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# EFFECT OF ELECTRON BEAM IRRADIATION DOSES IN ANTIOXIDANT ACTIVITY AND PHENOLICS CONTENT OF PORTUGUESE CHESTNUTS

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## KEYWORDS

Irradiated chestnuts, Electron beam irradiation, Antioxidant activity, Phenolics

## ABSTRACT

The objective of this study was to analyse the effect of electron beam irradiation (0.5, 1 and 3 kGy) on the antioxidant activity of Portuguese chestnuts (*Castanea sativa* Mill.) using different *in vitro* assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, reducing power, inhibition of  $\beta$ -carotene bleaching and inhibition of lipid peroxidation using thiobarbituric acid reactive species (TBARS). Total phenolics were determined by spectrophotometric assays. Irradiated samples seemed to preserve phenolics content and revealed higher antioxidant activity than the control sample. The most indicated dose to maintain antioxidants content, and to increase reducing power and lipid peroxidation inhibition was 1 kGy.

## INTRODUCTION

Sweet chestnut (*Castanea sativa* Mill.) woodland covers more than 2.5 million hectares in Europe, with a distribution area ranging from the Southern Mediterranean to Central, Atlantic and Eastern Europe (Díaz-Varela et al. 2011). Portugal is one of the most important chestnut producers with nearly 25% of European production. Trás-os-Montes region represents 75.8% of Portuguese chestnut crops and 84.9% of chestnut orchards area (23,338 ha). The best development conditions are found at altitudes higher than 500 m and winter low temperatures, as in the "Terra Fria Transmontana" region (Northeast of Portugal) in which 12,500 ha are used for chestnut cultivation. Due to the high economic value of chestnuts, it is important to develop conservation methodologies that allow the complete maintenance of their properties and also to fulfil the phytosanitary international regulations for exported products. The previously applied methods included mainly low temperature, controlled atmosphere storage and fumigation with methyl bromide (MeBr) for insect disinfestation. Methyl bromide was the most widely used fumigant for chestnuts post-harvest disinfestation, but induces the depletion of the ozone layer and has deleterious effects on health, so it was banned after the Montreal Protocol. In the European Union its use is forbidden since March 2010. Other conservation techniques such as hot

water dip, still represent low efficiency, affect chemical composition and may induce mould growth. Irradiation may represent a breakthrough on this issue, since it has been used with promising results (Antonio et al. 2011; Fernandes et al. 2011; Kwon et al. 2004). As far as we know, electron beam irradiation was only tried on chestnuts once, aiming to kill larvae (Todoriki et al. 2006). Electron beam radiation might be a clean, environment friendly, comparatively cheap procedure and an effective phytosanitary treatment. Nevertheless, it must be studied in detail since results may vary with fruit species, exposure time and beam intensity.

Chestnuts are important sources of polyphenolic antioxidants that have high free radical scavenging properties being associated to protective effects against coronary heart disease, cancer, neurodegenerative diseases and osteoporosis. Our research group has already determined the antioxidant properties of non-irradiated *Castanea sativa* Mill. (Barreira et al. 2008) and in samples exposed to gamma-radiation (Antonio et al. 2011).

Herein, we describe the influence of the electron beam irradiation process (at three different doses 0.5, 1 and 3 kGy) in antioxidant properties (radical-scavenging activity, reducing power, inhibition of  $\beta$ -carotene bleaching and inhibition of lipid peroxidation using thiobarbituric acid reactive species) and phenolics content of chestnuts immediately after irradiation.

## MATERIALS AND METHODS

### Samples and samples irradiation

Chestnuts samples (*Castanea sativa* Mill., Judia variety) were obtained directly from a local producer of Trás-os-Montes, Northeast of Portugal. They were divided in four groups to be exposed to different radiation doses (0, 0.5, 1 and 3 kGy) with 27 units per group (about 0.5 kg) placed into polyethylene plastic bags, being 0 kGy the non-irradiated, control samples. To estimate the dose during the irradiation process three types of dosimeters were used. A standard dosimeter, a graphite calorimeter, and two routine Gammachrome YR and Amber Perspex dosimeters, from Harwell company (U.K.).

After irradiation, 3 fruits from each group were separated from the skins and lyophilized (Labconco, FreeZone, Missouri, USA), reduced to a fine dried powder (20 mesh), and mixed to obtain a homogenate sample and analyzed.

## Antioxidant activity assays

The sample (1.0 g) was extracted twice with methanol (30 mL) for 1 h. After filtration and evaporation of the methanol, the extracts were re-dissolved in methanol at a concentration of 50 mg/mL and analysed for phenolics and flavonoids content, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity, reducing power inhibition of  $\beta$ -carotene bleaching and inhibition of lipid peroxidation using thiobarbituric acid reactive species.

### Determination of antioxidants content

For phenolics, an aliquot of the extract solution (0.5 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm (AnalytikJena 200 spectrophotometer). Gallic acid was used to calculate the standard curve ( $9.4 \times 10^{-3}$ – $1.5 \times 10^{-1}$  mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

### DPPH radical scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc.). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30  $\mu$ L) and aqueous methanolic solution (80:20 v/v, 270  $\mu$ L) containing DPPH radicals ( $6 \times 10^{-5}$  mol/L). The mixture was left to stand for 30 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: % RSA =  $[(A_{\text{DPPH}} - A_{\text{S}}) / A_{\text{DPPH}}] \times 100$ , where  $A_{\text{S}}$  is the absorbance of the solution when the sample extract has been added at a particular level and  $A_{\text{DPPH}}$  is the absorbance of the DPPH solution.

### Reducing power

The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48 wells, as also deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in the Microplate Reader described above.

### Inhibition of $\beta$ -carotene bleaching

A solution of  $\beta$ -carotene was prepared by dissolving this compound (2 mg) in chloroform (10 mL). Two mL of this solution were pipetted into a round bottom flask. After the chloroform was removed at 40 °C under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled

water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm.  $\beta$ -carotene bleaching inhibition was calculated using the following equation: (Absorbance after 2 h of assay/Initial Absorbance)  $\times$  100.

### Inhibition of lipid peroxidation using thiobarbituric acid reactive species

Porcine (*Sus scrofa*) brains were obtained from officially slaughtered animals, dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000 g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different concentrations of the sample solutions (0.2 mL) in the presence of FeSO<sub>4</sub> (10 mM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28%, w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: inhibition ratio (%) =  $[(A - B) / A] \times 100\%$ , where A and B were the absorbance of the control and the sample solution, respectively.

### Statistical analysis

Three replicates of each sample were used and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with  $\alpha = 0.05$ . This analysis was carried out using SPSS v. 18.0 program.

## RESULTS AND DISCUSSION

Irradiated samples seemed to preserve phenolics since these samples present higher concentrations of the mentioned compounds than the control sample (non-irradiated) (Table 1). Moreover, all the irradiated samples (0.5, 1 and 3 kGy) revealed higher antioxidant activity (lower EC<sub>50</sub> values) than control sample in all the assayed methods. The most indicated dose to maintain antioxidants content, and to increase reducing power and lipid peroxidation inhibition was 1 kGy. It was possible to correlate DPPH scavenging activity and reducing power with phenolics content ( $R^2=0.5607$  and  $0.9138$ , respectively). Nevertheless, these antioxidants are not correlated with the results obtained using lipid peroxidation inhibition assays ( $\beta$ -carotene bleaching inhibition and TBARS assays, Table 2,  $R^2 < 0.45$ ). This could be due to the lipidic environment in which these methods take place. Therefore, other

antioxidants rather than phenolics are certainly responsible for the antioxidant activity observed.

Table 1. Antioxidant activity (EC<sub>50</sub> values<sup>a</sup>) and phenolics content in irradiated and non-irradiated chestnuts.

	EC	E 0.5	E 1	E3
Phenolics (mg GAE/g extract)	3.61 ± 0.57 <sup>d</sup>	4.06 ± 0.93 <sup>c</sup>	8.16 ± 0.34 <sup>a</sup>	5.60 ± 0.50 <sup>b</sup>
Flavonoids (mg CE/g extract)	2.34 ± 0.25 <sup>a</sup>	0.40 ± 0.05 <sup>b</sup>	0.31 ± 0.06 <sup>c</sup>	0.24 ± 0.06 <sup>c</sup>
DPPH scavenging activity (mg/ml)	25.12 ± 1.11 <sup>a</sup>	23.27 ± 2.61 <sup>b</sup>	15.93 ± 0.71 <sup>c</sup>	13.81 ± 1.67 <sup>d</sup>
Reducing power (mg/ml)	7.05 ± 0.96 <sup>a</sup>	6.31 ± 0.59 <sup>b</sup>	2.81 ± 0.10 <sup>d</sup>	5.36 ± 0.27 <sup>c</sup>
β-carotene bleaching inhibition (mg/ml)	6.00 ± 0.43 <sup>b</sup>	2.54 ± 0.37 <sup>c</sup>	1.94 ± 0.20 <sup>d</sup>	6.95 ± 1.09 <sup>a</sup>
TBARS inhibition (mg/ml)	10.63 ± 1.72 <sup>a</sup>	4.06 ± 1.28 <sup>c</sup>	1.66 ± 0.41 <sup>d</sup>	7.82 ± 2.77 <sup>b</sup>

<sup>a</sup> EC<sub>50</sub>: Extract concentration corresponding to 50% of antioxidant activity (for DPPH, β-carotene linoleate and TBARS assays) or 0.5 of absorbance (for reducing power assay).

Table 2. Correlations established between phenolics and antioxidant activity EC<sub>50</sub> values of irradiated and non-irradiated chestnuts.

	EC <sub>50</sub> value (mg/ml)	Phenolics (mg GAE/g)	Flavonoids (mg CE/g)
DPPH scavengin g activity	Linear equation	Y=- 0.2793x+10.811	Y=0.1151x-0.429
	R <sup>2</sup>	0.5607	0.4290
Reducing power	Linear equation	Y=- 1.0581x+11.051	Y=0.2956x-0.7705
	R <sup>2</sup>	0.9138	0.3211
β-carotene bleaching inhibition	Linear equation	Y=- 0.3511x+6.8859	Y=0.1494x+0.169 6
	R <sup>2</sup>	0.1747	0.1424
TBARS inhibition	Linear equation	Y=- 0.2988x+7.1625	Y=0.154x-0.1106
	R <sup>2</sup>	0.3738	0.4472

## CONCLUSIONS

Overall, irradiated samples seemed to preserve phenolics content and revealed higher antioxidant activity (lower EC<sub>50</sub> values) than the control sample. The most indicated dose to maintain antioxidants content, and to increase reducing power and lipid peroxidation inhibition was 1 kGy

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## REFERENCES

- Díaz-Varela R.; Álvarez P.; Varela E.; Iglesias S. 2011. "Prediction of Stand Quality Characteristics in Sweet Chestnut Forests in NW Spain by Combining Terrain Attributes, Spectral Textural Features and Landscape Metrics". *Forest Ecology and Management*, 261, 1962-1972.
- Antonio A.L.; Fernandes A.; Barreira, J.C.M.; Bento A.; Botelho, M.L.; Ferreira, I.C.F.R. 2011. "Influence of Gamma Irradiation in the Antioxidant Potential of Chestnuts (*Castanea sativa* Mill.) Fruits and Skins." *Food and Chemical Toxicology*. 49, 1918-1923.
- Fernandes, A.; Barreira, J.C.M.; António, A.; Bento, A.; Botelho, M.L.; Ferreira I.C.F.R. 2011. "Assessing the Effects of Gamma Irradiation and Storage Time in Energetic Value and in Major Individual Nutrients of Chestnuts." *Food and Chemical Toxicology*. 49, 2429-2432.
- Kwon, J.; Kwon, Y.; Byun, M.; Kim, K. 2004. "Competitiveness of Gamma Irradiation with Fumigation for Chestnuts Associated with Quarantine and Quality Security." *Radiation Physics and Chemistry*, 71, 41-44.
- Todoriki, S.; Hasan, M.; Miyashita, A.; Imamura, T.; Hayashi, T. 2006. "Assessment of Electron Beam-Induced DNA Damage in Larvae of Chestnut Weevil, *Curculio sikkimensis* (Heller) (Coleoptera: Curculionidae) Using Comet Assay." *Radiation Physics and Chemistry*, 75, 292-296.
- Barreira, J.C.M.; Ferreira, I.C.F.R.; Oliveira, M.B.P.P.; Pereira, J.A. "Antioxidant Activities of the Extracts from Chestnut Flower, Leaf, Skins and Fruit." 2008. *Food Chemistry*, 107, 1106-1113.

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