Assessing the effects of gamma irradiation and storage time in energetic value and in major individual nutrients of chestnuts

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Abstract

Chestnut (*Castanea sativa* Miller) is an important food resource all over the world. In the present study, it is intended to evaluate if the application of gamma irradiation doses ≤ 3 kGy maintain chestnuts chemical and nutritional profiles unaffected. Furthermore, possible interactions among irradiation dose and storage time were accessed using linear discriminate analysis (LDA). The nutritional composition was evaluated through determination of proteins, fat, ash, carbohydrates and energetic value. The chemical composition was focused in the main nutrients found in chestnuts: sugars- sucrose, fatty acids- palmitic, oleic, linoleic and linolenic acids, tocopherols- γ-tocopherol. The obtained results seem to indicate that the irradiation treatment did not affect the nutritional and chemical quality of chestnut fruits. Otherwise, storage time exerted more evident influence in those parameters. The application of gamma irradiation emerges as a promising technology for chestnuts chemical quality, but food safety issues has to be evaluated in order to recommend its application as a useful conservation alternative.

Keywords: Irradiated chestnuts; Gamma irradiation; Energetic value; Nutritional parameters; Linear Discriminant Analysis

1. Introduction

Chestnut (*Castanea sativa* Miller) is an important food resource in several countries. In Europe, chestnut is regaining interest, with an increase in production area from 81,511 (2005) to 87,521 ha (2008). Portugal is among the major producers, with annual values of over 20,000 t (FAOSTAT, 2010). The Trás-os-Montes region concentrates over 75% of all Portuguese production, being chestnut one of the most important economic resources (Portuguese Agricultural Statistics, 2009).

Due to the high value of chestnuts, it is important to develop conservation methodologies that allow the complete maintenance of their properties. The previously applied methods include fumigation (carbon disulfide, phosphine, methyl bromide), low-temperature and controlled atmosphere storage, irradiation and submerging in icy water. Methyl bromide was the most widely used fumigant for chestnuts post-harvest disinfestation (UNEP, 2006), but induces the depletion of the ozone layer and has deleterious effects on health, so it was banned after the Montreal Protocol (Roy et al., 2008). In the European Union its use is forbidden since March 2010 (Official Journal of the EU, 2008). Temperature related methods may be time consuming and present low efficiency (UNEP, 2006). The immersion in cold or hot water affect the chestnut chemical composition and may induce the development of moulds (Jermini, 2006; UNEP, 2006).

The application of gamma irradiation seems to be a promising technology since it may achieve various effects (depending on the absorbed radiation dose) like reduced storage losses, extended shelf life and/or improved microbiological and parasitological safety of foods. This technology had already been applied to the main commodities such as tuber and bulb crops, stored grains, dried ingredients, meats, poultry and fish, or fruits (Farkas, 2006), having the additional advantage of being harmless to the environment. However, irradiation efficacy varies significantly within different fruit species, demanding continuous exposure

time (doses) and geometry (dose uniformity) studies (Belchior et al., 2007; Kim et al., 2007). In a previous study (Fernandes et al., 2011), low doses (0.27 and 0.54 kGy) of gamma irradiation were applied to chestnuts and it was found that this methodology did not affect the profile and composition in important nutrients (sugars, fatty acids and tocopherols). Furthermore, application of the same doses also seemed to be advantageous for chestnuts antioxidant potential (Antonio et al., 2011). In the present study, it is intended to evaluate if the application of higher irradiation doses (≤ 3 kGy) still maintain chestnuts chemical and nutritional profiles unaffected. Since these profiles are widely characterized (Barreira et al., 2009a; Barreira et al., 2009b; Borges et al., 2007; Borges et al., 2008; Vasconcelos et al., 2007; Vasconcelos et al., 2010), the analyses were focused in the main components of each nutritional group: sugars- sucrose, fatty acids- palmitic, oleic, linoleic and linolenic acids, tocopherols- γ-tocopherol. Furthermore, the effects of gamma irradiation on energetic contribution and proximate analysis of chestnuts stored at 4^{o} C for different periods were evaluated, in order to understand the possible interactions among these two main factors (irradiation and storage time).

2. Materials and methods

2.1. Standards and reagents

Ferrous ammonium sulphate (II) hexahydrate (0.001 M), sodium chloride and sulphuric acid (0.8 N) were purchased from Panreac S.A. (Barcelona, Spain) with purity pa (pro-analysis). Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also γ-tocopherol and D(+)-sucrose standards. Racemic tocol, 50 mg/ml, was purchased from Matreya (PA, USA). All other chemicals and solvents were of analytical grade and purchased from common

sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Samples and samples irradiation

Chestnuts samples were obtained in an industrial unit (Agroaguiar Lda.) of Trás-os-Montes, Northeast of Portugal. They were divided in five groups to be exposed to different radiation doses (0, 0.25, 0.50, 1.00 and 3.00 kGy) with fifteen units per group.

To estimate the dose rate it was used a chemical solution sensitive to ionizing radiation, Fricke dosimeter, using the procedure described in a previous study (Antonio et al., 2011).

After irradiation geometry dose rate estimation, the groups 2 to 5 were placed into polyethylene plastic bags and irradiated with 0.25 ± 0.05, 0.50 ± 0.10, 1.00 ± 0.20 and 3.00 ± 0.30 kGy, respectively. Group 1 was not irradiated, being the control sample. Prior to analysis, all the samples were lyophilized (Ly-8-FM-ULE, Snijders, Holland).

2.3. Energetic value

The samples were analysed for proximate composition (dry matter, proteins, fat, carbohydrates and ash) using the AOAC procedures (AOAC, 1995). The crude protein content of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ^{o}C. Total carbohydrates were calculated by difference. Total energy was calculated according to the following equations: Energy (kcal) = 4 × (g proteins +g carbohydrates) + 9 × (g fat).

2.4. Major individual nutrients

2.4.1. Analysis of sucrose

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as described by (Barreira et al., 2010). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ^{o}C (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1 ml/min. Sugar identification was made by comparing the relative retention times of sample peaks with standards. Quantification was made by the internal standard method and the results are expressed in g per 100 g of dry weight (dw).

2.4.2. Analysis of palmitic, oleic, linoleic and linolenic acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Fernandes et al., 2011). The equipment was a GC 1000 (DANI) with a split/splitless injector, a FID and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 oC, held for 2 min, then a 30 ^{o}C/min ramp to 125 ^{o}C, 5 ^{o}C/min ramp to 160 ^{o}C, 20 ^{o}C/min ramp to 180 ^{o}C, 3 ^{o}C/min ramp to 200 ^{o}C, 20 ^{o}C/min ramp to 220 ^{o}C and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 ^{o}C. Split injection (1:40) was carried out at 250 ^{o}C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.4.3. Analysis of γ-tocopherol

Tocopherols content was determined following a procedure previously described by the authors (Fernandes et al., 2011). The HPLC system described above was connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 × 4.6 mm) normal-phase column from YMC Waters operating at 30 ^{o}C. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 ml/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in the samples are expressed in mg per 100 g of dry weight (dw).

2.4.4. Statistical analysis

An analysis of variance (ANOVA) with Type III sums of squares was performed using the GLM (General Linear Model) procedure of the SPSS software, version 18.0 (SPSS, Inc.). The dependent variables were analyzed using 2-way ANOVA, with the main factors "irradiation dose" (ID) and "storage time" (ST). When a (ID×ST) was detected, the two factors were evaluated simultaneously by the estimated marginal means plots for all levels of each single factor. Alternatively, if no statistical significant interaction was verified, means were compared using Tukey's honestly significant difference (HSD) multiple comparison test.

In addition, a linear discriminant analysis (LDA) was used to assess the influence of either different storage times or irradiation doses on proximate composition profiles as well as in major individual nutrients (sucrose, palmitic, oleic, linoleic and linolenic acids and γ-tocopherol). A stepwise technique, using the Wilks' λ method with the usual probabilities of *F* (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination procedures, where before

selecting a new variable to be included, it is verified whether all variables previously selected remain significant (Maroco, 2003; López et al., 2008). With this approach, it is possible to identify the significant variables obtained for each sample. To verify which canonical discriminant functions were significant, the Wilks' λ test was applied. A leaving-one-out cross-validation procedure was carried out to assess the model performance.

All statistical tests were performed at a 5% significance level. All the assays were carried out in triplicate. The results are expressed as mean values with standard deviation (SD).

3. Results and discussion

Table 1 shows the proximate composition and energetic value data reported as mean value of each irradiation dose over three different storage times, as well as mean value of five irradiation doses within each storage time. Apart from carbohydrates, storage time (ST) ×irradiation dose (ID) interaction was a significant ($P < 0.05$) source of variation for all the performed analytical assays. Hence, despite the least squares means are presented for the main effects, no multiple comparisons could be performed.

Likewise, storage time ($P < 0.001$) and irradiation dose ($P < 0.025$, except for protein content) show a significant effect. However, from the analysis of the plots of the estimated margins means some general conclusions can be drawn. For instance, dry matter, protein and ash contents were higher for 30 days of ST, while carbohydrates, fat and energy contents were superior in samples no submitted to storage. The different ID did not induce any particular tendency in the proximate composition profiles.

Portuguese chestnuts chemical composition has been studied by our group (Barreira et al., 2009a; Barreira et al., 2009b) and by other research groups (Borges et al., 2007; Borges et al., 2008; Vasconcelos et al., 2007; Vasconcelos et al., 2010). Sucrose emerges as the main sugar

(97 to 100%), palmitic (14 to 20%), oleic (23 to 31%), linoleic (42 to 52%) and linolenic (5 to 9%) acids were the most abundant fatty acids, while γ-tocopherol (88 to 100%) was the main tocopherols isoform. Therefore, to evaluate the effects of irradiation dose and storage time we focused in these major molecules.

Table 2 shows chestnuts major individual nutrients. The non stored samples revealed higher palmitic acid levels and lower linoleic acid, linolenic acid and sucrose values. In other way, the samples stored for a 30 days period gave lower γ-tocopherol values. Furthermore, samples irradiated with 3.00 kGy demonstrated the highest linoleic acid contents. Generally, the acquired results showed that the gamma radiation dose used (0.25 ± 0.05, 0.50 ± 0.10, 1.00 ± 0.20 and 3.00 ± 0.30 kGy) did not produce an obvious effect in the assayed parameters, while storage time exerted more evident influence in these values.

To verify this conclusion, the results were evaluated through a linear discriminant analysis (LDA). All independent variables selected by the stepwise procedure of the discriminant analysis were statistically significant according to the Wilks'λ test ($P < 0.05$).

The LDA was performed considering different sets of the assayed parameters (proximate composition, individual compounds or both components simultaneously), in order to find which one permitted the best classification performance. For simplicity matters, only the results obtained when all parameters were considered together are presented.

Regarding storage time, the stepwise LDA resulted in a discriminant model with two significant ($P < 0.001$ for the Wilks' λ test) discriminant functions. These two functions explained 100.0% of the variance of the experimental data (the first explained 82.5% and the second 17.5%) (**Figure 1**).

The first function separates primarily *0 days* and *30 days* (means of the canonical variance (MCV): *0 days* = -3.290, *15 days* = 0.016 and *30 days* = 3.274), and was more powerfully correlated with ash, protein, carbohydrates and dry matter. The second function supported the

separation of *15 days* from the other storage times (MCV: *0 days* = -0.867, *15 days* = 1.746 and *30 days* = -0.879) and showed to be more correlated with fat, γ-tocopherol and linoleic acid. The model showed a very satisfactory classification performance allowing to correctly classifying 97.0% of the samples for the original groups and 96.3% for the cross-validation procedure.

Regarding irradiation dose, the stepwise LDA resulted in a discriminant model with three significant ($P < 0.005$ for the Wilks' λ test) discriminant functions. These three functions explained 98.6% of the variance of the experimental data (the first explained 60.9%, the second 21.4% and the third 16.1%) (**Figure 2**).

The first function was only able to poorly separate 0.50 kGy from the remaining doses (MCV: *0 kGy* = -0.811, *0.25 kGy* = 0.426; *0.50 kGy* = 1.421; *1.00 kGy* = -0.400; *3.00 kGy* = -0.635), being more strongly correlated with sucrose. The second and third functions demonstrated very weak discriminant power, reflected in the classification performance of the model which allowed only to correctly classifying 59.3% of the samples for the original groups and 52.6% for the cross-validation procedure.

The obtained results seem to indicate that the irradiation treatment did not affect the nutritional and chemical quality of chestnut fruits. In order to attend food safety issues it is now necessary to assess the efficacy of this methodology in order to recommend its application as a useful alternative.

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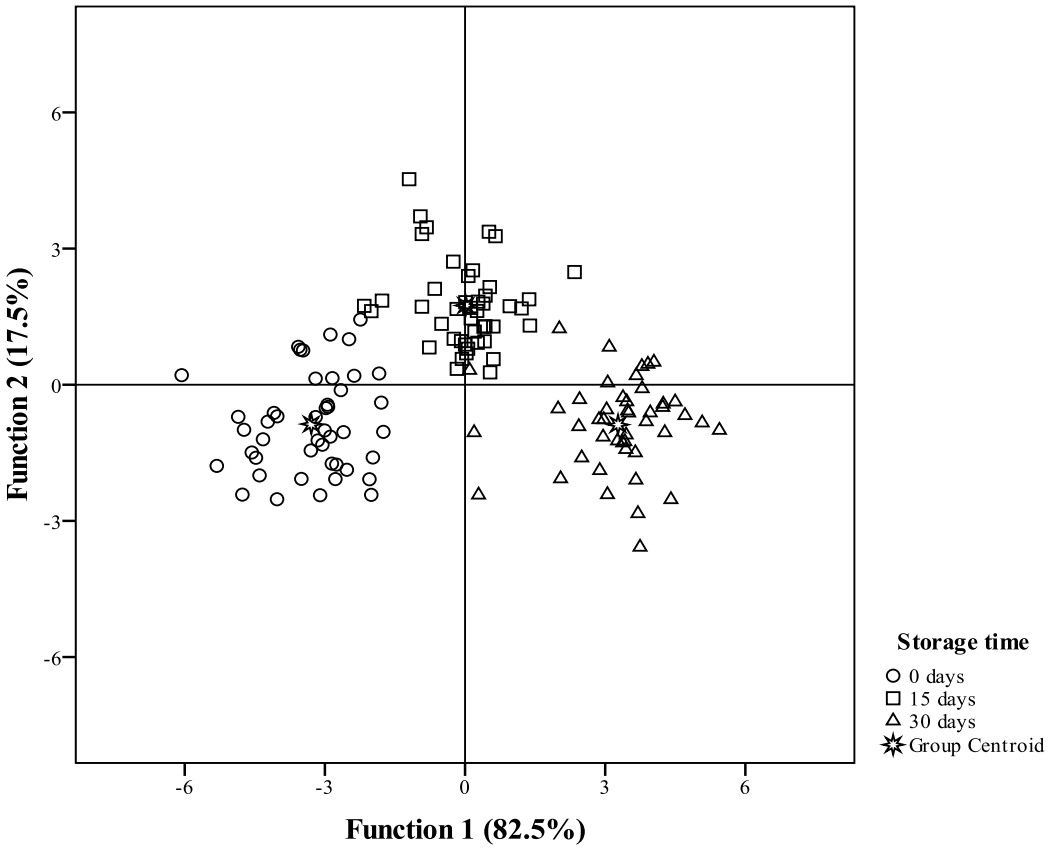

Figure 1. Discriminant scores scatter plot for the two defined canonical functions.

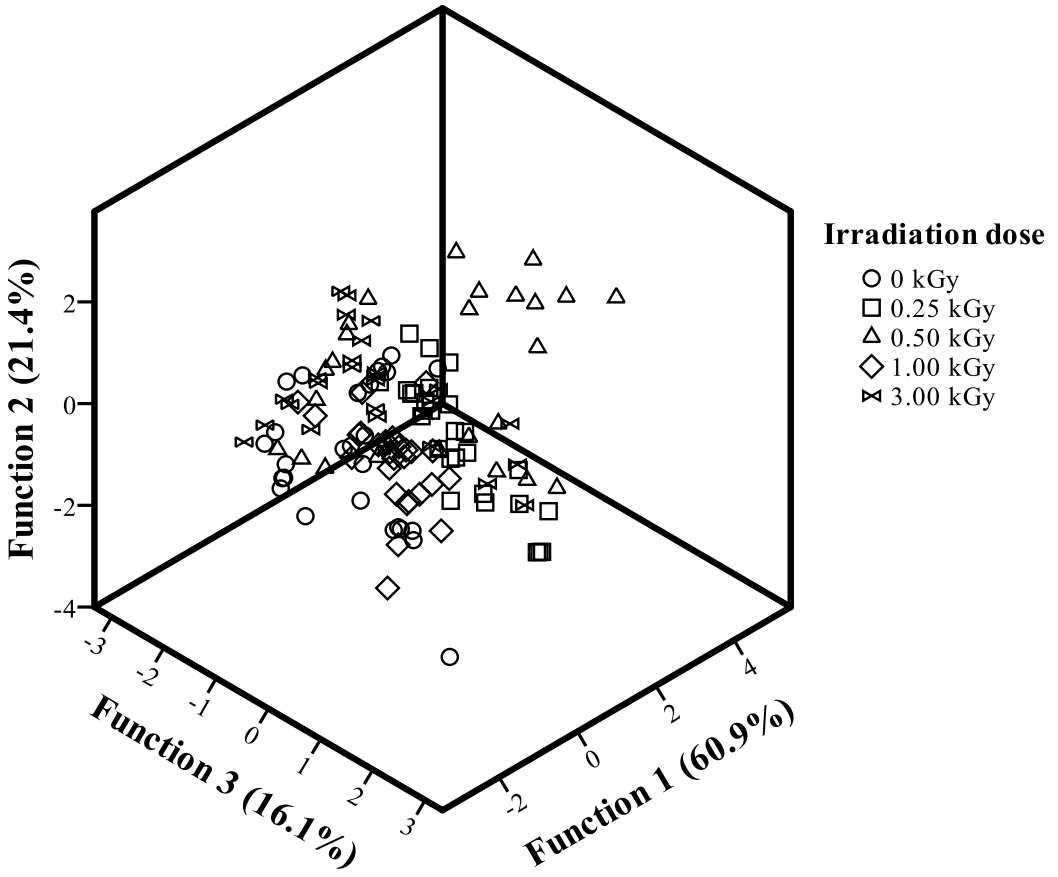

Figure 2. Discriminant scores scatter plot for the first three canonical functions.

Table 1. Chestnuts energetic value according with irradiation dose (ID) and storage time (ST).

		Dry matter (g/100 g fw)	Fat (g/100 g dw)	Protein (g/100 g dw)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)	Energetic value (kcal/100 g dw)
ST	0 days	51±3	2.0±0.5	4±1	2.0±0.2	92±1 a	402±3
	15 days	52±2	1.1±0.2	6±1	2.3±0.2	91±1 b	396±2
	30 days	58±4	1.1±0.4	7±1	2.7±0.1	90±1 c	394±2
	P-value (n=45)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ID	0.00 kGy	55±3	1.4±0.5	6±1	2.4±0.4	90±1 ab	398±5
	0.25 kGy	52±2	1.6±0.4	6±2	2.4±0.4	90±2 b	398±4
	0.50 kGy	56±6	1.3±0.4	6±2	2.3±0.3	90±2 ab	397±4
	1.00 kGy	53±5	1.3±0.4	6±2	2.4±0.3	91±1 ab	397±4
	3.00 kGy	53±4	1.4±0.4	6±2	2.3±0.3	91±2 a	398±2
	P-value (n=27)	<0.001	0.001	0.227	0.003	0.022	0.002
ST×ID	*P*-value	<0.001	<0.001	0.044	<0.001	0.113	<0.001

Table 2. Chestnuts major individual nutrients according with irradiation dose (ID) and storage time (ST).

		Sucrose (g/ 100 g dw)	C16:0 (g/100 g dw)	C18:1 (g/100 g dw)	C18:2 (g/100 g dw)	C18:3 (g/100 g dw)	γ-Tocopherol (mg/100 g dw)
ST	0 days	21±3	18±3	28±3	44±4	7±1	0.9±0.2
	15 days	30±8	16±1	25±2	48±2	8±1	1.0±0.1
	30 days	32±5	15±1	25±3	49±2	8±1	0.8±0.1
	P-value (n=45)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ID	0.00 kGy	23±3	17±2	27±3	46±3	7±1	0.8±0.1
	0.25 kGy	29±7	17±3	26±3	46±5	7±1	1.0±0.2
	0.50 kGy	35±11	17±3	26±2	46±4	7±1	0.9±0.2
	1.00 kGy	26±5	16±2	26±2	47±3	8±1	0.9±0.1
	3.00 kGy	27±6	15±2	25±3	49±3	8±1	0.8±0.1
	P-value (n=27)	<0.001	<0.001	0.002	<0.001	0.002	<0.001
ST×ID	*P*-value	<0.001	0.035	<0.001	<0.001	<0.001	<0.001