



Analytical Methods

A combined methodology to detect γ -irradiated white sesame seeds and evaluate the effects on fat content, physicochemical properties and protein allergenicity

Panagiotis Zoumpoulakis^a, Vassilia J. Sinanoglou^{b,*}, Anthimia Batrinou^c, Irini F. Strati^b, Sofia Miniadis-Meimaroglou^d, Konstantinos Sflomos^a

^a Laboratory of Molecular Analysis, Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., 11635 Athens, Greece

^b Instrumental Food Analysis Laboratory, Department of Food Technology, Technological Educational Institution of Athens, Ag. Spyridonos, 12210 Egaleo, Greece

^c Biotechnology Laboratory, Department of Food Technology, Technological Educational Institution of Athens, Ag. Spyridonos, 12210 Egaleo, Greece

^d Food Chemistry Laboratory, Department of Chemistry, University of Athens, Panepistimiopolis Zographou, 15701 Athens, Greece

ARTICLE INFO

Article history:

Received 15 April 2011

Received in revised form 27 May 2011

Accepted 14 September 2011

Available online 19 September 2011

Keywords:

White sesame

γ -Irradiation

Radiolytic products

Lipids

Colour

Sesame allergens

ABSTRACT

White sesame seeds (*Sesamum indicum* L.) were studied with the purpose to evaluate γ -irradiation effect at 2.5, 5.0 and 10.0 kGy on lipid and fatty acid content, colour and protein allergenicity and to identify whether sesame oil was extracted from irradiated seeds by using High Resolution Nuclear Magnetic Resonance spectroscopy (HR-NMR). The fat decreased significantly, whereas triglyceride and phospholipid content was significantly decreased by the increase of irradiation dose. Monounsaturated and polyunsaturated fatty acids and unsaturated/saturated fatty acids ratio decreased. The presence of radiolytic decomposition products, shown by NMR, formed after a 2.5 kGy dose, confirmed that the lipid content decrease was a result of the irradiation process. The allergenicity of storage seed proteins was not significantly affected by irradiation up to 10.0 kGy. Colour parameter a^* increased after 5.0 kGy, while L^* values decreased significantly after 2.5 kGy. Conclusively, irradiation could be applied at doses <2.5 kGy while sustaining the sesame seed nutritional benefits.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

White sesame, (*Sesamum indicum* L.), a member of the Pedaliaceae family, is recognised as one of the oldest crops in the world, originating from India; where it has been used for more than 5000 years. The crop has since spread over many parts of the world, owing to its high content of excellent quality oil (42–54%) and protein (22–25%) (Orriño & Morgan, 2007) and has also been listed among neglected and underutilised crop species by the International Plant Genetic Resources Institute (IPGRI) as a crop with high potential (Were, Onkware, Gudu, Welander, & Carlsson, 2006). Sesame is most commonly found in Middle Eastern dishes, bakery products, dips, salad dressings, and vegetarian foods, used in the form of whole or crushed seeds, oil or meal. In Greece, sesame seeds are used in traditional vegetarian dishes (e.g. tahini, a form of sesame butter and halva) recommended as a part of a healthy diet or for fasting.

However, sesame is an emerging food allergen in the EU and US and carries a significant risk of severe reactions including anaphylaxis (Stutius et al., 2010). Food allergies have become an

important health problem all over the world. Adverse health effects due to allergic reactions to foods or food ingredients occur in about 1–3% of the population and about 4–6% of children (including food intolerances). Due to globalisation, the increased consumption of ethnic foods containing sesame seeds is paralleled with an increase in reported sesame-induced allergic reactions (Hsiao et al., 2006). Within the EU (European Community Directive 2003/89/EC), sesame is classified as an allergen for which food labelling is mandatory. The technology of food irradiation is seen by the industry as a means of ensuring food safety, since it exposes foods to ionising radiation that kills insects, moulds and bacteria. Sesame seed belongs to the category of spices and seasonings, for which irradiation is applied to ensure a safer and more plentiful food supply by extending shelf life (Lee, Kausar, & Kwon, 2008).

The need to eliminate bacterial pathogens from food products should always be balanced with product quality maintenance. Therefore, the evaluation of the irradiation effects on product nutritional value and quality is of equal importance to the determination of effective ionising radiation doses required for pathogen elimination. The present study focuses on three main aspects: firstly to determine the profiling and composition of lipids and fatty acids of sesame seeds, since they constitute a rich source of the nutritionally important lipids (e.g. phytosterols, lecithin) and

* Corresponding author. Tel.: +30 210 5385523.

E-mail address: v.sinanoglou@yahoo.gr (V.J. Sinanoglou).

unsaturated fatty acids; secondly to evaluate the effect of γ -irradiation on these beneficial compounds and physicochemical properties of sesame seeds and thirdly to test whether sesame oil can be identified as deriving, or not, from irradiated seeds using NMR spectroscopy techniques. Finally, the effect of γ -irradiation was assessed on the allergenicity of the major sesame allergens, which are seed storage proteins and constitute an increasing allergy risk. For this purpose sesame seeds were processed with ^{60}Co γ -irradiation at 2.5, 5.0 and 10.0 kGy irradiated doses.

2. Materials and methods

2.1. Reagents and standards

Analytical-grade solvents were obtained from Merck (Darmstadt, Germany). All reagents used were of analytical grade and they were purchased from Mallinckrodt Chemical Works (St. Louis, MO) and from Sigma Chemical Co. (Sigma–Aldrich Company, UK).

The lipid standards used for TLC–FID (thin layer chromatography–flame ionisation detector) and NMR spectroscopy were: cholesteryl oleate, cholesterol, tristearoyl-glycerol, lauric acid, oleic acid, linoleic acid, 1,2-distearoyl-glycerol, 1-monostearoyl-glycerol, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, lyso-phosphatidylcholine, phosphatidylinositol and phosphatidylserine standards of the Sigma Chemical Co. (Sigma–Aldrich Company, Dorset, Great Britain and St. Louis, MO).

Fatty acid methyl esters used as GC standards were: lauric acid M-E, cis-5,8,11,14,17-eicosapentaenoic acid M-E, and cis-4,7,10,13,16,19-docosahexaenoic acid M-E, (purity $\geq 98\%$) purchased from Sigma Chemical Co. (Sigma–Aldrich Company, UK); Matreya Bacterial Acid Methyl Esters CPTM Mix; Supelco™ 37 Component FAME Mix C4–C24; Supelco PUFA No. 1, Marine Source.

2.2. Radiation process

Samples of white sesame seeds (*S. indicum* L.) of Indian origin were purchased from a leading Greek supermarket chain. The samples were sealed in own-branded plastic bags of 50 g (all samples selected were of the same lot number). Irradiation was carried out in the ELVIONY S.A plant (Mandra Attikis, Greece). For γ -radiation, a cobalt-60 source irradiator was used. The activity of the source was 150 kCi, with a dose rate of 1 kGy/h. The samples were irradiated under an air atmosphere. The doses applied were 2.5, 5.0 and 10.0 kGy. The temperature during the irradiation treatment was maintained at approximately 25 °C (room temperature). Dosimetry was performed using a Harwell Perspex polymethylmethacrylate Amber 3042 dosimeter (PMMA Instruments, Harwell, UK) placed on the surface of the irradiated samples. Triplicate samples in two repetitions of irradiation process ($n = 6$) were carried out per dose.

2.3. Proximate analysis

Moisture content was determined by oven drying at 105 ± 2 °C to constant weight. Ash was determined by heating in a muffle furnace at 550 °C to constant weight.

Total lipids were extracted according to the Bligh and Dyer method (Bligh & Dyer, 1959). After phase equilibration, the lower chloroform layer (Total Lipids; TL) was removed and dried in a rotary vacuum evaporator at 32 °C. The extracted lipids were redissolved in chloroform/methanol (9:1, v/v), containing t-butyl-hydroquinone (0.1%) as an antioxidant and finally stored at 0 °C until used. Afterwards, representative aliquots of all of the above mentioned samples were evaporated in pre-weighed vials to constant weight to determine the lipid content.

2.4. Lipids and fatty acids analysis

Lipid classes were separated on silicic acid-coated quartz rods, chromarods (Type SIII) (5 mm silica gel-coated quartz rod, Iatron Labs, Tokyo, Japan) and afterwards they were quantified using a thin layer chromatography–flame ionisation detection system. TLC–FID analysis was performed by an Iatroscan thin-layer chromatograph (Model MK-6 TLC/FID-SPD Analyser Iatron Laboratories, Tokyo, Japan) equipped with a flame ionisation detector and connected to a personal computer for collecting the chromatograms. The neutral solvent system consisted of n-hexane:diethyl-ether:formic acid (n-H:DE:FA) (60:15:1.5 by vol.) while the polar solvent system consisted of chloroform:methanol:water (C:M:W) (50:20:2, by vol.). The determination of weight percentages of individual neutral and polar lipid classes was achieved according to the procedure described by Sinanoglou, Strati, and Miniadis-Meimaroglou (2011).

Fatty acid methyl esters (FAME) of total lipids were prepared according to the procedure described by Sinanoglou and Miniadis-Meimaroglou (1998). Both quantitative and qualitative analysis were performed on an Agilent 6890 Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionisation detector, according to the procedure described by Sinanoglou et al. (2011). DB-23 capillary column (60 m $\times$ 0.25 mm i.d. 0.15 μm film) [50%-Cyanopropyl)-methylpolysiloxane] (Agilent Technologies) was used.

2.5. NMR spectroscopy

NMR spectra were acquired on a Varian 600 MHz spectrometer under the same experimental conditions at 25 °C, using CDCl_3 99.96% D and 5 mm NORELL 509-UP-7 NMR tubes. The same sample concentration for quantitative ^1H NMR and ^1H 2D DOSY experiments was fixed at 5 mg of fat sample in 500 μl CDCl_3 . For the ^{13}C NMR and the 2D homo and heteronuclear experiments sample concentration was increased to 20 mg of fat sample in 500 μl CDCl_3 in order to reduce experimental time.

For the ^1H NMR, the Spectral width (SW) was set to 4807 Hz. For the acquisition of quantitative spectra, the Relaxation Delay (RD) was determined from T1 measurements and was set to 15 s, which corresponds to a maximum time of 5T1. For the ^{13}C NMR spectra, SW was set to 40,323 Hz and RD = 1.5 s. 2D NMR COSY, HSQC and HMBC experiments were performed using standard Varian sequences and gradient sequence pulses. Processing was performed using MestReNova software. For all spectra, zero filling up to 32 Kb data points and an exponential multiplication of the FID with a line broadening of 0.5 Hz was performed before Fourier transformation.

For the 2D DOSY ^1H NMR experiments, a stimulated echo bipolar gradient pulse sequence including a spoiler gradient of -7.92 G cm^{-1} was used with a diffusion gradient time of 2 ms, and a diffusion delay of 400 ms. Sequence parameters were adapted in order to have the intensity of typical NMR signals of triacylglycerols strongly decreased (at least divided by 50) at 95% of the full gradient strength. SW was set to 6000 Hz, and minimum and maximum gradient values were set 1000 and 15,000, respectively, with 15 number of increments and 256 number of scans. Ninety degrees pulse was adjusted to 7.58° and the process of DOSY was performed using standard Varian routines.

2.6. Colour measurement

Colour values such as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values were measured with a tristimulus chromameter (model CR-400, Minolta, Tokyo, Japan) calibrated

with a white standard. Three random readings per sample were taken and averaged.

2.7. Sesame protein allergenicity

For the quantitative analysis of sesame allergens in the samples, a commercially available ELISA immunoassay (BioKits Sesame protein Test Kit, Neogen) was used based on specific antibodies to sesame (*S. indicum*) storage proteins which form the major sesame allergens. The test is a non-competitive double-antibody (sandwich) enzyme-linked immunosorbent assay (ELISA) utilising antibodies to sesame proteins coated onto micro-wells. The ELISA results in development of a blue colouration (which turns yellow when 'stopped'), the level of which is proportional to the amount of sesame present in the sample. Quantitative determinations were made by constructing a standard curve with standards of known sesame allergen concentration (6.25, 12.5, 25.0, 50.0, 100 mg/kg). Samples were extracted using Tris buffered saline. After appropriate dilutions, 100 µl of the sample extract was added to each antibody coated well and incubated for 60 min at room temperature (RT). A wash step (ELISA kit ready to use wash buffer) was repeated five times, and then 50 µl of anti-sesame-biotin (biotinylated anti-sesame antibody) were added to the wells for an incubation of 60 min at RT. After a second wash cycle, 50 µl of avidin peroxidase (HRP) reagent was added in each well and incubated for 15 min at RT. A third wash cycle was performed and 100 µl of chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine) were added. After the completion of the final incubation (45 min at RT), 50 µl of phosphoric acid stop solution was added in each well and the absorbance was measured at 450 nm with the ELISA reader (Neogen Microwell Reader).

2.8. Statistical analysis

Measurements were obtained in triplicate and values were averaged and reported along with the standard deviation (SD). All data concerning lipid and fatty acid composition were analysed with One-Way ANOVA Post Hoc Tests and pairwise multiple comparisons were conducted with the Tukey's honestly significant difference test. Probabilities less than 0.05 were considered as statistically significant ($P < 0.05$). All statistical calculations were performed with the SPSS 10.0 (Chicago, IL, USA) statistical software for Windows.

3. Results and discussion

3.1. Proximate analysis of neutral and polar lipid composition

Irradiation caused insignificant change of moisture and ash content ($P > 0.05$) (Table 1). Sesame seed fat content was found 54.72% (wet basis) (Table 1). In agreement with our results, it is reported that the fat content of four sesame cultivars ranged from 54.3% to 56.1% (Uzun, Arslan, Karhan, & Toker, 2007). Sesame fat content is considered important in the food industry due to its flavour and stability and can be affected by genotype within the same species and also by environmental factors (Uzun et al., 2007).

Fat content was significantly ($P < 0.05$) reduced on irradiation (control, 54.72%; 10.0 kGy, 49.59%) (Table 1). In accordance with the above results, Bhat and Sridhar (2008) reported that crude lipid was significantly ($P < 0.05$) reduced on electron beam-irradiated lotus (*Nelumbo nucifera*) seeds (control, 3.68%; 30 kGy, 2.72%).

The lipids from the control (non-irradiated) and irradiated sesame seeds were analysed by TLC–FID to investigate if there was any change in the lipid profile and composition due to irradiation. Neutral lipids (NL), the major fraction, constituted about 97.0% of

Table 1

Moisture, ash and fat content (g/100 g wet weight), as well as neutral and polar lipids profile (% of TL) of non-irradiated and irradiated sesame seeds as determined by Iatroscan.

Samples	0 kG	2.5 kG	5.0 kG	10.0 kG
Moisture	1.97 ± 0.03a	1.98 ± 0.03a	1.99 ± 0.06a	2.03 ± 0.04a
Ash	3.28 ± 0.07a	3.09 ± 0.30a	3.43 ± 0.24a	3.17 ± 0.03a
Fat	54.72 ± 0.81a	53.59 ± 0.60a	51.86 ± 0.56b	50.19 ± 0.60c
NL% of TL	97.04 ± 0.23a	97.06 ± 0.24a	97.08 ± 0.19a	97.11 ± 0.25a
TG	95.32 ± 0.35a	95.04 ± 0.24a	93.11 ± 0.20b	90.74 ± 0.19c
FFA	0.69 ± 0.02a	0.83 ± 0.03b	2.41 ± 0.02c	4.36 ± 0.01d
Phytosterols	0.55 ± 0.04a	0.58 ± 0.03a	0.60 ± 0.03ab	0.61 ± 0.04b
DG	0.27 ± 0.02a	0.29 ± 0.03a	0.44 ± 0.02b	0.63 ± 0.02c
MG	0.21 ± 0.01a	0.30 ± 0.03b	0.52 ± 0.02c	0.77 ± 0.02d
PL% of TL	2.96 ± 0.23a	2.94 ± 0.24a	2.92 ± 0.19a	2.89 ± 0.25a
PA	0.13 ± 0.01a	0.13 ± 0.01a	0.16 ± 0.01b	0.19 ± 0.01c
PE	0.35 ± 0.02a	0.34 ± 0.02ab	0.34 ± 0.02ab	0.32 ± 0.01b
PS	0.11 ± 0.02a	0.11 ± 0.01a	0.11 ± 0.01a	0.10 ± 0.01a
PI	0.51 ± 0.02a	0.51 ± 0.02a	0.49 ± 0.01ab	0.48 ± 0.01b
PC	1.78 ± 0.03a	1.77 ± 0.03a	1.72 ± 0.02b	1.67 ± 0.02c
I-PC	0.08 ± 0.01a	0.08 ± 0.01a	0.10 ± 0.01b	0.13 ± 0.01c

Values are the mean of three determinations from two individual experiments ($n = 6$).

Means in the same row bearing different letters differ significantly ($P < 0.05$).

the total lipids and polar lipids (PL) represented 3.0% of the total lipids (Table 1). In agreement with our results, it is reported that the major lipid fractions in all sesame species were NL, representing 91.0–96.0% of the total sesame lipids (Abou-Gharbia, Shehata, & Shahidi, 2000). No significant differences were observed between NL and PL percentages as well as individual lipid classes' profile of the control and irradiated samples.

Sesame seed NL mainly consisted of triglycerides (TG) followed by phytosterols, free fatty acids (FFA), diglycerides (DG) and monoglycerides (MG). Irradiation, especially 10.0 kGy, caused significant ($P < 0.05$) decomposition in TG that was reflected in a more than six fold increase in FFA and appreciable increase in DG and MG (Table 1). In accordance with the above findings, Abou-Gharbia et al. (2000) reported that roasting of sesame seeds at 200 °C for 20 min caused some hydrolysis of TG and an increase in MG and FFA. Yoshida, Shigezaki, Takagi, and Kajimoto (1995) observed that microwaving of sesame seeds caused some hydrolysis of TG and an increase in DG and FFA. Similar results in food items reported by the same research group showed that irradiation caused a significant increase in the contents of FFA, indicating their liberation due to irradiation (Sinanoglou, Batrinou, Konteles, & Sflomos, 2007).

Concerning the PL, phosphatidylcholine (PC) was by far the largest component comprising 60.14% of total PL, while the proportion of phosphatidylinositol (PI) and phosphatidylethanolamine (PE) were 17.23% and 11.82%, respectively (data not shown, but calculated from Table 1). The remaining PL [phosphatidic acid (PA), phosphatidylserine (PS) and lyso-phosphatidylcholine (I-PC)] were found to be less than 12% of the total PL. Abou-Gharbia et al. (2000) reported that in Egyptian sesame seeds PC was found as the major PL component, and constituted about 55.0% of total PL, with PI, PE + PS and PA representing, 18.6%, 11.9% and 13.7% of the total PL, respectively, which are comparable to the above data.

At 10.0 kGy, a significant ($P < 0.05$) decrease in the PE, PI and PC percentages was observed (Table 1). Irradiation appeared to cause significant ($P < 0.05$) degradations in the above PhL into PA, which increased from 4.56% (of the total PL) in the control seeds to 6.47% in the case of irradiated seeds at a dose of 10.0 kGy (data not shown, but calculated from Table 1). Shadyro, Yurkova, Kisel, Brede, and Arnhold (2004) reported that γ -irradiation leads to the formation of phosphatidic acid in the course of radiolysis of natural glycerophospholipids. Abou-Gharbia et al. (2000) reported that roasting and microwaving process of sesame seed appeared to

cause significant degradations of PC, PE, PS and PI into PA, which increased dramatically. 1-PC, which can be considered a degradation product of PC, was significantly ($P < 0.05$) increased from about 2.55% (of the total PL) in the control seeds to about 4.40% in case of irradiated seeds at the dose of 10.0 kGy (data calculated from Table 1).

From a nutritional perspective, the quantitative individual neutral and polar lipid composition, expressed as g/100 g of the edible portion, was determined in non-irradiated and irradiated samples. Total phytosterol content remained unchanged ($P > 0.05$) at 0.30–0.31 g per 100 g of sesame seeds (data calculated from Table 1). Phytosterols are compounds that have a chemical structure very similar to cholesterol and, when present in the diet in sufficient amounts, reduce blood levels of cholesterol, enhance the immune response and decrease risk of certain cancers (Katan et al., 2003). Consumption of 1.8–2.0 g/day of plant sterols has been shown to lower both total LDL and cholesterol concentrations by 10–15% in a variety of different population groups (Katan et al., 2003). According to Phillips, Ruggio, and Ashraf-Khorassani (2005), sesame seeds had a higher total phytosterol content than other nuts and seeds consumed as snack foods.

Sesame seeds are an excellent source of dietary PC, providing 970 mg of PC per 100 g of seeds (data calculated from Table 1). In spite of the significantly ($P < 0.05$) PC content decrease (from 0.97 g/100 g of edible portion in the control sample to 0.84 g/100 g in the highest irradiated sample), its concentration was found to exceed the PC recommended daily intake (RDI) in the human diet (550 mg for men and 450 mg for women) (Sinanoglu et al., 2011).

Thus, irradiation did not affect sesame seeds as an excellent source of dietary phytosterol and lecithin for their beneficial effects in the human diet as well as for industrial and biomedical applications.

3.2. Fatty acid composition

The fatty acid profiles of total lipids of non-irradiated and irradiated sesame seeds at different doses are shown in Table 2. All sesame seeds lipid had oleic (C18:1 ω -9) and linoleic (C18:2 ω -6) acid as the most predominant among the unsaturated fatty acids, as well as palmitic (C16:0) and stearic (C18:0) acid as the most predominant among the saturated ones, which all together comprised about 96.5% of the total fatty acids. Sesame seed was lower in oleic acid (37.04%) and higher in linoleic acid (44.09%), in accordance with the results reported for East African sesame cultivars studied by Were et al. (2006), for sesame seeds studied by Lee et al. (2008) and for four sesame cultivars studied by Uzun et al. (2007).

Concerning the sums of the fatty acids, Σ PUFA occurred in the highest percentage followed by Σ MUFA, while Σ SFA seemed to have a lower percentage (Table 2). Therefore, sesame seeds were found to contain a higher proportion of MUFA and PUFA than SFA. Sankar, Sambandam, Ramakrishna, and Pugalendi (2005) reported that addition of sesame oil or other MUFA-rich nuts to the diet significantly improves the blood lipid profile. Due to its high proportion of unsaturated fatty acids, sesame seed oil is favourable for healthy nutrition.

At 2.5 kGy, C16:0 and C18:0 percentages increased significantly ($P < 0.05$), whereas C16:1 ω -9, C18:1 ω -9 and C24:1 ω -9 percentages presented slight, but insignificant ($P > 0.05$), decrease (Table 2). On the contrary, the percentages of MUFA (C18:1 ω -7 and C20:1 ω -9) and of PUFA (C18:2 ω -6, C18:3 ω -6, EPA and C22:5 ω -6) decreased significantly ($P < 0.05$). The above changes resulted in insignificant effect in the Σ MUFA while the significant ($P < 0.05$) percentage increase of Σ SFA was equal to the significant percentage decrease of Σ PUFA.

At 2.5 and 5.0 kGy, the C16:0, C18:2 ω -6 and C18:3 ω -6 percentages differed insignificantly, but showed significant change at 10.0 kGy (Table 2). On the other hand the differences in C16:1 ω -9 and C17:0 percentages were only significant between control samples and irradiated at 5.0 kGy. C20:1 ω -9 and C20:5 ω -3 percentages decreased significantly up to 5.0 kGy and then remained unchanged at 10.0 kGy. No significant difference was determined in the percentage of C18:1 ω -9, the first most abundant fatty acid in seeds, between control and irradiated samples. Hong, Kim, Cho, and Park (2010) reported that oleic acid in a food matrix seemed to be stable when subjected to irradiation at doses ranging from 0 to 60 kGy because antioxidants in food may prevent the action of free radicals. A significant, but not linear, increase in C18:0 and C19:0, as well as a decrease in C18:1 ω -7, C24:1 ω -9 and C22:5 ω -6, were observed with increasing doses of γ -irradiation.

All the above results demonstrated that the changes were not homogeneous for the different fatty acids and could be dependent on the specific effect of radiation process in the individual fatty acids in accordance with the irradiated item origin.

Another interesting feature was the formation of C18:2 ω -6*cis*-9, *trans*-11 geometrical isomer of C18:2 ω -6*cis*-9, *cis*-11 at a radiation dose of 10.0 kGy, as a minor component, but in measurable amounts, according to the statistical analysis (Table 2). Geißler, Brede, and Reinhardt (2003) reported that in γ -irradiating barley grains with doses of 10–100 kGy, a dose dependent isomerization of the naturally occurring *cis*-unsaturated fatty acids such as oleic, *cis*-vaccenic, linoleic and linolenic acid was found.

As Σ SFA increase and Σ PUFA decrease synchronously following irradiation of sesame seeds, PUFA/SFA and UFA/SFA ratios decreased ($P < 0.05$) compared to control samples. For the control samples and those irradiated at a dose of 2.5 kGy, the C18:1 ω -9/C18:2 ω -6 ratio increased ($P < 0.05$), whereas no significant differences were observed between groups as a result of irradiation.

A comparison of per cent levels of main fatty acids and their sums in total lipids may result in a misleading notion concerning their stability towards irradiation effect. In a characteristic example in the present study, on a percentage basis, irradiated seeds presented a significant C16:0 increase and an insignificant C18:1 ω -9 change (Table 2). However, on a weight basis, it was observed that the increase of irradiation dose resulted in an insignificant C16:0 content change and a significant ($P < 0.05$) C18:1 ω -9 content decrease (Table 2). A similar observation concerns the percentages of C18:0 (% of TL) that differed significantly in all irradiated samples, but their concentrations (expressed as g/100 g edible portion) were found quite similar between the control and irradiated samples at 2.5 kGy, as well as between irradiated samples at 5.0 and 10.0 kGy (Table 2). In accordance with the above results Gölge and Ova (2008) reported that the effect of irradiation dose up to 5 kGy on C16:0 and C18:0 was statistically insignificant for pine nuts. Non-irradiated seeds provided the highest ($P < 0.05$) contents of TFA, MUFA and PUFA, which is related not only to the individual fatty acid percentages, but to their higher fat content (Tables 1 and 2). Irradiation caused a significant gradual decrease of MUFA and PUFA content as irradiation dose increased, but a significant SFA content increase occurred only at doses > 5.0 kGy (Table 2).

3.3. NMR analysis

High-resolution NMR spectroscopy is a method of choice in order to provide insight into the nature of mixtures present in oils and fats (Hidalgo & Zamora, 2003).

For a long time, the determination of fatty acids' composition by NMR has been based on the ^{13}C nucleus with time consuming quantification techniques. However, due to its higher sensitivity and natural abundance, proton high field NMR (at least 500 MHz)

Table 2

Fatty acids composition in total lipids of non-irradiated and irradiated sesame seeds.

Fatty acids	0 kG	2.5 kG	5.0 kG	10.0 kG
<i>Fatty acids composition [% (w/w)] in total lipids</i>				
C10:0	0.12 ± 0.01a	0.11 ± 0.00a	0.11 ± 0.00a	0.12 ± 0.01a
C14:0	0.13 ± 0.00a	0.14 ± 0.00b	0.13 ± 0.00c	0.14 ± 0.00d
C16:0	10.65 ± 0.25a	11.87 ± 0.13b	11.98 ± 0.15b	12.65 ± 0.24c
C16:1 ω -9cis	0.18 ± 0.01a	0.17 ± 0.01a	0.14 ± 0.01b	0.13 ± 0.01b
C17:0	0.06 ± 0.01a	0.06 ± 0.01a	0.09 ± 0.01b	0.08 ± 0.01b
C18:0	4.73 ± 0.05a	4.96 ± 0.05b	5.94 ± 0.08c	6.18 ± 0.05d
C18:2 ω -6cis	44.09 ± 0.30a	42.99 ± 0.42b	42.22 ± 0.42bc	41.68 ± 0.41c
C18:1 ω -9cis	37.04 ± 0.36a	36.94 ± 0.38a	36.72 ± 0.37a	36.32 ± 0.37a
C18:1 ω -7cis	1.04 ± 0.01a	0.98 ± 0.01b	0.91 ± 0.01c	0.86 ± 0.01d
C18:2 ω -6c-9, tr-11	–	–	–	0.20 ± 0.01
C18:2 ω -6cis	44.09 ± 0.30a	42.99 ± 0.42b	42.22 ± 0.42bc	41.68 ± 0.41c
C18:3 ω -6cis	0.37 ± 0.01a	0.35 ± 0.01b	0.34 ± 0.01b	0.31 ± 0.01c
C19:0	0.44 ± 0.01a	0.46 ± 0.01b	0.50 ± 0.01c	0.52 ± 0.01d
C20:0	0.22 ± 0.01a	0.22 ± 0.01a	0.22 ± 0.01a	0.22 ± 0.01a
C20:1 ω -9cis	0.20 ± 0.01a	0.18 ± 0.01b	0.17 ± 0.01bc	0.16 ± 0.01c
C20:5 ω -3cis	0.19 ± 0.01a	0.15 ± 0.01b	0.11 ± 0.01c	0.10 ± 0.01c
C22:5 ω -6cis	0.38 ± 0.01a	0.29 ± 0.01b	0.27 ± 0.01c	0.19 ± 0.01d
C24:1 ω -9cis	0.19 ± 0.01a	0.18 ± 0.01a	0.16 ± 0.01b	0.14 ± 0.01c
$\Sigma\omega$:0 (Σ SFA)	16.34 ± 0.23a	17.82 ± 0.19b	18.97 ± 0.21c	19.92 ± 0.29d
$\Sigma\omega$:1 (Σ MUFA)	38.63 ± 0.38a	38.44 ± 0.39a	38.10 ± 0.38a	37.60 ± 0.38a
$\Sigma\omega$:n (Σ PUFA)	45.02 ± 0.30a	43.78 ± 0.42b	42.93 ± 0.43c	42.48 ± 0.42c
C18:1 ω -9cis/C18:2 ω -6cis	0.84 ± 0.01a	0.86 ± 0.01b	0.87 ± 0.01b	0.87 ± 0.01b
PUFA/SFA	2.76 ± 0.09a	2.46 ± 0.01b	2.26 ± 0.01c	2.13 ± 0.01d
UFA/SFA	5.12 ± 0.08a	4.62 ± 0.06b	4.27 ± 0.05c	4.02 ± 0.07d
<i>Main fatty acid and fatty acid sums composition expressed as g/100 g edible portion</i>				
C16:0	4.44 ± 0.23a	4.50 ± 0.12a	4.59 ± 0.14a	4.70 ± 0.11a
C18:0	2.02 ± 0.02a	2.01 ± 0.02a	2.28 ± 0.02b	2.30 ± 0.02b
C18:1 ω -9cis	15.81 ± 0.16a	14.95 ± 0.15b	14.08 ± 0.14c	13.51 ± 0.14d
C18:2 ω -6cis	18.82 ± 0.13a	17.40 ± 0.17b	16.19 ± 0.16c	15.50 ± 0.15d
$\Sigma\omega$:0 (SFA)	6.88 ± 0.11a	6.92 ± 0.09a	7.27 ± 0.07b	7.41 ± 0.12b
$\Sigma\omega$:1 (MUFA)	16.49 ± 0.22a	15.56 ± 0.16b	14.61 ± 0.16c	13.98 ± 0.14d
$\Sigma\omega$:n (PUFA)	19.22 ± 0.13a	17.72 ± 0.17b	16.46 ± 0.16c	15.80 ± 0.16d
TFA	42.58 ± 0.35a	40.19 ± 0.37b	38.35 ± 0.35c	37.19 ± 0.33d

Values are the mean of three determinations from two individual experiments ($n = 6$).Means in the same row bearing different letters differ significantly ($P < 0.05$).

can be a more powerful technique for fatty acid composition determination of edible oils.

3.3.1. Structure elucidation of triglycerides

In order to examine differences between non-irradiated and irradiated samples, we initially elucidated the structure of oleic and linoleic triglycerides, which constitute almost 80% of the total lipids present in the sesame oil, using 2D NMR techniques. The fatty acid profiles of total lipids of sesame seeds have oleic (C18:1 ω -9) and linoleic (C18:2 ω -6) acids as the most predominant ones among the unsaturated fatty acids. ^1H NMR spectrum is shown in Fig. 1a and assignment of its peaks is shown in Table 3. The full assignment, which was achieved using 2D COSY, TOCSY, HSQC and HMBC techniques, is by comparison with a previous study (Segaa et al., 2010). Moreover, we have managed to distinguish several peaks of oleic and linoleic triglycerides. More specifically, from the triplet peak at 2.73 ppm, which is attributed to bis-allylic protons of linoleic acid, vinylic protons of linoleic acid at 5.31 ppm can be separated from the corresponding of oleic acid at 5.33 ppm. Furthermore, the allylic protons of linoleic acid resonate at 1.97–1.99 ppm, while the allylic protons of oleic resonate at 2.01–2.03 ppm (Table 3).

3.3.2. Quantification of esterified fatty acids

Several methodologies have been described in the literature for fatty acid content determination through ^1H NMR spectra using mathematical approaches. In this study, we have implemented the methodology introduced by Barison et al. (2010) which is based on the fact that all fatty acyl chains are esterified to glycerol in order to form triglycerides. As the area of the signals in the ^1H NMR spectra is proportional to the number of corresponding hydrogens in the

sample, the fatty acid composition can be determined through the relation between the integrated peak areas from the characteristic signals of each fatty acyl chain and those from the glycerol backbone in the NMR spectra. Signals at 4.12 and 4.27 ppm, attributed to the α hydrogens, are suitable for this purpose, since they do not overlap.

This methodology can be applied in the specific samples, since they do not contain significant amounts DG or MG, which could bias our results. ^1H NMR chemical shifts of mono- and DG are different from those of TG and cannot be detected in the spectra (Fig. 1a). As shown earlier (Table 1), the concentration of MG was determined as 0.21% and DG as 0.27% for the non-irradiated samples. In general MG and DG exist in very small amounts in edible oils (Barison et al., 2010). Furthermore, absence of proton peaks around 11.50 ppm and absence of carbon peaks around 174–176 ppm, characteristic for the carboxylate proton and carbon correspondingly, proves the absence of free fatty acids in a detectable concentration for NMR.

Since C18-3 PUFA are not detected in the ^1H NMR spectra (absent triplet peak at 0.98 ppm, characteristic for the methyl hydrogens of the linolenic acid), we focused our study on the C18-2 linoleic and C18-1 oleic acids. More specifically, by using the methodology described elsewhere (Barison et al., 2010) we determined the % mol of linoleic, oleic acids as well as SFA in the non-irradiated samples. Results showed that unsaturated linoleic and oleic acids are calculated to a total of 84.55% mol, and SFA is calculated at 14.45% mol in the non-irradiated sample in accordance with the results of Table 2.

Evaluation of the corresponding concentrations in the irradiated samples was not possible due to the radiolytic degradation of triglycerides. This can be deduced from the ratio between the areas

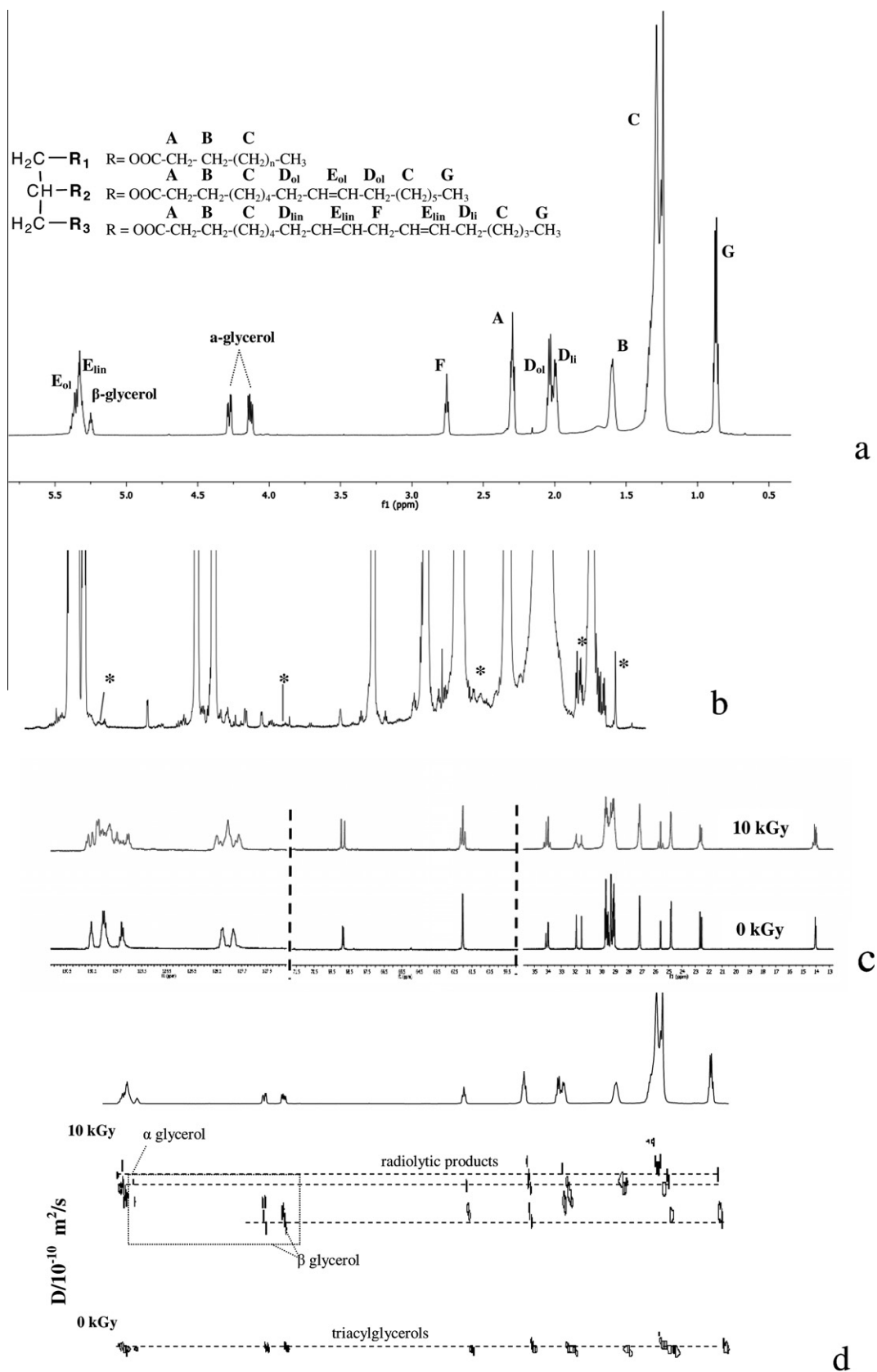
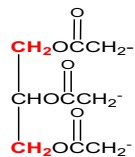
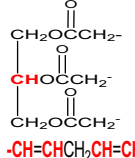
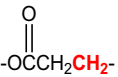
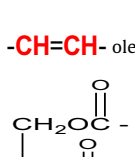
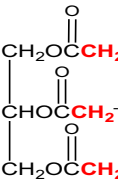
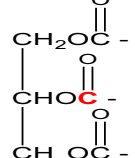
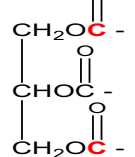


Fig. 1. (a) Assignment of ^1H NMR peaks of main triglycerides; (b) identification of sterol peaks using spiking experiments; (c) comparison of ^{13}C NMR spectra of non-irradiated and 10 kGy irradiated samples; (d) comparison of 2D DOSY ^1H NMR experiments, 0 kGy (down) and 10 kGy (up).

from the characteristic β - and α -proton signals of the glycerol moiety. This ratio was calculated to be 1:2 as expected for the

non-irradiated sample, and was reduced to 1:1.5 for the 10.0 kGy irradiated sample.

Table 3Assignment of ^1H and ^{13}C NMR peaks of major TG in non-irradiated sesame oil.

Moiety	^1H (ppm)	^{13}C (ppm)	Moiety	^1H (ppm)	^{13}C (ppm)
$-\text{CH}_3$	0.85 (m)	14.04		4.10 (dd, $J = 11.9, 5.9$ Hz) 4.26 (dd, $J = 11.9, 4.2$ Hz)	62.0
$-(\text{CH}_2)_n-$	1.23–1.30 (m)	22.5, 22.6, 29.0, 29.28, 29.65, 31.5, 31.8		5.23 (m)	68.85
	1.58 (m)	24.8		5.31 (m)	127.8, 128.0
$-\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2-$	1.98 (m)	27.1	$-\text{CH}=\text{CH}-$ linoleic	5.33 (m)	129.6, 129.9, 130.1, 172.7
$-\text{CH}_2\text{CH}=\text{CHCH}_2-$	2.02 (q, $J = 14.1, 7.2$ Hz)	27.1	$-\text{CH}=\text{CH}-$ oleic	-	-
	2.27 (m)	33.9, 34.1		-	173.1
$-\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}-$	2.73 (t, $J = 6.7$ Hz)	25.6		-	-

3.3.3. Formation of radiolytic products

Even though the profile of the ^1H NMR spectra is identical at all doses of irradiation, comparison of ^{13}C NMR peaks between non-irradiated and irradiated samples (Fig. 1b), provide clear evidence for the formation of new molecules, especially after the highest irradiation dose. The appearance of new peaks is obvious along almost the entire spectral width. More specifically, the ^{13}C spectral pattern of vinylic carbons of unsaturated fatty acids from ~127 to 130 ppm, α -carbons of glycerol at ~62 ppm, and aliphatic carbons from ~13 to 35 ppm are significantly changed after irradiation (Fig. 1c).

It is already known that the formation of new products occurs from the radiolytic degradation of free fatty acids and TG, to hydrocarbons and 2-alkylcyclobutanones (Lee et al., 2008). More specifically, irradiation of lipid-containing foods, initiates a radiolytic decomposition mechanism producing two main types of hydrocarbons, C_n-1 and C_n-2 coming from the α - and β -cleavages near the carbonyl group of the fatty acids (Dubravcic & Nawar, 1968). 2-alkylcyclobutanones have the same number of carbon atoms as the parent fatty acids from which they are formed with an alkyl group located in ring position 2. There are cyclic compounds formed by the loss of an electron from the oxygen on the carbonyl of fatty acids or TG, followed by a rearrangement process (Dubravcic & Nawar, 1968). Radiation induced hydrocarbons and 2-alkylcyclobutanones have been previously detected by gas chromatography/mass spectrometry (GC/MS) analysis and have been used as irradiation detection markers for food samples since they are not formed during cooking or as a result of oxidation of fatty acids (Lee, Byun, & Kin, 2000). As fatty acid moieties in

triglycerides are broken into the α and β positions when irradiated, the reduction of the α/β glycerol ratio, shown above, can be explained.

3.3.4. Identification of sterols

Spiking NMR experiments were followed to prove the existence of sterols in the lipid samples (Fig. 1c). More specifically, samples contained small amounts of stigmasterol, which can be detected from increase of the single ^1H NMR peak at 0.68 ppm and the characteristic doublets at 5.00 and 5.12 ppm. The single peak at 0.67 ppm can be attributed to β -sitosterol (Phillips et al., 2005). Both sterols are shown to remain untouched during irradiation, even at higher doses.

3.3.5. 2D diffusion-ordered spectroscopy (DOSY) ^1H NMR as a detection tool. 2D DOSY ^1H NMR experiments were performed in order to examine whether sesame oil can be identified as being derived from irradiated seeds or not. DOSY is a powerful technique based on the measurement of diffusion coefficients and is often described as “in tube chromatography” as it leads to a virtual separation of species.

Fig. 1d represents a comparison of the 2D DOSY spectra of the non-irradiated and the 10.0 kGy irradiated samples, where the formation of radiolytic products is apparent due to the difference in their diffusion rate. The signals formed at 10.0 kGy indicate the presence of hydrocarbon chains detached from the glycerol unit. It also provides evidence that cleavage occurred in the glycerol moiety giving signals of different diffusion rates for the α - (D /

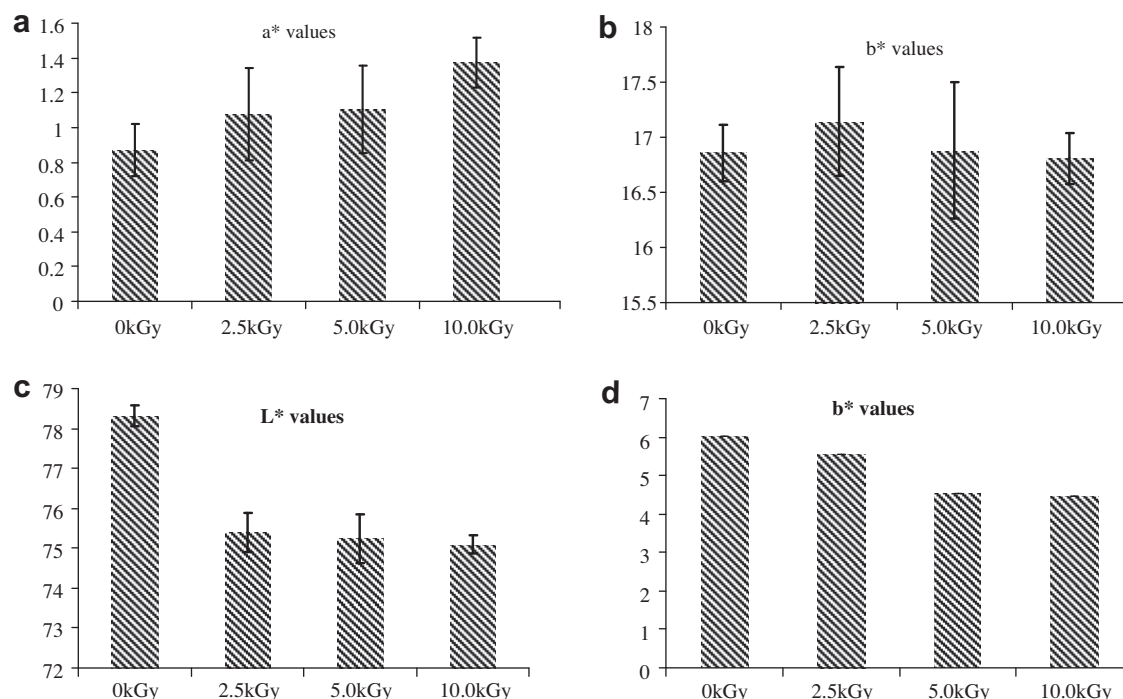


Fig. 2. Mean values of colour parameters a^* (a), b^* (b) and L^* (c), in non-irradiated (0 kGy) and irradiated sesame seeds (at 2.5, 5.0 and 10.0 kGy) and b^* (d) in non-irradiated and irradiated sesame oil extract. Values are the mean of three determinations from two individual experiments ($n = 6$) $\pm$ standard deviation.

10^{-10} 6.42–6.74 m^2/s) and b - ($D/10^{-10}$ 5.95 6.74 m^2/s) glycerol hydrogens.

3.4. Colour values

The effects of the irradiation doses on a^* , b^* and L^* values are shown in Fig. 2a–c. The a^* (redness) values of irradiated seeds compared to control samples, had a significant ($P < 0.05$) increase at 10.0 kGy. The b^* (yellowness) values of seeds were insignificantly ($P > 0.05$) affected. However, the L^* (lightness) values were significantly ($P < 0.05$) decreased between the control and irradiated samples. It has been reported that irradiation could affect the colour of a food product depending on the type and colour intensity of the food material (Gölge & Ova, 2008). Mexis and Kontominas (2009) reported that L^* and b^* values of cashew nuts were unaffected by irradiation up to a dose of 7 kGy, while a^* value increased at doses > 3 kGy reflecting a darkening of cashew nuts' colour. Sesame seeds contain the required reaction compounds (sugars and amine groups as found in protein molecules), to give the Maillard reaction products (Horrobin, Landman, & Ryder, 2003). Therefore, colour changes may be explained by the breakdown of glycosidic and peptidic linkages, promoted during irradiation, forming Maillard reaction browning products. The present study also determined the colour changes on extracted sesame oil since this is the main product derived from sesame seeds. It was observed that only the b^* values of extracted sesame oil were significantly decreased at the higher doses of 5.0 and 10.0 kGy, meaning that irradiation provoked decolourisation of the oil (Fig. 2d).

3.5. Sesame protein allergenicity

Fig. 3 shows results of quantification of allergenic proteins in analysed sesame samples. Values of allergenic proteins were estimated according to a constructed standard curve. ANOVA analysis has not revealed any significant differences ($P > 0.05$) among the mean values of allergenic protein concentration in sesame seeds between control and irradiated samples (2.5, 5.0 and 10.0 kGy).

Sesame allergy is a significant, serious, and growing problem. Evidence exists of the ability of protein and oil components of sesame to trigger immediate hypersensitivity through IgE antibody and delayed hypersensitivity through cell-mediated immune responses, respectively (Gangur, Kelly, & Navuluri, 2005). Sesame seeds contain two major families of seed storage proteins, 11S globulin (α -globulin) and 2S albumin (β -globulin), accounting for 80–90% of total seed proteins in sesame, and a smaller family 7S globulin accounting for 5% of total sesame protein (Orruño & Morgan, 2007). Storage proteins serve as a source of amino acids during germination and post-germinative growth of seedlings and are known food allergens in several plants, including peanut, walnut, Brazil

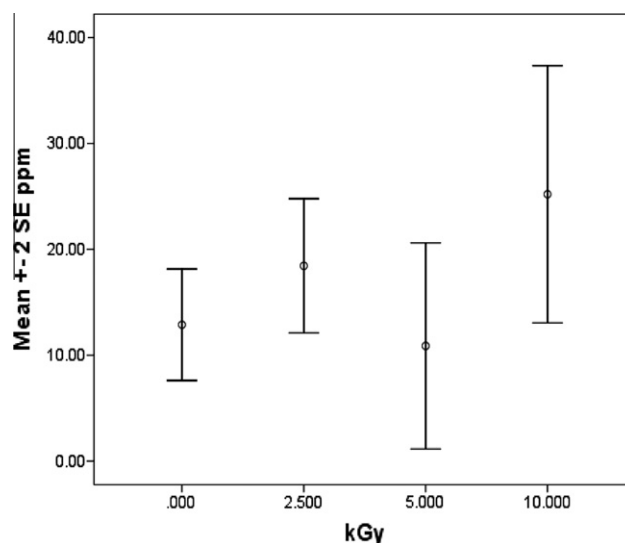


Fig. 3. Mean values of concentration (ppm) of allergenic proteins in non-irradiated (0 kGy) and irradiated sesame seeds (at 2.5, 5.0 and 10.0 kGy). Values are the mean of three determinations from two individual experiments ($n = 6$). Differences are not significantly different ($P > 0.05$). The standard error of mean increases linearly as irradiation dose increases.

nut, soybeans and sesame (Moreno, Maldonado, Wellner, & Mills, 2005). Most major seed allergens belong to the 2S albumin family and are compact globular proteins, rich in methionine and cysteine. Major white sesame 2S albumin (Ses i 1) was found to be extremely stable to acid conditions, thermal processing and in vitro gastrointestinal digestion and was also found to retain both linear and conformational epitopes following severe heat treatment. These properties are considered to be crucial for a protein to provoke an allergic reaction in a sensitised individual (Moreno et al., 2005; Wolff et al., 2004). Food-irradiation technology has been found to alter the structure of epitopes on certain food allergens, therefore reducing their allergenicity (Sinanoglou et al., 2007). However, the seed storage proteins have unusually high stability to denaturation and proteolysis, a property that may relate to their allergenicity. The abundance of cysteine residues in the 2S albumin precursor, which are involved in the formation of disulphide linkages, may certainly contribute to heat resistance (Wolff et al., 2004). This study confirms that the allergenicity of the sesame storage proteins was not significantly altered by the application of γ -irradiation for doses up to 10.0 kGy. It has been observed however, that the standard error of mean increased in a linear trend as irradiation doses increased, exhibiting the highest standard error in the highest dose (10.0 kGy) (Fig. 3). This may be interpreted by the assumption that irradiation caused conformational changes of the globular sesame seed allergenic proteins in a random way which resulted in either exposing some inner epitopes (increasing of allergenicity) or hiding epitopes (reducing allergenicity).

4. Conclusions

Sesame seeds are considered to be a health-promoting food containing a higher proportion of MUFA than SFA and also bioactive compounds such as lecithin and phytosterols. The combination of beneficial fatty acid profile and the rich nutritional composition of sesame makes it a unique and excellent food with functional components. Irradiation of sesame seeds at the doses studied showed significant effects on the total, neutral and polar lipids content, as well as on colour values. At irradiation doses of up to 2.5 kGy, the seeds retained the nutritional benefits of its fatty acids, in particular MUFA as well as of its phytosterols and lecithin. ^1H , ^{13}C and 2D ^1H DOSY NMR experiments indicated that the presented differences in irradiated samples are attributed to the effect of radiolytic decomposition of triglycerides. This becomes obvious after the 2.5 kGy dose. Moreover, the 2D DOSY technique could be applied as a qualitative detection tool since it provides information for the irradiated samples from low doses. Furthermore, the allergenicity of white sesame seed proteins was not significantly affected, even by the highest dose of irradiation (10.0 kGy). As the purpose of irradiation is mainly to ensure the microbiological safety of the product for human consumption, irradiation could be applied at doses up to 2.5 kGy, sustaining the quality of sesame seeds as assessed from proximate analysis and physicochemical assays.

Acknowledgements

This research was supported in part by the Special Research Account of National and Kapodistrian University of Athens under Project Number 70/4/8788.

References

Abou-Gharbia, H. A., Shehata, A. A. Y., & Shahidi, F. (2000). Effect of processing on oxidative stability and lipid classes of sesame oil. *Food Research International*, 33, 331–340.

Barison, A., Da Silva, C. W. P., Campos, F. R., Simonelli, F., Lenz, C. A., & Ferreira, A. G. (2010). A simple methodology for the determination of fatty acid composition

in edible oils through ^1H NMR spectroscopy. *Magnetic Resonance in Chemistry*, 48(8), 642–650.

Bhat, R., & Sridhar, K. R. (2008). Nutritional quality evaluation of electron beam-irradiated lotus (*Nelumbo nucifera*) seeds. *Food Chemistry*, 107, 174–184.

Bligh, E. D., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911–917.

Dubravac, M. F., & Nawar, W. W. (1968). Radiolysis of cleavage in simple triglycerides. *JAOCs*, 45, 656–660.

Gangur, V., Kelly, C., & Navuluri, L. (2005). Sesame allergy: A growing food allergy of global proportions? Review article. *Annals of Allergy, Asthma and Immunology*, 95, 4–11.

Geißler, C., Brede, O., & Reinhardt, J. (2003). Cis–trans-isomerization of unsaturated fatty acids during-irradiation of barley grains. *Radiation Physics and Chemistry*, 67, 105–113.

Gölge, E., & Ova, G. (2008). The effects of food irradiation on quality of pine nut kernels. *Radiation Physics and Chemistry*, 77, 365–369.

Hidalgo, F. J., & Zamora, R. (2003). Edible oil analysis by high-resolution nuclear magnetic resonance spectroscopy: Recent advances and future perspectives. *Trends in Food Science and Technology*, 14(12), 499–506.

Hong, S. I., Kim, J. Y., Cho, S. Y., & Park, H. J. (2010). The effect of γ -irradiation on oleic acid in methyl oleate and food. *Food Chemistry*, 121, 93–97.

Horrobin, D. J., Landman, K. A., & Ryder, L. (2003). Interior and surface colour development during wheat grain steaming. *Journal of Food Engineering*, 57, 33–43.

Hsiao, E. S., Lin, L. J., Li, F. Y., Wang, M. M., Liao, M. Y., & Tzen, J. T. (2006). Gene families encoding isoforms of two major sesame seed storage proteins, 11S globulin and 2S albumin. *Journal of Agriculture and Food Chemistry*, 54, 9544–9550.

Katan, M. B., Grundy, S. M., Jones, P., Law, M., Miettinen, T., & Paoletti, R. (2003). Efficacy and safety of plant sterols and sterols in the management of blood cholesterol levels. *Mayo Clinic Proceedings*, 78, 965–978.

Lee, H. J., Byun, M. W., & Kim, K. S. (2000). Detection of radiation induced hydrocarbons and 2-alkylcyclobutanones in irradiated perilla seeds. *Journal of Food Protection*, 63, 1563–1569.

Lee, J., Kausar, T., & Kwon, J.-H. (2008). Characteristic hydrocarbons and 2-alkylcyclobutanones for detecting γ -irradiated sesame seeds after steaming, roasting, and oil extraction. *Journal of Agriculture and Food Chemistry*, 56, 10391–10395.

Mexis, S. F., & Kontominas, M. G. (2009). Effect of γ -irradiation on the physicochemical and sensory properties of cashew nuts (*Anacardium occidentale* L.). *LWT – Food Science and Technology*, 42, 1501–1507.

Moreno, F. J., Maldonado, B. M., Wellner, N., & Mills, E. N. (2005). Thermostability and in vitro digestibility of a purified major allergen 2S albumin (Ses i 1) from white sesame seeds (*Sesamum indicum* L.). *Biochimica et Biophysica Acta*, 1752, 142–153.

Orruño, E., & Morgan, M. R. A. (2007). Purification and characterisation of the 7S globulin storage protein from sesame (*Sesamum indicum* L.). *Food Chemistry*, 100, 926–934.

Phillips, K. M., Ruggio, D. M., & Ashraf-Khorassani, M. (2005). Phytosterol composition of nuts and seeds commonly consumed in the United States. *Journal of Agriculture and Food Chemistry*, 53, 9436–9445.

Sankar, D., Sambandam, G., Ramakrishna, R. M., & Pugalendi, K. V. (2005). Modulation of blood pressure, lipid profiles and redox status in hypertensive patients taking different edible oils. *Clinica Chimica Acta*, 355, 97–104.

Segaa, A., Zanardia, L., Chiasserinia, L., Gabbriellia, A., Boccia, V., & Travaglia, V. (2010). Properties of sesame oil by detailed ^1H and ^{13}C NMR assignments before and after ozonation and their correlation with iodine value, peroxide value, and viscosity measurements. *Chemistry and Physics of Lipids*, 163, 148–156.

Shadyro, O., Yurkova, I., Kisel, M., Brede, O., & Arnhold, J. (2004). Formation of phosphatidic acid, ceramide and diglycerides on radiolysis of lipids: Identification by Maldi-TOF-Mass Spectrometry. *Free Radical Biology and Medicine*, 36, 1612–1624.

Sinanoglou, V. J., Batrinou, A., Konteles, S., & Sflomos, K. (2007). Microbial population, physicochemical quality and allergenicity of molluscs and shrimp treated with cobalt-60 γ -irradiation. *Journal of Food Protection*, 70, 958–966.

Sinanoglou, V. J., & Miniadis-Meimaroglou, S. (1998). Fatty acid of neutral and polar lipids of (edible) Mediterranean cephalopods. *Food Research International*, 31, 467–473.

Sinanoglou, V. J., Strati, I. F., & Miniadis-Meimaroglou, S. (2011). Lipid, fatty acid and carotenoid content of edible egg yolks from avian species: A comparative study. *Food Chemistry*, 124, 971–977.

Stutius, L. M., Sheehan, W. J., Rangsitienchai, P., Bharmatee, A., Scott, J. E., Young, M. C., et al. (2010). Characterizing the relationship between sesame, coconut and nut allergy in children. *Pediatric Allergy and Immunology*, 21, 1114–1118.

Uzun, B., Arslan, C., Karhan, M., & Tokar, C. (2007). Fat and fatty acids of white lupin (*Lupinus albus* L.) in comparison to sesame (*Sesamum indicum* L.). *Food Chemistry*, 102, 45–49.

Were, B. A., Onkware, A. O., Gudu, S., Welander, M., & Carlsson, A. S. (2006). Seed oil content and fatty acid composition in East African sesame (*Sesamum indicum* L.) accessions evaluated over 3 years. *Field Crops Research*, 97, 254–260.

Wolff, N., Yannai, S., Karin, N., Levy, Y., Reifien, R., Dalal, I., et al. (2004). Identification and characterization of linear B-cell epitopes of β -globulin, a major allergen of sesame seeds. *Journal of Allergy and Clinical Immunology*, 114, 1151–1158.

Yoshida, H., Shigezaki, J., Takagi, S., & Kajimoto, G. (1995). Variations in the composition of various acyl lipids, tocopherols and lignans in sesame seed oils roasted in microwave oven. *Journal of the Science of Food and Agriculture*, 63, 407–411.