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Induction of stress defense response and quality retention in minimally processed peaches through the application of gamma irradiation treatments

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ABSTRACT

Consumers demand fruit and vegetable products "on the go" that maintain color, flavor, nutritional and bioactive compounds almost equal to fresh products. Irradiation represents an effective technology traditionally used in fruit and vegetable as a phytosanitary treatment to preserve food quality and safety. In the present study we evaluated the effect of gamma irradiation treatments at several doses (0, 0.1, 0.3, 1.0, 2.5 kGy) on the quality and biochemical aspects of minimally processed peaches (*Prunus persica* L. Batsch) with low oxygen permeability packaging. During minimal processing, peach slices were treated in an antioxidant solution containing 1 % ascorbic acid and 0.5 % citric acid for 2 min. Trays of minimally processed peaches were irradiated packed inside cardboard boxes. Changes in color, firmness and polyphenoloxidase (PPO) and peroxidase (POD) activities of peaches were analyzed at different storage times (0, 7, 14 d) at 4 °C. In addition, the stress response of plant to these treatments was assessed by using the induction of heat shock proteins (HSP) as a biochemical marker. Results show that irradiation caused no substantial changes in chromatic parameters, although an immediate reduction in firmness and POD activity was observed, proportional to the increased doses applied. In turn, PPO activity remained stable while the activity of some of its isoenzymes decreased for doses higher than 0.3 kGy. The overexpression of HSP, only detected for irradiation doses of 0.1 and 0.3 kGy, in coincidence with the best performing treatments, constitutes a relevant finding not previously reported in fruit. Therefore, low doses of irradiation promoted the physiological and biochemical defense mechanisms of the fruit. HSP could thus be used in plant tissues as a biomarker of the stress brought about by exposure to irradiation, capable of preventing physiological damage. These preliminary results suggest that irradiation treatments (up to 1.0 kGy) in combination with modified atmosphere packaging (from 19 % and 10 % O_2 at 0 d to 3 % and 15 % CO_2 at 14 d of storage) could render high quality peach products with an extended shelf life.

1. Introduction

Peach (*Prunus persica* L. Batsch) is a climacteric fruit with a high content of carotenoids, phenolics, antioxidants and other bioactive compounds, increasingly appreciated by consumers because of their appealing color, unique flavor, nutritional and functional composition. However, shortly after harvest, peaches undergo relatively fast ripening and softening, which limit their shelf life (Melo et al., 2018). The postharvest behavior of peaches, as well as their physiological response to treatments, is strongly dependent on the variety, which determines the metabolic reconfiguration during ripening (Drincovich, 2021). Fruit and vegetables (F&V) can be conveniently presented in the form of minimally processed products (“on the go” or ready to eat or prepare), which are well regarded by nowadays consumers because of the preservation of freshness and nutritional properties, quite similar to fresh products (Denoya et al., 2021).

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Gamma irradiation (GI) is a non-thermal technology which is typically used in fresh fruit as a phytosanitary treatment in replacement to the controversial fumigation with methyl bromide, a substance with a scheduled prohibition under the Montreal Protocol (US EPA, 2020). This technology is becoming increasingly relevant in our country, given the recent modification of the Argentine Food Code (Resolution 13-E/2017) that promotes its application in different types of products, including fresh F&V (Código Alimentario Argentino, 2022). In this case, the maximum allowed doses are regulated according to the purpose of the irradiation: 1.0 kGy for delaying ripening, disinfecting insects and quarantine control, and 2.5 kGy for controlling spoilage microorganisms.

In minimally processed F&V, this treatment can also be used with preservation purposes (McDonald et al., 2012). The main advantage of this non-thermal treatment is its proved efficacy to maintain the quality and safety of produce without altering different quality attributes such as pigments, nutrients, bioactive compounds and flavor (Eustice, 2020). When applied at optimized doses, additional benefits of GI could be the improvement of the textural characteristics of minimally processed F&V (Wang and Meng, 2016), the increase in their antioxidant capacity (Lires et al., 2018) and the prevention of physiological disorders by inhibiting the activity of polyphenol oxidase (PPO) and peroxidase (POD), whose activities are linked to browning processes (Fan, 2012).

Since GI could be also regarded as an abiotic stress, a physiological response to this condition would be expected (Jacobo-Velázquez et al., 2021). Ionizing radiation targets DNA molecules either via direct interaction or via production of free radicals and reactive oxygen species (ROS), inducing therefore different reactions such as arrest of the cell cycle progression and repair of DNA lesions (Lee et al., 2001). In this regard, one of the cellular response to stress is represented by the induced synthesis of heat shock proteins (HSP), which protects cells against oxidative stress, preventing cell death and resulting in stress tolerance (Park et al., 2000). This phenomenon, which is well-known in tumor therapy because of the protection exerted in tumor cells against stress-induced lethal damage (Schmid and Multhoff, 2012), has been also reported in bacteria (Caillet et al., 2008; Trudeau et al., 2014), but is rather unexplored in plant tissues.

These proteins are usually present at low levels in cells of non-exposed tissues, and the relative increase of their concentration is part of the physiological response of any living tissue to thermal stress (Polenta et al., 2020). Indeed, their overexpression has been identified as an early marker of the response to a stress exposure (Basile et al., 2013), and has been linked to the acquired resistance of heat-treated commodities against chilling injury (Ré et al., 2017). In particular, HSP of low molecular weight (15–40 kDa) or "small HSP" exert their protective function by participating in the stabilization of proteins and membranes and in the refolding of proteins under stress conditions (Zeng et al., 2016).

The efficacy of irradiation treatments for the preservation of F&V has been mostly linked to its specific inhibitory effect on microorganisms and enzymes (Hussain et al., 2008). Therefore, its association with the stress physiology represents a novel approach, since the effect of the technology on the induction of the physiological mechanism of defense has been not widely explored in F&V.

Thus, the aim of this work was to evaluate the effect of different doses of gamma irradiation on the quality and biochemical aspects of minimally processed peaches, with a special emphasis on the induction of HSP, which constitutes a relevant part of the physiological basis of stress protection. The understanding of these phenomena could contribute to optimize GI application and improve the development of high-quality fruit products with extended shelf life.

2. Materials and methods

2.1. Fruit material and processing

Peaches (*Prunus persica* L. Batsch) cv. Granada (variety for industrial use) were harvested from experimental crops of EEA-INTA San Pedro, according to uniform size, skin background color and firmness. The selected fruit were transported to the pilot plant and stored at 0 °C until processing. The peaches were dipped in a solution of 20 mg L^{-1} HClO and cut into two halves (15 mm thick) between the epidermis and the stone, using a 7-inch ceramic knife (Accurato Ceramic Knife, Design Collection, Tramontina). The halves were cut into slices with the peel and treated by immersion in an antioxidant solution containing 1 % ascorbic acid and 0.5 % citric acid for 2 min, to prevent surface browning. The slices were randomly sorted in PET plastic trays (about 8 slices per tray) and packed at atmospheric pressure in *Cryovac BB2620* films with low gas permeability (O_2 transmission rate: 6–14 cm^3 m^{-2} 24 h^{-1} at 23 °C, 1 atm). Subsequently, the films were sealed by a manual tray sealer (PFS Manual plastic film sealer, model SF 300, China). The trays were randomly divided into different batches and placed inside corrugated cardboard boxes (300x400x500 mm). The boxes were stored at 0 °C.

2.2. Irradiation treatments and storage conditions

Trays of minimally processed peaches were irradiated packed inside cardboard boxes at different doses (0, control; 0.1; 0.3; 1.0 and 2.5 kGy) and room temperature (~ 23 °C) using ^{60}Co as gamma irradiation source at the Semi-Irradiation Plant Industrial (PISI), *Comisión Nacional de Energía Atómica* (Ezeiza, Buenos Aires) (Table 1). The boxes were placed on racks to receive a dose rate of ~ 2 kGy h^{-1}. Midway through the process, boxes were rotated 180° to ensure uniformity. During treatments, alanine dosimeters were placed at minimum and maximum dose locations which had been previously determined by dose mapping for each case. In addition, a temperature monitoring was carried out during the exposure at 1.0 kGy, inserting a data logger inside a peach slice. After irradiation, boxes were transported back to the lab in Styrofoam containers refrigerated with ice packs. Subsequently, they were stored at 4 °C to be evaluated at different storage days: 0 (2 h after irradiation), 7 and 14. A gas analyzer (GC6000 Headspace analyser, Systech Illinois, USA), equipped with a dual infrared detector, was used to analyze the gas composition of the container headspace in the trays.

2.3. Color

The chromatic parameters of peach fruit were determined with a colorimeter (Model CR-400, Konica Minolta Sensing, Inc. Osaka, Japan), using the CIEL * a * b * scale. The values of L *, a * and b * were converted to the L *, h° and C * system, where L* represents lightness, h° represents hue or saturation of color and C * represents chroma. The colorimeter was set for illuminant D_{65}, observer angle at 2° and calibrated using a standard white ceramic plate. Two measurements were made on each fruit slice in different areas of the surface.

Table 1
Description of the GI treatments and dosimetric parameters applied on minimally processed peaches.

GI treatments					
Requested dose (kGy)	Minimum dose (D_{min})	Maximum dose (D_{max})	D_{max}/D_{min} ratio	Irradiation time (h)	Dose rate (kGy h^{-1})
0 (Control)	–				
0.1	0.16	0.17	1.06	0.05	2.0
0.3	0.36	0.43	1.19	0.15	2.0
1.0	1.23	1.35	1.10	0.53	1.9
2.5	2.93	3.28	1.12	1.33	1.9

2.4. Firmness

A puncture test was performed on the midsection of each peach slice using a texture analyzer (Model TA.XT Plus, Stable Micro Systems, LTD, Surrey, UK) and the software Texture Exponent 32. A 3 mm diameter cylindrical probe was used, with a penetration depth of 5 mm, and a test speed of 1.5 mm s^{-1}. Firmness was measured as the maximum force (N) during the determination at the force x distance curve.

2.5. PPO and POD enzymes

2.5.1. Protein extraction

Proteins were extracted from peach pericarp following the method of Hurkman and Tanaka (1986) with some modifications. Briefly, two grams of pericarp were taken from fruit slices. The pericarps were thoroughly mixed in the presence of 1 mL extraction buffer [100 mmol L^{-1} Tris/HCl pH 8.0, containing 1 mmol L^{-1} EDTA, 1 mmol L^{-1} PMSF, and 2 % (v/v) β-mercaptoethanol] and 4 mL of phenol saturated with 100 mmol L^{-1} Tris buffer (pH 8.0), and then centrifuged at 21,000 × *g* for 15 min at 4 °C. The phenolic phase was recovered, mixed with four volumes of 0.1 mol L^{-1} ammonium acetate (AMA), and incubated overnight at − 20 °C. Protein pellets were obtained by centrifugation at 21,000 × *g* for 20 min at 0 °C. Pellets were then washed twice with AMA, once with cold acetone (80 % v/v), and dried at room temperature. The dried residue was redissolved directly in electrophoretic sample buffer [25 mmol L^{-1} Tris pH 6.8, 1 % (w/v) SDS, 10 % (v/v) glycerol, 5 % (v/v) β-mercaptoethanol, and 0.002 % (w/v) bromophenol blue].

2.5.2. Determination of enzymatic activity in solution

PPO activity was determined by using tert-butylcatechol as substrate, which generates as product a quinone more stable than catechol (the traditional PPO substrate) (Denoya et al., 2016). Cathecolase activity of PPO was evaluated using a spectrophotometric method based on the initial velocity increase in absorbance at 400 nm due to the production of 4-tert-butyl-benzoaquinone, using a UV–visible spectrophotometer (SPECTROstar Nano, BMG LABTECH GmbH, Germany). The mixture consisted of a 250 μL volume of 50 mmol L^{-1} phosphate buffer pH 6.5 with 4.95 mmol L^{-1} 4-tert-butylcatechol, and 25 μL of the enzyme extract. One enzyme unit (U) is defined as the amount of enzyme required to change absorbance at 400 nm by 0.01 per min at 30 °C.

POD activity was determined by the change in absorbance at 470 nm due to guayacol oxidation in the presence of hydrogen peroxide (Zheng and Van Huystee, 1992). The reaction mixture consisted of a 220 μL volume of 100 mmol L^{-1} phosphate buffer pH 6.2 with 20 mmol L^{-1} guayacol, 60 μL of enzyme extract and 2 μL of 5 mmol L^{-1} H_2O_2. One enzyme unit (U) is defined as the amount of enzyme required to change absorbance at 470 nm by 0.001 per min at 30 °C (Molar extinction coefficient of guayacol: 26.6 $mmol^{-1}$ L cm^{-1}).

2.5.3. Detection of PPO and POD isoenzyme activity by SDS-PAGE

Since PPO activity is not adversely affected by low SDS concentration (Maki and Morohashi, 2006), separation of enzyme from the plant extract using SDS-PAGE is possible (Cheng et al., 2007). According to this, the same procedure was used to measure the POD activity. For this, 25 μL of each protein extract were loaded onto each well of a 1.5 mm thick polyacrylamide slab gels, which were run at a constant current of 40 mA for 90 min in a Protean II electrophoresis system (BIORAD). For PPO staining, gels were inserted in absorbent papers soaked previously in a 0.5 % (w/v) catechol solution. After a brief blot on the papers, these were dried at 37 °C for 5 min. For POD staining, the gels were stained for activity bands by immersing in 0.2 % (w/v) o-dianisidine in 80 % methanol at pH 6.0, followed by addition of 30 mmol L^{-1} H_20_2. Stained bands indicating peroxidase isoenzyme activity appeared within 20 min at 37 °C and the gels were rinsed in distilled water (Neves and Lourenço, 1998).

2.6. Heat shock proteins

2.6.1. Protein extraction

Idem procedure of *Section 2.5.1* . In addition, the samples were boiled for 2 min before being loaded onto a gel and submitted to electrophoresis.

2.6.2. Electrophoretic analysis

SDS-PAGE was carried out according to the procedure of Laemmli (1970). For analytical purposes, 40 μg of the extract prepared in *2.6.1.* were loaded onto each well of a 0.75 mm thick gel. Proteins were separated by using 12.5 % homogeneous polyacrylamide slab gels. The electrophoresis was run in a Protean II electrophoresis system (BIORAD) at a following voltage steps: 100 V for 60 min and 130 V for 30 min. To estimate the molecular weight (MW) of the different protein bands, proteins of known MW were used, in a range between 10 and 245 kDa (PanReac AppliChem, *Protein Marker VI* prestained). Protein bands were visualized by staining with a 0.1 % (w/v) solution of Coomasie Brilliant Blue R-250.

2.6.3. Immunoblotting

For western blot analysis, separated polypeptides were transferred (50 min at 100 V) onto a nitrocellulose membrane (0.45 μm) by using a Mini Protean II Electrophoresis System (BIORAD). The polyclonal antiserum raised against HSPC1 (dilution 1:750) was used as primary antibody. The secondary serum was anti-rabbit IgG obtained from goat, conjugated with alkaline phosphatase (BIORAD, dilution 1:1500). Membranes were revealed with nitroblue tetrazolium chloride and 5-bromo- 4-chloro-3-indolyl phosphate. Rabbit immunization for the production of polyclonal antibodies was carried out as described by Polenta et al. (2007).

2.7. Image analysis

Gels and membranes were analyzed with a Bio-Rad GS-800 Imaging Calibrated Densitometer and digitally processed by Quantity One 1-D Analysis software. Lane- and band-based functions were used to determine apparent molecular weights and relative and absolute amounts of proteins.

2.8. Experimental design and statistical analysis

A completely randomized factorial design (5 ×3) was applied for the experiments. The factors were: Irradiation dose (0; 0.1; 0.3; 1.0 and 2.5 kGy) and refrigerated (4 °C) storage time (0, 7 and 14 d). The experimental unit was each tray. All the treatments (each combination of irradiation dose and storage time) and analyses were performed in triplicate. Two trays from each treatment were analyzed to carry out firmness and color determinations, using a total of sixteen slices for each combination of irradiation dose and storage time (eight slices from each experimental unit). A pooled sample was prepared from each tray to carry out biochemical determinations (enzyme activities and HSP analysis). The mean and standard error of the experimental values for each treatment were calculated. Differences were tested for significance by analysis of variance, which was performed using the General Linear Model procedure from SAS OnDemand software (SAS Institute, Cary, NC, USA). Least significance difference (LSD) test at 5 % probability was performed for the data presented in tables and figures.

3. Results

3.1. Temperature monitoring and packaging atmosphere

To discard any indirect thermal effect brought about by irradiation, internal temperature of peaches was monitored during the GI exposure. Fig. 1 shows the temperature monitoring of peach slices submitted to GI

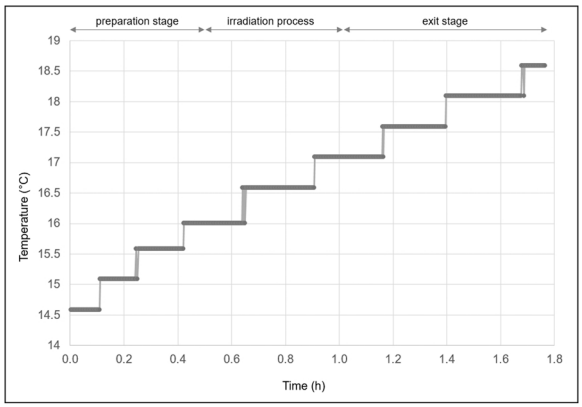


Fig. 1. Temperature mapping of minimally processed peaches during time of application of GI treatments at 1.0 kGy (1.76 h), including preparation stage (0.49 h), gamma ray exposure (0.53 h) and withdrawal stage (0.74 h).

of 1.0 kGy, which includes the previous preparation stage, the in situ application of gamma rays and the subsequent withdrawal stage. Considering a dose rate of 1.9 kGy h^{-1}, the total time of the irradiation process was 0.53 h, registering an increase around 1.0 °C. Table 2 describes the gas composition present in the packed trays for each day of refrigerated storage studied. Immediately after the GI treatments, O_2 consumption and CO_2 production increased, which was pronounced during refrigerated storage. After 14 d of storage, the O_2 composition varied from 19 % to 10 %, while the CO_2 composition changed from 3 % to 15 %.

3.2. Chromatic parameters and firmness

Results indicate that no substantial changes in the chromatic parameters were induced in minimally processed peaches submitted to the different irradiation doses during the storage times evaluated (Table 3). However, it is important to highlight that, immediately after treatments, samples irradiated at 0.1, 0.3 and 1.0 kGy presented an increase of 8–9 % in luminosity values compared to control samples while, after 7 and 14 d of storage, those submitted to the lowest doses (0.1 and 0.3 kGy) showed luminosity values similar to untreated fruit. Samples irradiated at the highest dose (2.5 kGy) showed a decrease of 10 % in L * parameter with significant differences with the rest of the treatments, especially after 14 d of storage. In relation to C * parameter, data show that the values decreased slightly by 5 % in total, for increasing irradiation doses, an effect that was enhanced in samples exposed to doses higher than 0.3 kGy and stored for 14 d. Regarding the h° parameter, samples irradiated at 0.1 kGy presented the highest values immediately after treatment. Similarly to all treatments, there was a tendency to decrease with the storage time, although h° values of fruit exposed to this dose were always the highest compared to other treatments.

In terms of firmness, GI treatments induced the softening of peach tissues, in a rate proportional to the applied dose (Fig. 2). Results show that firmness ranged from 2.16 N (control samples) to 1.66 and 1.44 N in samples irradiated at 0.1 and 0.3 kGy respectively, with the highest reduction observed in fruit exposed to 1.0 and 2.5 kGy (0.74 N; 0.58 N).

Table 2
Analysis of the gas composition in the packed trays for each day studied of refrigerated storage (0, 7 and 14 d).

Storage day	O_2 concentration (%)	CO_2 concentration (%)
0	19.1 ± 0.1	3.4 ± 0.2
7	11.3 ± 1.5	10.9 ± 1.6
14	10.0 ± 1.3	15.4 ± 1.6

This initial reduction was maintained throughout the storage, with the sample irradiated at 2.5 kGy and stored for 14 d presenting the lowest firmness value. The induction of tissue softening is clearly due to the effect of GI, since no differences were observed for each treatment among the different storage times.

3.3. PPO and POD enzyme activity

Data showing the effect of GI on enzyme activities of minimally processed peaches measured immediately after treatments are presented in Fig. 3-A (PPO) and Fig. 3-B (POD). Results evidence that PPO activity was not particularly affected by the irradiation doses applied, showing however a slight decrease as the dose increases, with the lowest value corresponding to the sample treated at 1.0 kGy. In the case of POD, activity decreased from 19 % to 65 % as the irradiation dose increased, with a greater effect for more intense treatments. The lowest activity corresponded to peaches treated at the highest dose (2.5 kGy). Interestingly, the results for the relative concentration of PPO and POD isoenzymes in activity gels (PAGE) presented a similar trend. In each case, specific bands corresponding to the two isoenzyme forms were observed in the molecular weight region between 0 and 135 kDa (Fig. 4). For PPO, the activity of isoenzyme "1" markedly decreased from the 0.3 kGy dose treatments on, while the activity of isoenzyme "2" only decreased in samples irradiated at 2.5 kGy (Fig. 4A and C). In the case of POD, the activity of isoenzyme "3" considerably decreased in samples exposed to the highest doses (1.0 and 2.5 kGy), while the activity of isoenzyme "4" progressively decreased with the increasing irradiation doses (Fig. 4-B). The analysis by activity gels helps appreciate the inactivation of each particular isoenzymes, which is clearly evidenced by a decreased in the band intensity and by the higher values on the gray scale (bits x pixel) in the densitometric analysis (Fig. 4-C).

3.4. HSP overexpression

Fig. 5-A shows the SDS-PAGE analysis of protein extracts from minimally processed peaches treated with the different irradiation doses (0; 0.1; 0.3; 1.0; 2.5 kGy). This methodology made possible the detection of a group of proteins induced by irradiation, whose molecular weight ranges from 15 to 40 kDa, which is compatible with the characteristics of small HSP. The location of these proteins in a region of the gel with a low density of proteins made it feasible the complementation by densitometry. From this analysis, it was possible to estimate the relative amounts of protein induced by each treatment (Fig. 5-B), which revealed a significant increase in the intensity of bands, corresponding to proteins from 11 to 20 kDa, particularly in samples irradiated at doses of 0.1 and 0.3 kGy. Moreover, as shown in western blot analysis (Fig. 5-C), a low basal level of small HSP was detected in control samples, while this level only increased in the processed peaches exposed to doses of 0.1 and 0.3 kGy, but not in samples submitted to higher doses (1.0 and 2.5 kGy).

4. Discussion

4.1. Impact of GI treatments on overall quality parameters

The present research showed that the internal fruit temperature increased by around 1.0 °C during GI treatment at 1.0 kGy, which is in agreement to the estimated value reported in the literature, considering the fact that an absorbed dose of 1.0 kGy is equivalent in thermal energy to an increase in water temperature by 0.24 °C (FAO/WHO, 1989; Loaharanu and Murrell, 1994). It is important to mention that the water composition of peaches (yellow and raw) is estimated at almost 88.9 % (USDA National Nutrient Database, 2019). The slight difference in temperature can be explained by the fact that the irradiation treatment is not applied under refrigerated condition, remaining the samples at room temperature. In this sense, this level of temperature increment

Table 3
Chromatic parameters of the CIEL * C * h° system determined in minimally processed peaches at different irradiation doses (0; 0.1; 0.3; 1.0; 2.5 kGy) during 0, 7 and 14 d of storage at 4 °C.

Chromatic parameter	Storage at 4 °C (days)	Dose (kGy)				
		0	0.1	0.3	1.0	2.5
L *	0	62.53 bA	68.26 aA	67.85 aA	67.86 aA	63.79 bA
	7	65.64 abA	66.75 aA	64.16 abB	63.23 bB	64.10 abA
	14	65.61 abA	69.29 aA	66.89 abAB	64.98 bB	58.95 cB
C *	0	49.12 aA	46.16 bA	47.50 abA	46.51 abA	46.40 abA
	7	46.69 aA	45.78 abA	45.78 abAB	44.12 bAB	45.55 abA
	14	46.93 abA	47.04 aA	45.03 abB	44.62 abB	44.41 bA
h°	0	81.04 bA	83.35 aA	81.65 bA	81.67 bA	80.74 bA
	7	81.00 aA	81.63 aB	79.44 bB	81.43 aA	80.77 aA
	14	78.74 bcB	81.20 aB	79.98 abB	80.29 abA	77.79 cB

Different lowercase letters show significant differences ($P < 0.05$) between treatments at each evaluation time according to Least Significance Difference Test (LSD).; Different capital letters represent differences ($P < 0.05$) due to storage for each treatment according to LSD.

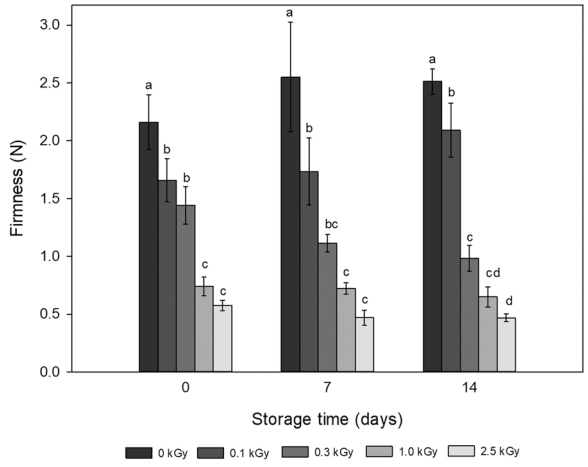


Fig. 2. Textural changes expressed as firmness (N) determined in minimally processed peaches at different irradiation doses (0; 0.1; 0.3; 1.0; 2.5 kGy) during 0, 7 and 14 d of storage at 4 °C. Each column shows the mean ± SD, n = 8. Different case letters show significant differences ($P < 0.05$) among treatments at each evaluation time. The statistical analysis revealed no differences ($P < 0.05$) for each treatment within the different storage times.

discards any induction of HSP associated to a thermal shock, for which it would be necessary a rise higher than 5–10 °C (Brodl, 1989). From the quality point of view, this minor increase in the temperature of the product, in addition to the room temperature of the fruit during processing, would guarantee the preservation of a fresh-like state after the treatment.

In turn, the combination of GI applied at low doses (0.1 and 0.3 kGy) with the packaging with low oxygen permeability film had positive effect on the product, allowing to stabilize and preserve the chromatic parameters, and limit the development of the typical alterations caused by oxidation processes. However, it is important to mention that the positive effect of the packaging could be overcome by the application of higher doses (1.0 and 2.5 kGy in the present study) leading to deleterious effects. It is known that doses of these levels or higher could break conjugated bonds of the chromophore structures provoking color alterations, together with the acceleration of the ripening process (Negut et al., 2012).

Previous studies comparing GI to traditional quarantine treatments reported that the application of irradiation doses at 0.3 kGy or lower was effective to maintain the chromatic parameters of fresh cherries, apricots and peaches, in contrast to the use of methyl bromide, which resulted in marked color alterations (Jesus Filho et al., 2018). Other important changes, such as softening and internal degradation were only evident for doses above 0.6 kGy, depending on the crop material (Drake and Neven, 2002). In this sense, loss of firmness is an immediate response induced by GI, which is dependent on dose, variety and ripening stage upon treatment (Wall, 2015; Mditshwa et al., 2017).

From the commercial point of view, the application of low doses (0.1 and 0.3 kGy) may have a positive effect for consumers, since stone fruit are harvested in a unripen stage, with a high level of firmness in order to allow the proper postharvest handling. Therefore, GI can help reach more quickly the optimal firmness values for consumption, rendering a homogeneous product at the time that is required. In previous studies, low doses of gamma irradiation (0.5 kGy) were used on papaya fruit as

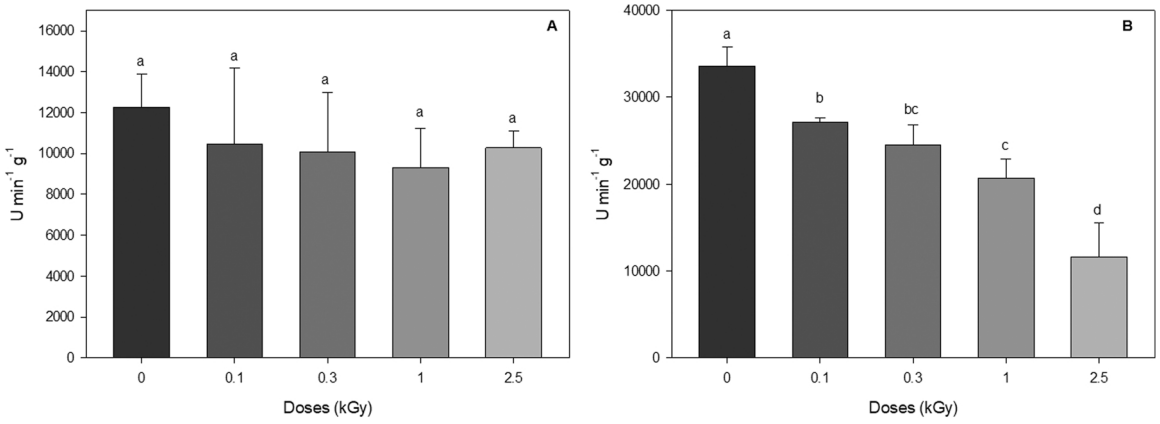
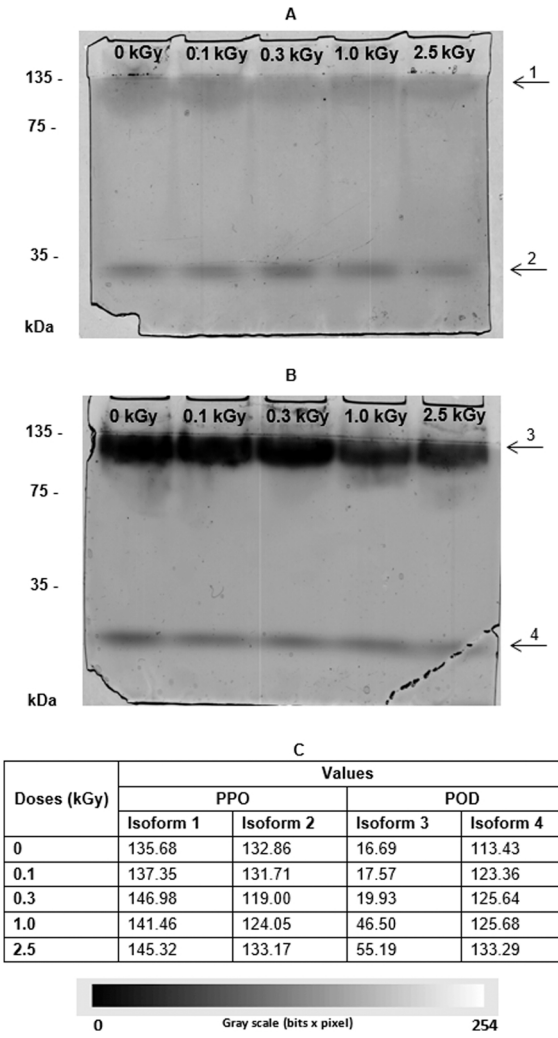


Fig. 3. Enzymatic activity in solution (U min^{-1} g^{-1}) determined in minimally processed peaches at different irradiation doses (0; 0.1; 0.3; 1.0; 2.5 kGy). (A) PPO activity, (B) POD activity. Different case letters show significant differences ($P < 0.05$) among treatments.



C

Doses (kGy)	Values			
	PPO		POD	
	Isoform 1	Isoform 2	Isoform 3	Isoform 4
0	135.68	132.86	16.69	113.43
0.1	137.35	131.71	17.57	123.36
0.3	146.98	119.00	19.93	125.64
1.0	141.46	124.05	46.50	125.68
2.5	145.32	133.17	55.19	133.29

Fig. 4. Identification of isoenzymatic activity determined by SDS-PAGE blot in minimally processed peaches at different irradiation doses (0; 0.1; 0.3; 1.0; 2.5 kGy). (A) PPO isoenzyme activity (U min^{-1} g^{-1}), (B) POD isoenzyme activity (U min^{-1} g^{-1}), (C) Densitometric analysis of the molecular weight region of the PPO and POD enzyme isoforms determined on the gels in (A) and (B).

an eliciting stress to induce the onset of ripening and improve, within a short time, the commercial firmness of fruit (D'Innocenzo and Lajolo, 2007). However, when samples are exposed to higher radiation doses (> 1.0 kGy), high levels of free radicals and reactive oxygen species (ROS) are generated leading to damage of cellular materials functionality.

4.2. Stress response induced by GI treatments: overexpression of HSP and activity of alteration enzymes

The most relevant finding of the study was the evidence that the application of low-dose GI treatments exerts in minimally processed peaches a response similar to that associated to stress physiology of plants. By electrophoretic and immunological analyses, it was possible to detect the overexpression of the so-called small HSP, which was only verified in fruit exposed to low doses of irradiation. This response had been previously linked in plant tissues to the protective response against heat (Aghdam et al., 2015), but to our knowledge, no previous study reported their association to the exposure to GI treatments. Interestingly, the induction was only verified in product exposed to the 0.1 and 0.3 kGy, in agreement with the best performing treatments. Other studies also reported an optimal radiation dose in fruit of around 0.5 kGy, which proved capable to slow down the rate of fruit decay (Zhao et al., 2020).

The induction of HSP brought about by GI has been rather unexplored in fruit, since the effectiveness of this treatment has been traditionally associated to the microbial inactivation and the concomitant amelioration of the phytosanitary quality. However, by taking into account the physiological response, there would be an optimal intensity, beyond which the effect would be deleterious, similarly to what we had previously found for the case of heat treatments (Polenta et al., 2020). In agreement with that finding, HSP could be used as a biochemical marker associated to treatment effectiveness, to establish the optimal irradiation dose. Therefore, the protective defense elicited by GI would be similar to that elicited by other stress treatments, which within a certain range of intensity, are able to induce physiological and biochemical defense mechanisms, preventing physiological damage such as chilling injury and decay. This finding can open a new insight for the irradiation of fruit and vegetable products, and provide with an analytical tool able to predict and optimize the effectiveness of treatments. As previously stated, this tolerance would be part of the evolutionary adaptation of plant tissues to different external disturbances (Sun et al., 2010; Sung et al., 2014).

A similar mechanism was also reported in microorganisms, where part of the response to the application of GI treatments was also the induction of HSP (Caillet et al., 2008). The increase in HSP was highly dependent on the dose of gamma rays (it was only verified for low doses), which would evidence the cytoprotective function against ionizing radiation stress. From a practical point of view, this response can be considered as a negative aspect of irradiation, since low doses could enhance the resistance of pathogenic and alteration bacteria to this kind of treatments.

The present study also evidenced the effect of GI on the activity enzymes related to alteration, which was dependent on the particular enzyme. In the case of POD, the activity decreased as the irradiation dose applied was increased, a behavior different from what is usually found with thermal treatments, associated to its well-known thermal resistance (Neves and Lourenço, 1998). The inactivation of enzymes by GI could be associated to a direct effect on the hydrogen and electrostatic bonds, disrupting the secondary and tertiary structure, as well as to an indirect effect linked to the generation of ROS, which together can interfere with the normal biological function if the active site is compromised (Latorre, et al., 2010). The inactivation of this enzyme probably contributed to prevent the development of browning reactions in treated slices.

In minimally processed fruit, the mechanical processing is known to provoke the rupture of the fruit epidermis with the concomitant exposure of internal tissues, which increases the respiration rate and accelerates the metabolism and the deterioration rate of the produce. Therefore, in the present research, we adopted the use of low oxygen permeability film packaging, which proved useful in previous studies to decrease the rate of respiration and the ethylene production (Janave and Sharma, 2005; Mathew et al., 2007). It is well known that the respiration rate depends on the oxygen partial pressure of the modified atmosphere packaging (Gomes et al., 2012). The respiration rate of the peach slices was reduced because of the low oxygen atmosphere generated inside the package. The low permeability of the film to oxygen in combination with the respiration of the fruit inside the package, reduced the percentage of the gas (from 19 % to 10 % O_2 at 14 d of storage-Table 2). In a previous study, Gunes et al., (2000) found that the application of low-dose irradiation treatments (up to 1.2 kGy) had minimal effects on the respiratory physiology of vegetable tissues. There were no significant changes in O_2 consumption and CO_2 production measured up to 72 h after the treatments. This shows that low doses of GI would not modify the respiratory metabolism of minimally processed fruit.

5. Conclusions

Gamma Irradiation can be considered as a promising technology to

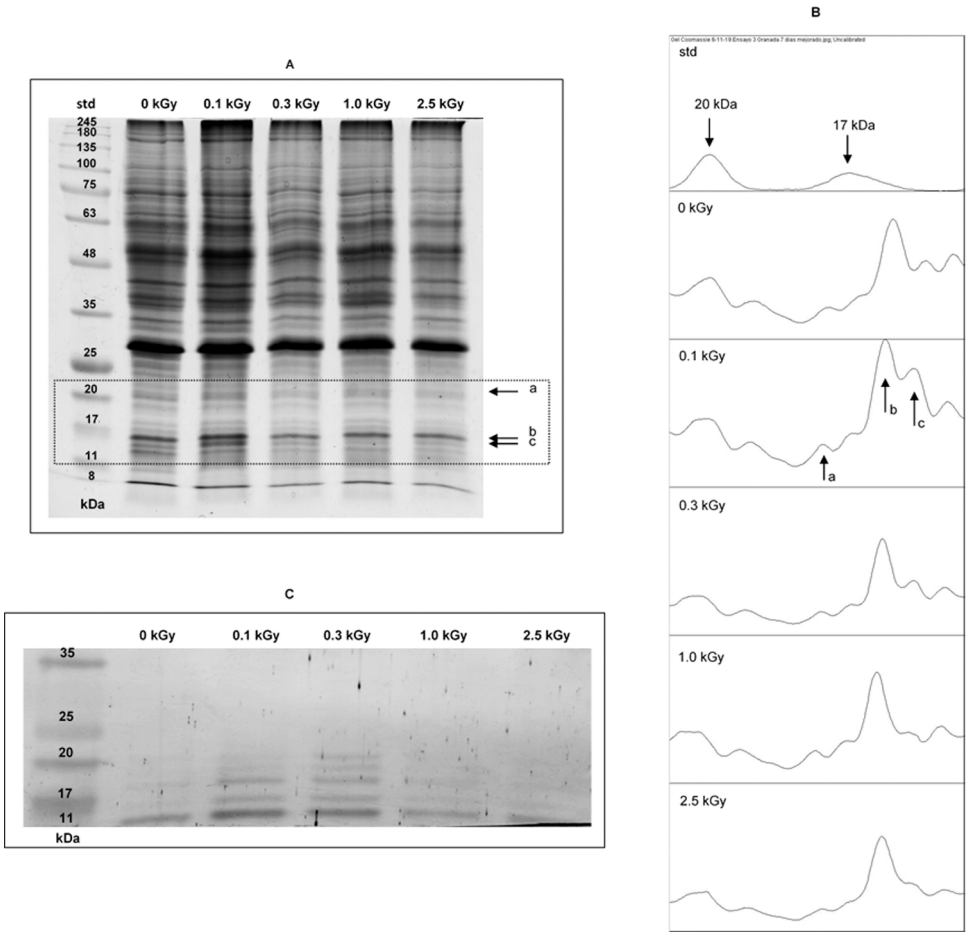


Fig. 5. Detection of HSP overexpression from minimally processed peaches at different irradiation doses (0; 0.1; 0.3; 1.0; 2.5 kGy). (A) SDS/PAGE of protein extracts (B) Densitometric analysis of the low molecular weight region of the gel (indicated by a dotted line in Fig. 5 A). (C) Western blot analysis of protein extracts.

improve the quality and extend the shelf life of minimally processed peaches, provided it is applied at low doses (0.1–0.3 kGy) and combined with low oxygen permeability packaging. The exposure to low doses of 0.1 and 0.3 kGy induced the overexpression of specific proteins in response to irradiation stress. In addition to their beneficial effect, these proteins could be used as biomarkers to optimize and select the most suitable treatments. To the best of our knowledge, there are currently no reports of this effect in plant tissues; then, new perspectives on the application and optimization of irradiation treatments in fresh or minimally processed fruit and vegetables are opened up.

CRediT authorship contribution statement

Analía C. Colletti: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Gabriela I. Denoya:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. **Claudio O. Budde:** Investigation, Methodology, Resources. **Julieta Gabilondo:** Investigation, Methodology, Resources. José A. Pachado: Methodology, Resources. **Sergio R. Vaudagna:** Formal analysis, Supervision, Writing – review & editing. **Gustavo A. Polenta:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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