependent upon DNA fragmentation, which was higher in case of 5 kGy. Hence, from this

difference in migration patterns, a rough dose estimate can also be made, which can be helpful for regulatory authorities to check administration of required radiation doses to different types of foods. Typical pictures of slides are given in Fig. 2 showing the DNA comet assay of fresh turkey meat.

The present investigation was actually the follow-up study of some earlier analyses, which were carried out for the detection of radiation treatment of animal tissues; good results were reported for samples of beef, fish and game etc [11, 16]. In some other studies on fresh and frozen beef, veal, fresh meat of cow and frozen turkey, it was reported that there were only the cells with comets in the irradiated samples, whereas no intact or apparently intact cells could be observed in case of irradiated samples. The round stains or stains with slight comets were always present in unirradiated samples, thus fulfilling the basic criteria for discerning unirradiated and irradiated food samples [11, 15, 17-19].

Experimental

Preparation of Food Samples

The samples of fresh meat of turkey and frozen chicken were purchased from local market of Karlsruhe (Germany) and were stored in ice during transportation. In laboratory, 200 g of tissues were separated from each chicken and turkey for making six packets (three for chicken and three for turkey) using small polyethene bags. One sample from each food was kept as controlled (labeled as 0 kGy) and other two samples from each food were kept for employing radiation dose of 1 and 5 kGy to turkey and 1 and 3 kGy to chicken meat.

Irradiation of Food Samples

The samples were irradiated by 10 MeV electron beam (Circe III linear accelerator, Thomson-CSF Linac, St. Aubin, France). The Dosimetry for measuring the employed radiation doses was done using GAF DM-1260 radiochromic films (International Specialty Products, Wayne, USA) [20]. A filter photometer (Ciba Corning Halstead, Essex, UK) was used for the evaluation of dosimetric films using the wavelength of 405 nm. The applied doses were kept between the minimum and maximum limits as endorsed by International Consultative Group on Food Irradiation (ICGFI) [21]. After irradiation by electron beam accelerator, all the samples from both the foods were stored in deep freezer at -20 °C up to complete analysis.

Un-irradiated (0 kGy)

Irradiated to 1 kGy

Irradiated to 5 kGy

Fig. 2: Typical DNA Comet Assay for Samples of unirradiated and irradiated Fresh Turkey; Silver staining; anode to the right; microscope object x 20.

Preparation of Single Cell Suspension

About 1 g samples of muscle tissues of frozen chicken and fresh turkey were scratched into very thin slices with the help of a sharp scalpel. The tissue samples for each type of food were put into small beakers containing 5 mL cold Phosphate Buffer Saline (PBS) solution and stirred at 500 rpm for 5-7 minutes by putting them at tray containing ice. Homogeneous mixture was filtered first through 200 μm and then through 100 μm sieves. The small beakers containing the filtrate were placed into the crushed ice for 3-5 minutes. The supernatant was used as single cell suspension for both the types of food samples.

DNA Comet Assay

DNA comet assay method was carried out as previously described [9-15, 17, 18] for finding out the appropriate conditions for the foods under present investigations. A 100 μL aliquot of supernatant was taken as cell suspension and was thoroughly mixed with 1 mL of warm low melting agarose (0.8 % in PBS at 45 °C). 100 μL of this mixture was then spread uniformly on pre-coated slides avoiding air bubbles. Lysis of the cells was carried out in a TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.4) containing 2.5 % SDS. Lysis time of 15 minutes was used. After lysis, the slides were conditioned in TBE buffer for 5 minutes and placed side by side into electrophoretic chamber having about 2 liters of TBE buffer. Electrophoresis was performed at 2V/cm for about 2 minutes for all the samples under study. The cells on the slides were fixed for 10 minutes and then silver stained for 30-40 minutes. The microscope slides were then evaluated under the ordinary transmission microscope (objective ×10 or magnification ×100) for the observation of migration patterns DNA.

Conclusions

The present investigations showed that radiation treatment of the samples of frozen chicken and fresh turkey could be identified precisely. The discrimination between controlled and irradiated frozen chicken's samples and fresh turkey's samples up to several days were possible with the help of relatively cheaper, rapid and simple comet assay method. Therefore, the method can be used reliably as a routine screening test for the control of radiation treatment of poultry both for commercial and regulatory purposes.

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References

1. A. Anellis, E. Shattuch, M. Morin, B. Srisara, S. Qvale, D. B. Rowely, and E. W. Ross-Jr., *Applied Microbiology,* **34,** 823 (1977).
2. A. Anellis, D. B. Rowely, and E. W. Ross- Jr., *Journal of Food Protection,* **42,** 927 (1979).
3. J. P. Dubey, R. J. Brake, K. D. Murrell, and R. Fayer, *American Vetnary Research,* **47,** 518 (1986).
4. WHO, *Safety of Nutritional adequacy of Irradiated Food,* World Health Organization, Geneva (1994).
5. H. Delincée, *Trends in Food Science & Technology,* **9**, 73 (1998).
6. H. Cerda, B. von Hofsten, and K. J. Johanson, in: *Recent advances of new methods of detection of irradiated foods,* Proceedings of workshop, Ancona, Commission of the European Communities, Luxembourg, **EUR 14315** (1993).
7. V. J. McKelvey-Martin, M. H. L. Green, P. Schmezer, B. L. Pool-Zobel, M. P. De Meo and A. Collins, A European review, *Mutation Research,* **228,** 47 (1993)
8. E. Rojas, M. C. Lopez, M. Valverde, *Journal of Chromatography B,* **722,** 225 (1999).
9. H. M. Khan, and H. Delincée, *Radiation Physics Chemistry,* **52**, 141 (1998).
10. H. Delincée, *Radiation Physics Chemistry,* **52**, 135 (1998).
11. A. A. Khan, H. M. Khan, and H. Delincée, *European Food Research & Technology,* **216, 88** (2003).
12. A. A. Khan, H. M. Khan H. Delincée, *Radiation Physics Chemistry,* **63,** 407 (2002).
13. A. A. Khan, H. M. Khan H. Delincée, *Food Control,* **16,** 141 (2005).
14. A. A. Khan, H. M. Khan and M. A. Wasim, *Journal of the Chemical Society of Pakistan,* **27**, 65 (2005).

15. A. A. Khan, H. M. Khan, *Journal of Radioanalytical and Nuclear Chemistry*, **275**, 337 (2008).
16. H. Nilson, and H. Cerda, *Analys av bestraalade livsmedei verksamnetsrapport under perioden 1989/90-1992/93,* SLV (National Food Administration) rapport, nr. **17,** Uppsala, Sweden.
17. H. Cerda, H. Delincée, H. Haine, and H. Rupp, *Mutation Research,* **375**, 167 (1997).
18. H. Cerda, *Lebensmittel-Wissenschaft und – Technologie*, **31**, 89 (1998).
19. H. Cerda, and G. Koppen, *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A (European Food Research and Technology),* **207,** 22 (1998).
20. M. C. Sayler, T. T. Tamargo, W. L. McLaughlin and H. M. Khan, *Radiation Physics Chemistry,* **27,** 41 (1986).
21. ICGFI, *ICGFI Document No. 15, ICGFI, Joint FAO/IAEA Division*, International Atomic Energy Agency, Vienna, Austria (1993).
22. A. A. Khan and H. Delincée, *Bericht der Bundesforschungsanstalt für Ernärung, Karlsruhe,* **BFE-99-01**, 316 (1999).