Identification of Radiation-Processed Edible Grains and Seeds using Single Cell Microgel Electrophoresis Assay: A Comparative Analysis

Khan Ashfaq Ahmad[1], Kashif Ayesha[2] and Shahid Muhammad Kashif[3,4]*

1. Department of Chemistry, Govt. Postgraduate College, Haripur, University of Haripur, Haripur 22620, KP, PAKISTAN
2. Department of Senior Health Care, Eulji University, Uijeongbu-si 11759, REPUBLIC OF KOREA
3. Faculty of Civil and Architecture, National Polytechnic Institute of Cambodia (NPIC), Phnom Penh 12409, CAMBODIA
4. Research Institute of Environment and Biosystem, Chungnam National University, Daejeon 34134, REPUBLIC OF KOREA

*mkbutt2000@gmail.com

Abstract

The DNA Comet assay (microgel electrophoresis method) is a recommended technique for detection of radiation treatment in food containing DNA material. It involves isolating cells/nuclei (DNAs) from both un-irradiated and irradiated samples of food and embedding them on microscope slides, lysing the cells/nuclei, performing electrophoresis, staining the cells/nuclei and then evaluating the patterns of migration of DNA in agarose gel. In this study, the assay was applied to identify irradiated grains and seeds commonly consumed by humans and animals such as wheat, melon seeds, sesame seeds, red lentils and pinto beans. Samples were irradiated within a permissible dose range and microscope slides were evaluated under an ordinary transmission microscope. The controlled samples showed intact membrane cells/nuclei while irradiated samples displayed dose-dependent comets with varying shapes, lengths and sizes of the tail.

On the basis of comet morphology, this method enables rapid screening and differentiation of food samples exposed to different radiation doses, providing a valuable tool for food quality and safety assessment. The study's main results demonstrate the effectiveness of the DNA Comet assay in distinguishing between irradiated and un-irradiated food samples, complementing the microscopic results obtained.

Keywords: DNA Comet assay, Microgel electrophoresis, Food irradiation, Grain and seed analysis, Dose-dependent comets.

Introduction

Food irradiation is a process that involves exposing food items to gamma rays, electron beams, or X-rays to enhance their safety and quality as well as to extend their shelf life for human consumption[1,17]. This method, often referred to as cold pasteurization, offers an environmental friendly solution and serves as a viable alternative to chemical preservatives which can have detrimental effects on human and animal health and are subjected to widespread bans[5,6].

* *Author for Correspondence*

The purpose of irradiating food materials is to prolong their shelf life, to eliminate pathogenic and spoilage microorganisms and to eradicate pests and insects[16,20,32]. With the increasing utilization of radiation processing in the food and agricultural sectors, global regulatory bodies such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) are actively working towards establishing standards and guidelines for the implementation of regulations regarding food irradiation[4,14,36].

The DNA comet assay, also known as microgel electrophoresis, is extensively utilized to detect and examine alterations in DNA material caused by ionizing radiation[7]. Exposure of DNA-containing foods to ionizing radiation can result in various types of DNA damage including single and double strand breaks, modifications in bases, strand fragmentation and separation of regions within the DNA double helix[11]. The DNA comet assay, a dependable method, is employed to identify and measure such damages induced by ionizing radiation in food items containing DNA[15,18,28]. In some earlier studies, the assay has been successfully applied to investigate the effects of irradiation on meat and vegetables[9,15,33].

The methodology has also been adapted and extensively verified for plant-based materials, particularly those utilized in the context of food and animal feed[31,34]. The comet assay has primarily been utilized to identify plant-derived food products that have undergone treatment with ionizing radiation. In one of the studies, Nikolova et al[29] demonstrated the application of a neutral comet assay with shortened electrophoresis time and a reduced voltage specifically for plant-based food materials. This modification was necessary due to the notable observation of DNA damage typically resulting from exposing plant foods to the permitted doses of ionizing radiation.

Similarly, Glei et al[13] have proved the effectiveness of the method in detecting radiation treatment. Several of the previous investigations have effectively distinguished between samples of lentils, sesame seeds, sunflower seeds, rose pepper, perilla seeds, figs and soybeans that have been irradiated and those that have not been irradiated[8,22,30]. Some other researchers conducted a study that demonstrated the capability of combining the DNA comet assay with image

analysis to quantify the radiation dose applied to irradiated citrus fruits including lemons, grapefruits, oranges and mandarins.

The primary objective of present study was to evaluate the irradiation status of a variety of agricultural commodities such as wheat grains, melon fruit seeds, sesame seeds, red lentils and pinto beans. The findings of this study are highly significant as they contribute to the field by expanding the existing methods utilized to identify both un-irradiated and irradiated food items. This research holds particular value as it provides valuable insights into the development of monitoring techniques applicable to a wide range of food products. With an improved ability for the determination of irradiation status of these items, this study represents a significant advancement in ensuring the implementation of robust food safety and quality control measures across the industry.

Material and Methods

Samples for the analysis: Various types of grains including wheat (*Triticum aestivum*), red lentils (*Lens culinaris*), pinto (*Phaseolus vulgaris*) beans, melon (*Cucumis melo*) seeds and sesame (*Sesamum indicum*) seeds were obtained from a local grocery store in Leipzig, Germany. Additionally, samples of freshly harvested wheat grains were collected directly from nearby crop fields in Eggenstein-Leopoldshafen, Karlsruhe, Germany. These grains and seeds were selected within their typical size ranges: melon seeds (8.0-11.5 mm in length), red lentils (5.0–7.5 mm wide), pinto beans (10-15 mm in length), sesame seeds (3-4 mm in length) and wheat grains (5.0-8.5 mm in length). Fig. 1 shows the appearance of the grains and seeds used in this study.

Irradiation of food samples: The food samples were irradiated using 10 MeV electrons generated by a Circe III linear accelerator (Thomson-CSF Linac) located at the Max Rubner Institute, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany. The dose rate was 10^7 Gy/s (pulsed). To determine the actual radiation doses received by the samples, radio-chromic film dosimeters (GAF DM-1260) were employed. These dosimeters were placed alongside the samples during irradiation and the change in absorbance of the dosimetric films was measured spectrophotometerically at 405 nm using a filter photometer (Ciba Corning).

To estimate the radiation doses delivered to the food samples, a linear calibration curve was used within the range of 0 to 35 kGy. Each food sample was exposed to selected doses of radiation, covering the permissible range for pest and insect disinfestation in accordance with the recommendations of the International Consultative Group on Food Irradiation (ICGFI). The applied radiation doses to the food samples were 0, 0.5, 1, 3 and 5 kGy.

Figure 1: The pictures of (a) melon seeds (8.0-11.5 mm in length), (b) red lentils (5.0–7.5 mm wide), (c) pinto beans (10-15 mm in length), (d) sesame seeds (3-4 mm in length) and (e) wheat grains (5.0-8.5 mm in length), subjected to radiation treatment to combat insect/pest infestation and then identification of controlled and treated samples using DNA comet assay.

Table 1
Optimised working conditions for different parameters of assay for food samples

Food sample	Amount mg/ml PBS	Sedimentation time (min)	Lysis time (min)	Staining time (min)	Electrophoretic time (min)
Red lentils	300/3	15	25	80	2
Wheat	500/5	17	15	60	2
Fresh wheat	500/5	17	15	60	2
Melon seeds	300/4	20	20	120	2
Sesame seeds	300/3	15	15	80	2
Pinto beans	600/5	30	25	80	2

DNA Comet assay: The comet assay was performed following a previously reported study and European standards[7,8]. Briefly, weighed amounts of controlled and treated food samples were taken. The outer skins (peelings) of red lentils, pinto beans, wheat grains and melon and sesame seeds were thoroughly removed to obtain the inner embryonic portions of the samples. These portions were then crushed into a fine powder using a mortar and pestle. The powdered materials from each sample were transferred to small beakers containing a specified volume of ice-cold solution of phosphate buffer saline (PBS). These smaller beakers were placed in crushed ice and stirred at a rate of 500 rpm for 5-10 minutes using small magnetic stirrers.

The cell suspensions from different samples were sequentially filtered through 200 µm and 100 µm nylon sieve cloth filters. The resulting suspension was then left on ice for a certain sedimentation time to allow the undesired contaminating material (debris) to settle. The supernatant, without the settled debris, was used as the cell extract for ongoing steps.

For the assay, the working conditions were optimized for parameters such as sedimentation time, lysis time, staining time, electrophoresis time and the amounts of food samples used for analysis. After electrophoresis, the DNA material (cells/nuclei) on the microscopic slides was stained using the solutions for silver staining for visual inspection under microscope. Duplicate microscope slides were developed for each sample in the assay. The optimized working conditions for each type of food are shown in table 1.

Evaluation of DNA comet assay: The results of the experiments were observed using an ordinary transmission microscope to examine the migration patterns of DNA material under the influence of the anode on the right side of the electrophoretic chamber. In untreated food samples, the presence of round or oval stains of DNA (with or without faintly dispersed tails or aura) indicated no radiation treatment. However, damaged or fragmented DNAs exhibited stains in the form of different comets characterized by varying shapes, sizes, necks, heads and lengths of the tails[10,27]. These comet patterns indicated damage to the DNA material within the cells/nuclei, indicating that the food samples had been exposed to irradiation treatment. The variations in the morphological patterns of the resulting comets provided information about the extent of DNA damage such as fragmentation, double or single-strand breaks caused by specific doses of ionizing radiation.

Regarding DNA damages and then different migration patterns towards anode, the literature has suggested the classification of comets of DNAs based on their morphology[21,24]. According to this classification, the comets with short tails and relatively little DNA degradation were classified as type 1. Other types included type 2 (long tail), type 3 (long tail wider at the end), type 4 (long tail separated from the head of the comet) and type 5 (almost no DNA left in the head of the comet, with the tail appearing as a cloud far apart from the head of comet).

Some of the other studies have also shown that sizes and shapes of the comets were found to be dependent on the radiation dose applied to the food samples and a linear increase in comet size was observed with an increase of radiation dose[18,19]. The migration patterns of intact or fragmented cells/nuclei (DNAs) were captured using photomicrophotography.

Results and Discussion

The assay results for identifying controlled and irradiated samples were analyzed through visual inspection of developed microscope slides using an ordinary transmission microscope. The findings revealed a gradual increase in DNA fragmentation with longer exposure of the samples to ionizing radiation (absorbed dose). Microscopic examination of the cells/nuclei in the subjected material from plant foods allowed for the assessment of intactness or the degree of DNA damage.

By evaluating the intact cells or grading the comets in grains and seed samples, it was possible to differentiate between irradiated and un-irradiated samples. This distinction is evident in the photomicrographs of DNA stains on microscope slides, as shown in figs. 2–5. Through this examination, it was observed that higher absorbed doses resulted in a shift towards higher-grade comets indicating incremental DNA damage, as seen in the doses of 0.5, 1 and 5 kGy. According to a study, it has been reported that there is a direct relationship between the absorbed dose and the length of the comet's tail[12].

In other words, as the absorbed dose increases, the length of the comet's tail is observed to grow proportionally. This

finding suggested that the extent of radiation exposure plays a crucial role in determining the size and length of the comet's tail. The study provides valuable insights into the impact of absorbed dose on the visual characteristics of comets and sheds light on the intricate relationship between radiation and celestial phenomena.

The current study focuses on treating food samples within the permissible dose range of ionizing radiation, aiming to gain experience in detecting irradiated food samples. The primary objective of radiation processing is to employ an environment friendly technique to extend the shelf life of food samples and to prevent pest and insect infestation in grains and seeds during post-harvest storage.

Comet assay of melon and sesame seeds: The identification of γ-irradiated samples of papaya, melon and watermelon has been carried out in previous studies[24]. In the present investigation, the comet assay was performed on both un-irradiated and irradiated melon seed samples. The results are presented in fig. 2 as pictures (a), (b), (c), (d) and (e). The controlled (un-irradiated) samples (Fig. 2a) exhibited staining of the nuclei/DNA in the form of round or oval stains, indicating the presence of intact cells or nuclei. This observation confirms that the samples were not treated with ionizing radiation.

In contrast, the irradiated samples subjected to doses of 0.5, 1, 3 and 5 kGy exhibited comet formations with a complete absence of intact cells/nuclei, similar to the controlled (un-irradiated) samples. Figure 2b illustrates the results for the 0.5 kGy dose, where the DNA material was primarily condensed towards the heads of the comets, while the density of DNA material was faint towards the tails.

Compared to the 0.5 kGy dose, the length and size of the comets were larger in samples irradiated with doses of 1, 3 and 5 kGy as shown in figure 2 (c), (d) and (e) respectively.

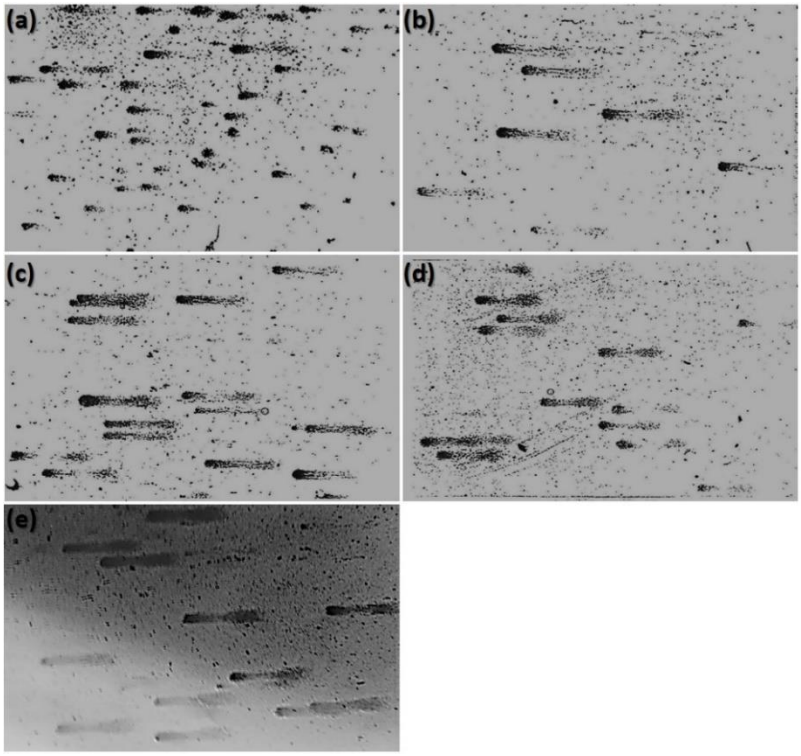

Figure 2: DNA comet assay of (a) un-irradiated and irradiated melon seeds to the (b) 0.5 kGy, (c) 1 kGy, (d) 3 kGy and (e) 5 kGy. Silver staining, anode to the right.

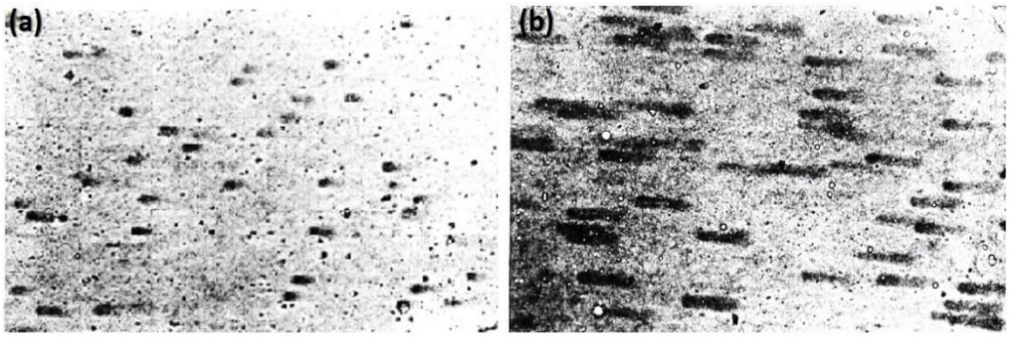

Figure 3: DNA comet assay of (a) un-irradiated and (b) irradiated sesame seeds to 1 kGy. Silver staining anode to the right.

In these cases, the tails of the comets were broader with a wider dispersion of DNA material. This difference in comet characteristics reflected the amount of radiation doses delivered to the samples of melon seed. A linear increase in the densities of DNA material in the comet's tails was observed with an increase in the radiation dose delivered to the irradiated samples. Similarly, an assay was conducted on un-irradiated and irradiated samples of sesame seeds. The difference between the controlled and irradiated (1 kGy) samples is depicted in figure 3 (a) and (b).

DNA Comet assay of red lentils: Figure 4 shows the DNA comet assay of controlled and irradiated samples of red lentils seeds. In the un-irradiated samples, numerous intact cells and nuclei were observed, exhibiting a range of sizes with some larger round stains. The controlled samples, upon visual inspection, predominantly contained intact cells, accompanied by a few comets. Conversely, the irradiated samples displayed a uniform migration pattern of comets. A direct correlation between comet size and the applied radiation doses was evident, enabling a rough estimation of the dosage. Figure 4 illustrates samples from the (a) un-irradiated, (b) 0.5 kGy-irradiated and (c) 5 kGy-irradiated cases.

The signs of polyploidy were observed in both the controlled and irradiated samples of red lentils. This sort of occurrence is typical in the seeds and grains of plant-based food items and prior studies have also identified it in various types of some other plant seeds[3]. It was noteworthy that the nuclei exhibited significant variations in sizes and susceptibility to ionizing radiation[12].

In summary, this study demonstrated that both un-irradiated and irradiated samples of red lentils could be easily identified using a simple microscope assay. Another advantage of this analysis was the presence of a clean background with very fewer contaminating debris. As a result, shorter sedimentation and lysis times of 15 min and 25 min respectively, could be employed for a rapid analysis. This approach yielded reliable results without the need for developing frequency histograms or manually counting stained cells or nuclei under the microscope which can be a tedious and time-consuming task. Even low doses of radiation, such as 0.5 kGy, could be identified with a quick visual assessment.

Comet assay of pinto beans: In the irradiated samples of pinto beans, distinct comets of varying shapes and sizes were observed which were directly influenced by the applied radiation doses. These comets differed significantly from the round or conical stains seen in the un-irradiated samples which were consistent in size and shape. The relationship between the shape and size of the comets and the radiation doses was evident as depicted in the photomicrographs (a), (b) and (c) of fig. 5, representing the un-irradiated sample and samples irradiated to 0.5 and 5 kGy respectively.

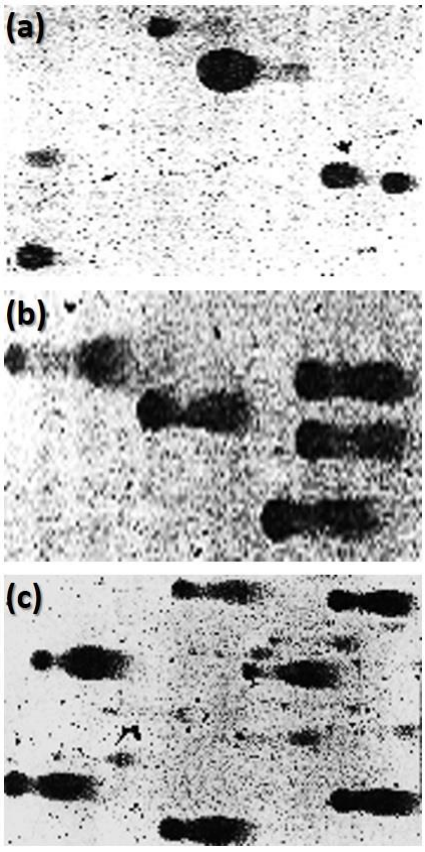

Figure 4: DNA comet assay of samples of (a) un-irradiated and irradiated red lentil seeds to (b) 0.5 kGy and (c) 5 kGy. Silver staining, anode to the right.

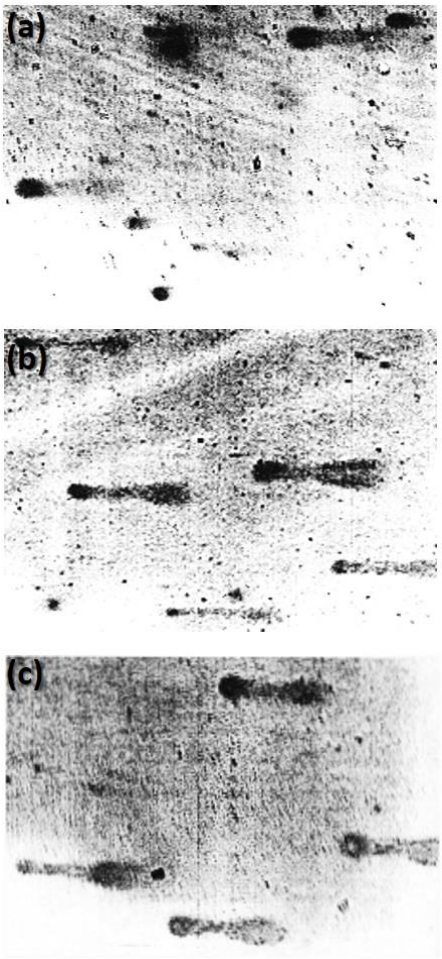

Figure 5: DNA comet assay of the samples of pinto beans (a) un-irradiated and irradiated to (b) 0.5 kGy and (c) 5 kGy. Silver staining, anode to the right.

It is worth noting that the inclusion of longer lysis times in our study had a substantial impact on the effectiveness of the comet assay test. The decision to extend the lysis times proved to be advantageous and contributed significantly to the overall success of the analysis. The successful application of the comet assay test for detecting radiation treatment in Brazilian beans of the carioca and macacar varieties irradiated at 1 and 10 kGy respectively, using frequency histograms of DNA comets was demonstrated in an earlier study[35].

In the present work, we applied a modified version of the assay under different working conditions specifically for pinto beans, which resulted in improved and relatively faster analysis. Unlike the previous study, an approach to eliminate the need for comparing frequency histograms, typically requires measuring the length and number of comets.

Additionally, the present conditions of the test easily discriminated between controlled and irradiated samples, even at lower doses (0.5 and 1 kGy) used for insect/pest disinfestations in contrast to the carioca and macacar varieties where the test was performed for the radiation doses of 1 and 10 kGy. These findings highlighted the effectiveness and discriminative capability of the comet assay for pinto beans, even at the level of insect/pest disinfestations.

Comet assay of wheat: The identification of radiation treatment in wheat samples during this study was unsuccessful. This trend was attributed to the presence of similar migration patterns of nuclei/DNAs which appeared solely in the form of comets in both un-irradiated and irradiated samples. Morphologically, the comets exhibited elongated tails and had small heads with thin necks. In the un-irradiated samples, no typical intact cells were found that could serve as evidence of the absence of radiation treatment. Surprisingly, on the basis of the findings of the present study, the DNA material in the un-irradiated samples was likely to be degraded, which was unexpected because these samples were obtained from the market.

To verify that no pre-treatment of the grain samples had occurred, fresh samples were collected from wheat fields in Eggenstein-Leopoldshafen (Karlsruhe, Germany) during the wheat harvesting season. However, the subsequent analysis yielded the same migration patterns of DNAs/nuclei in both controlled and irradiated samples, reaffirming the inability of the assay for the detection of radiation treatment under the presently optimized conditions of the assay.

Similar situations have been observed in previous studies on plant foods (grains or seeds) where the application of the assay became limited due to high value of the comet parameter "% DNA in tail" in the controlled samples which masks the effect of radiation treatment[2,25,26].

In another study that focused on DNA damage caused by high doses of UV-C, a decrease in the number of nuclei with intact membranes was observed in both pea and wheat samples after the application of high UV-C doses. The analysis aimed to identify appropriate experimental conditions for the neutral Comet assay, specifically for pea and wheat, by varying the main steps of the protocol, such as lysis and electrophoresis time.

The optimal conditions determined for pea samples were 5 minutes of lysis and 5 minutes of electrophoresis at a set voltage of 0.5 V/cm while for wheat samples, the most optimal conditions were 15 minutes of lysis and 15 minutes of electrophoresis at a set voltage of 1 V/cm[29]. Therefore, as a recommendation, considering a neutral comet assay with modifications in lysis and electrophoretic times, wheat samples treated with electron beams could be considered for discriminating radiation treatment in a further study.

Future perspectives

The study has provided valuable insights into distinguishing irradiated from un-irradiated samples in various grains and seeds using the comet assay and silver staining. Future prospects include the development of even more specific and sensitive methods for detecting irradiation, especially in challenging cases like wheat. Researchers can work on refining the comet assay technique to overcome the limitations observed in the study, particularly in the case of wheat. This may involve modifications in staining techniques, microscopy procedures, or other aspects of the assay.

In addition to the comet assay, other researchers can explore alternative techniques for irradiation detection in wheat and other grains. This could include molecular biology methods, spectroscopy, or advanced imaging technologies.

To ensure the reliability and reproducibility of the results, further studies should focus on validating the developed methods and establishing standardized protocols for irradiation detection in grains and seeds. This will be important for implementing these techniques in food safety and quality control.

As the study contributes to food safety and quality control, future prospects also include collaboration with regulatory bodies to potentially include these detection methods into food safety regulations. This could lead to stricter control and monitoring of irradiated food products.

Investigating the long-term stability of DNA migration patterns on microscope slides is important for practical application. Future work can explore the development of storage techniques that maintain the integrity of these patterns for extended periods. Moreover, a quantitative study can enhance the findings by providing statistical data such as the percentage of successful differentiation between irradiated and un-irradiated samples, thus offering a more precise assessment of the assay's effectiveness and reliability across different grain and seed types.

While this study has made significant progress in identifying irradiation treatment in common grains and seeds, there is room for further research. Future research should focus on refining the assay technique, exploring alternative detection methods, validating developed methods, collaborating with regulatory bodies for potential inclusion in food safety regulations and addressing long-term stability and quantitative analysis to enhance accuracy and applicability. These prospects are essential for advancing food safety, quality control and regulatory compliance in the food industry, particularly in challenging cases like wheat.

Conclusion

In conclusion, this study aimed to distinguish between un-irradiated and irradiated samples of commonly consumed grains and seeds including melon seeds, sesame seeds, red lentils seeds, pinto beans and wheat grains. Utilizing the comet assay, we sought a straightforward visual analysis method to differentiate between controlled and irradiated samples. Silver staining of the DNA played a crucial role in facilitating the identification process, as the stained patterns provided excellent visibility under a standard transmission microscope. It was observed that the silver staining of cells/nuclei/DNAs was long-lasting and the resulting DNA migration patterns on microscope slides could be stored at room temperature for several months without fading.

The results demonstrated successful differentiation between un-irradiated and irradiated samples for the four types of foods (melon, sesame, red lentils seeds and pinto beans) analyzed. However, discrimination between un-irradiated and irradiated samples of wheat proved challenging. Both controlled and irradiated samples exhibited the same migration patterns of DNA material only exhibiting the comets in both the cases.

Hence, no intact cells/nuclei/DNAs (typically as a round or oval shaped) were found in the un-irradiated samples of wheat and this pattern became the cause of hindrance in the path of identification of radiation treatment during this study. Despite repeated experiments, this limitation persisted within the current conditions of the comet assay.

In summary, this research successfully identified irradiation treatment in several common grains and seeds, offering valuable insights into food safety and quality control. However, further investigations may be necessary to develop more specific methods for detecting irradiation in wheat samples.

Acknowledgement

One of the authors, Mr. Ashfaq Ahmad Khan wishes to thank Dr. Henry Delincée, Mrs. Sigrid Delincée (late) and Mr. Knoerr for technical assistance at laboratory and e-beam radiation facility at Max Rubner Institute, Karlsruhe, Germany. One of the authors AAK is also grateful to Ministry of Science and Technology, Islamabad (Pakistan) for the grant of fellowship for advance study at German institute.

References

1. Alanazi S.F., Evaluating the effect of X ray irradiation in the control of food bacterial pathogens, *J. King Saud Univ. - Sci.*, **35**, 102367 **(2023)**

2. Angelis K.J., Dušinská M. and Collins A.R., Single cell gel electrophoresis: Detection of DNA damage at different levels of sensitivity, *Electrophoresis*, **20**, 2133–2138 **(1999)**

3. Ari E., Djapo H., Mutlu N., Gurbuz E. and Karaguzel O., Creation of variation through gamma irradiation and polyploidization in Vitex agnus-castus L., *Sci. Hortic.* (Amsterdam), **195**, 74–81 **(2015)**

4. Awuchi C.G., HACCP, quality and food safety management in food and agricultural systems, *Cogent Food Agric.*, **9**, 2176280 **(2023)**

5. Azizi-Lalabadi M., Moghaddam N.R. and Jafari S.M., Pasteurization in the food industry, Thermal Processing of Food Products by Steam and Hot Water, 247–273 **(2023)**

6. Bermudez-Aguirre D. and Niemira B.A., A review on egg pasteurization and disinfection: Traditional and novel processing technologies, *Compr. Rev. Food Sci. Food Saf.*, **22**, 756–784 **(2023)**

7. CEN, EN 13784:2001, Foodstuffs–DNA Comet assay for the detection of irradiated foodstuffs–Screening method, European Committee for Standardization **(2002)**

8. Cerda H., Delincée H., Haine H. and Rupp H., The DNA `comet assay' as a rapid screening technique to control irradiated food, *Mutat. Res. Mol. Mech. Mutagen.*, **375**, 167–181 **(1997)**

9. Chauhan S.K., Kumar R., Nadanasabapathy S. and Bawa A.S., Detection Methods for Irradiated Foods, *Compr. Rev. Food Sci. Food Saf.*, **8**, 4–16 **(2009)**

10. Delincée H., Khan A.A. and Cerda H., Some limitations of the comet assay to detect the treatment of seeds with ionising radiation, *Eur. Food Res. Technol.*, **216**, 343–346 **(2003)**

11. Dunkenberger L., Reiss K. and Del Valle L., Comet Assay for the Detection of Single and Double-Strand DNA Breaks, In mmunohistochemistry and Immunocytochemistry: Methods and Protocols, ed., Del Valle L., 263–269 **(2022)**

12. Eric M., Detection of Irradiated Foods, Food Irradiation Research and Technology, 123–145 **(2012)**

13. Glei M., Schneider T. and Schlörmann W. Comet assay: an essential tool in toxicological research, *Arch. Toxicol.*, **90**, 2315–2336 **(2016)**

14. Indiarto R., Irawan A.N. and Subroto E., Meat Irradiation: A Comprehensive Review of Its Impact on Food Quality and Safety, *Foods*, **12**, 1845 **(2023)**

15. Jiang N. et al, An Overview of Comet Assay Application for Detecting DNA Damage in Aquatic Animals, *Agriculture*, **13**, 623 **(2023)**

16. Khan A.A. and Shahid M.K., Identification of radiation processing of different plant foods of Pakistan origin using the rapid technique of Electron Spin Resonance (ESR) spectrometry, *Radiat. Phys. Chem.*, **204**, 110667 **(2022)**

17. Khan A.A. and Shahid M.K., Detection of radiation treatment of spices using Electron Spin Resonance Spectroscopy Technique, *J. Spices Aromat. Crop.*, **30**, 107–116 **(2021)**

18. Khawar A. et al, Evaluation of irradiation in foods using DNA Comet assay, *J. Food Sci. Technol.*, **48**, 106–109 **(2011)**

19. Kotsanopoulos K.V., Authenticity of Maize/Corn, Authenticity of Foods of Plant Origin, 138–160 **(2022)**

20. Kumar L. and Gaikwad K.K., Advanced food packaging systems for space exploration missions, *Life Sci. Sp. Res.*, **37**, 7–14 **(2023)**

21. Kumaravel T.S., Vilhar B., Faux S.P. and Jha A.N., Comet Assay measurements: a perspective, *Cell Biol. Toxicol.*, **25**, 53–64 **(2009)**

22. Lee M.K. et al, Radiosensitivity of lentil beam (Lens culinaris L) to gamma-irradiation, *J. Radiat. Ind.*, **10**, 211–217 **(2017)**

23. Marín-Huachaca N., Delincée H., Mancini-Filho J. and Villavicencio A.L.C.H., Use of the DNA Comet Assay to detect beef meat treated by ionizing radiation, *Meat Sci.*, **71**, 446–450 **(2005)**

24. Marín-Huachaca N.S., Mancini-Filho J., Delincée H. and Villavicencio A.L.C.H., Identification of gamma-irradiated papaya, melon and watermelon, *Radiat. Phys. Chem.*, **71**, 193–196 **(2004)**

25. Menke M., Angelis K.J. and Schubert I., Detection of specific DNA lesions by a combination of comet assay and FISH in plants, *Environ. Mol. Mutagen.*, **35**, 132–138 **(2000)**

26. Menke M., Meister A. and Schubert I., N-Methyl-N-nitrosourea-induced DNA damage detected by the comet assay in Vicia faba nuclei during all interphase stages is not restricted to chromatid aberration hot spots, *Mutagenesis*, **15**, 503–506 **(2000)**

27. Møller P. et al, Visual comet scoring revisited: a guide to scoring comet assay slides and obtaining reliable results, *Mutagenesis*, **38**, 253–263 **(2023)**

28. Nandhakumar S. et al, Evaluation of DNA damage using single-cell gel electrophoresis (Comet Assay), *J. Pharmacol. Pharmacother.*, **2**, 107–111 **(2011)**

29. Nikolova I., Georgieva M., Stoilov L., Katerova Z. and Todorova D., Optimization of Neutral Comet Assay for studying

DNA double-strand breaks in pea and wheat, *J. Biosci. Biotechnol.*, **2**, 151–157 **(2013)**

30. Park J.H. et al, Comet assay for rapid identification of irradiated perilla seeds and soybeams with gamma rays and electron-beams, *J. Radiat. Ind.*, **13**, 119–126 **(2019)**

31. Pereira A.D., de Andrade S.F., de Oliveira Swerts M.S. and Maistro E.L., First *in vivo* evaluation of the mutagenic effect of Brazilian green propolis by comet assay and micronucleus test, *Food Chem. Toxicol.*, **46**, 2580–2584 **(2008)**

32. Sahoo M., Aradwad P., Panigrahi C., Kumar V. and Naik S.N., Irradiation of Food, Novel Technologies in Food Science, 333–373 **(2023)**

33. Stefanova R., Vasilev N.V. and Spassov S.L., Irradiation of food, current legislation framework and detection of irradiated foods, *Food Anal. Methods*, **3**, 225–252 **(2010)**

34. Valencia-Quintana R. et al, Effect of Pesticide Exposure over DNA Damage in Farmers from Los Reyes, Michoacan in Mexico, *Toxics*, **11**, 122 **(2023)**

35. Villavicencio A.L.C.H., Mancini-Filho J. and Delincée H., Application of different techniques to identify the effects of irradiation on Brazilian beans after six months storage, *Radiat. Phys. Chem.*, **52**, 161–166 **(1998)**

36. Vinha A.C.M.F. da and Silva C.A. de A.S.E., Overview of irradiation: advantages to foods of plant origin, *South Florida J. Heal.*, **3**, 248–262 **(2022)**.

(Received 12th December 2023, accepted 08th Febraury 2024)