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RAPID AND SIMPLE SCREENING TESTS TO DETECT THE RADIATION TREATMENT OF FOODS

Henry DELINCÉE

Institute of Nutritional Physiology, Federal Research Centre for Nutrition
Engesserstr. 20, D-76131 Karlsruhe, Germany

ABSTRACT

A number of analytical detection methods for the irradiation treatment of foods have been developed in recent years. Most of these methods require relatively expensive equipment and/or extended sample preparation time. Therefore, simple and low-cost tests would be of advantage to rapidly screen foodstuffs for evidence of their having been irradiated. Two such rapid approaches are described in this contribution: one promising test is the microgel electrophoresis of single cells ("comet assay") which visualises DNA fragmentation in irradiated foods and the second test is the estimation of radiolytic formed gases such as carbon monoxide and hydrogen in irradiated meat by simple electrochemical gas sensors.

KEYWORDS

Food irradiation; irradiation identification; DNA fragments; radiolytic gases; meat; chicken; pork

INTRODUCTION

At the 8th IMRP, 1992 in Beijing, the considerable developments in analytical detection methods for the irradiation treatment of foods, particularly due to intensive international co-operation, were summarized (Delincée, 1993). Today, we are standing on the threshold of a new era which will see a number of these detection methods, not only introduced as official methods on a national level, but also recognized as international standards. Examples are ESR-spectroscopy of foodstuffs containing bone (e.g. meat, fish, froglegs) or cellulose (e.g. berries, nuts), thermoluminescence measurements on food contaminated with silicate minerals (e.g. herbs and spices) or GC-analysis of lipid-derived radiolytic products such as hydrocarbons or 2-alkylcyclobutanones (e.g. meat). However, these methods require sophisticated and relatively expensive equipment and sample preparation and analysis may be quite time-consuming. It would be desirable to have rapid, simple and low-cost screening tests, which although probably not providing an unequivocal detection, would immediately indicate a possible irradiation treatment. Suspected samples could then be analyzed by the more sophisticated and validated techniques.

In this report two such rapid detection tests are described. The first method relies on radiation-induced fragmentation of DNA, the very sensitive cellular target upon irradiation. DNA fragments are visualised using the so-called "comet assay", a microgel electrophoresis of single cells or nuclei (Cerda *et al.* 1993). The "comet assay" is simple and quick, the electrophoretic separation only requiring 2 - 5 minutes. Following silver staining, the

migration pattern of DNA indicates a possible irradiation treatment. The second test estimates trapped gases in frozen foods, which are formed by the irradiation treatment and can be detected by simple electrochemical gas sensors. So, for example, the amount of carbon monoxide can be rapidly estimated by expelling the trapped gas from the frozen food by quick microwave heating and registering the CO content with a sensor as used to estimate CO in ambient air in workplaces or in an underground garage. Similarly, other gases such as hydrogen, hydrogen sulphide or ammonia can be detected.

EXPERIMENTAL

Foods were purchased in local shops and irradiated either with ^{60}Co-γ-rays (Gammacell 220, AECL, dose rate ~0.1 Gy/s) or 10 MeV electrons (Circe III linear accelerator at our centre). Mechanically recovered poultry meat was kindly provided by a French company, unirradiated as well as irradiated (Sadat and Vaissenaix, 1990).

The DNA "comet assay" was performed as described by Cerda *et al.* (1993) but modified according to the instructions of Cerda in the recent inter-laboratory blind trial (Delincée 1994b). Thus, lysis of the cells was carried out with 2.5% SDS in a 45 mM Tris-borate buffer, 1 mM EDTA (pH 8.) and on electrophoresis the same TBE buffer but devoid of SDS was used. Lysis time was 10 min and submarine electrophoresis was performed at 2 V/cm for 2 min. Following silver staining of DNA (Delincée 1993e,1994b), the slides were observed in a transmission microscope and documented by photography or image analysis.

The gas evolution method was performed as previously described (Delincée *et al.* 1994, Delincée 1994a), employing a gas circuit with several electrochemical gas sensors. "Pac II" sensors for CO and H_2S were supplied by "Dräger" (Lübeck) and "Statotector" sensors for CO, H_2 and NH_3 by "Gesellschaft für Gerätebau" (Dortmund).

RESULTS AND DISCUSSION

DNA "comet assay"

During the last few years several experiments with the DNA "comet assay" to detect various irradiated foods have been carried out (Cerda *et al.* 1993, Cerda 1993, Delincée 1993b,e, 1994b, Delincée and Marchioni 1993, Delincée *et al.* 1994, Leffke *et al.* 1993). Unirradiated samples, if not exposed to other DNA-fragmenting treatments such as blanching or cooking, always show a number of intact cells without "comets". In irradiated samples, however, the radiation-induced DNA fragments always migrate forming "comets", thus no intact cells can be observed in an irradiated sample. With increasing radiation dose the shape of the "comet" is changed giving an indication about the height of the applied dose value. Temperature fluctuations, such as freezing/thawing cycles may also yield DNA "comets". On one slide, therefore, various "comets" may be observed, but the lowest degree of DNA damage will determine the classification of the sample. Using this classification principle, a recent inter-laboratory study with frozen chicken and pork cells irradiated with 0; 1; 3 and 5 kGy yielded a very high rate (> 90%) of identification (Delincée 1994b), although most laboratories were rather inexperienced with this technique. An experienced experimenter will frequently be able to decide whether the sample has been irradiated or not just by a glance at the slide, and even to make a proper estimation of dose.

In addition to chicken and pork, the "comet assay" has provided good identification results in our laboratory with frozen beef meat, beef liver and veal. Experiments with sea-food, like

oysters and mussels, are encouraging. However, with shrimps no well-defined cells or nuclei could hitherto be isolated, probably due to the blanching treatment of shrimps on board the sea vessel directly after being caught. Experiments with fruit and vegetables are progressing.

Gas evolution method

More than 20 years ago Pratt and Kneeland (1972) reported on low-molecular headspace gases such as hydrogen and carbon monoxide in canned radiation sterilized foods. These gases were derived from the various food components, such as carbohydrates, fats and proteins (see also Elias and Cohen, 1977). Almost twenty years later, Japanese authors described the estimation of these low-molecular gases, such as hydrogen and carbon monoxide, by gas chromatography (GC) for detection of various irradiated foods (Dohmaru *et al.* 1989, Furuta *et al.* 1992). Their research was followed up by Roberts *et al.* (1992, 1994), Hitchcock (1993, 1994) and Delincée (1993c,d, 1994a), the latter two authors employing specific gas sensors instead of GC. For the work in this report, four electrochemical gas sensors for carbon monoxide, hydrogen, hydrogen sulphide and ammonia were used. These gas sensors are usually employed to monitor gas concentrations in ambient air at working places.

Gas evolution for irradiated chicken breast chops (Fig. 1) showed a marked increase in carbon monoxide and hydrogen content with radiation dose. High increases in CO and H_2 content were also observed in commercial irradiated mechanically recovered poultry meat, although this sample had already been stored for more than five months. Hydrogen is virtually not detected in unirradiated samples. On the other hand, hydrogen is more rapidly lost on storage than carbon monoxide. In experiments with frozen irradiated (3 kGy) chicken legs, the carbon monoxide content only slightly decreased during 2 months storage at -18°C, whereas the hydrogen content was reduced to one tenth compared to the value measured immediately after irradiation. Similar results have been observed by other authors (Furuta *et al.* 1992, Hitchcock 1994, Roberts *et al.* 1994). However, the hydrogen content was still above the detection limit, thus the samples could be identified as irradiated. In non-frozen chicken legs, even the carbon monoxide diffuses so rapidly that an increase present immediately after irradiation can no longer be measured after a 14-day storage period. This gas evolution method is still at the research stage and more experiments are needed to study the influence of different structures and chemical compositions of the food; possible influence of temperature fluctuation during frozen storage; and for various storage periods and also interference or cross-reactivity factors of the various gas sensors. The method does, however, seem applicable to a number of foodstuffs as a rapid screening test.

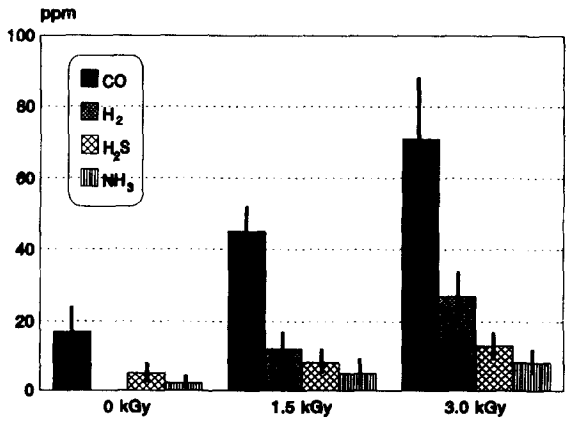

Fig. 1. Gas evolution from irradiated frozen chicken breast chops after 19 days storage at −18°C (mean values of 10 replications, sensor values in ppm represent part of the detected gas in the total gas stream in the applied circuit)

CONCLUSION

The DNA "comet assay" and the gas evolution method presented in this paper are potentially rapid and simple screening tests for identifying irradiated food. They contribute towards the simplification of food control, thereby enhancing consumer confidence in the proper surveillance of radiation processing.

REFERENCES

Cerda, H. (1993). Analysis of DNA in fresh meat, poultry and fish. Possibility of identifying irradiated samples. In: *Changes in DNA for the detection of irradiated food.* Strasbourg 25-26 May, 1992, (H. Delincée, E. Marchioni and C. Hasselmann, eds.), *EUR 15012*, pp. 5-6. Commission of the European Communities, Luxembourg.

Cerda, H., B. v. Hofsten and K.J. Johanson (1993). Identification of irradiated food by microelectrophoresis of DNA from single cells. In: *Recent advances on detection of irradiated food*, Ancona, 24-26 Sept. 1991, (M. Leonardi, J.J. Belliardo and J.J. Raffi, eds.), *EUR 14315*, pp. 401-405. Commission of the European Communities, Luxembourg.

Delincée, H. (1993a) Control of irradiated food: Recent developments in analytical detection methods. *Radiat. Phys. Chem.* 42, 351-357.

Delincée, H. (1993b). Micro gel electrophoresis of chicken DNA to detect radiation processing. In: *Changes in DNA for the detection of irradiated food.* Strasbourg, 25-26 May 1992, (H. Delincée, E. Marchioni and C. Hasselmann, eds.), *EUR 15012*, p. 7. Commission of the European Communities, Luxembourg.

Delincée, H. (1993c). Ein einfacher Schnelltest für bestrahltes Hühnerfleisch. *Bundesgesundheitsbl.* 36, 378- 379.

Delincée, H. (1993d). A rapid and simple screening test to detect the radiation treatment of fat-containing foods. In: *New Developments in Food, Feed and Waste Irradiation* (G.A. Schreiber, N. Helle und K.W. Bögl, eds.), *SozEp Heft 16/1993*, pp. 99-102. Bundesgesundheitsamt, Berlin.

Delincée, H. (1993e). Detection of the irradiation treatment of foods using micro gel electrophoresis of DNA. In: *New Developments in Food, Feed and Waste Irradiation* (G.A. Schreiber, N. Helle and K.W. Bögl, eds.), *SozEp Heft 16/1993*, pp. 112-116. Bundesgesundheitsamt, Berlin.

Delincée, H. and E. Marchioni (1993). Intercomparison study with micro gel elektrophoresis of DNA for detection of irradiated chicken: a preliminary trial. In: *Changes in DNA for the detection of irradiated food.* Strasbourg 25-26 May, 1992, (H. Delincée, E. Marchioni and C. Hasselmann, eds.), *EUR 15012*, pp. 8-14. Commission of the European Communities, Luxembourg.

Delincée, H. (1994a). A rapid and simple screening test to identify irradiated food using multiple gas sensors. *International Meeting on Analytical Detection Methods for Irradiation Treatment of Food*, Belfast, UK, 20-24 June 1994, Royal Society of Chemistry, London (in press).

Delincée, H. (1994b). Application of the DNA "Comet Assay" to detect irradiation treatment of foods. *International Meeting on Analytical Detection Methods for Irradiation Treatment of Food*, Belfast, UK, 20-24 June 1994, Royal Society of Chemistry, London (in press).

Delincée, H., P. Funk and D. Roig (1994). Fortschritte bei der Entwicklung von einfachen Schnelltests für bestrahlte Lebensmittel. In: *4. Deutsche Tagung Lebensmittelbestrahlung*, Hamm, 6.-7. April 1994. *SozEp Heft 1994*, Bundesgesundheitsamt, Berlin, (in press).

Dohmaru, T., M. Furuta, T. Katayama, H. Toratani and A. Takeda (1989). Identification of irradiated pepper with the level of hydrogen gas as a probe. *Radiat. Res.* 120, 552-555.

Elias, P.S. and A.J. Cohen, eds. (1977). *Radiation chemistry of major food components.* Elsevier, Amsterdam.

Furuta, M., T. Dohmaru, T. Katayama, H. Toratani and A. Takeda (1992). Detection of irradiated frozen meat and poultry using carbon monoxide gas as a probe. *J. Agric. Food Chem.* 40, 1099-1100.

Hitchcock, C.H.S. (1993). Determination of hydrogen using a novel hydrogen-specific electronic sensor: A potential method for detecting irradiated food. *J. Sci. Food Agric.* 62, 301-305.

Hitchcock. C.H.S. (1994). Determination of hydrogen in ice and in irradiated frozen chicken. *International Meeting on Analytical Detection Methods for Irradiation Treatment of Food*, Belfast, UK, 20-24 June 1994, Royal Society of Chemistry, London (in press).

Leffke, A., N. Helle, K.W. Bögl and G.A. Schreiber (1993). DNA-Electrophoresis of single cells - A method to screen for irradiated foodstuffs. In: *New Developments in Food, Feed and Waste Irradiation* (G.A. Schreiber, N. Helle and K.W. Bögl, eds.), *SozEp Heft 16/1993*, pp. 117-121. Bundesgesundheitsamt, Berlin.

Pratt, G.B. and L.E. Kneeland (1972). Irradiation induced headspace gases in packaged radiation sterilized food. *Technical Report 72-55-FL*, US Army Natick Laboratories, Natick, Mass.

Roberts, P.B., D.M. Chambers and G.W. Brailford (1992) Progress on detection methods in New Zealand. In: *Co-ordinated research programme on analytical detection methods for irradiation treatment of foods (ADMIT).* Report of second research co-ordination meeting, Budapest, Hungary, 15-19 June 1992, p. 78, Joint FAO/IAEA Division, Vienna.

Roberts, P.B., D.M. Chambers and G.W. Brailford (1994). Gas evolution as a rapid screening method for detection of irradiated foods. *International Meeting on Analytical Detection Methods for Irradiation Treatment of Food*, Belfast, UK, 20-24 June 1994, Royal Society of Chemistry, London (in press).

Sadat, T. and M. Vassenaix (1990). Use of a linear accelerator for decontamination of deboned poultry meat. *Radiat. Phys. Chem.* 36, 661-665.