LWT - Food Science and Technology 59 (2014) 572–576

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effects of γ-irradiation on the α-tocopherol and fatty acids content of raw unpeeled almond kernels (*Prunus dulcis*)

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ARTICLE INFO

Article history:
Received 4 May 2011
Received in revised form
10 March 2014
Accepted 25 April 2014
Available online 4 May 2014

Keywords:
Almonds
Gamma irradiation
Fatty acids
α-Tocopherol

ABSTRACT

The present study evaluated fatty acid composition and α-tocopherol content of almond as a function of irradiation dose in order to determine dose levels causing minimal undesirable changes to almonds. Raw unpeeled almonds variety Tuono (*Prunus dulcis* (Mill.) D. A. Webb) were irradiated using ^{60}Co source at dose of 0.5, 1.5, 3, 6, 8 and 10 kGy.

Both control and irradiated samples were kept frozen and immediately analyzed. The data obtained showed no change in fatty acid compositions up to a dose of 10 kGy; on the contrary, a general trend observed is that increasing the dose of irradiation resulted in the decrease of α-tocopherol content. The study has shown that irradiation is an effective tool in simultaneous preservation of α-tocopherol, that is the main vitamin-E active compound, and fatty acids content in almonds at low doses.

1. Introduction

The main cause of post-harvest losses of stored dried fruits and nuts is the proliferation of larvae and insects in stored products (Thomas, 2001). Researches carried out world-wide in the past four decades have shown that ionizing radiation processing could be an effective alternative to chemical treatment of foods (Gupta, 2001). Food irradiation is currently used for disinfestation, inhibition of sprouting, delay of fruit ripening, pasteurization and sterilization (Chauhan, Kumar, Nadanasabapathy, & Bawa, 2009; Grolichova, Dvoak, & Musilovaet, 2004; Sabato, da Silva, da Cruz, Salmieri, Relav& Lacroix, 2009; Sánchez-Bel, Egea, Romojaro, & Martìnez-Madrid, 2008; Waje et al., 2009).

Radiation processing has already found its area of commercial application, governments have approved the process, the food industry is using it and consumers respond favorably where the irradiated product is available on the market. Food preservation by γ-irradiation is gaining importance because it provides longer shelf lives without impairing wholesomeness.

Irradiation treatment does not cause any significant change in the proximate composition of dried fruits and nuts, and any adverse effects on either nutritional value or the sensory quality (Akingbohungbe, 1994; Narvaiz, Lescano, & Kairiyama, 1992).

Numerous scientific studies have demonstrated the beneficial effects for human health of the consumption of nuts, these being an important component of a balanced diet due to their high nutritional value. In spite of their high fat content, they possess elevated levels of mono and poly-unsaturated fatty acids and large quantities of vitamin E and fiber.

Almonds (*Prunus dulcis*), like all other nuts, are globally consumed for their desirable sensory and nutritional attributes (Su, Venkatachalam, Teuber, Roux, & Sathe, 2004). They are typically high in fat but their fatty acid profile is beneficial in relation to risk of coronary heart disease. Frequent almonds consumption is also associated with lower risk of diabetes and cancer. Quality of almonds may be substantially reduced if the product is subjected to insect damage during post-harvest storage on the ground and/or pre-harvest insect attack while the crop is still on the tree. Even more, growth of an aflatoxigenic *Aspergillus* species and production of aflatoxin may render almonds unfit for consumption. In both cases post-harvest preservation strategies are necessary to prevent mycotoxin production and/or insect growth The ionizing radiation treatment can be an effective method to kill insects and diminished

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http://dx.doi.org/10.1016/j.lwt.2014.04.055
0023-6438/

aflatoxin concentrations might be due to fungal destruction (Mexis, Badeka, Chouliara, Riganakos, & Kontominas, 2009).

The irradiation of food stuffs up to an overall dose of 10 kGy is permitted in numerous countries for commercial food processing (Lacroix & Quattara, 2000). According to the literature the lowest dose needed to control insects is about 0.5 kGy (Burbitt, Hungate, & Toba, 1989; Mansour & Al-Bachir, 1995). As for of mold growth control it has been shown that gamma irradiation at dose levels above 5 kGy is effective in reducing population on the surface of peanut kernels.

In addition, it was reported that at doses higher than 3 kGy both mycelium growth and toxin production of *Aspergillus flavus* were completely inhibited in ground nutmeg and peanuts (El-Bazza, Mahmoud, Roushdy, Farrag, & El-Tablawy, 1996; Hilmy, Chosby, & Matsuyama, 1995).

The applicability of the ionizing radiation treatment and its effects on the quality characteristics in nuts were discussed in many papers. According to P. Sanchez-Bel et al., irradiation of almonds up to 7 kGy does not cause significant changes neither in the sensorial quality nor in the contents of proteins, fiber, water or lipids with respect to the control samples; Mexis F.S et al. showed that the lipid oxidation parameters and volatile compounds of almonds increase proportionally to the dose but organoleptic remain acceptable up to a dose of 3 kGy. Similar results were obtained by Gölge and Ova (2008) on pine nuts.

The aim of this study was to evaluate any changes in fatty acid composition and α-tocopherol content, never assessed before simultaneously, of raw unpeeled almond kernels irradiated in dose range of 0.5–10 kGy.

2. Plant material and methods

Shelled almonds (*P. dulcis* (Mill.) D.A. Webb) variety Tuono were used for the experiments. The drupes were collected at the end of August 2009 in a local farm located in Naro, Agrigento (Sicily), and dried fruits were transferred to laboratory in polypropylene bags under cool conditions.

Unpeeled almond kernels were obtained from shells by hand-processing; they were crushed, crumbled, pounded in a mortar, packaged in polyethylene bags (50 micron thickness) and irradiated in the dose range 0.5–10 kGy.

2.1. Irradiation

Irradiation of the almond samples, (10 g each, packaged in polyethylene bags) with gamma rays was carried out at the ISOF-CNR Bologna, in the ^{60}Co Nordion Gammacell 220 (Canada), having a dose rate of about 7.5 Gy/min; irradiation chamber temperature during irradiation was 25 °C. The bags containing the almond samples were enclosed in a plastic chamber with wall thickness of 0.4 g/cm^2 which is suitable for establishing electronic equilibrium. The dose rate of the Gammacell for the reference geometry was determined with the alanine reference transfer standard dosimeters from RISØ high dose reference laboratory, with an expanded uncertainty of 2.8% at 95% confidence level. The applied doses were 0.5, 1.5, 3, 6, 8, and 10 kGy. The non irradiated samples were kept separated as control lots. Both control and irradiated samples were kept frozen and immediately analyzed.

2.2. Oil extraction

Almond oil was obtained using the Welmann method (G.S.C.L. 1976) from crushed unpeeled almond kernels (5 g) and 100 mL of ethyl ether. The extract obtained was washed twice with two aliquots of distilled water, dried with anhydrous sodium sulfate, and then was evaporated.

Almond oil thus obtained was used to determine the content of fatty acids and vitamin E, without prior lipid extraction (Mexis et al., 2009; Mexis & Kontominas, 2009).

3. Determination of fatty acid methyl esters

The fatty acid composition was determined according to the official method EEC (2568/91) Annex Xa Annex Xb, modified by Rec EEC *n*. 796/2002 method for the measurement of the characteristics of olive oil and olive-residue oil.

GC/MS analysis were carried out using a Thermo Scientific DSQ II single quadrupole system in EI (Electron Ionization) mode, working in full scan. The temperature of ion source and injector were 250 °C and 280 °C, respectively. The capillary column used was a AT-5MS (5% phenyl- methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm, Alltech Associates, Inc., Deerfield, Illinois, USA). The oven temperature was programmed from 130 to 170 °C at 10 °C/min, from 170 to 230 °C at 2 °C/min, from 230 to 280 °C at 40 °C/min, followed by 2 min under isothermal conditions. Helium was used as the carrier gas at a flow rate of 1 mL/min; 1 μl of sample was injected using a split ratio of 1:40. Identification of the individual components was performed using Thermo Scientific Xcalibur Data system software for Windows. Identification of fatty acid methyl esters was carried out using the mass spectrum libraries (Wiley7, Nist 02). Triplicate samples were prepared for every treatment and two determinations were carried out in triplicate sample ($n = 3 \times 2 = 6$).

4. Determination of α-tocopherol

4.1. Reagents and standards

Methanol, ethanol, water and ciclohexane HPLC grade were purchased from Carlo Erba (Milan, Italy). The standards α-tocopherol and α-tocopherol acetate were purchased from Sigma Aldrich (Milan, Italy).

α-tocopherol acetate was selected as the internal standard owing to its availability and its structural similarity to the compound assayed.

Stock standard solutions of α-tocopherol and α-tocopherol acetate were prepared in ethanol and stored at −4 °C in amber colored glass bottles to protect them from direct light exposure. Every 48 h the working standard solutions were prepared from stock solutions and stored at −4 °C, since standards are highly susceptible to oxidation and degradation.

The linear range was calculated by testing seven different concentrations of α-tocopherol. The peak-area ratio between α-tocopherol and α-tocopherol acetate versus the standard mass of α-tocopherol showed linear behavior in the concentration range between 0.07 μg/mL and 1.2 μg/mL ($r^2 = 0.997$); the Limit of Quantitation (LOQ) value was found to be 0.07 μg/mL; the Limit of Detection (LOD) was calculated as the concentration corresponding to three times the standard deviation of the background noise, and the value was 0.05 μg/mL.

The within-run precision of α-tocopherol was measured using the relative standard deviation (RSD) in 10 replicates of almond oil (51.3 mg/100 g extracted oil as mean value, RDS % = 3.4).

The standard addition method was used for testing the accuracy of the method. Standard of α- tocopherol was added to three aliquots of a diluted sample at three concentration levels in order to calculate the recovery rates (recovery %). Each analysis was carried out in triplicate. Results of recovery studies are presented in Table 1 and demonstrate the good recovery for α-tocopherol. These results

Table 1
% Recoveries of α-tocopherol from a spiked almond diluted sample.

	Present (μg/g)	Added (μg/g)	Found (μg/g)	Recovery %
α-tocopherol	0.16	0.08	0.25	104.8
		0.24	0.40	100.0
		0.32	0.49	102.0

confirmed no interferences effects due to the complexity of the matrix.

4.2. Sample preparation

Almond oil samples were diluted in ciclohexane (1:100, v/v) and 400 μl each were transferred to a screw-capped test tube together with 660 μl of the internal standard solution (α-tocopherol acetate in ethanol) and diluted to 2 mL with ethanol. Internal standard concentration in 2 mL ethanol solution was 100 μg/mL.

After shaking, the samples were filtered through a 0.45 μm pore size filter and directly injected into the HPLC system. Reversed phase HPLC with fluorescence detection was used to determine α-tocopherol content in sample oils (Gimeno, Castellote, Lamuela-Raventos, de la Torre, & Lopez- Sabater, 2000). The instrumentation was an Agilent 1100 Series liquid chromatograph included a binary pump (Model G1312A; Agilent, Agilent Technologies; Hewlett–Packard, Waldbronn, Germany), a fluorescence detector (Model G1312A; Agilent), a Rheodyne 7125 injection valve fitted with a 20 μl loop and a column temperature controller (Thermosphere TS-130; Phenomenex, Torrance, California).

Separation was achieved using a C18 5 μm particle size column (Luna C18 (2) 100A, 150 × 4.6 mm; Chemtek Analytica Srl, Anzola Emilia (BO), Italy), equipped with a security guard cartridge C18 (4 × 3.0 mm; Chemtek Analytica Srl, Anzola Emilia (BO), Italy), which was maintained at 40 °C. The mobile phase was methanol-water (96:4, v/v) and the isocratic elution was performed at a flow rate of 1.2 mL/min; fluorescence excitation and emission wavelengths were 290 nm and 340 nm, respectively, during the chromatographic run. An adequate signal-to-noise ratio was obtained with a photomultiplier gain of 15 and a response time of 4 s.

Mobile phase was prepared fresh daily and degassed ultrasonically for 20 min before use. The run time was 20 min and the retention times for α-tocopherol and α-tocopherol acetate were 11.7 and 16.7 min, respectively (Fig. 1).

5. Results and discussion

5.1. Fatty acid composition

Fatty acid composition of non-irradiated and irradiated raw unpeeled almonds variety Tuono (*P. dulcis* (Mill.) D. A. Webb) is shown in Table 2, expressed as the mean percentage value of each fatty acid with respect to the total fat content.

The main fatty acids were oleic ($C_{18:1\ n-9}$), linoleic ($C_{18:2\ n-9,12}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), elaidic ($C_{18:1\ n-9}$) and palmitoleic ($C_{16:1\ n-9}$). Traces of heptadecanoic ($C_{17:0}$) and arachidic ($C_{20:0}$) fatty acids were also detected but not included in Table 2. The content of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in non-irradiated unpeeled almond oils was 9.17, 79.59 and 11.24% respectively. Significant variations in oleic acid and linoleic acid content in almonds have been noted by several researchers (Mexis et al., 2009; Miraliakbari & Shahidi, 2008; Sabudak, 2007; Venkatachalam & Shridhar, 2006). Part of these variations may be due to cultivar differences.

The fatty acids compositions reported in our study are in good agreement with those reported in the literature for almonds variety Tuono (García-López, Grané-Teruel, Berenguer-Navarro, García-García, & Martín- Carratalá, 1996; Özcan, Ünver, Erkan, & Arslan, 2011; Piscopo, Romeo, Petrovicova, & Poiana, 2010). Data reported show that the harvest time and so the ripeness significantly influenced the fatty acids content. In fact, it is possible to observe an increase in oleic fatty acid content and a decrease of palmitic and linoleic fatty acids, with increasing harvest time (Piscopo et al., 2010). This trend was due to the biosynthesis of triglycerides during the almond ripening. This process induces the increasing of the oleic acid amount and so a rise of unsaturated/saturated acids and oleic/linoleic acids values. From these results the almonds seem to be very important for the human nutrition, because a MUFAs rich diet can regulate the lowdensity of lipoprotein cholesterol and total cholesterol levels as reported in literature (Sabate, Bell, & Fraser, 1996; Sabate & Hook, 1996; Zacheo, Cappello, Gallo, Santino, & Cappello, 2000). In addition, unsaturated fatty acids contribute to

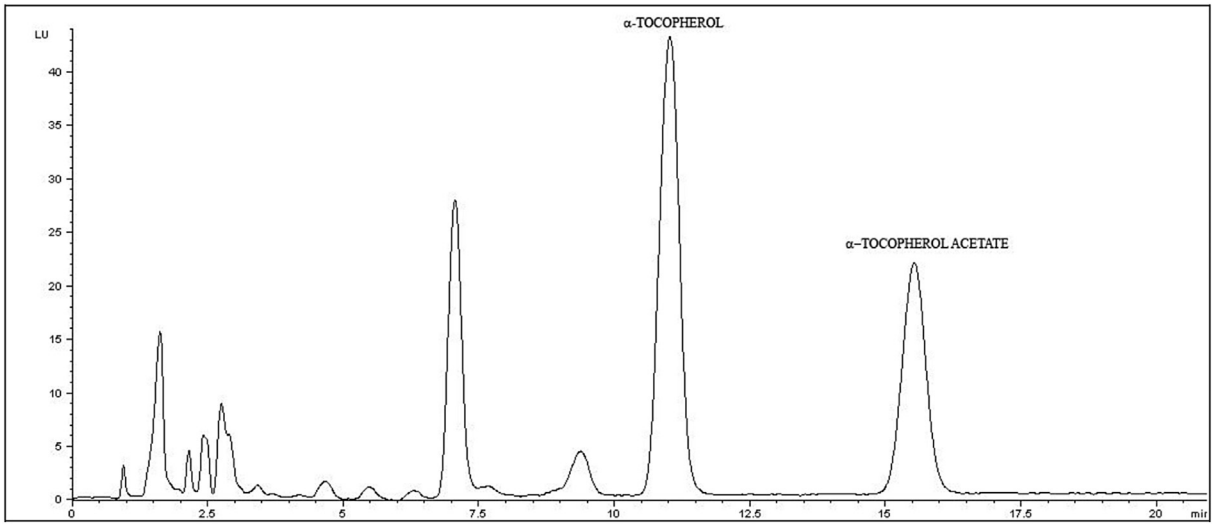

Fig. 1. A typical chromatogram of a sample of oil obtained from non-irradiated almonds. The retention times for α-tocopherol and α-tocopherol acetate were 11.7 and 16.7 min, respectively.

Table 2
% Fatty acid composition of oils obtained from control and irradied raw almonds, as function of irradiation dose. Values are the mean of six determinations ($n = 3 \times 2 = 6$), ±S.D.

Fatty acid	Control (0)	Irradiation dose (kGy)					
		0.5	1.5	3	6	8	10
Palmitic acid ($C_{16:0}$)	6.22 ± 0.63	6.23 ± 0.29	6.26 ± 0.28	6.26 ± 0.40	6.23 ± 0.08	6.22 ± 0.67	6.25 ± 0.06
Stearic acid ($C_{18:0}$)	2.95 ± 0.14	2.93 ± 0.25	2.92 ± 0.26	2.95 ± 0.16	2.97 ± 0.15	2.94 ± 0.32	2.95 ± 0.33
Total saturated	**9.17 ± 0.75**	**9.16 ± 0.54**	**9.18 ± 0.54**	**9.21 ± 0.56**	**9.20 ± 0.23**	**9.16 ± 0.99**	**9.20 ± 0.39**
Palmitoleic acid ($C_{16:1\ n-9}$)	0.32 ± 0.04	0.30 ± 0.05	0.32 ± 0.04	0.28 ± 0.03	0.30 ± 0.03	0.33 ± 0.08	0.32 ± 0.03
Oleic acid ($C_{18:1\ n-9}$)	77.32 ± 0.94	77.22 ± 0.86	77.23 ± 0.44	77.25 ± 0.62	77.30 ± 0.31	77.30 ± 2.05	77.29 ± 0.35
Elaidic acid ($C_{18:1\ n-9}$)	1.95 ± 0.13	2.01 ± 1.07	1.90 ± 0.04	1.99 ± 0.07	1.97 ± 0.17	1.94 ± 0.22	1.96 ± 0.24
Total Monounsaturated	**79.59 ± 1.11**	**79.53 ± 1.98**	**79.45 ± 0.52**	**79.52 ± 0.72**	**79.57 ± 0.51**	**79.57 ± 2.35**	**79.57 ± 0.62**
Linoleic acid ($C_{18:2\ n-9,12}$)	11.24 ± 0.63	11.31 ± 0.40	11.37 ± 0.39	11.27 ± 0.23	11.23 ± 0.22	11.27 ± 1.13	11.23 ± 0.28
Total Polyunsaturated	**11.24 ± 0.63**	**11.31 ± 0.40**	**11.37 ± 0.39**	**11.27 ± 0.23**	**11.23 ± 0.22**	**11.27 ± 1.13**	**11.23 ± 0.28**

the beneficial associations of frequent nuts intake observed in prevention of coronary heart disease (CHD), diabete, and decreases in other cardiovascular disease (CVD) risk factors (Kris-Etherton, Hu, Ros, & Sabaté, 2008).

The analysis of variance (*F*-test) was carried out on all the original data, to detect any dependence on dose of the percentage fatty acid composition. For each fatty acid under test, no significant difference was found at a confidence level of 95% among unirradiated and irradiated samples, no matter the dose applied.

5.2. α-Tocopherol content

Sample preparation for the analysis of α-tocopherol in foods generally includes saponification of the matrix followed by extraction of the analytes from the unsaponifiable matter (Lim, Woo, Kim, Jong, & Lee, 2007; Paixao & Campos, 2003; Panfili, Fratianni, & Irano, 2003).

However, the saponification causes pronounced losses of α-tocopherol even in protective conditions such as darkness and high nitrogen (Rupérez, Barbas, Castro, Martínez, & Herrera, 1998). In some cases, the direct solvent extraction (Mendoza, Pons, Bargallo, & Lopez-Sabater, 2003; Ryynänen, Lampi, Salo-Väänänen, Ollilainen, & Piironen, 2004) or the Soxhlet extraction (González, Pablos, Martín, León-Camacho, & Valdenebro, 2001; Ramadan & Moersel, 2002) of the vitamins, without previous saponification, is carried out. For the analysis of vitamin E in oils, the sample can be injected directly into the LC system after appropriate dilution in an organic solvent (Gliszczynska-Swiglo & Sikorska, 2004; Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Seppanen, Rahmani, & Csallany, 2003).

So, direct analysis after dilution, unlike saponification and extraction, simplifies the procedure and shortens the analysis. Moreover, many oil samples can be analyzed several times without altering the chromatographic efficiency or the column efficiency, which remains high.

Table 3
α-Tocopherol oils content (mg/100 g extracted oil) and % α-tocopherol content obtained from control and irradied raw almonds, as function of irradiation dose. Values are the mean of six determinations ($n = 3 \times 2 = 6$), ±S.D.

Irradiation dose (kGy)	α-tocopherol (mg/100 g)	% α-tocopherol content
Control (0)	50.6 ± 0.6	100
0.5	43.9 ± 0.5	86.7
1.5	42.2 ± 0.2	83.4
3	34.5 ± 1.2	68.2
6	9.2 ± 2.7	18.2
8	6.4 ± 0.4	12.6
10	UDL	0

UDL: Under Detection Limit.

Fig. 1 shows a typical chromatogram of a sample of non-irradiated almond oil. Dilution of the oil in ciclohexane in a 1:100 ratio resulted in chromatogram with α-tocopherol diluting separately from α-tocopherol acetate that is used as internal standard (I.S.).

α-tocopherol contents (mg/100 g extracted oil) of non-irradiated and irradiated raw unpeeled almonds variety Tuono (*P. dulcis* (Mill.) D. A. Webb) are reported in Table 3 and are expressed as mg/100 g of extracted oil.

Many published studies mention that α-tocopherol is the main vitamin-E active compound present in different varieties of almonds. The average content of α-tocopherol in non-irradiated unpeeled almond oils was 50.6 mg/100 g extracted oil. The results reported in Table 3 show that the almonds variety Tuono used in our study have a higher content of α-tocopherol compared with that found in other varieties (Kornsteiner, Wagner, & Elmadfa, 2006; Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004; Matthäus & Özcan, 2009).

Table 3 shows α-tocopherol contents of oil samples as function of irradiation doses absorbed by almonds; non irradiated samples at 0 KGy were considered as control.

A general trend observed is that increasing the dose of irradiation resulted in the decrease of α-tocopherol content. The results obtained in our study are in good agreement with observation in earlier studies other food materials (Bhatti, Ashraf, Shahid, Asi, & Mehboob, 2010; Camargo et al., 2012; Ma, Lu, Liu, & Ma, 2013). The α-tocopherol content of irradiated almond samples in the doses range 0.5–8 kGy ranged from 86.7% to 12.6%. At 10 kGy has been observed total reduction of α-tocopherol.

During the irradiation treatment, the temperature was maintained at 25° C; no ozone significant levels in irradiation environment has been detected, this means that the sample damage may be due exclusively to radiation.

6. Conclusion

The obtained results, measured no longer than 24 h after the irradiation treatment, showed that 0.5–10 kGy induced no significant change in fatty acid composition of almonds.

After the irradiation process the content of alpha-tocopherol is gradually decreased from 87.3, 81.8, 31.9 and 16.6 to radiation doses of 1.5, 3, 6 and 8 kGy, respectively, up to 10 kGy when the content of alpha-tocopherol was under detection limit.

So regarding these nutritional parameters, almonds irradiation treatment looks like a valid preservation method at low doses for insect control, but for higher doses aimed at microbial decontamination, tocopherol losses should be considered. It would be advisable to continue these studies along storage time.

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