Influence of gamma radiation on the antioxidant properties of edible chestnuts

A. L. António[#1], I. C. F. R. Ferreira[#2], A. Bento[#3], P. Teubig [*4], M. Luísa Botelho[*5]

[#] *CIMO/Agricultural School, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5301-855 Bragança, Portugal.*

[1]amilcar@ipb.pt; [2]iferreira@ipb.pt; [3]bento@ipb.pt

[*] *Unit of Physics and Accelerators, Nuclear and Technological Institute Estrada Nacional 10, 2686-953 Sacavém, Portugal*

[4]pteubig@itn.pt; [5]mlb@itn.pt

Abstract

As seasonal products chestnut have to be postharvest treated to increase the shelf-life. Several problems are associated with traditional preservation methods. These are a decreasing in quality due to dehydration and contamination with insects and microbes including secondary metabolites, *e.g.:* mycotoxin. The most common preservation method for chestnuts is the use of chemical fumigation with methyl bromide. Methyl bromide is a toxic agent and has been banned according to the Montreal Protocol due to its adverse effects on human health and the environment. Following the recommendations of a scientific committee of the United Nations Environmental Program (UNEP), Methyl Bromide Technical Options Committee (MBTOC), food irradiation is a possible feasible option. This preliminary study evaluated the influence of gamma irradiation in the antioxidant potential of chestnut fruits and skins. Results showed that irradiation of chestnuts at low irradiation doses, 0.27 kGy and 0.54 kGy, could affected the skin and fruit properties differently.

Keywords: *Castanea sativa* Mill.; Irradiated chestnut; Gamma irradiation; Antioxidant activity.

Introduction

There are two main problems related to chestnuts preservation: weight losses due to dehydration and development of insects and microorganisms. Methyl bromide (MeBr) fumigation has been used traditionally for chestnuts preservation. However, according to Montreal Protocol it was banned due to their harmful environment and health effects. Another alternative conservation process is heat treatment, but it is time consuming and has a low efficiency. Therefore, an alternative conservation process is urgently needed. Food irradiation has been successfully used for fruit disinfestations [1], [5], [12]. This technique has recently been considered as an alternative to fumigation, as it reduces considerably the amount of product lost during post-harvest period due to rotting, resulting from the development of fungi and moulds. Also, this technology is environmentally friendly, in contrast to the traditional use of fumigants (*e.g.:* methyl bromide), not leaving any type of chemical residues on fruits or environment.

Nevertheless, irradiation is a method that must be studied in detail, since the results vary significantly within different fruit species, exposure time (doses) and geometry (dose uniformity) [6], [7].

As far as we know, little research has been done in the irradiation of chestnut fruits, and particularly on Portuguese varieties

nothing has been reported. Furthermore, our research group has reported the antioxidant potential of different extracts of *Castanea sativa* Mill. (flowers, leaves, skins and fruits) [8]. Herein, we describe the influence of irradiation process (two different doses) in antioxidant properties of fruits and skins stored at 4 °C for 2 months.

Methodology

Samples. Chestnut cv. Longal samples were obtained from Trás-os-Montes, in the Northeast of Portugal. Chestnuts were divided in three samples (control, sample 1, sample 2) with fifteen units per sample.

Irradiation. The irradiations were performed in a ^{60}Co experimental equipment (Precisa22, Graviner Lda, UK). Sample 1 was irradiated 1 h and sample 2 was irradiated 2 h.

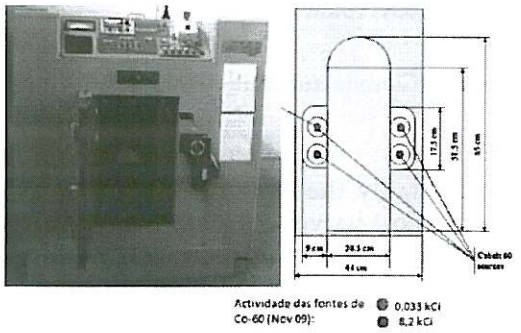

Fig. 1 – Irradiation chamber: activity of sources and dimensions [7]

The ^{60}Co irradiation facility, shown in Fig. 1, consists of a rectangular cavity with the following dimension: 65 cm x 50 cm x 20 cm (h x d x w) and surrounded with a lead protection barrier. Four ^{60}Co sources, with an activity of 305 TBq (8.233 kCi) in November 2009, are positioned in stainless-steel tubes located in the lateral walls of the chamber, in positions directly facing each other, about 30 cm above the chamber floor. The movement of the sources in the 50 cm long tubes is controlled by an automatic mechanism.

A dosimetric study was performed using Fricke solution as reference dosimeter, within the range of 40 to 400 Gy. The Fricke dosimeter is widely used in the calibration of radiation processing and provides a reliable means of absorbed doses measurement in water, based on an oxidation process of ferrous ions to ferric ions in acidic aqueous solution by ionizing radiation. The change in absorbance of the solution was measured using a spectrophotometer at 305 nm [9], [10], [11].

Five dosimeters of pyrex glass tubes with 15 mL of the Fricke solution were used. This dosimeter volume was chosen in accordance with the thickness of chestnut fruit samples. Irradiations were performed on the 4th level of the cobalt-60 experimental chamber at the corners and the centre of the rectangle (the approximately area occupied by the sample bag).

Fricke solutions were placed on the corners and central points of a rectangle wooden tray at positions equal to the position of the sample bags (as shown in Fig. 2).

After irradiation, the absorbance, *Ai*, of the irradiated solution was determined

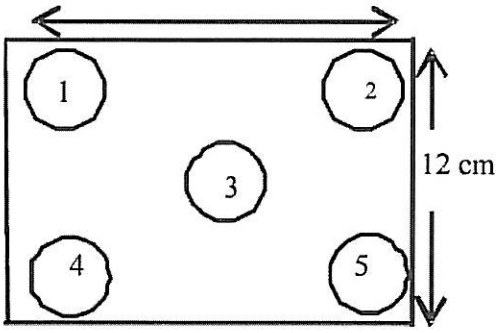

Fig. 2: Irradiated area and dosimeter positions

using a spectrophotometer (Shimadzu mini UV 1240) at 305 nm, using a non-irradiated solution as a reference blank.

The equation used for estimating the absorbed dose, D:

$$D = \frac{275 \times \Delta A}{1 + 0.007\,(T - 25)} \quad (Gy)$$

where ΔA is the difference in absorbance at 305 nm, between the irradiated and the non-irradiated solution and T is the solution temperature (in °C) during the spectrophotometric measurements.

After irradiation geometry dose rate estimation, the chestnut samples 1 and 2 were placed into plastic bags and irradiated for 1 h and 2 h , respectively.

Antioxidant activity assays. The samples were stored at 4 °C for 0 days, 30 days and 60 days. A sub-sample from each of the treatments was obtained at each time point and analyzed (control, sample 1, sample 2 - Fig. 3).

Fruits were separated from the skins (Fig. 3) and the samples were dried in an oven at ~ 30 °C. A fine dried powder (20 mesh) (1.5 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 20 mg/mL and analysed for phenolics and flavonoids contents, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power and inhibition of β-carotene bleaching [8].

Total phenolics were determined by the *Folin-Ciocalteu* colorimetric assay [8]. Total flavonoids were determined spectrophotometrically using the method based on the formation of a complex flavonoid-aluminum [8]. Results were expressed as gallic acid equivalents (GAE) and catechin equivalents (CE), respectively.

DPPH radical-scavenging activity was measured using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc). The reaction mixture consisted of extract solution and aqueous methanolic solution containing DPPH radicals. The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The presence of reducers (i.e. antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm we can monitor the Fe^{2+} concentration; a higher absorbance at 700 nm indicates a higher reducing power. Decolourization of β-carotene was monitored spectrophotometrically at 470 nm. The β-carotene undergoes a rapid discoloration in the absence of an antioxidant since the free linoleic acid radical attacks the β-carotene molecule, which looses the double bonds and, consequently, looses its characteristic orange colour. Classical antioxidants can donate hydrogen atoms to quench radicals and prevent decolourization of carotenoids [8].

Fig. 3 – Chestnuts samples: Control (without irradiation), Sample 1 (0.27 kGy), Sample 2 (0.54 kGy)

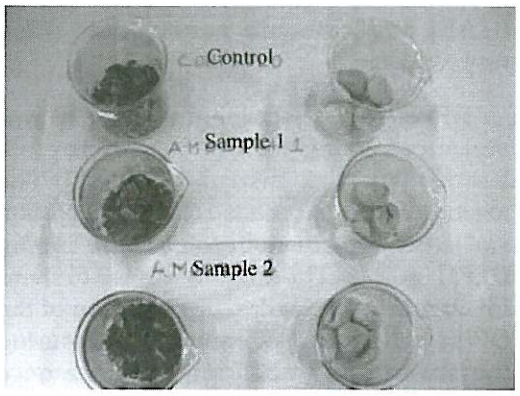

Fig. 4: Chestnuts after peeling: Control (without irradiation), Sample 1 (0.27 kGy), Sample 2 (0.54 kGy)

Results and Discussion

A. Irradiation

An estimation of dose was performed using Fricke chemical dosimeter solution as described above.

The estimated values for the different positions are presented in Table 1.

TABLE 1: Dose distribution

Position	Dose rate (kGy/h)
1	0.30
2	0.29
3	0.29
4	0.23
5	0.23
D_{mean}	0.27 ± 0.04

In this experimental setup, the dose uniformity ratio, the ratio of maximum to minimum absorbed dose in the production lot, obtained is similar to one (D_{max}/D_{min} = 1.3).

Samples were exposed to 1 and 2 h of irradiation, therefore, using the average dose rate this would equivalent to 0.27 and 0.54 kGy, respectively.

B. Antioxidant potential

The chestnut skins had higher phenolic and flavonoid contents (Fig. 5 and 6), as well as higher antioxidant activity (lower EC_{50} values) than chestnut fruits (Table 2 and Table 3), which is in agreement to our previous results [8].

Table 2: Anti-oxidant activity in fruits

Fruits	DPPH scavenging activity EC_{50} (mg/mL)	Reducing Power EC_{50} (mg/mL)	β- carotene bleaching inhibition EC_{50} (mg/mL)
0 Days			
Control	33.00 ± 0.80	7.27 ± 0.00	1.27 ± 0.02
Sample 1	54.58 ± 0.97	9.92 ± 0.02	1.88 ± 0.28
Sample 2	48.57 ± 2.03	8.56 ± 0.02	3.42 ± 0.46
30 Days			
Control	18.08 ± 0.33	3.34 ± 0.02	1.20 ± 0.03
Sample 1	15.50 ± 0.61	3.49 ± 0.05	2.08 ± 0.39
Sample 2	16.43 ± 0.46	3.50 ± 0.01	1.96 ± 0.04
60 Days			
Control	11.77 ± 0.15	3.50 ± 0.04	1.23 ± 0.04
Sample 1	15.77 ± 0.31	3.55 ± 0.08	1.19 ± 0.05
Sample 2	17.35 ± 0.61	3.75 ± 0.06	1.20 ± 0.03

Table 3: Anti-oxidant activity in skins

Skins	DPPH scavenging activity EC_{50} (mg/mL)	Reducing Power EC_{50} (mg/mL)	β-carotene bleaching inhibition EC_{50} (mg/mL)
0 Days			
Control	57.22 ± 4.84	33.52 ± 0.02	46.42 ± 2.88
Sample 1	97.30 ± 2,77	53.46 ± 0.08	27.21 ± 0,00
Sample 2	46.42 ± 2.87	27.21 ± 0.00	59.41 ± 0.08
30 Days			
Control	43.49 ± 3.13	26.32 ± 3.41	241.97 ± 8.02
Sample 1	49.32 ± 0.37	28.09 ± 0.18	199.83 ± 9.24
Sample 2	38.00 ± 0.83	22.62 ± 0.18	44.16 ± 5.90
60 Days			
Control	33.91 ± 1.49	23.32 ± 0.71	95.93 ± 4.97
Sample 1	33.59 ± 1.40	22.50 ± 0.91	73.78 ± 6.57
Sample 2	29.10 ± 0.74	21.96 ± 0.49	47.49 ± 1.99

The flavonoid content of both skin and fruit showed the same tendency for non-irradiated and for the irradiation treatments (0.27 and 0.54 kGy) during the entire storage (0 days, 30 days and 60 days) The phenolic and antioxidant potential content of the skins increased after a dose of 0.27 kGy and a decrease after 0.54 kGy of irradiation in comparison to the control sample at day 0. Irradiated fruits revealed a decrease in phenolic content and antioxidant potential relatively to control. The gamma radiation dose used (sample 1, 0.27 ± 0.04 kGy; sample 2, 0.54 ± 0.04 kGy) did not shown significant influence in those parameters. Along storage (up to 60 days) the studied parameters followed the same tendency in control and irradiated sample fruits.

The results indicate that the dose of 0.27 kGy of irradiation seemed to be more adequate to maintain antioxidant potential of skins than the dose of 0.54 kGy. The higher dose decreased the antioxidant potential of the fruits.

conclusions

In food irradiation the dose distribution inside the chamber and the dose uniformity ratio must be well characterized to control the irradiation process.

The results highlighted that the material could be rotated to obtain a better uniform dose, as is a standard practice in commercial units. However, the dose uniformity ratio

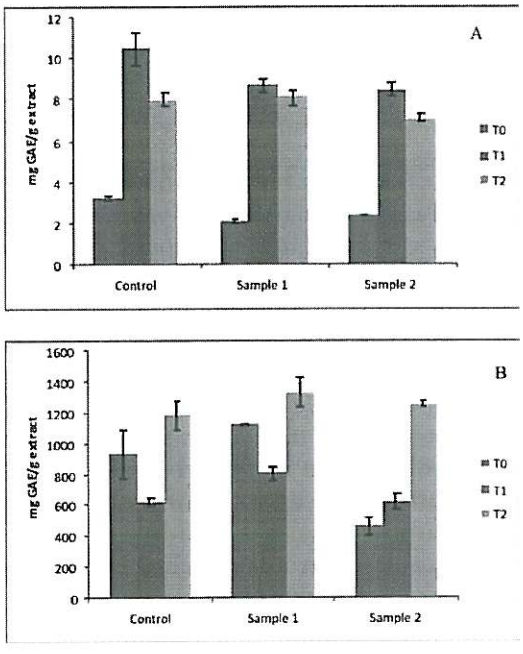

Fig. 5 – Phenolic content in chestnut fruits (A) and skins (B): T0 – 0 days; T1 – 30 days; T2 – 60 days

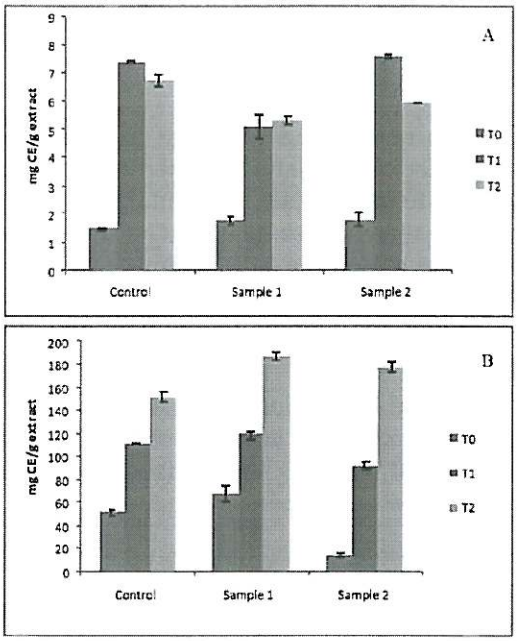

Fig. 6 – Flavonoid content in chestnut fruits (A) and skins (B): T0 – 0 days;

T1 – 30 days; T2 – 60 days

obtained is in conformity with the good practices for food irradiation.

In this preliminary study we can suggest that a variation of 0.27 kGy could affect the skin and fruit properties in different ways, maybe due to different chemical composition of these parts. However, along storage time the control and irradiated samples follow the same tendency. Further studies will be done in order to elucidate the influence of irradiation in chemical composition and nutritional value of chestnuts fruits. The study will be extended to include all positions in the irradiation chamber and to consider more expose doses.

Acknowledgment

This work was supported in part by the Ministry of Economy and Innovation of Portugal with the Project nº 13198/2010 "*CHESTNUTSRAD – An alternative treatment for chestnuts preservation*".

References

1. UNEP, Montreal Protocol on substances that deplete the ozone layer: Report of the Methyl Bromide Technical Options Committee, pp. 205-206, 310-313, 2006.
2. WHO - World Health Organization, Safety and nutritional adequacy of irradiated food, Geneva: , 1994.
3. Directive 1999/2/EC. (22 de February de 1999). Directive of the European Parliament and of the Council. OJ L 66 of 13.03.1999.
4. CODEX STAN 106-1983. General Standard for Irradiated Food. pp. 1-3, rev.1-2003
5. CAC/RCP 19-1979 Rev 2. (2003). Recommended International Code of Practice for Radiation Processing of Food. pp. 1-7, 2003.
6. J. Kim, R. Moreira, Y. Huang, M. Castell-Perez, 3-D dose distributions for optimum radiation treatment planning of complex foods. J. Food Eng. 79, pp. 312-321, 2007.
7. A. Belchior, M. L Botelho, P. Vaz, Monte Carlo simulations and dosimetric studies of an irradiation facility. Nuclear Instruments and Methods in Physics Research A 580 pp. 70-72, 2007.
8. J.C.M. Barreira, I.C.F.R. Ferreira, M.B.P.P. Oliveira, J.A. Pereira. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chemistry 107, pp.1106-1113, 2008.
9. IAEA, Dosimetry for food irradiation, Technical Report Series nº 409, International Atomic Energy Agency, 2002
10. AMERICAN SOCIETY FOR TESTING AND MATERIALS, Practice for Using the Fricke Reference Standard Dosimetry System, ASTM E1026, Annual Book of ASTM Standards, Vol. 12.02, Philadelphia, PA, 2000.
11. K. Sehested, The Fricke Dosemeter, N. H. Bary Ed., New York, MarcelDekker, 1970
12. P. Pinto, S. Cabo Verde, M. J. Trigo, A. Santana M. L. Botelho. Food Irradiation: Microbial, Nutritional,, and Functional Assessment. In Radionuclide Concentrations in Food and the Environment. CRC Taylor & Francis. Pp. 411-438.