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Application of the microbiological method DEFT/APC to detect minimally processed vegetables treated with gamma radiation

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ABSTRACT

Marketing of minimally processed vegetables (MPV) are gaining impetus due to its convenience, freshness and apparent health effect. However, minimal processing does not reduce pathogenic microorganisms to safe levels. Food irradiation is used to extend the shelf life and to inactivate food-borne pathogens. In combination with minimal processing it could improve safety and quality of MPV. A microbiological screening method based on the use of direct epifluorescent filter technique (DEFT) and aerobic plate count (APC) has been established for the detection of irradiated foodstuffs. The aim of this study was to evaluate the applicability of this technique in detecting MPV irradiation. Samples from retail markets were irradiated with 0.5 and 1.0 kGy using a ^{60}Co facility. In general, with a dose increment, DEFT counts remained similar independent of the irradiation while APC counts decreased gradually. The difference of the two counts gradually increased with dose increment in all samples. It could be suggested that a DEFT/APC difference over 2.0 log would be a criteria to judge if a MPV was treated by irradiation. The DEFT/APC method could be used satisfactorily as a screening method for indicating irradiation processing.

1. Introduction

In recent decades, consumers have been living in a society where convenience and high-quality food products are at a premium. One of the responses of the food industry to this trend has been production of minimally processed vegetables. These vegetables combine their fresh-like and healthy characteristics (preserved during storage by a natural packaging system) with a minimal time of preparation before consumption both at consumer and catering levels (Watada et al., 1996).

Minimally processed vegetables have a physical structure which is susceptible to microbiological invasion, present before, during or after harvest. Possible contamination sources are seeds, soil, irrigation water, animals, manure/sewage sludge use, harvesting, processing and packaging (Brackett, 1999 and Francis et al., 1999).

The use of food irradiation is increasing throughout the world as a suitable and safe process to reduce the number of microorganisms and disinfestate various food products, such as herbs and spices, meat and fish products and fruits and vegetables (Prakash and Foley, 2004).

Appropriate techniques for detection of irradiated foods and determination of applied dose requirements are needed to guarantee proper consumer information, to prevent misuse of technology and to facilitate trade of irradiated foods (Farkas, 2006 and Schreiber et al., 1993).

The characteristics of microbial population of irradiated foods have been used for developing detection methods for irradiated foods (Delincée, 1998). A microbiological method based on the use of the direct epifluorescent filter technique (DEFT) and the conventional aerobic plate count (APC) has been used for the detection of several foodstuffs, such as spices, poultry and meat (Boisen et al., 1992 and Wirtanen et al., 1993).

DEFT is a method originally developed for the rapid enumeration of microorganisms in raw milk samples (Pettipher et al., 1980). The method is based on a membrane filtration that captures the microorganisms, followed by a staining with a fluorochrome, acridine orange. After staining, the membrane is rinsed and mounted on a microscope slide, which can be easily visualized and counted with an epifluorescent microscope. The complete procedure can take as little as 30 min.

The DEFT count enumerates the total number of contaminating microorganisms, irrespective of viability. The APC indicates the number of viable microorganisms capable of forming colonies on an agar plate and is expressed as colony forming units (CFU). For a non-irradiated sample, the counts by DEFT are in close agreement with those by APC, because nearly all the cells present are alive.

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However, when the APC of an irradiated sample is compared with the DEFT count of the same sample, the APC is found to be considerably less than that obtained by DEFT, and the difference indicates that the sample could have been irradiated. (Jones et al., 1994, 1996).

Attempts to combine radiation treatment with other agents and treatments are of utmost importance in enhancing the microbiological effectiveness, reducing energy requirement of food preservation and in improving the product quality. Also, applied doses should be low enough to not affect sensorial characteristics of products (Farkas, 1989 and Thayer and Rajkowski, 1999). In this study, the DEFT/APC method was first used to detect irradiation processing of minimally processed vegetables.

2. Materials and methods

2.1. Samples

Samples of minimally processed lettuce, chard, watercress, escarole, chicory, spinach and cabbage were purchased in their original sealed packages from local retail markets in São Paulo, Brazil. Ten samples of each minimally processed vegetable were tested in triplicate. Manufacture date met to the day of analysis.

2.2. Irradiation

The samples were irradiated using a multi-purpose ^{60}Co gamma ray facility installed at Instituto de Pesquisas Energéticas e Nucleares-IPEN/CNEN (São Paulo, Brazil). The temperature was around 6 °C. The applied doses were 0 (control), 0.5 and 1.0 kGy. The dose rate was around 3.0 kGy/h. Harwell Gammachrome YR dosimeters were used for radiation dose measurement.

2.3. Analysis

2.3.1. DEFT/APC procedure

Samples were tested immediately after irradiation. Analysis was performed according to EN 13783 (2001), using Swinnex filters holders instead of funnels for filtration. Unirradiated and irradiated vegetables (10 g) were stirred vigorously with 90 mL of sterile peptone saline diluent from Merck and serially diluted with the same diluent (10^{-1}–10^{-6}). For prefiltration of suspensions, 25 mm diameter fast quantitative filter paper (Nalgon) were mounted in a Swinnex filter holder (Millipore Ltd., London) and autoclaved before use. A new device was used for each dilution of sample. For the DEFT procedure, after serial dilution, 10^{-1} or 10^{-2} diluted solutions were filtered through a new Swinnex filter holder containing an 11 μm polypropylene filter above a 0.6 μm polycarbonate filter. After that, staining and rinsing steps were performed. For APC (viable aerobic microorganisms count), 1.0 mL of each dilution were transferred to a sterile Petri dish and added 15 mL of plate count agar (Difco), previously melted and cooled to 47 °C (±2 °C). The plates were incubated upside down at 30 °C (±1 °C) for 72 h and then expressed as $\log_{10}$ CFU/g.

2.3.2. Calculation

The DEFT count per gram (X) of vegetables was calculated by using the mean number of DEFT units per microscope field (N/n), the microscope factor (MF) and dilution factor (DF) of the sample Eq. (1).

$$X = \text{DEFT count/g} = (N \times \text{MF} \times \text{DF})/n \quad (1)$$

The log (DEFT/APC) calculation was expressed as the difference (Dc) between the DEFT count and APC count using the logarithmic values ($Dc = \log \text{DEFT} - \log \text{APC}$).

3. Results and discussion

Vegetables analyzed in this study, non-treated by irradiation, had mesophilic bacterial populations (viable microorganisms) ranging from 10^6 to 10^8, even after the minimal processing they were subjected to. These microorganisms were decreased to the level of 10^3 with 1.0 kGy gamma radiation treatment. Irradiation treatment guaranteed at least one log cycle reduction of aerobic microorganisms for each radiation dose applied.

Most of the pathogenic bacteria, including *Salmonella* and *Escherichia coli,* are mesophiles, thus high mesophilic populations indicate there were propitious conditions during processing/storage to its proliferation. Also, several studies (Goularte et al., 2004; Lee et al., 2006 and Sommers and Boyd, 2006) have already demonstrated that doses up to 1.0 kGy could result in a 90% reduction for these two pathogenic bacteria.

The results of both non-irradiated and irradiated minimally processed vegetables are presented in Table 1 and Fig. 1. Table 1 shows the influence of radiation doses on log DEFT and log APC counts.

All the vegetables studied showed DEFT counts nearly at the same level despite irradiation treatment, however, APC counts reduced gradually with the incremental radiation dose. Even at the lowest radiation dose, 0.5 kGy, the viable count (log APC) was reduced by approximately two log units compared with that found in control samples, while DEFT count remained at the same level. Irradiation treatment with a dose of 1.0 kGy showed a similar viable count reduction compared with that obtained with 0.5 kGy (Table 1).

The log DEFT/APC (*Dc*) was determined for each vegetable and this difference increased with increasing radiation doses (Fig. 1). This is due to that the log DEFT count remained similar in the same range of values despite irradiation treatment, whereas log APC count decreased gradually.

Samples irradiated with 0.5 kGy showed the lowest *Dc* and most of them could not exceed a 2.0 log difference. Only spinach and lettuce samples irradiated with 0.5 kGy reached this value. Otherwise, samples irradiated with 1.0 kGy exceed a 2.0 log difference. Samples of spinach, lettuce and cabbage reached the highest values, 2.51, 3.42 and 3.62, respectively. Watercress and chard samples irradiated with a 1.0 kGy showed the lowest difference log DEFT/APC, 2.01 and 2.03, respectively (Fig. 1).

Table 1
Logarithmic microbial counts of minimally processed vegetables irradiated.

Samples		Radiation doses (kGy)		
		0	0.5	1.0
Chard	log DEFT	6.48 ± 0.19	6.29 ± 0.11	6.43 ± 0.15
	log APC	7.08 ± 0.14	4.32 ± 0.21	4.40 ± 0.17
Watercress	log DEFT	6.62 ± 0.09	6.62 ± 0.13	6.64 ± 0.19
	log APC	7.11 ± 0.22	5.00 ± 0.14	4.63 ± 0.13
Lettuce	log DEFT	6.29 ± 0.16	6.09 ± 0.09	6.03 ± 0.23
	log APC	6.49 ± 0.18	3.76 ± 0.09	2.61 ± 0.11
Chicory	log DEFT	6.69 ± 0.20	6.57 ± 0.08	6.48 ± 0.18
	log APC	7.14 ± 0.10	4.90 ± 0.19	4.28 ± 0.10
Escarole	log DEFT	6.03 ± 0.09	6.18 ± 0.24	6.32 ± 0.16
	log APC	6.08 ± 0.24	4.28 ± 0.13	4.11 ± 0.14
Spinach	log DEFT	6.50 ± 0.10	6.59 ± 0.15	6.59 ± 0.19
	log APC	7.02 ± 0.20	4.59 ± 0.14	4.08 ± 0.09
Cabbage	log DEFT	6.51 ± 0.07	6.56 ± 0.16	6.54 ± 0.14
	log APC	7.06 ± 0.18	4.84 ± 0.20	2.92 ± 0.10

*Mean values ± SD (standard deviation).

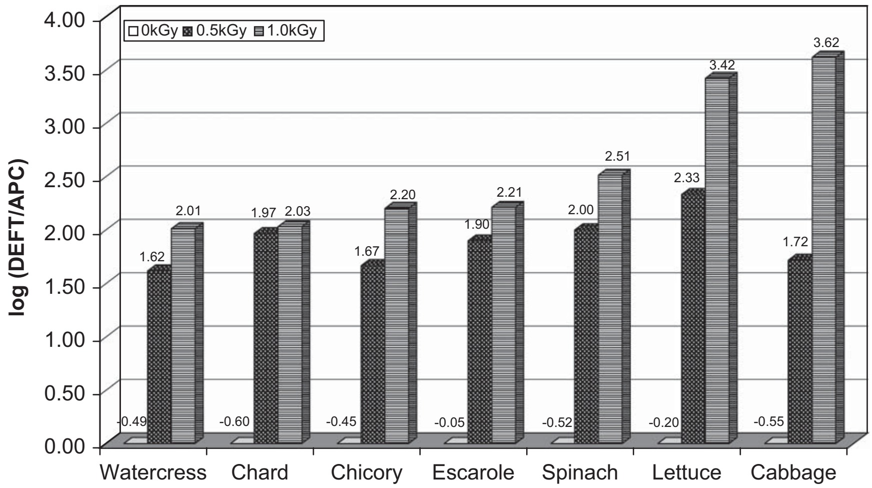

Fig. 1. Histogram of log (DEFT/APC) of minimally processed vegetables irradiated at 0.5 and 1.0 kGy.

No studies were found in literature about DEFT/APC application to detect irradiation treatment of minimally processed vegetables. Previous research carried out with cereal grains and beans (Oh et al., 2002a, b) agreed with our results, since they found a *Dc* value between 2.0 and 3.0 for doses of 0.5 kGy or over. Other research, when applying DEFT/APC methodology with spices and meat (Boisen et al., 1992; Wirtanen et al., 1993, 1995; Jones et al., 1995 and Hammerton and Banos, 1996), found a *Dc* higher than those obtained in the present study, usually higher or equal to 3.0 log. A probable reason for this would be the use of low radiation doses and/or a low-initial microbial load.

Analyzing the previous data, it would appear that for doses of 1.0 kGy or higher, we could suggest that a log DEFT/APC difference of around 2.0 log could be a relative criterion of judging a sample as unirradiated or irradiated.

4. Conclusions

The DEFT/APC method would be a cost effective and easy-to-use technique for the screening of irradiation treatment and for indicating the hygienic status of vegetable samples. It would appear that the DEFT/APC screening method could easily identify MPV that had been irradiated with doses of 1.0 kGy or higher, resulting in a log DEFT/APC over 2.0 log. Therefore, as DEFT/APC is a screening assay, it is necessary to confirm positive results with a confirmatory method, e.g. thermoluminescence or ESR of cellulose.

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