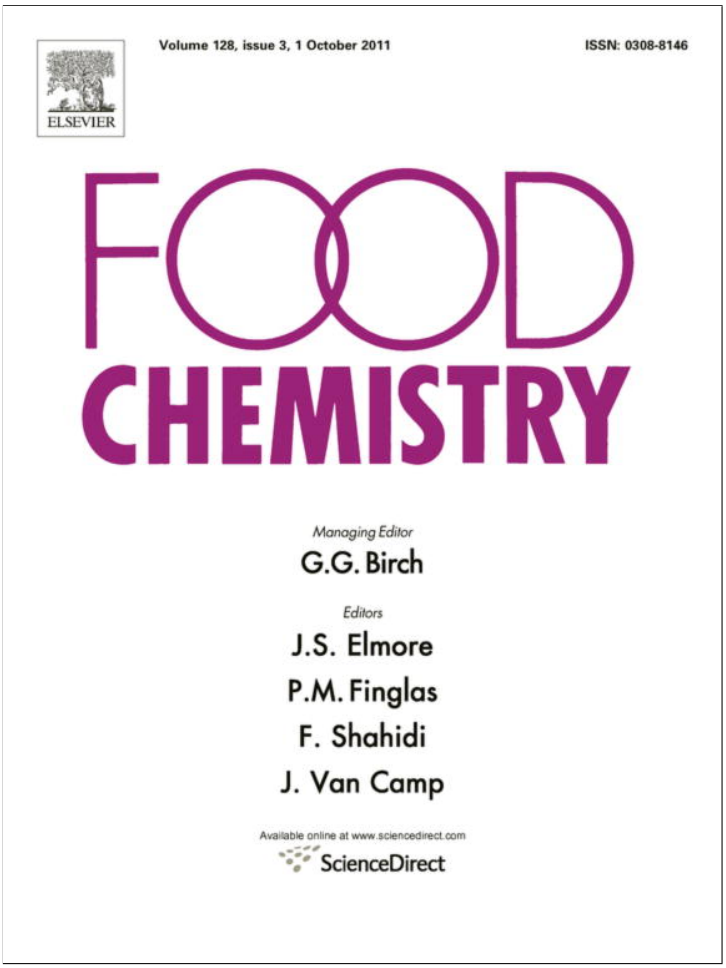

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Effect of γ-radiation on green onion DNA integrity: Role of ascorbic acid and polyphenols against nucleic acid damage

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ABSTRACT

The effect of γ-radiation on green onion DNA integrity, phenol content, oxygen radical absorbance capacity, employing pyrogallol red and fluorescein as probes, as well as ascorbic acid content has been evaluated. Measurements using thiazole orange-DNA fluorescence and agarose gel electrophoresis show that γ-radiation does not lead to an apparent DNA change in green onion. However, it was readily cleaved upon irradiation from the previously isolated nucleic acid. Furthermore, green onion exposure to γ-radiation produces slight increases in the polyphenol concentrations (163–188 μM Trolox eq.) and a decrease in the oxygen radical absorbance using fluorescein (from 245 to 200 Trolox eq.) Interestingly, a high ascorbic acid content (364 μM), which decreases by 40% after γ-ray exposure was measured by using pyrogallol-red-based oxygen radical absorbance capacity induction times from green onion aqueous extracts. Thus, our results suggest that ascorbic acid present in green onion plays a fundamental role in the plant antioxidant response toward γ-radiation exposure, while polyphenols remain largely unchanged, as revealed from oxygen radical absorbance capacity, employing pyrogallol red.

1. Introduction

Gamma rays (γ) are powerful electromagnetic radiations with energies close to 100 keV and high frequency $\approx 10^{19}$ Hz, which are produced in atomic processes involving sub-atomic particles. γ-radiation is considered to be part ionising radiation and it has been demonstrated to produce irreversible DNA damage and as such, exposure of living organisms to this radiation can induce mammalian cell death (Cosa, Vinette, McLean, & Scaiano, 2002; Mathieu, Richard, Ballester, Chancerelle, & Multon, 1999; Ogawa et al., 1998). In the 1960s, γ-irradiation was introduced in North America as a methodology for food preservation. Indeed, controlled exposure to γ-irradiation has proven an efficient methodology against pathogens and is used to sterilise medical material (Shokyu, 2002), frozen food (Abreu et al., 2009), ready-to-eat food (Sommers & Fan, 2002), and in fruit and vegetable pathogen control (Kwon & Yoon, 1985; Prakash, Inthajak, Huibregtse, Caporaso, & Foley, 2000). In for example, celery and other vegetables, including onions, food shelf-life is extended upon γ-radiation exposure using a dose range between 1.0 kGy to 7.0 kGy (One Gy is equal to the amount of γ-radiation necessary to deposit 1.0 J of energy in 1.0 kg of matter) which efficiently kills *Escherichia coli* bacteria (Fan, Niemira, & Sokorai, 2003; Niemira & Fan, 2006). Lower energy doses ca. 0.15 kGy are enough for inhibiting sprouting during storage of onions (*Allium cepa*). However, food exposure to γ-radiation can provoke changes in some sensorial properties (Fan & Sokorai, 2008) as well as in proteins (Edwards, Ruiz, Silva, & Lissi, 2002; Maity et al., 2009), vitamins (Kilcast, 1994; Reyes & Cisneros-Zevallos, 2007), membranes (Mishra, 2004), DNA (Srinivasan et al., 2007), etc., affecting food integrity and its nutritional contribution. In fact, ionising radiation such as γ-radiation is capable of producing hydroxyl radicals from water in aqueous media (Czapski, 1971; Schwarz, 1981), which, in later steps, can abstract a hydrogen atom or add to unsaturated structures, (i.e., nucleic acids, lipids, and proteins) promoting the formation of carbon-centred radicals that, in the presence of oxygen, will cause free radical chain oxidation. Specifically, the Recommended Canadian Code of Practise for Food Irradiation (Health Products and Food Branch, Health Canada in cooperation with Canadian Food Inspection Agency), states: "Food irradiation is the treatment of food products with ionising radiation in order to control food-borne pathogens, reduce microbial load and insect infestation. In fact, γ-radiation has been proven to be effective in germination inhibition of root crops, extending the durable life of perishable product."

Vegetables and fruits have numerous compounds able of acting as free radical scavengers, such as polyphenols and ascorbic acid,

Abbreviations: GO, green onion; I-GO, γ irradiated green onion; NI-GO, non-γ irradiated green onion; ORAC-PGR, absorbance oxygen radical capacity pyrogallol red; ORAC-FL, absorbance oxygen radical capacity fluorescein; Trolox eq, trolox equivalents; AAPH, 2,2′-azo-bis(2-amidinopropane) dihydrochloride; TO, thiazole orange tosylate; ROO, peroxyl radical.

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which are especially important. The intake of natural products rich in antioxidants, such as fruits and vegetables, is recommended due to their health benefits (Bland, 1995; Rice-Evans & Diplock, 1993; Surh, 2003). Several methodologies have been developed to evaluate the antioxidant content and activity for pure and complex mixtures (Liu, 2010; Perez, Leighton, Aspee, Aliaga, & Lissi, 2000). Most of them are not influenced by the presence of ascorbic acid in the media. Nevertheless, López-Alarcón et al. (Atala, Vasquez, Speisky, Lissi, & López-Alarcón, 2009), have recently reported that one oxygen radical absorbance capacity (ORAC) assay; ORAC-pyrogallol red is suitable to evaluate simultaneously the antioxidant capacity and ascorbic acid content by using peroxyl radicals produced from AAPH thermal decomposition as an oxidant source. This is very important for vegetables rich in ascorbic acid, since this compound has been described as a protecting agent toward thymine oxidation promoted by γ-radiation (Shadyro, Lagutin, Edimecheva, Brinkevich, & Kagiya, 2008). Similar to celery or garlic (Prakash et al., 2000), green onions have a high ascorbic acid content and are one of the vegetables commonly exposed to γ-radiation in order to extend their shelf-life and eliminate pathogens (Fan et al., 2003). Despite this fact no work has been performed in order to elucidate the effect of γ-radiation exposure of commercially available green onions on their DNA integrity. As a first attempt to evaluate the effect of γ-radiation on GO, a radiation dose of 1.2 kGy was used, specifically selected to be in the range of commercially employed doses (*vide supra*), yet low enough to deal as much as possible with primary radiation-induced changes; DNA integrity before and after γ-radiation exposure was evaluated; surprisingly no cleaved or any detectable (with the systems employed in this work) change on the nucleic acid was observed. These results pointed out the presence of efficient sacrificial radical compounds, such as polyphenols and ascorbic acid, that are capable of reacting with radicals generated during GO γ-radiation exposure, thus preventing DNA damage. Antioxidant polyphenols and ascorbic acid content of GO extracts, before and after γ-ray exposure were evaluated in order to elucidate how the concentration of these compounds changes by γ-ray exposure. Interestingly, our results indicate that GO-DNA is protected by ascorbic acid, since its content decreases by at least 45% after γ-radiation exposure. The decrease of vitamin C and the relationship with the remarkable DNA protection toward γ-radiation observed for commercially available GO, as well as the antioxidant ability and polyphenol content of these extracts, are discussed in the present contribution.

In order to monitor DNA damage, we combine gel techniques, with some of the fluorescence sensors we have studied in the past, which provide methods for rapid determination of DNA damage. These have been compared with conventional techniques including the comet assay (Cosa, Focsaneanu, McLean, McNamee, & Scaiano, 2001; Cosa, Focsaneanu, McLean, & Scaiano, 2000; Cosa et al., 2002; Trevithick-Sutton, Mikelsons, Filippenko, & Scaiano, 2007).

2. Materials and methods

2.1. Chemicals

Thiazole orange tosylate, 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH), pyrogallol red, Trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid), fluorescein disodium salt, sodium carbonate, ascorbic acid, oxalic acid and ascorbate oxidase were purchased from Sigma–Aldrich (St. Louis, MO). The Promega Wizard® Genomic DNA Purification Kit used to isolate DNA from the green onion samples and Proteinase K were supplied by Fisher Scientific, Ltd. (Nepean, ON). Folin–Ciocalteu reagent was obtained from Merck (Darmstadt, Germany). All compounds were employed as received.

2.2. Samples and γ-radiation system

Green onion (GO, *Allium wakegi*) (Xiao & Parkin, 2006) plants were cultivated and harvested at an organic farm located near Ottawa, Canada, and used as fresh as possible without previous cold storage. In the case of GO taken from a market, the procedures for γ-irradiation and DNA extraction were the same. Each plant was cut in two equal parts and one of them was γ-irradiated with a final dose of 1.2 kGy. GO continuous radiolysis was performed at room temperature in a GammaCell40 (Health Canada, Ottawa) with a source of ^{60}Co and a dose rate of 0.8 Gy min^{-1}. Both parts, non-irradiated (NI-GO) and irradiated (I-GO), were further treated using the same procedure for DNA extraction.

2.3. DNA extraction

Briefly, 1.0 g of fresh GO leaf was frozen with liquid nitrogen and crushed in a mortar to obtain a fine powder. This leaf powder was transferred to a 12 mL centrifuge tube and could be used immediately or kept for one month at −80 °C until next DNA extraction. The DNA extraction for each plant (NI-GO and I-GO) was performed employing the Wizard® Genomic DNA Purification Kit from Promega and the procedure followed is very well described in the respective protocol for "Isolation of Genomic DNA from Plant Tissue" [http://www.promega.com/tbs/tm050/tm050.html]. Minimal changes were made in order to adapt the protocol to our system; for example, solutions of Proteinase K and RNase were added simultaneously. At the end of the procedure, a 0.8 mL solution containing DNA was obtained. DNA concentration was quantified by measuring its absorbance at 260 nm (using a molar absorption coefficient of 6600 $M^{-1}cm^{-1}$ per DNA base at 260 nm) and its purity was determined by using the 260/280 nm ratio in a Varian Cary-50 UV–visible spectrophotometer (Palo Alto, CA). The nucleic acid concentrations obtained were ca. 800 μM in both cases, NI-GO and I-GO. DNA solutions were employed for the intercalation study immediately after isolation or otherwise stored at −18 °C until their use within the next 2 days.

2.4. Fluorescence of thiazole orange as DNA intercalator

A fresh stock solution of thiazole orange tosylate (TO) 40 mM in DMSO was prepared daily. Then, DNA and TO solutions were mixed, in 2 mL centrifuge tubes to a total volume of 450 μL. Three different protocols were carried out to prepare our working solutions; the first ones were prepared keeping constant TO (5 μM) concentration and employing different DNA concentration from 0 to 50 μM; the second ones were made keeping constant DNA (50 μM) concentration and varying TO quantity from 0 to 20 μM. Finally, the third ones were prepared by changing both DNA and TO concentrations, but maintaining constant DNA:TO ratio as 10:1. Fluorescence measurements were carried out by using 10 mm and/or 2 mm optical path length quartz cuvettes in a Photon Technology International (PTI) spectrofluorometer (Birmingham, AL). Emission spectra were taken at 505 nm in triplicate at room temperature (Trevithick-Sutton et al., 2007).

2.5. Agarose gel electrophoresis

Agarose gels (1.25% w/v) were prepared in 1× TAE buffer. Approximately 6.0 μg of sample DNA was loaded per well. DNA markers used were Lambda DNA HindIII + EcoRI Ladder and O'GeneRuler 100 bp DNA Ladder Plus. HindIII + EcoRI Ladder has 21, 5, 3.5, 2, 1.5, and 1.4 kbp bands and O'GeneRuler has 3, 2, 1.5, 1.2,

1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 kbp bands. The samples were run at 130 V and visualised under UV light in a Bio-Rad Gel-doc trans-illuminator by ethidium bromide staining.

2.6. Green onion extracts

GO extracts were prepared by using 10 g of fresh vegetable and then frozen employing liquid nitrogen and crushed in a mortar to obtain a fine powder. This powder was transferred to an Erlenmeyer flask containing 100 mL of 3% w/v oxalic acid aqueous solution and the mixture stirred for 90 min. Then, two aliquots of 10 mL each were centrifuged at 950*g* for 20 min and the supernatant filtered through 0.22 μm pore diameter filter paper. The samples were analysed immediately after extraction.

2.7. ORAC-fluorescein and ORAC-pyrogallol red determination

A simplified kinetic model for the ORAC procedures is shown in Scheme 1.

In Scheme 1, reaction (1) corresponds to AAPH thermal decomposition, which depends only on the media temperature; thus at 37 °C peroxyl radicals are produced at 0.8 μM/min for [AAPH] = 10 mM (Niki, 1990). Reaction (2) involves the probe consumption (such as pyrogallol red, fluorescein and red alizarin (Cao, Alessio, & Cutler, 1993; López-Alarcón & Lissi, 2006; Martin, Aspee, Torres, Lissi, & López-Alarcón, 2009; Ou, Hampsch-Woodill, & Prior, 2001), which allows us to detect changes in the rate of consumption or induction time, depending on the probe reactivity and its concentration. Reaction (3) refers to the reaction between the antioxidant and the peroxyl radical, which will decrease or prevent probe consumption. Instead (4) implies the probable reaction between two peroxyl radicals rendering no radical products. Finally, we have also included in Scheme 1 the probable DNA oxidation by peroxyl radicals (5), which would be prevented by the action of antioxidants in the complex mixture during γ-radiation exposure.

The oxygen radical absorbance capacity (ORAC) measurements (PGR and fluorescein) were carried out according to the procedure described by López-Alarcón and Lissi, (2005,2006) Briefly, daily fresh stock solutions of fluorescein (10 μM) and pyrogallol red (PGR, 1 mM) were prepared in 75 mM phosphate buffer, pH 7.4, and AAPH (0.6 M) in Milli-Q water. A reaction mixture containing AAPH (10 mM), PGR (5 μM) and green onion extract (except for the control) was prepared in a quartz cuvette, and placed into a UV–visible spectrophotometer at 37 °C and the PGR consumption was followed at 540 nm. A similar procedure was performed for ORAC-FL, where PGR was replaced by fluorescein (70 nM), and its fluorescence decay profile at 515 nm was monitored (λ_{exc} = 493 nm). The consumption of fluorescein or pyrogallol red, both in the presence of AAPH, was estimated from fluorescence (F) or absorbance (A) measurements, respectively. Thus, from (F/F_0) or (A/A_0) ratios at different green onion extract volumes, the ORAC indices were obtained from the area under the curve (AUC) when almost 80% of the probe has been consumed and using the Eq. (1)

$$\mathrm{ORAC} = \frac{(\mathrm{AUC} - \mathrm{AUC}^0)}{(\mathrm{AUC}_{\mathrm{Trolox}} - \mathrm{AUC}^0)} f[\mathrm{Trolox}] \quad (1)$$

In this equation: AUC = area under the curve in the presence of tested GO extract, integrated between time zero and that corresponding to 80% of the probe consumption; AUC^0 = area under the curve for the control; $\mathrm{AUC}_{\mathrm{Trolox}}$ = area under the curve for Trolox (employed as reference compound); f = Dilution factor, equal to the ratio between the total volume of the working solution (target molecule plus AAPH, plus GO extract) and the added GO extract volume; [Trolox] = Trolox molar concentration.

$$\mathrm{AAPH} + \mathrm{O_2}\ (\Delta, \mathrm{heat}) \rightarrow 2\mathrm{ROO}\bullet + \mathrm{N_2} \quad (1)$$

$$\mathrm{ROO}\bullet + \mathrm{Probe} \rightarrow \mathrm{Probe\ consumption} \quad (2)$$

$$\mathrm{ROO}\bullet + \mathrm{AH} \rightarrow \mathrm{ROOH} + \mathrm{A}\bullet \quad (3)$$

$$2\mathrm{ROO}\bullet \rightarrow \mathrm{no\ radicals} \quad (4)$$

$$\mathrm{ROO}\bullet + \mathrm{DNA} \rightarrow \mathrm{DNA\text{-}Ox} \quad (5)$$

Scheme 1. Simplified kinetic model for ORAC assays. Also included is the DNA oxidation by peroxyl radicals, a process that our results suggest is prevented.

2.8. Ascorbic acid determination

Stock solutions of ascorbic acid at 6.0 mM concentration were prepared daily in ethanol. Working solutions of ascorbic acid 1.0 mM were prepared by diluting stock solution in 75 mM phosphate buffer, pH 7.4. Ascorbic acid content in GO was estimated from calibration curve containing PGR induction times, measured at 540 nm, *vs*. ascorbic acid concentration from 0 to 20 μM. In order to probe the real presence of ascorbic acid in GO, the extract was incubated in the presence of ascorbate oxidase and then the ORAC-PGR measured. The enzyme was reconstituted in 4 mM phosphate buffer, pH 6.2 (2 mL), then mixed with GO or ascorbic acid solutions in buffer (enzyme final concentration = 0.09 U/mL) for 40 min at room temperature. After this pre-incubation, the solutions were thermostated at 37 °C, and PGR and AAPH were added.

2.9. Total phenolic compounds determination

Total phenol content in extracts of green onions was determined according to the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965), using Trolox as standard. Briefly, appropriate dilutions of the samples (1 mL) were added to Folin–Ciocalteu reagent (5 mL, 0.2 N). After 5 min, sodium carbonate (75 g/L) was added. The mixture was incubated for 2 h and the absorbance of the resulting blue colour was measured at 740 nm in a Varian Cary-50 UV–visible spectrophotometer. Quantification was carried out on the basis of the standard curve prepared with Trolox and the results were expressed as Trolox equivalents (μM).

3. Results and discussion

3.1. Fluorescence of intercalated TO in Green Onion DNA

Fig. 1A shows the TO fluorescence spectra when intercalated in non irradiated GO-DNA (Cosa et al., 2001; Trevithick-Sutton et al., 2007). From the integrated fluorescence spectra at different DNA concentrations, a new plot containing areas under the curve for irradiated and non-irradiated GO as a function of DNA concentration was obtained (see Fig. 1B). This figure clearly shows that there is no significant difference for TO fluorescence after GO irradiation. In fact, agarose gel electrophoresis experiments do not show any detectable DNA cleavage and/or change on its conformation (Fig. 2). It is important to mention that those results cannot be explained in terms of a lower DNA content upon γ-irradiation since no differences in the nucleic base content was observed after GO irradiation. However, control experiments carried out using calf thymus DNA (CT-DNA) in aqueous solutions did not show TO fluorescence after γ-irradiation, which are in agreement with electrophoresis results where any detectable DNA structure change was observed (see Fig. 2).

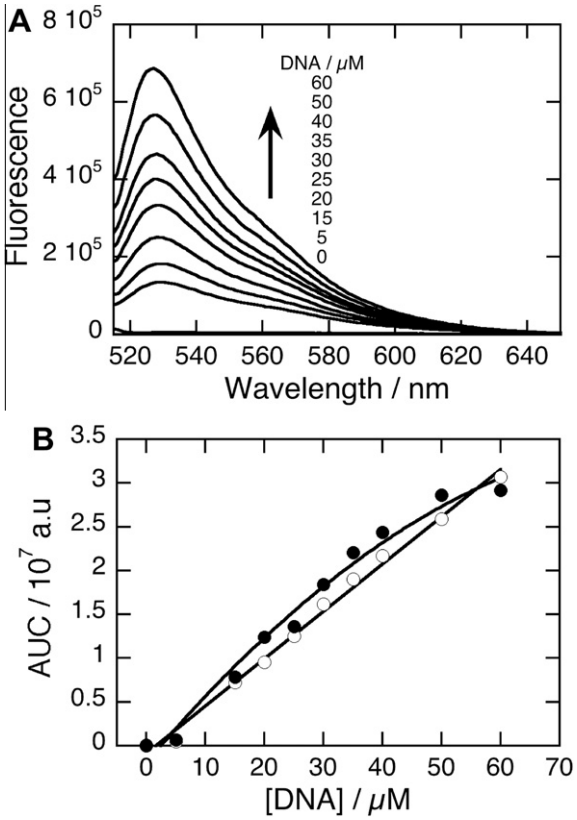

Fig. 1. (A) TO fluorescence spectra obtained at different non-irradiated GO-DNA concentrations. (B) Area under the curve of TO fluorescence spectra in the presence of non-irradiated (○) and irradiated (●) GO-DNA. Fluorescence spectra of TO (5 μM)-GO DNA aqueous solutions were measured by using 505 nm as excitation wavelength.

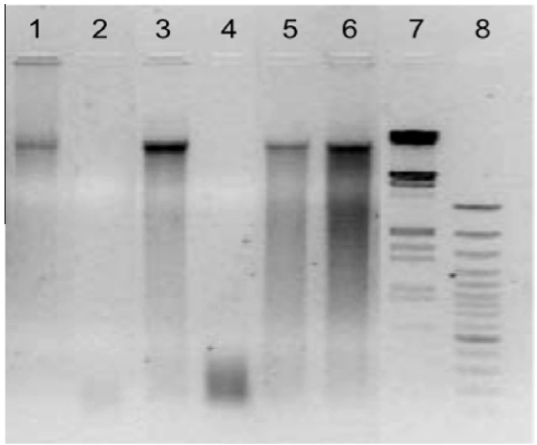

Fig. 2. Agarose gel electrophoresis experiment. Lanes 1–4 correspond to aqueous solutions of DNA: (1) non-irradiated (NI) and (2) γ-irradiated (I) from CT; (3 and 4) NI and I DNA isolated from the plant and exposed to γ-radiation in solution, respectively; (5) not exposed and (6) exposed GO; (7) Hind III + EcoRI Ladder with DNA fragments from 0.56 (bottom) to 21 kb (top) and (8) O'GeneRuler 100 bp DNA Ladder Plus with a range between 0.1 and 3 kbp of DNA bands.

The results suggest that for GO the TO intercalation in DNA takes place, since the nucleic acid double-strand does not break, or it occurs with a very low quantum yield (Cadet, Douki, Gasparutto, & Ravanat, 2005; Gulston, Fulford, Jenner, de Lara, & O'Neill, 2002; Trevithick-Sutton et al., 2007; Tuteja, Ahmad, Panda, & Tuteja, 2008; Yokoya et al., 2008. Cells have a complex enzyme system that protects them from oxidative damage which may occur (Rasmussen & Singh, 2007). It has been postulated that plant cells repair their DNA faster (one day after irradiation) and more efficiently than animal (Schoenmakers, van der Meulen-Muisers, & Koornneef, 1994). In our samples, DNA was extracted within 2–3 h after γ-irradiation; then, we assume that the DNA repair could not be completed. Thus, we rationalise that antioxidant compounds in GO are capable of preventing DNA damage and that they do so in a sacrificial manner. Similar claims have been made to justify the lack of DNA-cleavage by γ-radiation in other vegetables (Chang et al., 2010; Jagetia, Rajanikant, Baliga, Rao, & Kumar, 2004;Wan et al., 2006). Indeed, an efficient DNA cleavage was observed when GO-DNA previously isolated was exposed to γ-radiation (Fig. 2). In order to confirm this possibility, polyphenol content, ascorbic acid and antioxidant ability measurements for irradiated or non-irradiated GO were carried out.

3.2. Total phenolic content in green onion extracts

Table 1 contains the total phenol content for green onion extracts determined by using the Folin–Ciocalteu method (FC) (Singleton and Rossi, 1965). The data shows a slight increase in the polyphenol content for irradiated-GO (I-GO, 188 Trolox eq.) in comparison with non-irradiated GO (NI-GO, 164 Trolox eq.), similar to data reported for dry rosemary leaf powder polyphenol content upon γ-radiation (Pérez, Calderóna, & Crocia, 2007). Nevertheless, polyphenol contents are in agreement with those previously reported for green onion (Prakash, Singh, & Upadhyay, 2007; Tsai, Tsai, & Ho, 2005).

3.3. ORAC-fluorescein values

ORAC-fluorescein has been largely employed as a methodology to evaluate the antioxidant capacity of pure compounds or complex mixtures (Alarcon, Campos, Edwards, Lissi, & López-Alarcón, 2008; Lucas-Abellán et al., 2008; Martin et al., 2009; Otaolaurruchi, Fernández-Pachón, Gonzalez, Troncoso, & García-Parrilla, 2007; Ou et al., 2001). In our specific case, the addition of I-GO and NI-GO extracts to fluorescein and AAPH mixtures, elicited a neat induction time dependent on the GO concentration, which can be interpreted as probe protection ((Ou et al., 2001), see Fig. 3).

After the induction time has ended, the observed fluorescein rate of consumption was barely slower than the respective rate for the control (no GO added), suggesting the presence of a very low quantity of reactive compounds able to compete with fluorescein for peroxyl radicals. This result is fully consistent with the poor reactivity of GO extracts toward DPPH free radical and H_2O_2 reported by Benkeblia (2005). Particularly, ORAC-FL has been described as a methodology most related with the stoichiometry of reaction (Alarcon et al., 2008) rather than reactivity; this assay can be used to estimate the antioxidant capacity for the compounds present in the complex mixture. Thus, ORAC-FL values reported in Table 1 for NI-GO and I-GO decrease after γ-irradiation. These data cannot be explained in terms of lower polyphenols

Table 1
Selected chemical values for irradiated (I-GO) and non-irradiated (NI-GO) fresh green onion extracts.

Sample	Polyphenol content[a]	ORAC-FL[a]	ORAC-PGR[b]	Ascorbic acid content in μg/g of solid
NI-GO	164 ± 10	245 ± 17	2.0	645 (364)[c]
I-GO	188 ± 5.0	200 ± 14	1.0	372 (213)[c]

[a] Trolox equivalents, μM.
[b] Calculated without considering the induction time, upper limit values (see text).
[c] Values in parentheses are concentrations expressed in μM of ascorbic acid.

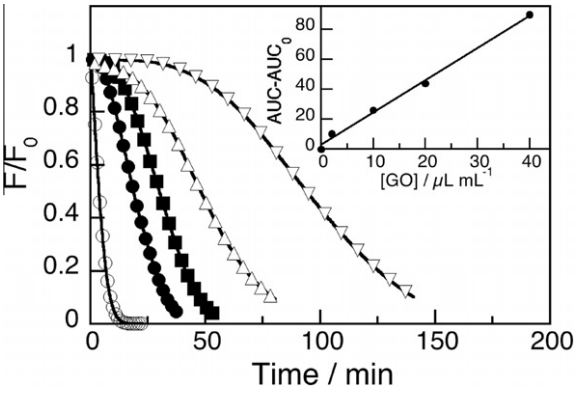

Fig. 3. Effect of green onion extract on 70 nM fluorescein consumption followed by fluorescence emission at 515 nm (λ_{exc} = 493 nm) induced by 10 mM AAPH. Green onion extract: control (○), 2.0 μL/mL (●); 10 μL/mL (■); 20 μL/mL (△); 40 μL/mL (▽). Inset: dependence of the area under the curve after 80% of reaction versus no irradiated green onion concentration. All measurements were carried out in 75 mM phosphate buffer pH 7.4 at 37 °C.

content in the solution (see Table 1). A probable explanation could be obtained by taking into account a decrease of any other compound not measurable by FC assay but able to react with peroxyl radicals and/or capable of repairing phenoxyl radicals from polyphenols in the complex mixture also called secondary reactions, as described elsewhere by Lissi et al. (López-Alarcón, Aspee, & Lissi, 2007; Pino, Campos, López-Alarcón, Aspee, & Lissi, 2006; Pino & Lissi, 2001; Pino, López-Alarcón, Aspee, & Lissi, 2006).

3.4. ORAC-PGR and ascorbic acid determination

The reactivity of phenolic compounds toward peroxyl radicals can be estimated by using PGR as a probe. Indeed, Atala et al. (2009) and López-Alarcón and Lissi (2006) have proposed that this ORAC procedure is more influenced by the compound reactivity than reaction stoichiometry. Thus, the consumption of the PGR does not show induction times as observed for fluorescein assays. Nevertheless, ascorbic acid generates lag times in the consumption of PGR with a linear response at concentration lower than 10 μM (Atala et al., 2009). Interestingly, the addition of GO extracts to PGR mixtures show clear induction times (see Fig. 4A), proportional to the GO extract added volume as is shown in Fig. 4B.

The induction times could be related to the presence of ascorbic acid in the GO extract and, in order to probe this, ascorbate oxidase was added to the media. The results obtained show the elimination of the induction time following preincubation of the extract with ascorbate oxidase (Fig. 3C); two conclusions can be drawn from these results:

(i). Ascorbic acid is present in the complex mixture, and is responsible for the induction time observed here, and,
(ii). The polyphenols present in the media have very low reactivity toward peroxyl radicals as evidenced by their small ORAC-PGR values calculated after one discounts the ascorbic acid induction time.

In order to estimate the concentration of ascorbic acid in the GO extract, a calibration for ascorbic acid was performed (Fig. 5). The reaction stoichiometry obtained from these measurements was 1.7 in agreement with values reported previously (Atala et al., 2009). In fact, this curve shows a linear response for the induction time and ascorbic acid concentration as expected for concentrations lower than 10 μM. Thus, considering the linear region in the calibration curve (ascorbic acid *vs.* induction times, Inset

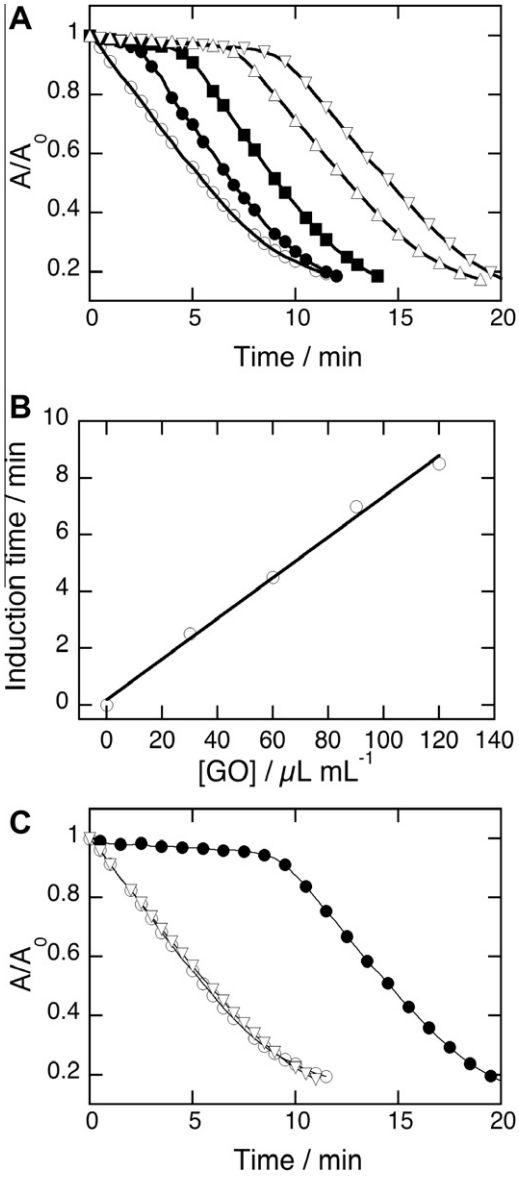

Fig. 4. (A) Effect of irradiated green onion extract on 5.0 μM of PGR consumption monitored by its absorbance loss at 540 nm induced by 10 mM AAPH. Green onion extract: control (○), 30 μL/mL (●); 60 μL/mL (■); 90 μL/mL (△); 120 μL/mL (▽). (B) Dependence of the induction time with the irradiated GO concentration. (C) Effect of ascorbate oxidase (0.09 U/mL) on 5.0 μM pyrogallol red consumption induced by 10 mM AAPH in the presence of non-irradiated GO extract. Green onion extract: control (○), 120 μL/mL (●); GO + ascorbate oxidase (▽). All measurements in 75 mM phosphate buffer.

Fig. 5) the ascorbic acid concentrations showed in Table 1 were obtained by interpolation of the induction times generated from NI-GO and I-GO samples. These values indicate that ascorbic acid concentration is reduced by at least 40% after γ-irradiation. Recently, a decrease of 42% in ascorbic acid concentration was found in baby-leaf spinach exposed to γ-radiation (Lester, Hallman, & Pérez, 2010). Authors proposed that this loss in ascorbic acid content is related to the direct production of radical oxygen species by the irradiation employed. It is important to mention that the presence of ascorbate oxidase in the reaction media does not change the ORAC-FL values for our samples to any major extent (data not shown). This result indicates that the polyphenols present in GO have a very low reactivity toward peroxyl radicals in comparison with PGR.

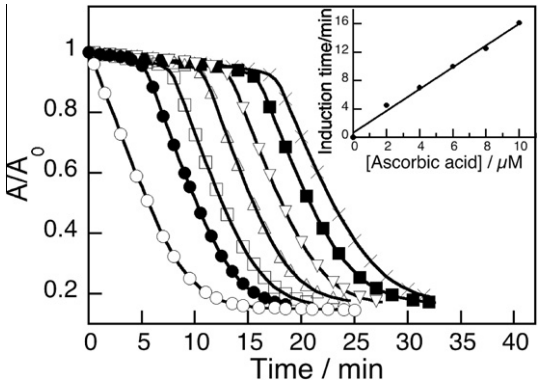

Fig. 5. Pyrogallol red (5 μM) consumption time profiles induced by peroxyl radicals from 10 mM AAPH thermal decomposition in the presence of different concentrations of ascorbic acid; control (○), ascorbic acid: 2.0 μM (●); 4.0 μM (□); 6.0 μM (△); 8.0 μM (▽); 9.0 μM (■); 10 μM (×). Inset: change in the PGR induction time produced by different ascorbic acid concentrations. All measurements were carried out in 75 mM phosphate buffer pH 7.4 at 37 °C.

4. Conclusions

In summary, our results indicate that ascorbic acid present in green onion acts as DNA protector against γ-radiation. In fact, ascorbic acid has proven to be effective against ovalbumin and ovomucoid aggregation and denaturation promoted by γ-radiation (Moon & Song, 2001). In addition, several studies have suggested ascorbic acid as protector agent against oxidative damage promoted by γ-radiation and it is consistent with the findings here (Jagetia et al., 2004; Mishra, 2004; Shadyro et al., 2008; Wan et al., 2006). These results have important relevance from a nutritional point of view, since even under γ-radiation accepted doses for vegetables such as GO, procedure used as a food preservation methodology, their exposure causes an extraordinary decreases in the ascorbic acid content, thus, in their nutritional properties.

The effect of γ-radiation on green onion DNA integrity, phenol content, antioxidant ORAC-PGR and ORAC-FL as well as ascorbic acid content was evaluated. Employing the TO-DNA intercalation fluorescence methodology it was possible to determine that γ-radiation does not modify the green onion DNA integrity when it is in the vegetable. Nevertheless, it was readily cleaved upon irradiation from the isolated nucleic acid, at a similar extent to calf thymus. Moreover, γ-radiation produces slight increases in the polyphenol concentrations (163–188 μM Trolox eq.), matched by a decrease in ORAC-FL (from 245 to 200). Interestingly, green onion extracts have a high ascorbic acid content (364 μM), which decreases after γ-radiation (213 μM). Thus, our results suggest that ascorbic acid present in green onion plays a fundamental role in the antioxidant response in the plant, since their polyphenols show a poor reactivity toward peroxyl radicals, as revealed by ORAC-PGR.

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