JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Effects of Electron-Beam Radiation on Nutritional Parameters of Portuguese Chestnuts (*Castanea sativa* Mill.)

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ABSTRACT: Chestnuts are a widely consumed fruit around the world, with Portugal being the fourth biggest producer in Europe. Storage of these nuts is an important step during processing, and the most widely used fumigant was banned in the European Union under the Montreal Protocol because of its toxicity. Recently, radiation has been introduced as a cheap and clean conservation method. Previous studies of our research group proved that γ radiation had no negative effect on the nutritional value of chestnuts; in fact, storage time had a much bigger influence on the chestnut quality. In the present study, we report the effect of a less ionizing radiation, electron beam, with doses of 0, 0.5, 1, 3, and 6 kGy in the nutritional value of chestnuts (ash, energy, fatty acids, sugars, and tocopherols), previously stored at 4 °C for 0, 30, and 60 days. The storage time seemed to reduce fat and energetic values but reported a tendency for higher values of dry matter. With regard to fatty acids, there was a higher detected quantity of C20:2 in non-irradiated samples and four fatty acids were only detected in trace quantities (C6:0, C8:0, C10:0, and C12:0). γ-Tocopherol decreased during storage time but did not alter its quantity for all of the radiation doses (as like α-, β-, and δ-tocopherol); in fact, these compounds were present in higher concentrations in the irradiated samples. Sucrose and total sugars were lower in non-irradiated samples, and raffinose was only detected in irradiated samples. Electron-beam irradiation seems to be a suitable methodology, because the effects on chemical and nutritional composition are very low, while storage time seems to be quite important in chestnut deterioration.

KEYWORDS: *Irradiation, electron beam, chestnuts, nutrients, storage time*

■ INTRODUCTION

Chestnuts are one of the oldest consumed fruits in Portugal; they were consumed many centuries before potatoes and other tubers became available.[1] Recently, the land occupied with chestnut trees in Europe rose from 81 511 ha (2005) to 87 521 ha (2008).[2] The Trás-os-Montes region, in the northeastern part of Portugal, produces 75% of the nation's chestnuts, being one of the region's main economic resources. Chestnuts consumption could be stimulated as a result of their antioxidant potential[3−5] and health benefits derived from compounds such as tocopherols and polyunsaturated fatty acids that have been found in these nuts,[6,7] being described as effective against cancer, atherosclerosis, and myocardial infarction, among many other diseases.[8,9] Our research group has already studied the nutritional value of chestnuts, determining that the major fatty acids were linoleic, linolenic, and palmitic acids.[10] γ-Tocopherol was the most predominant tocopherol,[6] while sucrose was the principal sugar.[7]

Although chestnuts may seem dry, they are perishable and have a limited shelf life, because of their high metabolic activity.[11] Also, during harvest period, they could become infested with two types of insects (*Curculio elephas* Gyllenhal and *Cydia splendana* Hübner) that cause losses for the producers and the industry, and because a significant part of the production is to export, it must also fulfill the international phytosanitary regulations, eliminating the presence of insects. Until 2010, the most common disinfestation method (elimination of insects) was methyl bromide, but under the Montreal Protocol guidelines, the European Union restricted its use for allegedly being toxic to the operators and polluting the environment.[12] There are several alternative disinfestation methods, such as temperature treatment, cold or hot water dip, and other fumigants,[13] but they still represent quite a number of limitations and disadvantages.

Recently, irradiation has become a promising alternative for chestnut conservation and disinfestation, especially in Korea, where these nuts are irradiated with a maximum of 0.25 kGy for sprout inhibition and with 0.50 kGy for insect disinfestations.[14,15] Some research groups are trying different types of radiation with different doses to guarantee pest-free chestnuts. Our research group has tested both low doses of γ radiation

Received: May 21, 2012
Revised: July 16, 2012
Accepted: July 18, 2012
Published: July 18, 2012

 © 2012 American Chemical Society dx.doi.org/10.1021/jf302230t | J. Agric. Food Chem. 2012, 60, 7754−7760

(0.27 and 0.54 kGy)[16] and higher doses (1 and 3 kGy),[17] concluding that none of the doses altered the nutritional value of chestnuts. There are available reports regarding the use of electron-beam radiation on chestnuts to kill *Curculio sikkimensis* larvae[18] and to destroy yeasts and molds;[19] nevertheless, nothing is known regarding the effects of electron-beam radiation on the nutritional parameters of these nuts. Herein, we report the effects of different doses [0 (control), 0.5, 1, 3, and 6 kGy] of electron-beam radiation and different storage periods [0 (assays conducted immediately after irradiation), 30, and 60 days] on the nutritional value of chestnuts and their sugar, fatty acid, and tocopherol composition.

MATERIALS AND METHODS

Standards and Reagents. Acetonitrile (99.9%), *n*-hexane (95%), and ethyl acetate (99.8%) were of high-performance liquid chromatography (HPLC) grade and purchased from Lab-Scan (Lisbon, Portugal). The fatty acid methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO), as well as the other individual fatty acid isomer, tocopherol (α, β, γ, and δ isoforms), and sugar [D(−)-fructose, D(+)-glucose anhydrous, D(+)-raffinose pentahydrate, D(+)-sucrose, and D(+)-trehalose] standards. Racemic tocol (50 mg/mL) was purchased from Matreya (Pleasant Gap, PA). 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, Greenville, SC).

Samples and Sample Irradiation. Chestnut samples were obtained in an industrial unit (Agroaguiar Lda.) of Trás-os-Montes, northeastern Portugal. The irradiation was performed at the Institute of Nuclear Chemistry and Technology (INCT) in Warsaw, Poland. The samples were divided into five groups: control (without irradiation), sample 1 (0.5 kGy), sample 2 (1 kGy), sample 3 (3 kGy), and sample 4 (6 kGy), with 15 units per group. 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 (Didcot, U.K.). The irradiation took place in an electron-beam irradiator of 10 MeV of energy, with a pulse duration of 5.5 μs, a pulse frequency of 440 Hz, an average beam current of 1.1 mA, a scan width of 68 cm, a conveyer speed in the range of 20−100 cm/min, and a scan frequency of 5 Hz. The absorbed dose was 0.53, 0.83, 2.91, and 6.10 kGy, with an uncertainty of 20% for the first two doses, 15% for the third dose, and 10% for the last dose. To read the Amber and Gammachrome YR dosimeters, spectrophotometric methods were used. For the graphite calorimeter dosimeter, the electrical resistance was read and converted in dose according to a previous calibrated curve. For simplicity, from now on, we refer only to the exact value for the dose: 0, 0.5, 1, 3, and 6 kGy.

From each group, three subgroups with five units were randomly selected: subgroup 1 was promptly analyzed; subgroup 2 was stored at 4 °C (in a refrigerator) for 30 days; and subgroup 3 was stored in the same conditions for 60 days (period long enough for collection, storage, calibration, and export to the final destination until further use). Prior to analysis, all of the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas City, MO), reduced to a fine dried powder (20 mesh), and mixed to obtain homogenate samples.

Energetic Value. The samples were analyzed for proximate composition (dry matter, proteins, fat, carbohydrates, and ash) using the Association of Official Analytical Chemists (AOAC) procedures.[20] 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 °C. Total carbohydrates were calculated by difference. The total energy was calculated according to the following equation: energy (kcal) = 4(grams of protein) + grams of carbohydrate) + 9(grams of fat).

Analysis of Free Sugars. Free sugars were determined by high-performance liquid chromatography coupled to a refraction index detector (HPLC−RI) as described previously by the authors.[7] The equipment consisted of an integrated system with a pump (Knauer, Smartline System 1000), a degasser system (Smartline Manager 5000), an autosampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). The data were analyzed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 °C (7971 R Grace oven). The mobile phase was 7:3 (v/v) acetonitrile/deionized water, at a flow rate of 1 mL/min. The 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 grams per 100 g of dry weight (dw).

Analysis of Fatty Acids. Fatty acids were determined by gas−liquid chromatography with flame ionization detection (GC−FID)/capillary column as described previously by the authors.[16] The equipment was a GC 1000 (DANI) with a split/splitless injector, a FID, and a Macherey-Nagel column (30 m × 0.32 mm inner diameter × 0.25 μm film thickness). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 30 °C/min ramp to 125 °C, a 5 °C/min ramp to 160 °C, a 20 °C/min ramp to 180 °C, a 3 °C/min ramp to 200 °C, a 20 °C/min ramp to 220 °C, and held for 15 min. The carrier gas (hydrogen) flow rate was 4.0 mL/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples

Table 1. Chestnut Nutritional Parameters and Energetic Values According to ID and ST (Mean ± SD)[a]

		dry matter (g/100 g of fw)	fat (g/100 g of dw)	protein (g/100 g of dw)	ash (g/100 g of dw)	carbohydrates (mg/100 g of dw)	energy (kcal/100 g of dw)
ST	0 days	58 ± 3	3 ± 1 a	6 ± 2	1.8 ± 0.4	89 ± 2	409 ± 4 a
	30 days	56 ± 5	3 ± 1 a	6 ± 2	2 ± 3	89 ± 3	408 ± 12 ab
	60 days	71 ± 5	2 ± 1 b	5 ± 2	2.0 ± 0.3	91 ± 2	404 ± 4 b
	p value (n = 45)	<0.001	<0.001	0.050	0.949	0.003	0.012
ID	0 kGy	63 ± 7	2.7 ± 0.5 b	5 ± 2	1.8 ± 0.5	90 ± 2	407 ± 4
	0.5 kGy	62 ± 9	2.8 ± 0.4 b	5 ± 2	1.7 ± 0.5	90 ± 2	407 ± 6
	1 kGy	63 ± 6	3.0 ± 0.5 ab	5 ± 2	1.9 ± 0.3	90 ± 2	408 ± 4
	3 kGy	60 ± 7	3.4 ± 0.5 a	5 ± 2	3 ± 4	89 ± 4	406 ± 15
	6 kGy	60 ± 10	2.8 ± 0.5 b	5 ± 2	1.6 ± 0.3	90 ± 2	408 ± 5
	p value (n = 27)	0.144	0.011	0.973	0.351	0.391	0.983
ST × ID	p value	0.021	0.060	0.023	0.385	0.033	0.478

[a]Results are reported as the mean value of each ID over the different STs, as well as the mean value of all STs within each ID. Therefore, SD reflects values in those samples (under different IDs or STs). In each column, different letters mean significant differences.

Table 2. Fatty Acid Profiles (Percentage) According to ID and ST (Mean ± SD)[a]

	ST				ID						ST × ID
compound	0 days	30 days	60 days	p value (n = 45)	0 kGy	0.5 kGy	1 kGy	3 kGy	6 kGy	p value (n = 27)	p value
C14:0	0.14 ± 0.05	0.14 ± 0.03	0.14 ± 0.02	0.877	0.14 ± 0.03	0.15 ± 0.04	0.14 ± 0.05	0.13 ± 0.06	0.15 ± 0.02	0.349	0.052
C15:0	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.129	0.09 ± 0.01	0.10 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.025	0.002
C16:0	17 ± 3	16 ± 1	16 ± 1	0.015	16 ± 1	16 ± 1	17 ± 3	17 ± 2	17 ± 2	0.132	<0.001
C16:1	0.3 ± 0.1	0.35 ± 0.05	0.4 ± 0.1	<0.001	0.3 ± 0.1	0.39 ± 0.05	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.003	0.009
C17:0	0.16 ± 0.02 a	0.14 ± 0.01 b	0.14 ± 0.01 b	<0.001	0.14 ± 0.01	0.14 ± 0.02	0.15 ± 0.03	0.15 ± 0.03	0.14 ± 0.02	0.086	0.118
C18:0	1.1 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	0.054	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	0.727	0.261
C18:1	33 ± 5	31 ± 5	32 ± 3	0.045	32 ± 2	31 ± 5	32 ± 3	34 ± 3	30 ± 6	0.009	<0.001
C18:2	41 ± 5	44 ± 3	43 ± 3	<0.001	43 ± 2	43 ± 3	42 ± 5	40 ± 3	44 ± 5	0.028	<0.001
C18:3	5 ± 2	6 ± 1	6 ± 1	0.545	6 ± 1	6 ± 1	5 ± 1	5 ± 1	6 ± 1	0.014	0.027
C20:0	0.5 ± 0.02 a	0.38 ± 0.04 b	0.40 ± 0.04 ab	0.017	0.39 ± 0.05	0.40 ± 0.04	0.5 ± 0.3	0.4 ± 0.1	0.4 ± 0.1	0.323	0.146
C20:1	0.6 ± 0.3	0.6 ± 0.1	0.6 ± 0.1	0.457	0.64 ± 0.05	0.6 ± 0.1	0.6 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.657	0.560
C20:2	0.1 ± 0.1	0.03 ± 0.01	0.03 ± 0.01	<0.001	0.03 ± 0.01	0.03 ± 0.01	0.1 ± 0.1	0.1 ± 0.1	0.05 ± 0.03	0.046	0.027
C20:3	0.1 ± 0.1	0.06 ± 0.01	0.06 ± 0.01	0.015	0.06 ± 0.01	0.06 ± 0.01	0.1 ± 0.1	0.06 ± 0.01	0.07 ± 0.01	0.003	0.007
C22:0	0.3 ± 0.1	0.28 ± 0.04	0.30 ± 0.05	0.566	0.28 ± 0.05	0.30 ± 0.04	0.3 ± 0.1	0.29 ± 0.05	0.31 ± 0.05	0.484	0.101
C23:0	0.08 ± 0.02 a	0.07 ± 0.01 b	0.07 ± 0.01 ab	0.043	0.07 ± 0.01 b	0.08 ± 0.01 ab	0.09 ± 0.02 a	0.07 ± 0.02 b	0.07 ± 0.01 ab	0.012	0.286
C24:0	0.16 ± 0.04	0.15 ± 0.02	0.16 ± 0.03	0.331	0.15 ± 0.03	0.16 ± 0.03	0.16 ± 0.04	0.15 ± 0.03	0.16 ± 0.03	0.646	0.474
SFA	20 ± 3	18 ± 1	19 ± 1	0.006	18 ± 2	19 ± 1	19 ± 4	19 ± 2	20 ± 2	0.186	0.001
MUFA	34 ± 5	32 ± 5	33 ± 2	0.054	33 ± 2	32 ± 4	33 ± 3	35 ± 3	31 ± 6	0.009	<0.001
PUFA	46 ± 6	50 ± 4	49 ± 3	0.001	49 ± 2	50 ± 4	48 ± 6	46 ± 3	49 ± 6	0.007	<0.001

[a]Results are reported as the mean value of each ID over the different STs, as well as the mean value of all STs within each ID. Therefore, SD reflects values in those samples (under different IDs or STs). In each column, different letters mean significant differences.

Table 3. Composition in Tocopherols (μg/100 g of dw) According to ID and ST (Mean ± SD)[a]

		α-tocopherol	γ-tocopherol	δ-tocopherol	total
ST	0 days	2 ± 2	1192 ± 185 a	19 ± 11	1213 ± 190 a
	30 days	2 ± 1	1149 ± 262 a	37 ± 61	1187 ± 255 a
	60 days	1.6 ± 0.5	825 ± 224 b	23 ± 17	850 ± 229 b
	p value (n = 45)	0.023	<0.001	0.089	<0.001
ID	0 kGy	1.4 ± 0.5	997 ± 265	41 ± 80	1039 ± 265
	0.5 kGy	3 ± 3	1121 ± 389	20 ± 12	1144 ± 391
	1 kGy	1.6 ± 0.5	1080 ± 198	27 ± 19	1109 ± 204
	3 kGy	1.2 ± 0.3	1029 ± 220	21 ± 9	1052 ± 221
	6 kGy	1.4 ± 0.5	1049 ± 288	21 ± 9	1072 ± 291
	p value (n = 27)	0.001	0.518	0.298	0.611
ST × ID	p value	0.003	0.218	<0.001	0.125

[a]Results are reported as the mean value of each ID over the different STs, as well as the mean value of all STs within each ID. Therefore, SD reflects values in those samples (under different IDs or STs). In each column, different letters mean significant differences.

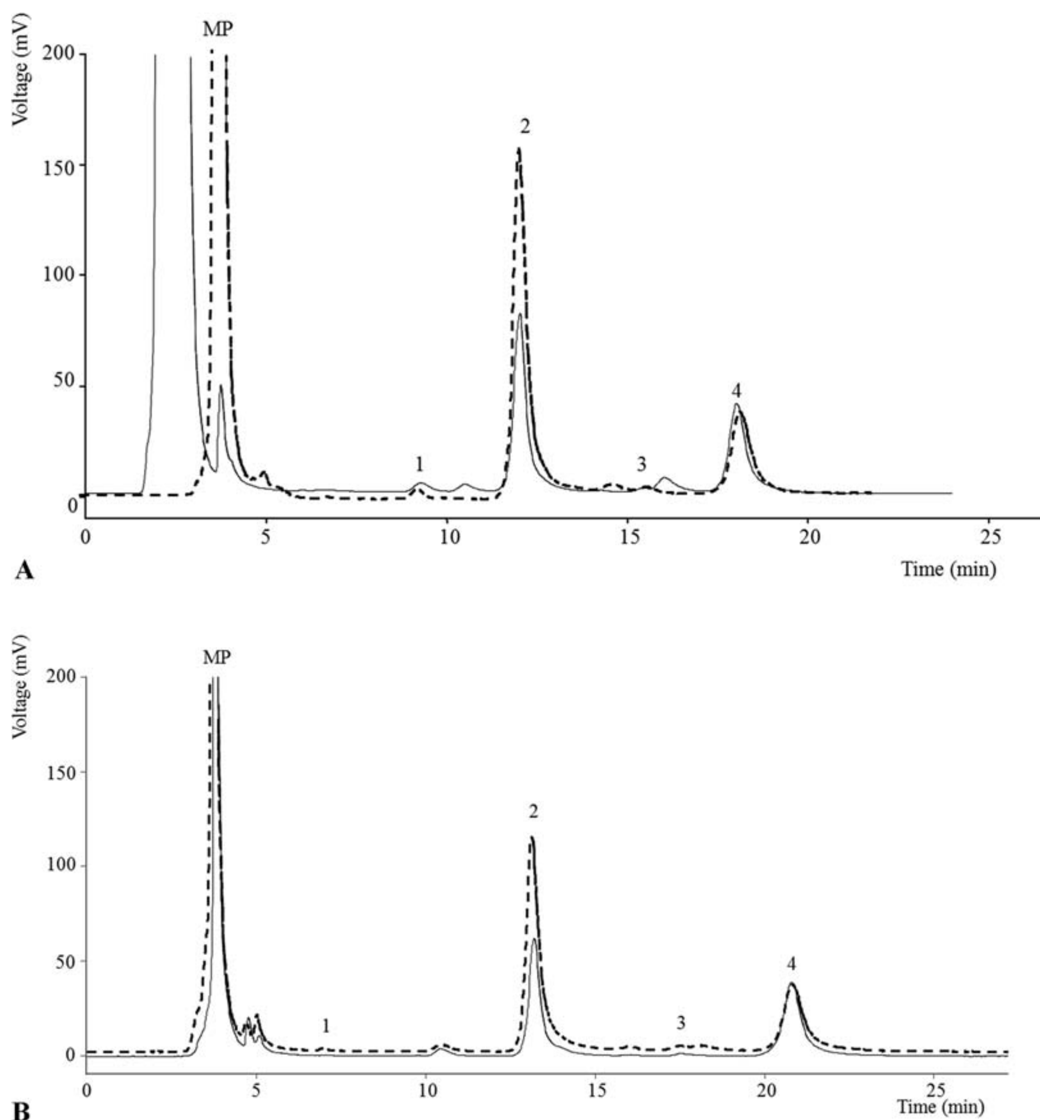

Figure 1. Tocopherol profile of (A) non-irradiated samples, after 0 days (- - -) and 60 days (—) of storage and (B) non-irradiated sample (- - -) and sample irradiated at 6 kGy (—) after 60 days of storage. MP, mobile phase; 1, α-tocopherol; 2, γ-tocopherol; 3, δ-tocopherol; and 4, tocol (IS).

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.

Analysis of Tocopherols. Tocopherol content was determined following a procedure previously described by the authors.[16] 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 °C. The mobile phase used was a mixture of

Table 4. Composition in Free Sugars (g/100 g of dw) According to ID and ST (Mean ± SD)[a]

		fructose	glucose	sucrose	raffinose	total sugars
ST	0 days	0.1 ± 0.1	0.1 ± 0.1	19 ± 2 b	0.1 ± 0.1	19 ± 2 b
	30 days	0.1 ± 0.1	0.1 ± 0.1	22 ± 2 a	nd[b]	23 ± 2 a
	60 days	0.2 ± 0.4	0.2 ± 0.4	23 ± 3 a	nd	23 ± 3 a
	p value (*n* = 45)	0.474	0.654	0.478	0.081	0.440
ID	0 kGy	0.2 ± 0.4	0.2 ± 0.4	22 ± 3	0.1 ± 0.1	22 ± 4
	0.5 kGy	0.07 ± 0.05	0.1 ± 0.1	22 ± 3	0.1 ± 0.1	22 ± 3
	1 kGy	0.2 ± 0.3	0.2 ± 0.4	21 ± 2	0.04 ± 0.05	21 ± 2
	3 kGy	0.1 ± 0.1	0.1 ± 0.1	21 ± 3	0.04 ± 0.05	21 ± 3
	6 kGy	0.2 ± 0.2	0.2 ± 0.2	22 ± 3	0.03 ± 0.05	22 ± 3
	p value (*n* = 27)	0.092	0.103	<0.001	<0.001	<0.001
ID × ST	*p* value	0.755	0.532	0.184	0.040	0.168

[a]Results are reported as the mean value of each ID over the different STs, as well as the mean value of all STs within each ID. Therefore, SD reflects values in those samples (under different IDs or STs). In each column, different letters mean significant differences. [b]nd = not detected.

Table 5. LDA Parameters Considering Different Grouping Variables

		correctly classified cases (%)			
grouping variable	assayed variables	original grouped	cross-validated grouped	number of defined functions	selected variables
ST	all	96.7	96.7	2	C6:0, C10:0, C12:0, C18:2, C23:0, fructose, raffinose, dry matter
ID		36.7	36.7	1	C12:0
ST	nutritional parameters	66.7	66.7	1	dry matter
ID					no variables were qualified
ST	fatty acids	80.0	76.7	2	C10:0, C12:0, C17:0, C23:0
ID		36.7	36.7	1	C12:0
ST	tocopherols	50.0	50.0	1	γ-tocopherol
ID		26.7	26.7	1	γ-tocopherol
ST	sugars	64.4	64.4	1	raffinose
ID					no variables were qualified

n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons to authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in the samples are expressed in milligrams per 100 g of dw.

Statistical Analysis. For each one of the storage times and irradiation doses, three samples were analyzed, with all of the assays also being carried out in triplicate. An analysis of variance (ANOVA) with Type III sums of squares was performed using the general linear model (GLM) procedure of the SPSS software, version 18.0. The dependent variables were analyzed using two-way ANOVA, with the main factors being "irradiation dose" (ID) and "storage time" (ST). When a (ID × ST) was detected, the two factors were evaluated simultaneously by the estimated marginal mean 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, fatty acid, tocopherol, or sugar profiles. 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 a new variable is selected to be included, it is verified whether all variables previously selected remain significant.[21,22] 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 of the assays were carried out in triplicate. The results are expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The effects of electron-beam irradiation (0, 0.5, 1, 3, and 6 kGy) and storage time (0, 30, and 60 days), as well as the interaction of both effects, were assessed by evaluating changes in nutritional composition of selected chestnut samples. Considering both effects together, it is possible to understand the influence of irradiation dose (ID) independent of storage time (ST) and vice versa, an essential requirement to consider electron-beam irradiation as a feasible conservation technique.

Table 1 shows the proximate composition and energetic value data reported as the mean value of each ID along the different storage times, as well as the mean value of each ST for the five irradiation doses. The ST × ID interaction was a significant ($p < 0.05$) source of variation for dry matter, protein, and carbohydrates. Among the remaining parameters, the effect of each individual main factor was only significant for fat content (in both cases) and energy value (only for ST). The allowed multiple comparisons pointed out a lower fat content and energy value after 60 days of storage, while the highest content of fat was quantified in samples irradiated with a 3 kGy ID. However, from the analysis of the plots (data not shown) of the estimated margin of the mean (EMM), it was also possible to identify a marked tendency for a higher dry matter value after 60 days of storage.

Table 2 shows the fatty acid composition data reported as the mean value of each ID along the different STs, as well as the mean value of all STs for the five IDs. Following the same reasoning, the multiple comparisons could only be performed on C17:0 (higher for 0 days), C20:0 (higher for 0 days), and C23:0 (higher for 0 days and 1 kGy). The plots (data not included) of the EMM also showed an increased value for C20:2 in the non-stored samples. Besides the 16 tabled fatty acids, 4 more (C6:0, C8:0, C10:0, and C12:0) were quantified in trace (<0.10%) quantities.

The results obtained for the tocopherol profile indicate a significant decrease in γ-tocopherol contents along ST (Table 3 and Figure 1A), which is in agreement with previous results in chestnuts submitted to γ irradiation (another type of radiation).[17] The applied ID did not cause any significant

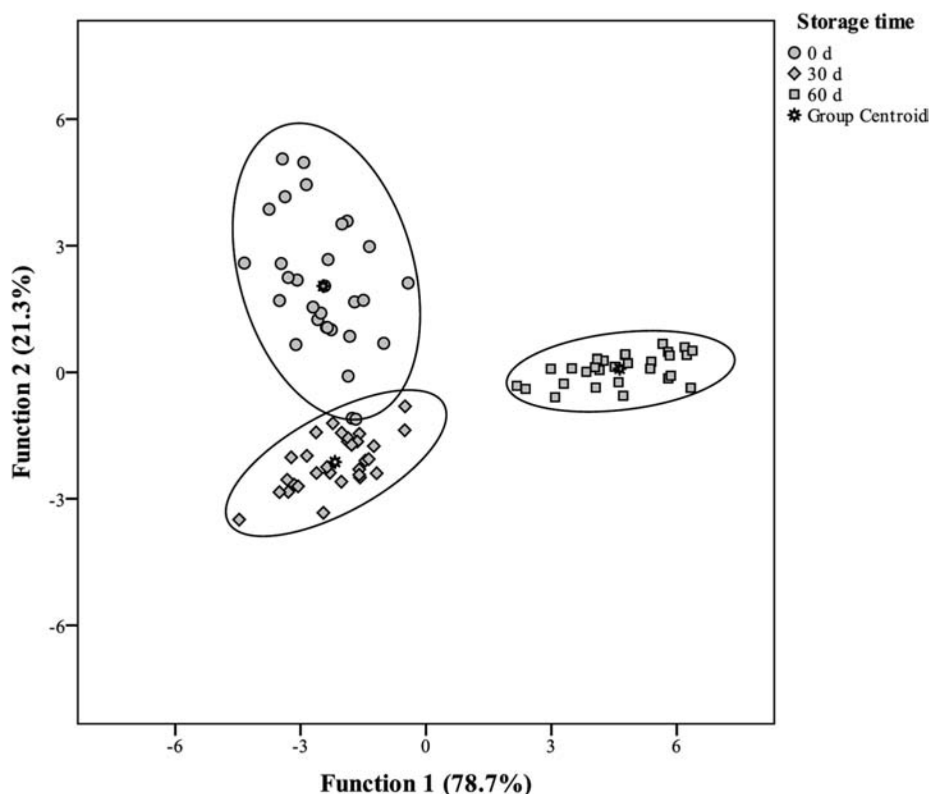

Figure 2. Discriminant score scatter plot of the canonical functions defined for all assayed parameter results.

change in tocopherol profiles, but the overall content tended to be higher in irradiated samples (Figure 1B). In another study,[16] the use of γ irradiation exerted a protective effect on vitamin E content that could be associated with the conversion of molecular to atomic oxygen, decreasing the oxidation of tocopherol molecules. Furthermore, vitamin E has a well-known stabilizing effect against oxidation.[23]

The results obtained for individual sugars are presented in Table 4. Similar to the former results, STs caused higher changes than IDs, despite the only statistical significant differences that might be pointed out were the lower values obtained for sucrose and total sugars in non-stored samples. Furthermore, raffinose was only found in non-stored samples, indicating that trisaccharide might have been hydrolyzed along time; this hypothesis is reinforced by the slight increase of fructose and glucose, despite galactose not being detected.

In general, the results are similar to those obtained in previous studies,[1,6,10,24−26] with water and carbohydrates as major components among nutritional parameters, oleic and linoleic acids as the main fatty acids, γ-tocopherol as the most abundant vitamin E isoform, and sucrose as the highest individual sugar.

Generally, the assayed electron-beam ID (0.5−6 kGy) seemed to produce less obvious effects than ST in all of the assessed parameters.

To confirm this assumption, 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 according with the analyzed groups of compounds (proximate composition, fatty acids, tocopherols, or individual sugars or all parameters simultaneously), to find which one permitted the best classification performance. The main outcomes for each case are presented in Table 5. As seen, the differences induced by ID showed higher discriminant ability than those caused by ST. When the results of all assayed parameters were included in the model, 96.7% of the cases were correctly classified; i.e., the differences verified among non-stored samples, samples stored for 30 days, or samples stored for 60 days were sufficient to separate the obtained values in distinct groups. In fact, only 3 of the 90 assayed cases were misclassified (3 non-stored samples were classified as having been stored for 30 days). The two defined functions (Figure 2) included 100.0% of the observed variance, with the first function separating 0 and 30 days from 60 days [means of the canonical variance (MCV): 0 days = −0.458, 30 days = −2.176, and 60 days = 4.635] mostly based on C10:0 and dry matter contents. The second function allowed for the separation of 0 and 30 days of STs (MCV: 0 days = 2.044, 30 days = −2.128, and 60 days = 0.085), showing a high correlation with raffinose contents. The model showed a very satisfactory classification performance, allowing us to correctly classifying 97.0% of the samples for the original groups and 96.3% for the cross-validation procedure. As already verified,[27] fatty acid profiles are important to evaluate differences induced by ST in chestnut samples, because 5 of the 8 selected variables in the analysis were fatty acids.

The LDA results in Table 5 highlighted the low discriminant ability of ID. Even with all parameters, the classification performance reached only 36.7%, the same value as that obtained for the analysis based only on the fatty acid profile. In the case of nutritional and sugar profiles, no variable was qualified for the analysis, proving the high similarity among the results obtained for different STs.

Overall, considering the effect of either ST or ID, the number of correctly classified cases in the LDA for fatty acid,

tocopherol, sugar, or nutritional parameters was not as high as in previous studies dealing with the application of γ irradiation as an alternative conservation methodology.[16,17,27] Therefore, in this particular subject, electron-beam irradiation seems to be a more adequate methodology, because the effects on chemical and nutritional composition were less detectable that those caused by γ irradiation. However, it is mandatory to perform further studies (for instance, biocide efficacy or food safety requirements) to consider its application as a useful alternative.

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Funding

The authors thank ON.2/QREN/EU Project 13198/2010 for financial support of this work to Fundação para a Ciência e a Tecnologia (FCT, Portugal) and COMPETE/QREN/EU for financial support to CIMO (Strategic Project PEst-OE/AGR/UI0690/2011). Amilcar L. Antonio and João C. M. Barreira also thank FCT, POPH-QREN, and FSE for their grants (SFRH/PROTEC/67398/2010 and SFRH/BPD/72802/2010, respectively). The authors also thank Prof. A. Chmielewski, General Director of the Institute of Nuclear Chemistry and Technology, Warsaw, Poland, for allowing electron-beam irradiations.

Notes

The authors declare no competing financial interest.

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