Pergamon

Radiat. Phys. Chem. Vol. 52, Nos 1–6, pp. 135–139, 1998

Printed in Great Britain

PII: S0969-806X(98)00062-0

0969-806X/98 $19.00 + 0.00

DETECTION OF IRRADIATED FOOD: DNA FRAGMENTATION IN GRAPEFRUITS

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ABSTRACT

Employing the simple microgel electrophoresis of single cells - 'comet assay' - on grapefruit seeds enabled a rapid identification of irradiated fruits. Fruits were exposed to radiation doses of 0, 0.1, 0.2, 0.3, 0.4 and 0.5 kGy covering the range of potential commercial irradiation for insect disinfestation and quarantine purposes. Seeds were isolated, crushed, and the cells embedded in an agarose layer. After lysis of the cells, they were subjected to microgel electrophoresis for 2.5 minutes, and then stained. Fruits irradiated with 0.2 kGy and higher doses showed typical DNA fragmentation, the DNA fragments stretching or migrating out of the cells forming a tail towards the anode, giving the damaged cells an appearance of a comet. With increasing dose a longer extension of the DNA from the nucleus towards the anode is observed. Undamaged cells will appear as intact nuclei without tails. The DNA comet assay is thus a rapid and inexpensive screening technique to detect irradiated grapefruits. Suspected samples may subsequently be analysed by officially validated methods for detection of irradiated foods.

KEYWORDS

Food irradiation; irradiation identification; DNA fragmentation; grapefruit

INTRODUCTION

Treatment of food with ionizing energy - 'food irradiation' - is now finally becoming a reality in many countries. [Loaharanu, 1995; IAEA, 1996]. The benefits include an improvement of food hygiene, spoilage reduction and extension of shelf life. Since ionizing radiation is targeting living organisms, rapidly dividing cells being particularly sensitive, irradiation is highly efficient to inactivate micro-organisms and parasites, and to eradicate insects. Irradiation of fruit is regarded as as versatile and effective replacement of chemical fumigants in the fight against insect pests. Using irradiation, quarantine requirements can be fulfilled, and trade barriers reduced [Moy, 1985; IAEA, 1991; WHO, 1994; Diehl, 1995]. To control this kind of processing, it is desirable

not only to rely on administrative control of facilities licensed for food irradiation and compulsory certification of treated foods, but also to be able to detect the irradiation treatment directly in the food itself. However, it must be clearly understood that the objective of radiation disinfestation of foods is not the treatment of the fruit, but the elimination of the insect pests. Hence, tests for identification of irradiated insects still present on the irradiated food, have also been proposed (Nation *et al.*, 1995).

Recent research on a world-wide level has lead to a number of analytical detection methods [Delincée, 1991; Raffi *et al.*, 1994; McMurray *et al.*, 1996], some of which now are established as European Standards, such as the EN-1784 - EN-1788:1996. It is conceivable that some of these methods may be applied to fruits which for disinfestation of insects has received a low radiation dose - usually far below 1 kGy. Probably, both the detection of fat-derived radiolytic products like hydrocarbons or 2-alkylcyclobutanones in the fat-containing part of the fruit, e.g. seeds, and the thermoluminescence of mineral debris deposited on the fruit surface may serve as suitable tools to control radiation processing. Other methods like electron spin resonance still need higher sensitivity as presently achieved to detect radiation treatment at these low doses applied for insect disinfestation. A drawback of the abovementioned techniques is their requirement for sophisticated and relatively expensive equipment, and sample preparation and analysis may be quite time-consuming. A promising simple technique for identification of low-dose irradiated fruit is the half-embryo test, which also was successfully checked in an interlaboratory trial [Kawamura *et al.*, 1996]. However, the latter method, although simple and of low-cost, lasts about 4 days. It would be desirable to have a more rapid, but still inexpensive test to check the radiation treatment.

Since the large molecule of DNA is an easy target for ionizing radiation, changes in DNA offer potential as a detection method [Delincée *et al.*, 1993; Delincée 1996]. A sensitive technique to detect DNA fragmentation is the microgel electrophoresis of single cells or nuclei, also called "comet assay" [McKelvey-Martin *et al.*1993; Fairbairn *et al.* 1995]. The application of the comet assay to food to detect irradiation has been described by Cerda *et al.* (1993, 1997). However, mostly foods treated with radiation doses higher than 1 kGy were studied. In this paper, grapefruits exposed to doses of 0.1 to 0.5 kGy, covering the range of potential commercial application for the use of irradiation for insect disinfestation and quarantine purposes of fruit, were studied. Grapefruit was used to represent the class of citrus fruit for which the half-embryo test is well established.

EXPERIMENTAL

Grapefruits (not seedless!) were purchased in local shops and irradiated with ^{60}Co-γ-rays (Gammacell 220, AECL, dose-rate ~0.1 Gy/s). The radiation dose levels were 0, 0.1, 0.2, 0.3, 0.4, and 0.5 kGy (nominal doses ± 15%). Seeds were collected and analysed using the DNA comet assay as described by Cerda *et al.* (1997). Briefly, about 250 mg of seeds were crushed with a mortar and pestle, and transferred to 3 ml ice-cold PBS. This suspension was stirred for 2-5 min at about 500 rpm, filtered through 200- and 100 μm nylon sieve cloth, and then let to sediment for about 45 min. 100 μl cell suspension was mixed with 1 ml warm 0.8% casting agarose gel solution, and 100 μl of this mixture was spread on a microscope slide (76 x 26 mm). The casted slides were immersed in lysis buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.4 containing 2.5% SDS) for 60 min. Electrophoresis was carried out using the same TBE buffer, but devoid of SDS, at a potential of 2V/cm for 2.5 min. Silver staining was employed to visualise DNA. Slides were evaluated with a standard transmission microscope.

Fig. 1. Microgel electrophoresis of cells from grapefruit seeds; silver staining; anode to the right; radiation dose from top to bottom: 0, 0.2 and 0.5 kGy.

RESULTS AND DISCUSSION

Photographs of slides from grapefruit cells subjected to the comet assay either after irradiation with doses of 0.2 and 0.5 kGy or without irradiation are shown in Figure 1. Just at a glance, the irradiated samples can be classified as such due to the long tails, representing DNA fragments migrating out of the cells towards the anode, giving the damaged cells an appearance of a comet. Unirradiated cells are virtually intact or show only very slight migration or stretching of DNA towards the anode. Sometimes also a few cells with longer comet tails can be observed in the unirradiated sample, but always accompanied by intact cells. On the contrary, intact cells are not seen in irradiated samples. It should be mentioned that lysis of cells walls has to be ensured. Otherwise, if cell walls are not permeable, DNA fragments are hindered to migrate and the analysis will provide false negatives. Whereas samples irradiated with 0.1 kGy were difficult to discern visually from the controls, fruits irradiated with 0.2 kGy or higher doses showed marked DNA fragmentation illustrated by longer comet tails. Probably, the use of an image analyzer could help to discern samples irradiated at the lower dose from the controls, by enabling an automated evaluation of a large number of cells and deriving appropriate and characteristic comet indices. But since the comet assay is intended as a simple, fast and inexpensive screening technique, the use of an image analyzer was not employed in this study. It ismay be concluded that the DNA comet assay is a simple low-cost and rapid test for detection of irradiated grapefruits. In case of positive identification the test could be supplemented by the more demanding officially validated methods for identification of the irradiation treatment.

ACKNOWLEDGMENTS

The skilful technical assistance of Mrs. U. Stadler-Prayle and S. Delincée is gratefully acknowledged.

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