



## Effects of Gamma Radiation on Raspberries: Safety and Quality Issues

S. Cabo Verde , M. J. Trigo , M. B. Sousa , A. Ferreira , A. C. Ramos , I. Nunes , C. Junqueira , R. Melo , P. M. P. Santos & M. L. Botelho

To cite this article: S. Cabo Verde , M. J. Trigo , M. B. Sousa , A. Ferreira , A. C. Ramos , I. Nunes , C. Junqueira , R. Melo , P. M. P. Santos & M. L. Botelho (2013) Effects of Gamma Radiation on Raspberries: Safety and Quality Issues, Journal of Toxicology and Environmental Health, Part A, 76:4-5, 291-303, DOI: [10.1080/15287394.2013.757256](https://doi.org/10.1080/15287394.2013.757256)

To link to this article: <http://dx.doi.org/10.1080/15287394.2013.757256>



Published online: 20 Mar 2013.



Submit your article to this journal [↗](#)



Article views: 362



View related articles [↗](#)



Citing articles: 12 View citing articles [↗](#)

## EFFECTS OF GAMMA RADIATION ON RASPBERRIES: SAFETY AND QUALITY ISSUES

S. Cabo Verde<sup>1</sup>, M. J. Trigo<sup>2</sup>, M. B. Sousa<sup>2</sup>, A. Ferreira<sup>2</sup>, A. C. Ramos<sup>2</sup>, I. Nunes<sup>1</sup>,  
C. Junqueira<sup>1</sup>, R. Melo<sup>1</sup>, P. M. P. Santos<sup>1</sup>, M. L. Botelho<sup>1</sup>

<sup>1</sup>Instituto Tecnológico e Nuclear, Instituto Superior Técnico, Universidade Técnica de Lisboa, Sacavém, Portugal

<sup>2</sup>INRB- L-INIA, UITA, Quinta do Marquês, Oeiras, Portugal

There is an ever-increasing global demand from consumers for high-quality foods with major emphasis placed on quality and safety attributes. One of the main demands that consumers display is for minimally processed, high-nutrition/low-energy natural foods with no or minimal chemical preservatives. The nutritional value of raspberry fruit is widely recognized. In particular, red raspberries are known to demonstrate a strong antioxidant capacity that might prove beneficial to human health by preventing free radical-induced oxidative stress. However, food products that are consumed raw, are increasingly being recognized as important vehicles for transmission of human pathogens. Food irradiation is one of the few technologies that address both food quality and safety by virtue of its ability to control spoilage and foodborne pathogenic microorganisms without significantly affecting sensory or other organoleptic attributes of the food. Food irradiation is well established as a physical, nonthermal treatment (cold pasteurization) that processes foods at or nearly at ambient temperature in the final packaging, reducing the possibility of cross contamination until the food is actually used by the consumer. The aim of this study was to evaluate effects of gamma radiation on raspberries in order to assess consequences of irradiation. Freshly packed raspberries (*Rubus idaeus L.*) were irradiated in a <sup>60</sup>Co source at several doses (0.5, 1, or 1.5 kGy). Bioburden, total phenolic content, antioxidant activity, physicochemical properties such as texture, color, pH, soluble solids content, and acidity, and sensorial parameters were assessed before and after irradiation and during storage time up to 14 d at 4°C. Characterization of raspberries microbiota showed an average bioburden value of 10<sup>4</sup> colony-forming units (CFU)/g and a diverse microbial population predominantly composed of two morphological types (gram-negative, oxidase-negative rods, 35%, and filamentous fungi, 41%). The inactivation studies on the raspberries mesophilic population indicated a one log reduction of microbial load (95% inactivation efficiency for 1.5 kGy), in the surviving population mainly constituted by filamentous fungi (79–98%). The total phenolic content of raspberries indicated an increase with radiation doses and a decrease with storage time. The same trend was found for raspberries' antioxidant capacity with storage time. Regarding raspberries physicochemical properties, irradiation induced a significant decrease in firmness compared with nonirradiated fruit. However, nonirradiated and irradiated fruit presented similar physicochemical and sensory properties during storage time. Further studies are needed to elucidate the benefits of irradiation as a raspberries treatment process.

Ensuring the security of current and future food supplies is one of the main challenges

facing governments globally, driven by the need to feed an increasing world population and

Received xxxxx

This work was developed within the Coordinated Research Project D6-RC-1163.2 financed by the International Atomic Energy Agency (IAEA) and EUP7 EUBerry Proj 265942.

Address correspondence to S. Cabo Verde, Instituto Tecnológico e Nuclear, Instituto Superior Técnico, Universidade Técnica de Lisboa, Estrada Nacional 10, 2686-953 Sacavém, Portugal. E-mail: sandracv@itn.pt

consumer demand for freshness and variety. However, there is also a need to address issues associated with the supply of safe and healthy food. Fresh fruit and vegetables are important components of a healthy and balanced diet; their consumption is encouraged in many countries by health agencies to protect against a range of diseases. The nutritional value of raspberry fruit is widely recognized and demanded by consumers, especially for protection against cardiovascular disorders, cancer, and other diseases, as well as for general health benefits (Milivojević et al., 2011). Red raspberries (*Rubus idaeus* L.), in particular, are known to demonstrate strong antioxidant capacity, mainly as a result of their high levels of anthocyanins and other phenolic compounds (Kähkönen et al., 2001; Kafkas et al., 2008; Çekiç and Özgen, 2010). Raspberries are highly perishable fruit with a storage life limited by rots (*Botrytis cinerea*), loss of firmness, and darkening of the attractive red color (Haffner et al., 2002). Most of the raspberries produced worldwide are processed as frozen and sold within different frozen fractions (Milivojević et al., 2011); however, there has been an increasing demand for fresh raspberries out of season (Milutinović et al., 2008).

Food products that are consumed raw are increasingly being recognized as important vehicles for transmission of human pathogens that were traditionally associated with foods of animal origin (Berger et al., 2010; EFSA and ECDC, 2012). Moreover, during growth, harvest, transport, and further processing and handling, these products may be contaminated with pathogens from human or animal sources (Lynch et al., 2009). Small fruits, such as raspberries, have been associated with several outbreaks of *Cyclospora cayetanensis* and norovirus (Herwaldt & Ackers, 1997; Herwaldt and Beach, 1999; Cotterelle et al., 2005; Falkenhorst et al., 2005; Hjertqvist et al., 2006; Maunula et al., 2009). However, despite the associated risk of foodborne diseases, the small fruits are not washed before marketing because of the negative effect on their quality and shelf life (Bialka and Demirci, 2007b).

Better methods of preventing contamination on the farm, or during packing or processing, or use of a terminal control such as irradiation might reduce the burden of disease transmission from fresh produce (Lynch et al., 2009). Irradiation may constitute an alternative technology for fresh food treatment (Trigo et al., 2006, 2009). Nonthermal technologies, like irradiation, have the ability to inactivate microorganisms at ambient or near-ambient temperatures, therefore avoiding the deleterious effects that heat has on flavor, color, and nutrient value of food. Irradiation has the advantage that products are processed in the final packaging stage, reducing the possibility of cross contamination until actual use by the consumer. The safety and wholesomeness of irradiated foods have been well established and revised from time to time (Diehl, 2002). The joint FAO/IAEA/WHO Experts Committee also confirmed that irradiation at 10 kGy and above does not produce any apparent toxicological hazards or nutritional or microbiological problems in food (FAO/IAEA/WHO, 1999). Reports are available showing that irradiation of food commodities, in the form of gamma rays or electron beams, is effective in overcoming quarantine barriers in international trade, as a mode of decontamination, disinfestation, and improvement of nutritional attributes and shelf life (Lacroix and Ouattara, 2000; Teets et al., 2008). However, to our knowledge the application of ionizing radiation to small fruits preservation has not been fully investigated. The aim of this study was to evaluate effects of gamma radiation on raspberries in order to assess the potential use of irradiation as a treatment process.

## MATERIAL AND METHODS

### Sampling and Packing

Raspberries (*Rubus idaeus* L., cv. Amira) were obtained from INRB experimental fields and collected for uniform degree of maturity in order to have a similar sample. Fruits (125 g) were packed in polystyrene boxes (Nutrip-PS) with a lid and holes for air circulation.

### Irradiation

Irradiation was carried out using  $^{60}\text{Co}$  experimental equipment (Precisa 22, Graviner, Lda, UK) in the Ionizing Radiation Unit located in the *campus* of Nuclear and Technological Institute, Sacavém, Portugal. Dosimetric studies were performed using an ionization chamber (FC65-P) to establish the irradiation geometry and estimate the dose rate (2.2 kGy/h). The obtained dose uniformity was 1.23. Thirty-six packages of raspberries (125 g each) were irradiated at several doses (0.5, 1, or 1.5 kGy) in three irradiation batches (4 packages/dose). After irradiation the raspberries were stored at 4°C until analysis.

### Microbiological Inactivation Studies

Microbiological analyses were performed on 25 g of fruit. Samples were blended on 100 ml physiological solution with 0.1% Tween 80 and homogenized (Stomacher 3500; Seaward, UK). Serial decimal dilutions were performed before plating. Aerobic mesophilic counts were carried out in triplicate on tryptic soy agar (Merk, Germany) at 30°C for 7 d. Microbiological counts were expressed as mean log colony-forming units (CFU) per gram.

All colonies (microbial population from nonirradiated and irradiated raspberries) were macroscopically (e.g., pigmentation, texture, shape), microscopically, and biochemically typed by gram staining, catalase activity, and oxidase production. The isolates were organized into typing groups according with *Bergey's Manual of Determinative Bacteriology* (Holt et al., 1994). The frequency of each phenotype was calculated based on the number of isolates and their characterization.

### Evaluation of Total Phenolic Content

Total phenolic compound content of was determined based on the Folin–Ciocalteu method (Singleton et al., 1999) using gallic acid as standard for the calibration curve. Twenty-five-gram fruit samples were homogenized in 100 ml of ultrapure water. One milliliter of homogenate ( $n = 3$ ) was added to 65 ml of

water and 5 ml of Folin–Ciocalteu reagent (FC). After shaking and incubating 5 min at room temperature, 15 ml sodium carbonate solution and water were added to 50 ml and content mixed. After an incubation period of 2 h at room temperature, the absorbance of the reaction mixture was measured at 765 nm using a Shimadzu ultraviolet (UV) 1800 spectrophotometer. The results were expressed as mg gallic acid equivalent (GAE) per g raspberries fresh weight (fw).

### Evaluation of Antioxidant Activity—Ferric Reducing/Antioxidant Power (FRAP Assay)

The assay was carried out according to the method described by Benzie and Strain (1996), which is based on the reduction of  $\text{Fe}^{3+}$ -TPTZ to a blue colored  $\text{Fe}^{2+}$ -TPTZ. FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a ratio of 10:1:1 at 37°C. Twenty-five-gram fruit samples were homogenized in 100 ml ultrapure water. One hundred microliters of homogenate ( $n = 3$ ) was diluted 10-fold in ultrapure water and added to FRAP reagent (3 ml) in a test tube. After 15 min of incubation at 37°C, the absorbance was measured at 593 nm. The antioxidant potential of the sample was determined from a standard curve using  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  at a concentration range between 0 and 1 mM. Results were expressed as milligrams of ferrous sulfate equivalent (FSE) per gram raspberries fresh weight (fw).

### Evaluation of Physicochemical Parameters

Textural attributes of raspberries before and after irradiation were analyzed using a texturometer TA-Hdi (Stable Micro System, UK) interfaced with “texture expert” analyzer. Six puncture tests per sample were performed with a penetrometer of 4 mm (load cell of 25 kg, velocity of 3.33 mm/s), and the maximum force (N) was estimated based on the

force–deformation curve. The titratable acidity (three measures/sample) was determined by titration and expressed in grams citric acid per 100 g raspberries fresh weight (fw). The pH was measured with a potentiometer Crison-micro pH 2002 (Crison Instruments SA, Barcelona, Spain) using a glass electrode (three measures/sample). Soluble solids content was performed with a hand-held refractometer ATAGO (Atago Co, Ltd, Tokyo, Japan) on extracted juice (three measures/sample) at 20°C. The superficial color was evaluated using a Minolta Chroma Meter CR 200b (Minolta Corp., Tokyo, Japan) using a measurement diameter of 8 mm and diffuse illumination/0° viewing angle (d/0) geometry. Data of  $L^*$ ,  $a^*$ , and  $b^*$  values, Hue angle ( $H^\circ = \arctan b/a$ ), and Chroma [ $C^* = (a^{*2} + b^{*2})^{1/2}$ ] were determined (six measures/sample).

### Sensory Analysis

A test panel made up of seven trained panelists was performed to assess the sensory quality of samples. The color, texture, aroma, and flavor were evaluated using a hedonic scale from 1 to 5 (1 = dislike extremely and 5 = like extremely).

### Assessment of Shelf-Life Extension

In order to evaluate shelf-life of fruit, physicochemical and sensory parameters were assessed after irradiation (0 d of storage; T0) and after 2 (T2) and 5 (T5) days of storage (4°C) as described previously. Microbiological analysis (total counts), total phenolic content, and antioxidant activity were performed after irradiation (0 d of storage; T0) and after 7 (T7) and 14 (T14) days of storage (4°C).

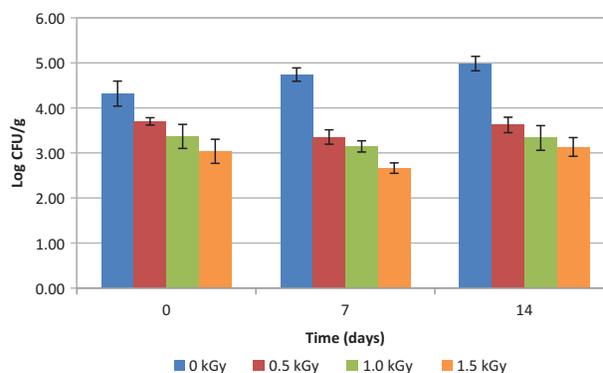
### Data Analysis

Descriptive statistics (mean and standard error) were used to analyze the obtained data. Confidence intervals for means values were estimated considering a significance level of  $p < .05$  and the number of replicates for each assay.

## RESULTS

The microbiological results indicated that raspberries presented an average bioburden of  $6 \times 10^4$  CFU/g. This mesophilic microbial population showed linear gamma radiation inactivation kinetics, with a maximum inactivation efficiency of 95% for 1.5 kGy. The performed microbial studies demonstrated a 1 log cycle reduction in total counts for 1.5 kGy that remained constant during the storage time as observed in Figure 1.

In order to evaluate the dynamics of raspberries' microbial community and its pattern with radiation doses, the microbiota from nonirradiated and irradiated raspberries were phenotypically characterized (Table 1). The initial microbial population was divided in 7 out of 11 defined morphological types indicating a considerable diversity. The most frequent morphological types were the gram-negative, oxidase-negative rods (35%) and filamentous fungi (41%). With an increase in radiation dose, fewer morphological types (3/11) were observed, suggesting a decrease of diversity with irradiation. During storage an alteration (type and number) of the contamination pattern was noted for nonirradiated raspberries, becoming less diverse with duration. The appearance of some morphological types for the higher irradiation doses not initially detected in the lower doses (e.g., phenotype VI–T7; 1.0 kGy and 1.5 kGy) may be explained



**FIGURE 1.** Average total mesophilic counts for non-irradiated and irradiated raspberries during storage time. Error bars correspond to 95% confidence intervals about mean values ( $n = 18$ ;  $\alpha = 0.05$ ) (color figure available online).

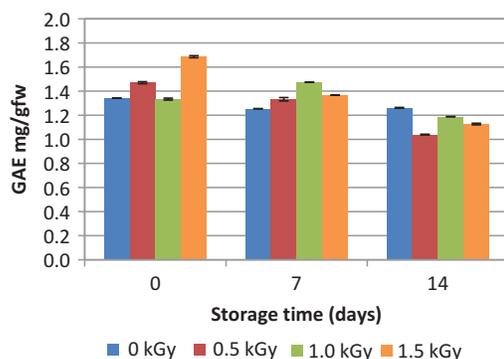
**TABLE 1.** Frequency of the Morphological Phenotypes of the Isolates (n = 1800) From Nonirradiated and Irradiated Raspberries With Storage Time

Phenotype	Relative frequency (%)															
	T0				T7				T14							
	0 kGy	0.5 kGy	1.0 kGy	1.5 kGy	0 kGy	0.5 kGy	1.0 kGy	1.5 kGy	0 kGy	0.5 kGy	1.0 kGy	1.5 kGy	0 kGy	0.5 kGy	1.0 kGy	1.5 kGy
Type I—gram-positive, catalase-positive cocci	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	13
Type II—gram-positive, catalase-negative cocci	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Type III—gram-negative, catalase-positive cocci	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Type IV—gram-negative, catalase-negative cocci	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Type V—gram-positive, endospore-forming bacilli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Type VI—gram-positive, non-endospore-forming, catalase-positive bacilli	5	14	1	4	0	0	15	19	100	0	3	0	0	0	0	0
Type VII—gram-positive, non-endospore-forming, catalase-negative bacilli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Type VIII—gram-negative, oxidase-positive bacilli	2	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Type IX—gram-negative, oxidase-negative bacilli	35	0	1	0	0	0	0	0	0	0	0	0	0	25	0	0
Type X—yeasts	5	0	0	17	33	6	10	3	0	0	2	9	0	0	0	0
Type XI—filamentous fungi	41	85	98	79	67	94	70	78	0	75	95	78	0	75	95	78

considering the inactivation of population with the increasing dose and experimental procedure. For nonirradiated and 0.5-kGy-irradiated samples, dilutions were performed to obtain a countable number of colonies, and thus, only the microorganisms in highest number were detected. For the higher doses, no dilutions were performed, assuming the inactivation of some microorganisms, and it was presumed the microorganisms in lower numbers are able to be detected.

The surviving microbiota of irradiated raspberries displayed homogeneity with storage time with prevalence of filamentous fungi. Among the fungal population the most frequent genus morphologically identified was *Cladosporium* (75%), in mycobiota of both nonirradiated and irradiated raspberries, correlated with storage time. Regarding gamma-radiation-surviving fungi, the genera *Penicillium* (22%; T0, 0.5 kGy), *Alternaria* (<1%; T0, 1.0 kGy, and T7, 1.0 kGy), *Fusarium* (<1%; T0, 1.0 kGy), and *Rhizopus* (<1%; T14, 1.0 kGy) were detected. The differences in total phenolics content and antioxidant activity of raspberries with irradiation and storage time are presented in Figure 2.

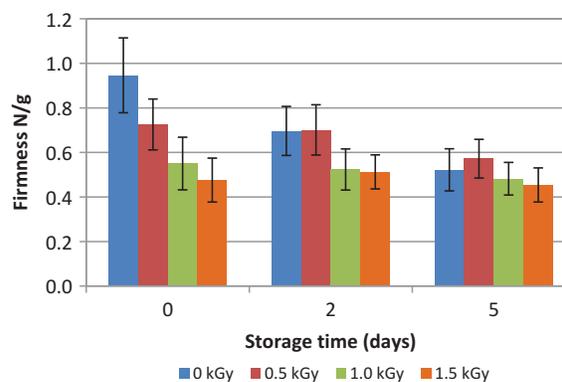
The total phenolic content of raspberries ranged between 1.24 and 1.34 mg GAE/g fresh weight basis for nonirradiated fruit, and from 1.04 to 1.69 mg GAE/g fresh weight basis for irradiated fruit. The results of total phenolic content of raspberries indicated a rise with radiation doses (T0) and decrease with the storage



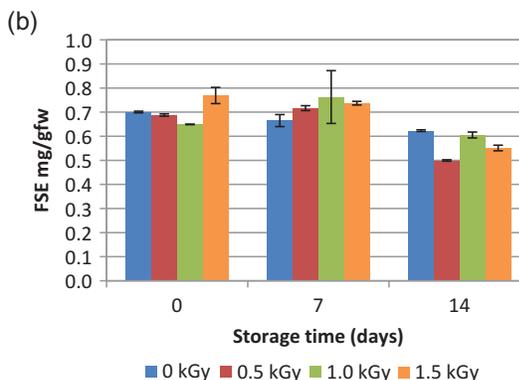
**FIGURE 2.** Effect of gamma radiation and storage time on total phenolic content (a) and antioxidant capacity (b) of raspberries. Error bars correspond to 95% confidence intervals about mean values ( $n = 3$ ;  $\alpha = 0.05$ ) (color figure available online).

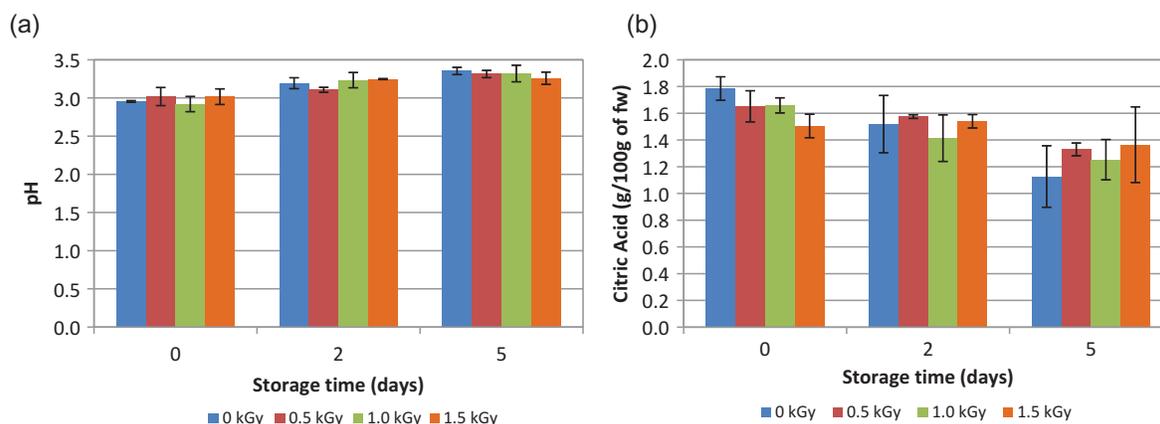
time. The same trend was noted for raspberries antioxidant capacity with storage time. The measured reducing power of raspberries ranged between 0.5 and 0.7 mg FSE/g fresh weight basis for nonirradiated fruit, and from 0.5 to 0.7 mg FSE/g fresh weight basis for irradiated fruit. This decreasing effect of storage was more noticeable in irradiated fruit.

The energy of irradiation affected the fruit firmness, being directly related with radiation dose (Figure 3). The results indicated a fall in raspberries texture for radiation doses of 1 and 1.5 kGy after irradiation (T0). Further, during the conservation period a negative effect on raspberries texture was also detected. However, with storage time the firmness of treated fruits with higher radiation doses (1 and 1.5 kGy) was not as extensively impaired (Figure 3).



**FIGURE 3.** Effect of gamma radiation and storage time on raspberries firmness (N). Error bars correspond to 95% confidence intervals about mean values ( $n = 6$ ;  $\alpha = 0.05$ ) (color figure available online).





**FIGURE 4.** Effect of gamma radiation and storage time on pH (a) and titratable acidity (b) of raspberries. Error bars correspond to 95% confidence intervals about mean values ( $n = 3$ ;  $\alpha = 0.05$ ) (color figure available online).

The obtained results showed an elevation of pH and reduction in titratable acidity with storage period for non-irradiated and irradiated raspberries (Figure 4). Regarding total soluble content, the obtained °Brix values were not markedly different between irradiated and nonirradiated raspberries during storage (data not shown).

In terms of fruit color, the irradiation at the applied doses seemed not to exert a pronounced effect. However, a decrease in color parameters was observed during storage for irradiated and non-irradiated fruit (Table 2). The variation in  $b^*$  was higher than those of  $L^*$  and  $a^*$ . In the chromaticity coordinates, where high  $b^*$  indicates toward yellow direction and

high  $a^*$  indicates toward red direction, during conservation the fruit appearance turn to a blue-based lighter red colour.  $L^*$  values represent lightness and stored raspberries had higher  $L^*$  values.

The sensory analysis indicated an effect of gamma irradiation and storage time on raspberries' firmness similar to the effect on texture. However, irradiated and nonirradiated fruit showed similar sensory characteristics during storage (Figure 5).

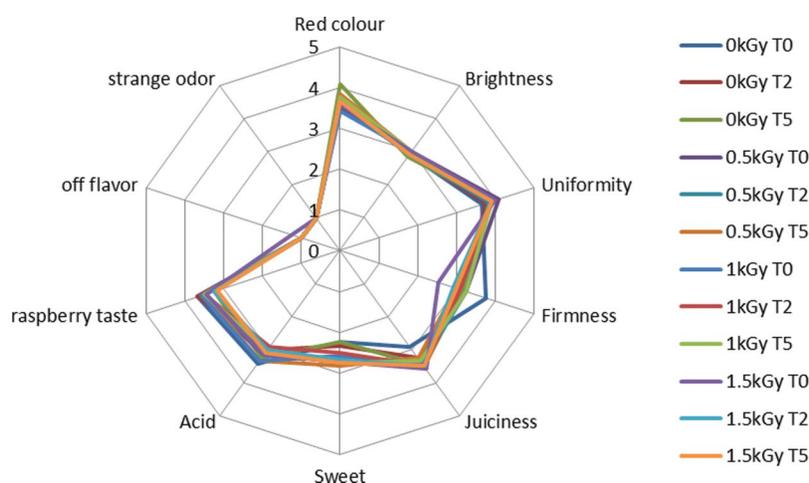
## DISCUSSION

According to the U.S. Food and Drug Administration (U.S. FDA, 2009), washing

**TABLE 2.** Effect of Gamma Radiation and Storage Time on Raspberry Color Parameters

Dose	Storage time (days)	$L^*$	$a^*$	$b^*$	Chroma	Hue angle
0 kGy	0	$30.1 \pm 0.5$	$21.9 \pm 1.6$	$7.4 \pm 0.8$	$23.2 \pm 1.8$	$18.2 \pm 0.6$
	2	$32.6 \pm 0.5$	$16.1 \pm 0.6$	$2.8 \pm 0.3$	$16.3 \pm 0.6$	$9.5 \pm 0.8$
	5	$30.3 \pm 0.7$	$15.1 \pm 0.8$	$2.8 \pm 0.3$	$15.1 \pm 0.8$	$10.4 \pm 0.8$
0.5 kGy	0	$30.4 \pm 0.3$	$20.2 \pm 1.0$	$6.3 \pm 0.4$	$21.2 \pm 1.0$	$17.3 \pm 0.7$
	2	$32.8 \pm 0.3$	$16.1 \pm 0.6$	$3.0 \pm 0.3$	$16.3 \pm 0.6$	$10.4 \pm 0.8$
	5	$31.6 \pm 0.7$	$15.2 \pm 0.7$	$3.0 \pm 0.3$	$15.5 \pm 0.7$	$10.8 \pm 1.0$
1.0 kGy	0	$31.2 \pm 0.5$	$20.4 \pm 1.0$	$6.7 \pm 0.5$	$21.5 \pm 1.1$	$17.9 \pm 1.0$
	2	$33.1 \pm 0.3$	$16.5 \pm 0.7$	$3.2 \pm 0.4$	$16.8 \pm 0.8$	$10.7 \pm 0.8$
	5	$30.8 \pm 0.6$	$16.3 \pm 1.1$	$3.2 \pm 0.6$	$16.6 \pm 1.2$	$10.3 \pm 1.3$
1.5 kGy	0	$30.7 \pm 0.4$	$20.2 \pm 1.2$	$5.5 \pm 0.6$	$21.0 \pm 1.3$	$14.8 \pm 0.8$
	2	$32.1 \pm 0.4$	$15.8 \pm 1.3$	$3.3 \pm 0.3$	$16.2 \pm 1.3$	$14.5 \pm 3.2$
	5	$31.2 \pm 0.9$	$16.6 \pm 0.8$	$3.6 \pm 0.4$	$17.1 \pm 0.8$	$11.8 \pm 1.0$

Note. Data are the means of 12 replications  $\pm$  standard error.



**FIGURE 5.** Sensory evaluation for irradiated and non-irradiated raspberries during storage time. Panel members (6 trained panellists) were asked to evaluate the colour (e.g. red colour; brightness, colour uniformity), texture (e.g. firmness, juiciness), flavor and aroma (e.g. sweet; acid, raspberry taste, off flavor, off aroma) on a scale from 1 = bad to 5 = excellent (color figure available online).

fresh food by the consumer with water or conventional disinfectants has a limited efficacy in the elimination of foodborne microorganisms. Yu et al. (2001) studied the effects of 5 disinfectants in reduction of *E. coli* O157:H7 on surface of strawberries, and reported that the most efficient chemical treatment was hydrogen peroxide with a reduction of 2.2 log CFU/g of bacterial population. Since washing alone is not a viable option, the use of alternative technologies needs to be investigated. There are some studies indicating that gaseous chlorine dioxide ( $\text{ClO}_2$ ) shows promise as a sanitizer for small fruits (Han et al., 2004; Sy et al., 2005). Sy et al. (2005) found that treatment with 4.1 to 8.0 mg/L  $\text{ClO}_2$  produced reduction in populations of yeast and moulds on blueberries, strawberries, and raspberries of 1.4 to 2.5, 1.4 to 4.2, and 2.6 to 3 log CFU/g, respectively. However this treatment may leave residues of  $\text{ClO}_2$  and chlorite on fruits. Other studies demonstrated usefulness of ozone as a decontaminant for small fruits, indicating maximum pathogen reductions on raspberries of 4.5 log CFU/g for *Salmonella* and 5.6 log CFU/g for *Escherichia coli* O157:H7 (Bialka and Demirci, 2007a, 2007b). Bialka and Demirci (2008) also using pulsed UV light on raspberries reported maximal decrease of 3.9 and 3.4 log for *Escherichia coli* O157:H7 and *Salmonella*, respectively.

The previously cited studies only reported the achieved reduction of artificially inoculated fruit with pathogenic bacteria. In the present study, the efficacy of gamma radiation doses on the inactivation of natural microbiota of raspberries was estimated. A reduction of 1 log of raspberries microbial population was obtained after irradiation at 1.5 kGy preserved until 14 d of storage. This apparent numerical reduction may be related to the low radiation doses applied and to the types of microorganisms that compose the raspberries microbiota. The effectiveness of irradiation treatment depends on several factors, including composition of food, number and type of microorganisms, and applied dose (Diehl and Josephson, 1994). In fact, it was noted that filamentous fungi were the predominant morphotype found on raspberries microbiota. In general, fungi are considered more resistant to radiation than the vegetative forms of bacteria. The sensitivity of fungi to gamma irradiation was determined by several investigators (Diehl, 1995; Refai et al., 1996; Aziz et al., 1997; Aziz and Moussa, 2002; Mahrous et al., 2003), who reported that the dose required for complete inhibition of fungi in different food and feed products ranged from 4 to 6 kGy.

Considering the performed morphological identification, the genus *Cladosporium* was the

most frequent isolated in nonirradiated and irradiated fruit, but fungi belonging to the genera *Penicillium*, *Fusarium*, and *Alternaria* were also detected. Tournas and Katsoudas (2005) demonstrated that the most common fungi found in commercial raspberries were *Botrytis cinerea*, and the genera *Fusarium*, *Cladosporium*, *Penicillium*, and *Rhizopus*. It should be noted that in the present study the analyzed raspberries were from a new variety (Amira) and directly collected from an experimental field, factors that may influence the mycoflora.

Regarding the resistance to irradiation, previous studies also showed a higher resistance of dematiaceous fungi (*Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia lunata*, etc.) to gamma radiation (Saleh et al., 1988). Ferreira-Castro et al. (2007) studied the effects of gamma radiation on corn samples artificially contaminated with *Fusarium* spp. and observed fungal growth in 80% of the samples irradiated to 5 kGy and a complete decontamination at 10 kGy. Aquino et al. (2007), in guarana samples, indicated that the fungal contamination was reduced by 85% to the acceptable limit using a dose of 5 kGy, but 20% of the genera *Cladosporium* and *Rhizopus* and 10% of *Penicillium* was recovered from samples irradiated with the dose of 5 kGy.

In previous studies on blueberries irradiation with a gamma radiation dose ranging from 0.5 to 3 kGy, a decrease in blueberries texture from 1.5 kGy was found (Trigo et al., 2006). These findings led us to select in this raspberries irradiation trial gamma radiation doses lower than 1.5 kGy, in order to determine a good compromise for fruit quality parameters. Although a decrease of fruit texture with the gamma radiation doses and conservation time was observed, the firmness of the treated raspberries with the higher doses of radiation (1 and 1.5 kGy), was not as severely affected during the storage period. This may be related to the known irradiation effect on the delay of fruit ripening (Pinto et al., 2006). The softening of irradiated fruits was attributed to solubilization of pectic substances, particularly protopectin (Somogyi and Romani, 1964). Similar to the

obtained results, other investigators showed a decrease of firmness of fresh raspberries during storage (Zhang and Quantick, 1998; Han et al., 2004). The pH increased and titratable acidity decreased over storage time for nonirradiated and irradiated raspberries. These results are consistent with those reported by Haffner et al. (2002) and Han et al. (2004) indicating that a fall in acidity during storage was indicative of fruit senescence.

The irradiation at the applied doses seemed not to exert a marked effect on raspberries color. Bialka and Demirci (2007a, 2007b) detected no significant changes in color coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ) in the decontamination of raspberries using aqueous and gaseous ozone, but the effect during storage was not examined. During storage (d 2) a change in raspberries color was found that remained constant until the end of shelf life (d 5). Related results were reported in raspberries storability experiments, denoting that after storage the berry color was darker (lower  $L^*$ ), less intense in red (lower  $a^*$ ) and less yellow (lower  $b^*$ ), and had a more red, bluish hue (lower Hue°) than the starting material (Haffner et al., 2002; Han et al., 2004). According to Han et al. (2004) the hue angle decreased during storage due to the synthesis of anthocyanins, a pigment contributing to the red color in strawberries and raspberries.

Modern consumers are increasingly interested in their personal health and expect foods purchased to be tasty, attractive, and safe and health promoting. Phenolic compounds are one of the most diverse groups of secondary metabolites in raspberries (Badjakov et al., 2008). An increase in total phenolics in irradiated fruits and vegetables was also reported (Benoit et al., 2000; Breitfellner et al., 2002; Fan, 2005; Lee et al., 2009; Hussain et al., 2010). Harrison and Were (2007) suggested this increase in total phenolics may be due to the release of phenolic compounds from glycosidic components and degradation of larger phenolic compounds into smaller ones by gamma irradiation. A declining trend of total phenolics after storage was also observed by Benoit et al. (2000) and Hussain et al. (2010) in control produce samples and in irradiated ones.

A wide variation was observed on total phenolic contents in raspberries, namely, 1.15 to 4.66 mg gallic acid equivalents/g fresh weight basis (Tosun et al., 2009; Milivojević et al., 2011). Our total phenolic results of control fruit are comparable with those in the literature, and to our knowledge there are no data available with reference to total phenolic contents in irradiated raspberries. Although it is worthwhile noting that genotype-dependent phenolic contents were determined in raspberries (Tosun et al., 2009), and the obtained results are dependent on solvents used for extraction (Alothman et al., 2012).

An irradiation-induced rise in antioxidant activity by ferric reducing/antioxidant power (FRAP) was observed in raspberries fruits. Similar increase in antioxidant activity by FRAP assay as a result of irradiation was reported in foodstuffs (Hussain et al., 2010; Fan, 2005). This radiation-induced elevation in total antioxidant activity may be the result of high total phenolic accumulation following radiation treatment. Toward the end of storage, FRAP values also showed a decreasing trend as previously reported following peach irradiation (Hussain et al., 2010). Data suggested that antioxidant activity of raspberries expressed a similar trend to those of total phenolic compounds. Phenolic compounds act as antioxidants by scavenging free radicals and chelating metals in foods (Zhang et al., 2006; Behgar et al., 2011). Connor et al. (2005a, 2005b) previously reported correlation among measures of total phenolic content, anthocyanin levels, and antioxidant capacity of *Rubus* species. The obtained values of antioxidant capacity could not be compared with the literature since data were expressed in milligrams of ferrous sulfate equivalent per gram of raspberries fresh weight. There are no stated values for antioxidant capacity for irradiated raspberries and the available values are results from application of other methods or standard curves.

Raspberries are highly perishable fruit with a storage life limited by loss of firmness and darkening of the attractive red color (Haffner et al., 2002); therefore, alternative treatments need to be explored for extending

the marketable life. Gamma irradiation has emerged as a potential alternate method of food preservation, diminishing the use of chemical preservatives. The application of this treatment has yielded satisfactory results in different aspects of food technology, such as disinfection, sterilization, inhibition of sprouting, and extension of storage life of fresh fruits and vegetables.

In summary, data indicate that an irradiation dose of 1.5 kGy did not result in a major impact on raspberries sensory and quality attributes with the a beneficial effect of reducing microbiota by 1 log (95% inactivation), and enhancement of phenolic compounds and antioxidant activity for 7 d of refrigerated storage. However, the study needs to be continued further to investigate the effects of higher irradiation doses on fruit, in an attempt to augment the reduction of the microbial population of raspberries. Due to health benefits of rich antioxidant fruits the applicability of irradiation technology as food safety measure may serve as a forward step to increase the variety, availability and acceptability of foods for immunocompromised patients and other target groups with special dietary needs.

## REFERENCES

- Alothman, M., Bhat, R., and Karim, A. A. 2009. Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends Food Sci. Technol.* 20: 201–212.
- Aquino, S., Gonçalves, E., Reis, T. A., Sabundjian, I. T., Trindade, R. A., Rossi, M. H., Corrêa, B., and Villavicencio, A. L. C. H. 2007. Effect of gamma irradiation on mycoflora of guarana (*Paullinia cupana*). *Radiat. Phys. Chem.*, 76: 1470–1473.
- Aziz, N. H., and Moussa, A. A. 2002. Influence of gamma-radiation on mycotoxin producing moulds and mycotoxins in fruits. *Food Control* 13: 281–288.
- Aziz, N. H., Attia, E. S. A., and Farag, S. A. 1997. Effect of gamma irradiation on the natural occurrence of *Fusarium* mycotoxins in wheat, flour, and bread. *Nahrung* 41: 34–37.

- Badjakov, I., Nikolova, M., Gevrenova, R., Kondakova, V., Todorovska, E., and Atanassov, A. 2008. Bioactive compounds in small fruits and their influence on human health. *Biotechnol. Equip.* 22: 581–587.
- Behgar, M., Ghasemi, S., Naserian, A., Borzoie, A., and Fatollahi, H. 2011. Gamma radiation effects on phenolics, antioxidants activity and in vitro digestion of pistachio (*Pistachia vera*) hull. *Radiat. Phys. Chem.* 80: 963–967.
- Benoit, M. A., D'Aprano, G., and Lacroix, M., 2000. Effect of gamma irradiation on phenylalanine ammonia-lyase activity, total phenolic content, and respiration of mushroom (*Agaricus bisporus*). *J. Agric. Food Chem.* 48: 6312–6316.
- Benzie, I. F. F., and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP Assay. *Anal. Biochem.* 239: 70–76.
- Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand P., and Frankel G. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ. Microbiol* 12: 2385–2397.
- Bialka, K. L., and Demirci, A. 2007a Efficacy of aqueous ozone for the decontamination *Escherichia coli* O157:H7 and *Salmonella* on raspberries and strawberries. *J. Food Prot.* 70: 1088–1092.
- Bialka, K. L., and Demirci, A. 2007b Utilization of gaseous ozone for the decontamination *Escherichia coli* O157:H7 and *Salmonella* on raspberries and strawberries. *J. Food Prot.* 70: 1093–1098.
- Bialka, K. L., and Demirci, A. 2008. Efficacy of pulsed UV light for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* spp. on raspberries and strawberries. *J. Food Sci.* 73: M201–M207.
- Breitfellner, F., Solar, S., and Sontag, G. 2002. Effect of gamma-irradiation on phenolic acids in strawberries. *J. Food Sci.* 67: 517–521.
- Çekiç Ç., and Özgen, M. 2010. Comparison of antioxidant capacity and phytochemical properties of wild and cultivated red raspberries (*Rubus idaeus* L.). *J. Food Compos. Anal.* 23: 540–544.
- Connor, A. M., McGhie, T. K., Stephens, M. J., Hall, H. K., and Alspach, P. A. 2005a. Variation and heritability estimates of anthocyanins and their relationship to antioxidant activity in a red raspberry factorial mating design. *J. Am. Soc. Hortic. Sci.*, 130: 534–542.
- Connor, A. M., Stephens, M. J., Hall, H. K., and Alspach, P. A. 2005b. Variation and heritabilities of antioxidant activity and total phenolic content estimated from a red raspberry factorial experiment. *J. Am. Soc. Hortic. Sci.*, 130: 403–411.
- Cotterelle, B., Drougard, C., Rolland, J., Becamel, M., Boudon, M., Pinede, S., Traoré, O., Balay, K., Pothier, P., and Espié, E. 2005. Outbreak of norovirus infection associated with the consumption of frozen raspberries, France, March 2005. *Euro Surveill.* 10: E050428.1.
- Diehl J. F. 1995. *Safety of irradiated foods*, 2nd ed. New York, NY: Marcel Dekker.
- Diehl, J. F. 2002. Food irradiation—Past, present and future. *Radiat. Phys. Chem*, 63: 211–215.
- Diehl, J. F., and Josephson, E. S. 1994. Assessment of wholesomeness of irradiated food: AQ review. *Acta Alimentaria* 2: 195–214.
- European Food Safety Authority and European Centre for Disease Prevention and Control. 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *EFSA J.* 10: 2597.
- Falkenhorst, G., Krusell, L., Lisby, M., Madsen, S., Böttiger, B., and Mølbak, K. 2005. Imported frozen raspberries cause a series of norovirus outbreaks in Denmark, 2005. *Euro Surveill.* 10: E050922.2.
- Fan, X. 2005. Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation. *J. Sci. Food Agric.* 85: 995–1000.
- FAO/IAEA/ WHO. 1999. High-dose irradiation: Wholesomeness of food irradiated with doses above 10 kGy. Report of a Joint FAO IAEA WHO Study Group. Rome, Italy: Food and Agriculture Organization of the United Nations.

- Food and Drug Administration. 2009. Outbreaks associated with fresh and fresh-cut produce. Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. In *Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce*, chap. IV. FDA. Available at <http://www.fda.gov/Food/ScienceResearch/ResearchAreas/SafePracticesforFoodProcesses/ucm090977.htm>
- Ferreira-Castro, F. L., Aquino, S., Greiner, R., Ribeiro, D. H. B., Reis, T. A., and Corrêa, B. 2007. Effects of gamma radiation on maize samples contaminated with *Fusarium verticillioides*. *Appl. Radiat. Isotopes* 65: 927–933.
- Haffner, K., Rosenfeld, H. J., Skrede, G., and Wang, L. 2002. Quality of red raspberry *Rubus idaeus* L. cultivars after storage in controlled and normal atmospheres. *Postharvest Biol. Technol.* 24: 279–289.
- Han, C., Zhao, Y., Leonard, S. W., and Traber, M. G. 2004. Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria ananassa*) and raspberries (*Rubus idaeus*). *Postharvest Biol. Technol.* 33: 67–78.
- Han, Y., Selby, T. L., Schultze, K. K., Nelson, P. E., and Linton, R. H. 2004. Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments. *J. Food Prot.* 67: 2450–2455.
- Harrison, K., and Were, L. M. 2007. Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of almond skin extracts. *Food Chem.* 102: 932–937.
- Herwaldt, B. L., and Ackers, M. L. 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. The Cyclospora Working Group. *N. Engl. J. Med.* 336: 1548–1556.
- Herwaldt, B. L., and Beach, M. J. 1999. The return of Cyclospora in 1997: Another outbreak of cyclosporiasis in North America associated with imported raspberries. Cyclospora Working Group. *Ann. Intern. Med.* 130: 210–220.
- Hjertqvist, M., Johansson, A., Svensson, N., Abom, P. E., Magnusson, C., Olsson, M., Hedlund, K. O., and Andersson, Y. 2006. Four outbreaks of norovirus gastroenteritis after consuming raspberries, Sweden, June–August 2006. *Euro Surveill.* 11: E060907.1.
- Holt, J. G., Kreig, N. R., Sneath, P. H. A., Staley, J. T., and Williams, S. T. 1994. *Bergey's manual of determinative bacteriology*, 9th ed. Baltimore, MD: Williams & Wilkins.
- Hussain, P. R., Wani, A. M., Meena, R. S., and Dar, M. A. 2010. Gamma irradiation induced enhancement of phenylalanine ammonia-lyase (PAL) and antioxidant activity in peach (*Prunus persica* Bausch, Cv. Elberta). *Radiat. Phys. Chem.* 79: 982–989.
- Kafkas, E., Özgen, M., Özoğul, Y., and Türemiş, N. 2008. Phytochemical and fatty acid profile of selected red raspberry cultivars: A comparative study. *J. Fruit Qual.* 31: 67–78.
- Kähkönen, M. P., Hopia, A. I., and Heinonen, M. 2001. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* 49: 4076–4082.
- Lacroix, M., and Ouattara, B. 2000. Combined industrial processes with irradiation to assure innocuity and preservation of food products e a review. *Food Res. Int.* 33: 719–724.
- Lee, J. W., Kim, J. K., Srinivasan, P., Choi, J., Kim, J. H., Han, S. B., Kim, D., and Byun, M. W. 2009. Effect of gamma irradiation on microbial analysis, antioxidant activity, sugar content and color of ready-to-use tamarind juiced during storage. *Food Sci. Technol.* 42: 101–105.
- Lynch, M. F., Tauxe, R. V., and Hedberg, C. W. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol. Infect.* 137: 307–315.
- Mahrous, S. R., Aziz, N. H., and Shahin, A. A. 2003. Influence of gamma-irradiation on the occurrence of pathogenic microorganisms and nutritive value of some cereal grains. *Isotope Radiat. Res.* 35: 551–586.
- Maunula, L., Roivainen, M., Keranen, M., Makela, S., Soderberg, K., Summa, M., von Bonsdorff, C.H., Lappalainen, M., Korhonen, T., Kuusi, M. and Niskanen, T. 2009. Detection of human norovirus from frozen

- raspberries in a cluster of gastroenteritis outbreaks. *Euro Surveill.* 14: 16–18.
- Milivojević, J. M., Nikolić, M. D., Dragišić Maksimović, J. J., and Radivojević, D. D. 2011. Generative and fruit quality characteristics of primocane fruiting red raspberry cultivars. *Turk. J. Agric. For.* 35: 289–296.
- Milutinović, M. D., Nikolić, M., Milivojević, J., Milutinović, M. M., and Đaković, G. 2008. Growing primocane raspberry cultivars in Serbia. *Acta Hort.* 777: 443–446.
- Pinto, P., Cabo Verde, S., Trigo, M. J., Santana, A., and Botelho, M. L. 2006. Food irradiation: Microbiological, nutritional and functional assessment. In *Radionuclide concentrations in food and the environment*, edited by M. Poschl and L. Nollet, 411–438. Boca Raton, FL: CRC Press Taylor & Francis.
- Refai, M. K., Niazi, Z. M., Aziz, N. H., and Khafaga, N. E. M. 2003. Incidence of aflatoxin B1 in the Egyptian cured meat basterma and control by  $\gamma$ -irradiation. *Food/Nahrung* 47: 377–382.
- Saleh, Y. G., Mayo, M. S., and Ahearn, D. G. 1988. Resistance of some common fungi to gamma irradiation. *Appl. Environ. Microbiol.* 54: 2134–2135.
- Singleton, V. L., Orthofer, R., and Lamuela-Raventos, R. M. 1999. Analysis of total phenolics and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.* 299: 152–178.
- Somogyi, L. P., and Romani, R. J. 1964. Irradiation induced textural changes in fruits and its relation to pectin metabolism. *J. Food Sci.* 29: 366–371.
- Song, K.-B. 2008. Inactivation of *Enterobacter sakazakii*, *Bacillus cereus*, and *Salmonella typhimurium* in powdered weaning food by electron-beam irradiation. *Radiat. Phys. Chem.* 77: 1097–1100.
- Sy, K. Y., McWatters, K. H., and Beuchat, L. R. 2005. Efficacy of gaseous chlorine dioxide for killing Salmonella, yeasts, and molds on blueberries, strawberries, and raspberries. *J. Food Prot.* 68: 1165–1175.
- Teets, A. S., Sundararaman, M., and Were, L. M. 2008. Electron beam irradiated almond skin powder inhibition of lipid oxidation in coked salted ground chicken breast. *Food Chem* 111: 934–941.
- Tosun, M., Ercisli, S., Karlidag, H., and Sengul, M. 2009. Characterization of red raspberry (*Rubus idaeus* L.) genotypes for their physicochemical properties. *J. Food Sci.* 74: C575–C579.
- Tournas, V. H., and Katsoudas, E. 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *Int. J. Food Microbiol.* 105: 11–17.
- Trigo, M. J., Sousa, M. B., Sapata, M. M., Ferreira, A., Curado, T., Andrada, L., Botelho, M. L., and Veloso, M. G. 2009. Radiation processing of minimally processed vegetables and aromatic plants. *Radiat. Phys. Chem.* 78: 659–663.
- Trigo, M. J., Sousa, M. B., Sapata, M. M., Ferreira, A., Curado, T., Andrada, L., Ferreira, E. S., Antunes, C., Horta, M. P., Pereira, A. R., Botelho, M. L., and Veloso, G. 2006. Quality of gamma irradiated blueberries. *Acta Hort.* (ISHS) 715: 573–578.
- Yu, K., Newman, M. C., Archbold, D. D., and Hamilton-Kemp, T. R. 2001. Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. *J. Food Prot.* 64: 1334–1340.
- Zhang, D., and Quantick, P. 1998. Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. *J. Hort. Sci. Biotechnol.* 73: 763–767.
- Zhang, J., Stanley, R. A., Adaim, A., Melton, L. D., and Skinner, M. A. 2006. Free radical scavenging and cytoprotective activities of phenolic antioxidants. *Mol. Nutr. Food Res.* 50: 996–1005.