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Detection of irradiated fresh fruits treated by e-beam or gamma rays

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Abstract

Since about 1990, the amount of commercially irradiated food products available worldwide has increased. Commercial irradiation of foods has been allowed in Brazil since 1973 and now more than 20 different food products are approved. Among these products are a number of fresh fruits which may be irradiated for insect disinfestation, to delay ripening and to extend shelf-life. Today, there is a growing interest to apply radiation for the treatment of fruits instead of using fumigation or e.g. vapour-heat treatments, and an increased international trade in irradiated fruits is expected. To ensure free consumer choice, methods to identify irradiated foods are highly desirable. In this work, three detection methods for irradiated fruits have been employed: DNA Comet Assay, the half-embryo test and ESR. Both electron-beam (e-beam) and gamma rays were applied in order to compare the response with these two different kinds of radiation. Fresh fruits such as oranges, lemons, apples, watermelons and tomatoes were irradiated with doses in the range 0, 0.50, 0.75, 1.0, 2.0 and 4.0 kGy. For analysis, the seeds of the fruits were utilized. Both DNA Comet Assay and the half-embryo test enabled an easy identification of the radiation treatment. However, under our conditions, ESR measurements were not satisfactory.

Keywords: Food irradiation; Detection; Electron beam; Gamma radiation; Half-embryo test; DNA comet assay; Electron spin resonance; Oranges; Lemons; Apples; Watermelons; Tomatoes

1. Introduction

A long and growing list of different food items, groups or classes has been approved for irradiation by an increasing number of countries (Clearances of Irradiated Food, http:www.iaea.org/icgfi). These approvals are based on numerous experiments with irradiated food and on the conclusions of expert groups; e.g. those convened by the World Health Organization (WHO). In 1994, WHO stated that irradiated food produced in accordance with established good manufacturing practice (GMP) can be considered safe and nutritionally adequate (WHO, 1994). The notion that radiation-processed food is safe and wholesome has been endorsed by major public health institutions around the world (Diehl, 1995). It was confirmed again in 1997 by a Joint FAO/IAEA/WHO Study Group for food irradiated with doses even >10 kGy (WHO, 1999).

However, consumers in many countries have remained sceptical about food preservation by ionizing radiation, probably mostly due to lack of information

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about what happens in food upon irradiation (Delincée, 1998a). Correct and comprehensive information about food irradiation and irradiated food must reach consumers in order to enable them to reach decisions based on well-founded reasons (Bruhn, 1995). In addition, the confidence of consumers in correct labelling can be bolstered by the availability of reliable and sensitive detection methods at the hands of food control agencies (Delincée, 1998a). The value of a detection method was recognized in 1988 at the international conference on the *Acceptance, Control of, and Trade in Irradiated Food*, in which it was recommended that governments should encourage research into methods of detection of irradiated food (Anon, 1989). This recommendation has led to increased international cooperation in the field of detection methods and a milestone was reached at the end of 1996, when five detection methods for irradiated food were adopted by the European Committee for Standardization (CEN). A number of other analytical procedures are in an advanced state of development, and new techniques are emerging (Delincée, 1998a).

In this work, three detection methods of irradiated fruits were investigated, namely the DNA Comet Assay, the half-embryo test and electron spin resonance (ESR). The DNA Comet Assay measures DNA fragmentation using microgel electrophoresis of single cells or nuclei, and has also been utilized to identify irradiated food (Cerda et al., 1997; Koppen and Cerda, 1997; Cerda, 1998a, b; Cerda and Koppen, 1998; Delincée, 1998b; Villavicencio et al., 2000). The method is now a draft European Standard for screening purposes (prEN 13784). The half-embryo test is based on the inhibition of shooting and rooting in seeds or grains due to irradiation, and is a sensitive, simple and inexpensive method (Kawamura et al., 1996; Villavicencio et al., 1997). ESR detects free radicals in the food either due to irradiation or to other compounds present. Radiation treatment produces radicals which can be detected in solid and dry parts of the food (Raffi et al., 1988; Stevenson and Gray, 1995; Desrosiers, 1996; De Jesus et al., 1996; Douifi et al., 1998). Irradiated foods containing cellulose may be identified by analyzing the ESR spectrum, since in addition to the central signal, a pair of lines appears to the left and right of the central signal. The spacing of this radiation-induced signal pair is about 6.0 mT and is symptomatic of radiation treatment having taken place (EN 1787, 2000).

The fruits selected for this study are approved for irradiation in Brazil, with the exception of apples, which however, are authorized in a number of other countries, e.g. Mexico. Both electron beam (e-beam) and gamma rays were applied in order to compare the response with these two different kinds of radiation.

2. Experimental

2.1. Samples

Oranges, lemons, apples, watermelons and tomatoes were purchased in local shops in São Paulo, Brazil.

2.2. Irradiation

The samples were irradiated at ambient conditions at dose levels of 0.5, 0.75, 1.0, 2.0 and 4.0 kGy using ^{60}Co gamma rays (Gammacell 220, AECL; dose rate 5.8 kGy/h) or electrons from an e-beam facility (Radiation Dynamics Inc., USA; $E = 1.5$ MeV, $I = 25$ mA).

2.3. Methodology

The DNA Comet Assay was performed as described by Cerda et al. (1997). The silver stained slides were evaluated in a standard transmission microscope. The half-embryo test was carried out as described by Kawamura et al. (1996). ESR measurements were done with a Bruker EMS 104 spectrometer with the settings according to the European Standard EN 1787, 2000.

3. Results and discussion

Employing DNA Comet Assay, increasing DNA fragmentation was characterized by various types of comets as already described by Cerda and Koppen (1998). The comets were classified as type 1: short tail, only slight degradation; type 2: long tail of even width, 2–3 times longer than type 1; type 3: long tail which widens at the end; and type 4: long tail almost separated from the head of the comet. All non-irradiated fruits exhibited comets of types 1 and 2 only, with very slight amounts of type 3 in apples and tomatoes (Fig. 1). With increasing dose, DNA fragmentation became obvious, increasing amounts of type 3 comets being observed. At higher doses also type 4 comets appeared in tomatoes, apples and watermelons. At a single glance, one could distinguish non-irradiated samples from samples irradiated with doses higher than 0.75 kGy. At lower doses, quantitative evaluation by image analysis could help to discriminate between irradiated and non-irradiated samples.

The response to gamma rays or e-beam was in principle very similar for the fruits tested here. Only a slightly more damaging effect on the DNA was observed for gamma rays in the case of watermelons and apples. Whether this difference is significant, needs to be confirmed by additional experiments.

Using the half-embryo test, shoot elongation and root growth was markedly inhibited already at a dose of 0.5 kGy, particularly for oranges and lemons. This very

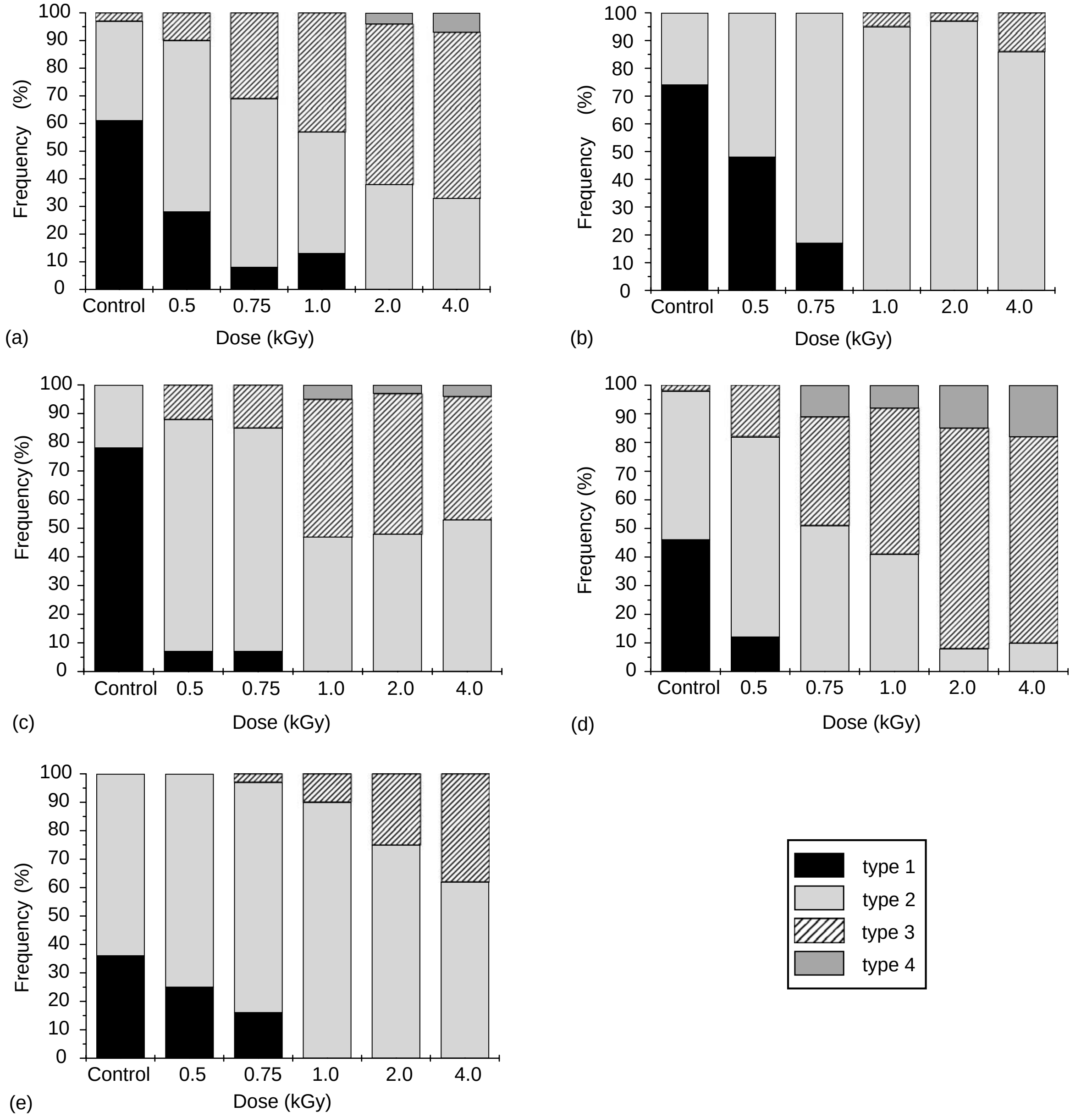

Fig. 1. Frequency of comet types of gamma-irradiated fruits cells: (a) orange; (b) apple; (c) watermelon; (d) tomato; and (e) lemon.

simple, however, long-lasting test (4–6 days) was capable to discriminate between irradiated and non-irradiated seeds of all fruits. For samples irradiated with doses at 0.5 kGy or higher, the retardation was similar, so a dose estimation could not be performed. There was no difference between the effect of gamma rays or e-beam.

ESR measurements yielded an increase in the central signal of the seeds or seed testas by irradiation, however, the typical satellite pair of signals symptomatic for cellulose were not observed in these experiments. More work is needed to arrive at conditions where successful detection by ESR measurements will be achieved.

4. Conclusions

The radiation treatment of fresh fruits, either by e-beam or by gamma rays, can be detected using various analytical methods. Both DNA Comet Assay and the half-embryo test are suitable procedures to identify irradiated fresh fruits. Similar responses were observed for e-beam and gamma rays.

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