Low dose γ-irradiation as a suitable solution for chestnut (*Castanea sativa* Miller) conservation: effects on sugars, fatty acids and tocopherols

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Running title: Influence of irradiation in sugars, fatty acids and tocopherols of chestnuts.

ABSTRACT

Along with dehydration, the development of insects and microorganisms is the major drawback in chestnut conservation. Irradiation has been regaining interest as an alternative technology to increase food products shelf-life. In the present work, the effects of low dose gamma irradiation on sugars, fatty acids and tocopherols composition of chestnuts stored at 4 ºC for different storage periods (0, 30 and 60 days) was evaluated. The irradiations were performed in a ^{60}Co experimental equipment, for 1 h (0.27 ± 0.04 kGy) and 2 h (0.54 ± 0.04 kGy). Changes in sugars and tocopherols were determined by high performance liquid chromatography (HPLC) coupled to refraction index (RI) and fluorescence detections, respectively, while changes in fatty acids were analysed by gas-chromatography coupled to flame ionization detection (GC-FID). Regarding sugars composition, storage time proved to have higher effect than irradiation treatment. Fructose and glucose increased after storage, with the corresponding decrease of sucrose. Otherwise, tocopherols content was lower in non-irradiated samples, without a significant influence of storage. Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids levels were not affected neither by storage nor irradiation. Nevertheless, some individual fatty acids concentrations were influenced by one of both factors, such as the increase of palmitic acid in irradiated samples or the decrease of oleic acid after 60 days of storage. Overall, the assayed irradiation doses seem to be a promising alternative treatment to increase chestnuts shelf-life, without affect the profile and composition in important nutrients.

KEYWORDS: Irradiated chestnuts; Gamma irradiation; Sugars; Fatty acids; Tocopherols.

INTRODUCTION

According to FAO, chestnut worldwide production is estimated in 1.1 million tons spread along 340 thousand hectares. China is the major producer with 800 thousand tons per year. Europe is responsible for about 12% of worldwide production, with relevance for Italy and Portugal, corresponding to 4% and 3%, respectively. Trás-os-Montes region represent 85% of Portuguese chestnut crops and 82% of chestnut orchards area (25603 ha) (*1*). As seasonal products chestnuts have to be postharvest treated to increase the shelf-life. The main storage problems with chestnuts are the presence of insect worms (*Cydia splendana* Hb, *Cydia fagliglandana* Zel. and *Curculio elephas* Gyll), and fungi development, mainly *Cyboria*, which blackens the flesh, but also *Rhizopus*, *Fusarium*, *Collectotrichum*, and *Phomopsis*, causing considerable product losses during post-harvest period (*2*). The most common preservation method for chestnuts is the use of chemical fumigation with methyl bromide, a toxic agent that is used under strict control according to the Montreal Protocol due to its adverse effects on human health and environment (*3*). Food irradiation is a possible alternative to substitute the traditional quarantine chemical fumigation treatment (*4-6*).

Carbohydrates are relevant components in chestnuts, especially starch, which is followed by sucrose. This disaccharide is one of the most important parameters in the assessment of fruit quality, once sugar content and composition is lowered or modified by conditions like storage temperature, relative humidity, harvest time, oxygen level or packaging (*7,8*).

The fatty acid composition of tree nuts is important from several perspectives including (1) nutritional quality [the MUFAs and PUFAs (notably the n-3 and n-6 fatty acids) being considered more desirable than the saturated fatty acids]; (2) possible health benefits offered by MUFAs and PUFAs, especially in relation to blood serum lipid profile (notably the decrease in undesirable low-density cholesterols VLDLs and LDLs); (3) desirable flavors often attributed to several fatty acids in the nut seeds; (4) contribution to texture; and (5)

importance in keeping quality (shelf life), especially the propensity for generating off-flavors upon oxidation of MUFAs and PUFAs (*13*). Chestnuts are sources of essential fatty acids, mainly linoleic acid, which play an important role in preventing cardiovascular diseases in adults and promoting the development of the brain and retina of infants (*10*).

Tocopherols are important lipophilic antioxidants with essential effects in living systems against aging (*11*), strengthening the immune system and reducing the risk of chronic diseases such as cancer and cardiovascular diseases (*12*). Furthermore, the oxidation of lipids in food is responsible for the formation of off-flavors and undesirable chemical compounds that may be detrimental to health, and tocopherols, as antioxidants, can stabilize fatty acids and thus prevent the food rancidity during storage (*13*). Vitamin E could also work as a reliable authenticity indicator, allowing the identification of chestnut varieties according to their tocopherol and tocotrienol profile (*14*).

Some studies on chestnuts irradiation were done on Asian (*15*) and Italian (*16*) varieties, but on Portuguese varieties nothing has been reported. The determination of the effective dose is an essential factor to achieve the necessary quality and safety conditions of the product. Doses too low could not be sufficient to eliminate the microbiological risks, whereas doses too high might lead to undesirable physico-chemical changes in the product. Those changes could affect compounds such as sugars, fatty acids and tocopherols (*7,10,14*).

Herein, the influence of irradiation process (at two different doses) in sugars, fatty acids and tocopherols profiles and quantities present in chestnuts stored at 4 ºC for 2 months, was evaluated for the first time in non-irradiated and irradiated samples.

MATERIALS AND METHODS

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), in air-saturated water (Milli-Q Millipore, model A10, USA).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 other individual fatty acid isomers, tocopherols (α-, β-, γ- and δ-isoforms) and sugars (D(-)-fructose, D(+)-glucose anhydrous, D(+)-raffinose pentahydrate, D(+)-sucrose, D(+)-trehalose) 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).

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 three groups: control (without irradiation); sample 1 (0.27 ± 0.04 kGy) and sample 2 (0.54 ± 0.04 kGy) with fifteen units per group.

The absorbed dose was confirmed with Fricke dosimeter, a reference standard dosimeter within the range 40 to 400 Gy, that 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 acid aqueous Fricke dosimeter solution was prepared following the standard procedure (*17*).

Five dosimeters of Pyrex® glass tubes were filled with 15 mL of Fricke solution, according to the thickness of chestnuts. Irradiations were performed on the 4th level of the Cobalt-60 Gammacell (Precisa 22, Graviner Manufacturing Company Ltd). The ^{60}Co irradiation facility consists of a rectangular cavity with 65 × 50 × 20 cm (h × d × w) and surrounded with a lead

protection barrier. Four ^{60}Co sources, with a total activity of 305 TBq (8.233 kCi) in November 2009, were 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 was controlled by an automatic mechanism.

Fricke dosimeters were placed at the corners and centre of a rectangle in an area approximately equal to the sample bag. After irradiation, the absorbance (Ai) of the irradiated solution was determined (Shimadzu mini UV 1240 spectrophotometer) set at 305 nm. The equation used to estimate the absorbed dose, D, was (*17*, *18*):

$D_{Fricke} = (278\ \Delta A) / ([1+0.007(T - 25)][1+0.0015(T' - 25)])$, where ΔA is the difference in absorbance at 305 nm, between irradiated and non-irradiated solution; T is the solution temperature (°C) during the spectrophotometric measurements and T' is the irradiation temperature (°C).

After irradiation geometry dose rate estimation, the groups 2 and 3 were placed into polyethylene plastic bags and irradiated for 1 h (0.27 ± 0.04 kGy) and 2 h (0.54 ± 0.04 kGy), respectively. From each group, three subgroups with three chestnuts were randomly selected. Subgroup 1 was promptly analysed, subgroup 2 was stored for 30 days and subgroup 3 was stored for 60 days. Prior to analysis, all the samples were lyophilized (Ly-8-FM-ULE) and powdered.

Analysis of free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as described by Barreira et al. (*7*). Lyophilized powder sample (1.0 g) was spiked with the melezitose as internal standard (IS, 5 mg/ml), and was extracted with 40 mL of 80% aqueous ethanol at 80 ºC for 30 min. The resulting suspension was centrifuged (Centorion K24OR refrigerated centrifuge) at 15,000*g* for 10 min. The

supernatant was concentrated at 60 ºC (rotary evaporator Büchi R-210) under reduced pressure and defatted three times with 10 mL of ethyl ether, successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 5 mL, and filtered through 0.2 µm nylon filters from Whatman for HPLC analysis. 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 x 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 7:3 (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 internal standard method. Sugars contents in the samples are expressed in g 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 (*19*). Fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v:v:v), during at least 12 h in a bath at 50 ºC and 160 rpm; then 3 mL of deionised water were added, to obtain phase separation; the FAME were recovered with 3 mL of diethyl ether by shaking in vortex, and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 µm nylon filter from Whatman. It was used a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel (50% cyanopropyl-methyl – 50%

phenylmethylpolysiloxane) 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 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. For each analysis 1 µL of the sample was injected in GC. Fatty acid identification was done 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.

Analysis of tocopherols

Tocopherols content was determined following a procedure previously described by the authors (*19*). BHT solution in *n*-hexane (10 mg/mL; 100 µL) and tocol solution in *n*-hexane (internal standard- IS; 50 µg/mL; 400 µL) were added to the lyophilized powder sample prior to the extraction procedure. The samples (~500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, *n*-hexane (4 mL) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000*g*) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with *n*-hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of *n*-hexane, dehydrated with anhydrous sodium sulphate, filtered through 0.2 µm nylon filters from Whatman, transferred into a dark injection vial and analysed by the HPLC system described above, 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 ºC. 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, and the injection volume was 20 µL. 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).

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. All the assays were carried out in triplicate.

RESULTS AND DISCUSSION

Table 1 shows the composition in free sugars reported as mean values of each irradiation dose (ID) over three different storage times (ST) and mean values of all ID within each ST. The obtained profiles are similar to previous studies on Portuguese cultivars (*10*), with sucrose as the main sugar, and low quantities of glucose and fructose. The oligosaccharides trehalose and raffinose were also detected (**Figure 1**).

The results show that ST×ID interaction was a significant ($P < 0.001$) source of variation for all the quantified sugars, with the exception of trehalose ($P = 0.085$), which proved to be present in higher values after 60 days of storage and when irradiated with 0.27 kGy. Likewise,

both main factors (ST and ID) show a significant effect ($P < 0.001$), except irradiation of raffinose ($P = 0.246$). Nevertheless, from the analysis of the plots of the estimated margins means, some general conclusions can be drawn. For instance, glucose and fructose were present in higher quantities in the samples that were stored for one or two months, whereas sucrose showed an opposite behavior (**Figure 1**). It can be assumed that this disaccharide was enzymatically hydrolyzed, releasing the corresponding monosaccharides. The irradiation treatment did not produce any particular effect in sugars composition. This is an important result, since sugars composition is often considered as the best storage quality indicator (*20*). A similar result was previously reported in unrelated food matrixes, like juices (*21*) and tropical fruits (*22*) after exposure to irradiation doses until 5 kGy.

Table 2 shows the composition of tocopherols reported as mean values of each ID over three different ST and mean values of all ID within each ST. γ-Tocopherol is evidently the most abundant isoform, remotely followed by δ-tocopherol and α-tocopherol, revealing a profile in isoforms without insaturations in the isoprenic side chain very similar to previously studied samples (*14*). The results show that ST×ID interaction was a significant ($P < 0.05$) source of variation for all the isoforms. Similarly, both main factors (ST and ID) show a significant effect ($P < 0.001$), except ST for γ-tocopherol ($P = 0.208$) and total tocopherols ($P = 0.788$). However, from the analysis of the plots of the estimated margins means, some general conclusions can be pointed out. For example, γ-tocopherol, δ-tocopherol and total tocopherols were higher in samples subjected to irradiation (**Figure 2**), highlighting a degradation/oxidation of these molecules on control (non-irradiated) samples.

Other studies are available in literature reporting the effects of irradiation on vitamin E content of animal (*23*) and vegetable (*24*) food products, but only in the form of α-tocopherol. Nevertheless, these reports mentioned different effects: significant decrease until 9.5 kGy and no effects at 1 kGy, respectively. The higher levels observed in the present work for the

irradiated samples in relation to control, could be related to the transformation of molecular oxygen present on the sample bag atmosphere into atomic oxygen, decreasing the oxidation of tocopherol molecules.

Table 3 shows the composition in fatty acids reported as mean value of each ID over three different ST and mean value of all ID within each ST. SFA presented amounts closely related to those found in a previous study (*10*), whereas lower quantities of MUFA and higher contents of PUFA have been revealed in the present study. The obtained profiles are mainly related with linoleic, oleic and palmitic acids. Besides the 17 presented fatty acids, five (C6:0, C8:0, C10:0, C20:5n3 and C22:1n9) more were quantified in trace (<0.10%) quantities. The results show that ST×ID interaction was a significant ($P < 0.005$) source of variation for all the quantified fatty acids. Likewise, both main factors (ST and ID) show a significant effect ($P < 0.005$). Nevertheless, from the analysis of the plots of the estimated margins means, some general conclusions can be noticed. For example, C14:0, C16:0, C16:1, C18:0, C23:0 were higher in irradiated samples; C18:0, C20:0, C20:1 and C23:0 were favored by storage, in particular for a 60 days period, whilst C16:1, C18:1 and C24:0 were lowered after chestnuts storage. Despite the mentioned particular effects of ID in some individual fatty acids, no linear effects were generally observed for SFA, MUFA and PUFA contents with the increase of ID. This is an interesting finding, since it reveals irradiation effects in food matrixes with a lipid profile different from the reported in former studies (*25*).

Food irradiation is a versatile process that can be applied to pasteurize, sterilize, replace chemical fumigation, inhibit sprouting, enhance quality or eliminate parasitic hazards. Regarding the applied dose, irradiation can be divided in three major groups: 1) low dose (up to 1 kGy): already applied to potatoes, onions, garlic, ginger root, chestnut, cereals and legumes, fresh and dried fruits, dried fish and meat, fresh pork, freshwater fish, etc. It is used

for sprouting inhibition, insect and parasite disinfestations and ripening delay; 2) medium dose (1 to 10 kGy): already applied to raw and frozen fish and seafood, fruits and vegetables, meat and poultry, spices and dried vegetable seasonings, etc. in order to extend the shelf-life, inactivate the spoilage and pathogenic bacteria and improve the technical properties of foods; 3) high dose (above 10 kGy): already applied to meat, poultry, seafood, sausages, prepared meals, hospital diets, spices, enzyme preparations, natural gum, gel, etc. with the objectives of industrial sterilization (in combination with mild heat) and decontamination of certain food additives and ingredients (*26*).

Overall, the applied irradiation doses did not affect significantly sugars or fatty acids composition. The main effect was observed on tocopherol levels, which were lower on non-irradiated samples, probably due to some degradation of this vitamin caused by higher amounts of molecular oxygen present in control sample bags. The assayed irradiation doses seem to be a promising alternative treatment to increase chestnuts shelf-life, without affect the profile and composition in important nutrients. Further work is necessary in order to evaluate the effects of higher doses on food safety parameters.

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Table 1. Composition in free sugars (g/100 g dw) according with irradiation dose (ID) and storage time (ST) (mean ± SD). In each column, different letters mean significant differences.

		Fructose	Glucose	Sucrose	Trehalose	Raffinose	Total
ST	0 days	0.27±0.05	0.31±0.07	20.06±0.82	0.19±0.04 ab	0.35±0.08	21.18±0.88
	30 days	0.63±0.26	0.95±0.12	18.19±1.20	0.17±0.04 b	0.32±0.05	20.26±1.35
	60 days	0.74±0.19	0.76±0.28	16.77±0.89	0.22±0.06 a	0.44±0.11	18.94±1.00
	P-value (n=27)	<0.001	<0.001	<0.001	0.015	<0.001	<0.001
ID	0 kGy	0.39±0.18	0.63±0.33	17.94±1.15	0.17±0.03 b	0.37±0.05	19.50±0.81
	0.27 kGy	0.75±0.34	0.85±0.37	18.64±1.80	0.23±0.06 a	0.40±0.13	20.86±1.10
	0.54 kGy	0.50±0.14	0.54±0.21	18.44±2.04	0.18±0.03 b	0.36±0.09	20.02±1.88
	P-value (n=27)	<0.001	<0.001	0.018	0.002	0.246	<0.001
ST × ID	*P*-value	<0.001	<0.001	<0.001	0.085	<0.001	<0.001

Table 2. Composition in tocopherols (μg/100 g dw) according with irradiation dose (ID) and storage time (ST) (mean ± SD).

		α-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols
ST	0 days	5.70±0.61	1078.70±79.45	38.19±5.72	1122.60±81.61
	30 days	7.64±2.17	1074.73±105.25	42.22±14.58	1124.59±116.49
	60 days	9.31±0.22	1043.12±178.61	57.21±15.98	1109.64±186.48
	P-value (n=27)	<0.001	0.208	<0.001	0.788
ID	0 kGy	6.79±0.93	915.48±88.21	38.51±14.37	960.77±82.35
	0.27 kGy	9.20±2.96	1134.34±41.96	57.99±15.52	1201.53±56.92
	0.54 kGy	6.67±1.77	1146.73±50.31	41.12±5.09	1194.52±53.73
	P-value (n=27)	<0.001	<0.001	<0.001	<0.001
ST × ID	*P*-value	0.024	0.002	0.031	0.003

Table 3. Composition in fatty acids (percentage) according with irradiation dose (ID) and storage time (ST) (mean ± SD).

	ST				ID				ST × ID
Compound	0 days	30 days	60 days	*P*-value (n=27)	0 kGy	0.27 kGy	0.54 kGy	*P*-value (n=27)	*P*-value
C12:0	0.11±0.03	0.08±0.01	0.09±0.01	<0.001	0.08±0.01	0.10±0.01	0.10±0.03	<0.001	<0.001
C14:0	0.26±0.06	0.22±0.03	0.29±0.06	<0.001	0.20±0.01	0.28±0.05	0.29±0.05	<0.001	<0.001
C15:0	0.16±0.03	0.14±0.01	0.15±0.02	0.004	0.13±0.01	0.17±0.02	0.15±0.03	<0.001	0.001
C16:0	14.65±0.26	13.90±0.60	15.26±0.90	<0.001	13.97±0.61	15.07±0.65	14.76±0.88	<0.001	<0.001
C16:1	0.34±0.07	0.29±0.04	0.39±0.10	<0.001	0.28±0.03	0.41±0.08	0.34±0.06	<0.001	<0.001
C17:0	0.22±0.02	0.20±0.01	0.24±0.02	<0.001	0.21±0.02	0.23±0.02	0.22±0.03	0.043	0.003
C18:0	1.08±0.20	1.05±0.16	1.80±0.57	<0.001	0.97±0.07	1.38±0.59	1.58±0.48	<0.001	<0.001
C18:1n9	20.53±1.45	23.08±5.56	17.66±3.01	<0.001	22.50±1.73	17.53±3.20	21.24±5.51	<0.001	<0.001
C18:2n6	53.20±1.32	52.46±3.90	53.80±1.04	<0.001	53.03±0.63	54.56±2.31	51.87±3.04	<0.001	<0.001
C18:3n3	7.68±0.42	6.68±1.49	8.29±0.52	<0.001	6.88±0.86	8.25±0.36	7.52±1.49	<0.001	<0.001
C20:0	0.33±0.01	0.32±0.03	0.40±0.05	<0.001	0.33±0.02	0.37±0.07	0.35±0.03	<0.001	<0.001
C20:1	0.49±0.08	0.60±0.02	0.59±0.04	<0.001	0.54±0.12	0.57±0.02	0.57±0.04	<0.001	<0.001
C20:2	0.07±0.01	0.09±0.02	0.09±0.01	<0.001	0.08±0.02	0.09±0.01	0.08±0.01	<0.001	<0.001
C20:3n3+C21:0	0.08±0.02	0.09±0.01	0.11±0.02	<0.001	0.08±0.02	0.11±0.02	0.09±0.01	<0.001	<0.001
C22:0	0.35±0.04	0.33±0.01	0.38±0.05	<0.001	0.32±0.01	0.38±0.05	0.36±0.04	<0.001	<0.001
C23:0	0.07±0.01	0.06±0.01	0.10±0.02	<0.001	0.06±0.01	0.09±0.02	0.07±0.01	<0.001	<0.001
C24:0	0.30±0.04	0.29±0.03	0.22±0.04	<0.001	0.24±0.05	0.29±0.03	0.28±0.05	<0.001	0.010
Total SFA	17.57±0.46	16.65±0.75	19.02±1.69	<0.001	16.58±0.63	18.44±1.47	18.23±1.40	<0.001	<0.001
Total MUFA	21.38±1.42	23.99±5.53	18.66±2.96	<0.001	23.33±1.77	18.52±3.13	22.17±5.50	<0.001	<0.001
Total PUFA	61.05±1.38	59.36±5.29	62.32±1.30	<0.001	60.10±1.24	63.04±2.70	59.60±4.47	<0.001	<0.001
Total fat	1.94±0.33	2.25±0.35	2.64±0.34	<0.001	2.50±0.39	1.99±0.19	2.33±0.54	<0.001	<0.001

FIGURE CAPTIONS

Figure 1. Sugars profile of non-irradiated samples after 0 days (——) and after 60 days (- - -) of storage at 4 ºC. 1- Fructose; 2-glucose; 3-sucrose; 4- trehalose; 5-melezitose (IS) and 6-raffinose.

Figure 2. Tocopherols profile of non-irradiated sample (——) and a sample irradiated with 0.54 kGy (- - -) after 60 days of storage. 1-α-Tocopherol; 2-γ- tocopherol; 3- δ-tocopherol and 4-tocol (IS).

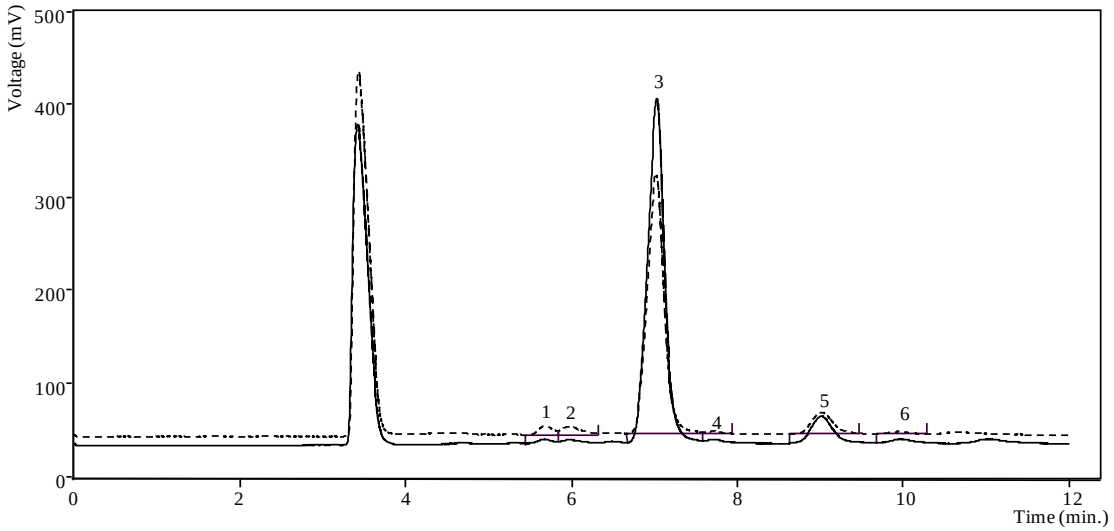

Figure 1.

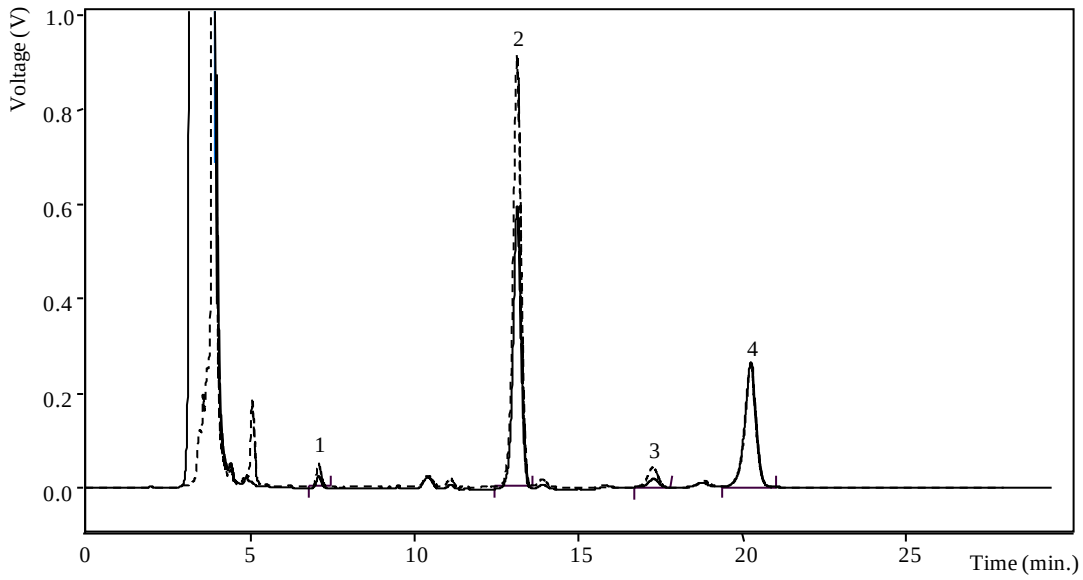

Figure 2.