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Gamma-irradiation of nuts – EPR characterization and effects on lipids and oxidative stability: I. Hazelnuts

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Gamma-irradiation is very fast, efficient, inexpensive, secure and safe method for sterilization of food products approved in many countries. Unfortunately, along with the benefits of such treatment, negative effects are possible because of reactive oxygen species and other radicals which can damage the biologically active components. In present study hazelnuts were gamma-irradiated at 10 kGy and 25 kGy. No significant difference between EPR spectra of irradiated samples at these two doses was determined, and the kinetic behavior of the central and satellite lines was the same for 10 kGy and 25 kGy. The presence of satellite lines allowed to identify radiation treatment even 230 days after irradiation. During the time, the free radical scavenging activity of 10 kGy irradiated hazelnuts decreased in comparison with the non-irradiated sample but it was not changed six months after irradiation, whereas in 25 kGy gamma-treated samples it continuously decreased up to six months. Concerning the lipids, no significant changes in fat content and fatty acids composition of irradiated hazelnuts were detected. Some increasing in acid value (from 0.7 to 1.8 mg KOH/g) and in specific absorption of conjugated dienes and trienes (from 1.1 to 2.3 and from 0.05 to 0.13, respectively) was observed for radiation doses in the order 0 kGy, 10 kGy, 25 kGy. However, no significant changes in the oxidative stability at different temperatures (80°C – 120°C) of autoxidation of oil from irradiated hazelnuts were found.

Key words: hazelnuts, gamma-irradiation, EPR, DPPH, fatty acids, oxidative stability

INTRODUCTION

In recent decades it was shown that a very fast, efficient, inexpensive, secure and absolutely safe method of sterilization is irradiation of the products with high-energy gamma-rays. Food irradiation has been approved in many countries, especially in cases to replace the addition of various chemicals such as sodium nitrite, as well as fumigants ethylene dibromide, ethylene oxide and methyl bromide for preservation. The maximum permissible dose for gamma-irradiation of food is 10 kGy [1], and China, USA and Canada do quarantine radiation processing of herbs and spices to 30 kGy [2]. Unfortunately, along with the benefits of such treatment, negative effects are possible because of changes in biologically active components as a result of reactive oxygen species and other radicals with a strong toxic effect on many important biological structures - cellular lipid membranes, proteins, etc.

Since ancient times hazelnuts have been a favourite part of the human diet and an important source of energy. Due to their excellent taste and increasingly demonstrated health benefits, they are currently considered fundamental to several dietary guidelines worldwide. Hazelnuts contain high content of monounsaturated fatty acids which have been proven to reduce the risk of cardiovascular disease and type-2 diabetes as well as to have a preventive effect on atherosclerosis [3]. In addition, they are a rich source of antioxidants and trace elements that are vital for the biological processes in the human body.

Only single studies have been found in literature concerning the effects of gamma-irradiation on hazelnuts, at that the data are not systematic and sometimes the results are conflicting. For that reason the aim of our investigation was to study the effect of gamma-irradiation on the fat content, fatty acids composition and oxidative stability of oil from gamma-treated hazelnuts. Doses of 10 kGy and 25 kGy gamma-rays were chosen based on recommended medium-dose and high-dose irradiation, respectively. In addition, EPR spectroscopy was used to determine kinetics of changes of gamma-rays induced free radicals in treated hazelnuts as well as their DPPH free radical scavenging activity.

MATERIALS AND METHODS

Samples and reagents

Hazelnuts were purchased from the local market in the year 2018 and were tested by Electron paramagnetic resonance (EPR) spectroscopy (see below) if they had already been treated by gamma-irradiation. If not, they were subjected to subsequent

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analyses. All reagents and solvents were of analytical grade (Merck, Darmstadt, Germany) and were used without additional purification. Reference fatty acid methyl esters and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma-Aldrich Co. (St. Louis, MO, USA).

EPR measurements

The EPR spectra were recorded as a first derivative of the absorption signal of an JEOL JES-FA 100 EPR spectrometer at room temperature. The spectrometer operated in X–band equipped with a standard TE_{011} cylindrical resonator. The hazelnuts shells were cut into small pieces suitable to insert in quartz EPR tube and were fixed in the cavity center. The EPR spectra were recorded at following conditions: modulation frequency 100 kHz, microwave power 0.4 mW, modulation amplitude 0.4 mT, sweep 15 mT, time constant 0.3 s and sweep time 2 min.

Gamma-irradiation of the hazelnuts

Two parallel samples of 100 g shelled hazelnuts were gamma-irradiated at 10 kGy and at 25 kGy in a mobile irradiation chamber (4.0 L volume) using Co-60 source with 8 200 Ci activity (equipment of the National Centre of Radiobiology and Radiation Protection, Sofia, Bulgaria). During the irradiation the chamber rotated on its vertical axe. For the study of the absorbed dose distribution Alanine dosimeters (Kodak BioMax) were used, measured by an ESR spectrometer E-scan Bruker and calibrated in units of absorbed dose in water. Three dosimeters were placed in each point.

Extraction of oil; determination of fat content

Portions of about 30 g (precisely weighted unshelled) hazelnuts – non-irradiated and gamma-ray treated at 10kGy and 25kGy, respectively, were ground and extracted with hexane in Soxhlet apparatus for 8 h [4]. The solvent was distilled in rotary evaporator and the residue was weighted to calculate the fat content by equation:

$$\text{Fat } \% = (m_{oil} / m_{nuts}) \times 100,$$

where *m* was the mass [g] of the residue (oil) and the initial sample (nuts), respectively. Then 10% stock solutions of oils in hexane were prepared for subsequent analyses.

Estimation of DPPH free radical scavenging activity by EPR spectroscopy

Extracts preparation: 7.5 mL absolutely pure ethanol and 2.5 mL distilled water were added to 0.5 g dry residue hazelnuts (non-irradiated and irradiated with 10 kGy and 25 kGy, respectively). These samples were incubated for 24 hours at room temperature without air access and then were filtered before further investigations. Freshly prepared extracts were used for each experiment.

Estimation of DPPH free radical scavenging activity: 1 mL hazelnut extract and 1 mL 0.002 M ethanolic solution of DPPH were mixed. Then EPR spectroscopy was applied for monitoring of the changes in spectrum intensity over a period of 4 hours. For the purpose, the mixture was transferred to a capillary tube in a definite time interval. The capillary tube was sealed and placed inside a standard EPR quartz tube that was placed in the EPR cavity. The control sample contained 1 mL ethanolic solution of DPPH and the same amount of ethanol instead of extract. The percent of the DPPH radicals scavenged by nut extracts was calculated according to the equation:

$$\text{Scavenged DPPH radicals } (\%) = [(I_0 - I) / I_0] \times 100,$$

where I_0 was the intensity of the second peak of DPPH signal of the control sample and I was the intensity of the second peak of the same EPR spectrum after addition of the tested substance.

Analysis of fatty acids composition

Fatty acids composition of the hazelnut oils was determined by gas chromatography (GC) of methyl esters (FAME) after transesterification of the corresponding oil with 1 % sulfuric acid in methanol [5]. Then FAME were purified by preparative silica gel G thin-layer chromatography (TLC) with a mobile phase of hexane-acetone (100:6, v/v) and eluted from the layer with diethyl ether. GC was performed on Shimadzu 17A (Shimadzu, Japan) gas chromatograph equiped with a flame ionization detector and Simplicity-wax column (30 m x 0.32 mm x 0.25 μm, Supelco). The column temperature was programmed from 170°C to 260°C with 2°C/min and held at the final temperature for 5 min. The injector and detector temperatures were 260°C and 280°C, respectively. Helium was the carrier gas at 0.5 mL/min flow rate; split 1:50; sample size 15 μg. The peaks identification was according to retention times of reference FAME. Analyses were performed in triplicate and the results were presented as relative percent of each fatty acid.

Determination of oxidative stability

Acid value (AV, presented as mg KOH/g oil) was determined by titration with ethanolic KOH [6]. Conjugated dienes and trienes were measured by their absorbance at 232 nm and 268 nm, respectively, in 1% oil solutions in iso-octane, using a Cecil Series 8000 UV/VIS double beam scanning spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) [7]. Peroxide value (PV, expressed as meq/kg oil) was estimated by modified iodometric method [8]. The Induction period (IP), as a measure

of the oxidative stability of oils, was determined using the following procedure: 2 g oil sample was oxidized, respectively, at 80°C, 100°C and 120°C, by blowing air at 50 mL/min flow rate in special reactive vessel. The oil oxidation kinetics was monitored. Aliquots were taken in fixed time intervals and the degree of oxidation was estimated by iodometric determination of the primary products (hydroperoxides) as peroxide value (PV). The Induction period (IP, in hours) was determined by method of tangents to two parts of the kinetic curves [9].

Statistics

Three measurements of two parallel samples were done. The results are presented as mean value ± standard deviation and have been compared by Student`s *t*-test (Microsoft Excel software).

RESULTS AND DISCUSSION

Gamma-irradiation and EPR investigations

Gamma-irradiation leads to the formation of free radicals in foodstuffs which are relatively stable and therefore easily detected with EPR spectroscopy. The features of the EPR method as speed of analysis, lack of continuous sample preparation and determination without the need of non-irradiated (control) sample makes it preferable to all other methods for investigation of irradiated foods [10]. The European Standard related to gamma-sterilization of nuts using EPR is EN 1787. It is based on two interlaboratory tests with pistachio nut shells. Detection of irradiated pistachio nuts has been validated for doses of 2 kGy and above and there are no limitations of stability of free radicals and their detection of irradiation for at least one year after treatment [11]. Before irradiation EPR spectra of hazelnut shell samples exhibited one singlet line, characterized with g-factor 2.0036 (Fig. 1). This line is observed in all non-irradiated foodstuffs of plant origin and is attributed to free radicals of semi-quinones, produced by oxidation of plant phenolic groups present in polyphenols or lignin [12]. Irradiated hazelnut samples at 10 kGy and 25 kGy exhibited typical "cellulose-like" EPR spectrum. It contained the irradiation specific line pair of the cellulose radical (satellite lines), spaced about 6 mT from each other (Fig. 1 A), and increased intensity of the central line. The satellite lines arise from C(5) carbon-centered cellulose radical [13]. Just the presence of "cellulose–like" EPR spectrum is considered as unambiguous evidence for previous radiation treatment of vegetable foodstuffs [11]. The second radiation induces signal which is a strong singlet with g-factor of 2.0052 and overlaps with natural weak line and "cellulose-like" EPR spectrum. This assumption for second radiation induced EPR singlet line with g = 2.0050 was confirmed by the different decay rate constant of disappearance of the two radiation-induced features [14]. As could be seen from Figure 1 gamma-irradiation did not induce a large difference in the amount of free radicals for the two irradiation doses since no significant difference in intensity of EPR spectra for 10 kGy and 25 kGy was observed. Comparison between Figures 1A and 1B revealed considerable decrease in intensity of the spectrum (note different spectrometer gain) 230 days after irradiation, nevertheless the satellite lines were still visible.

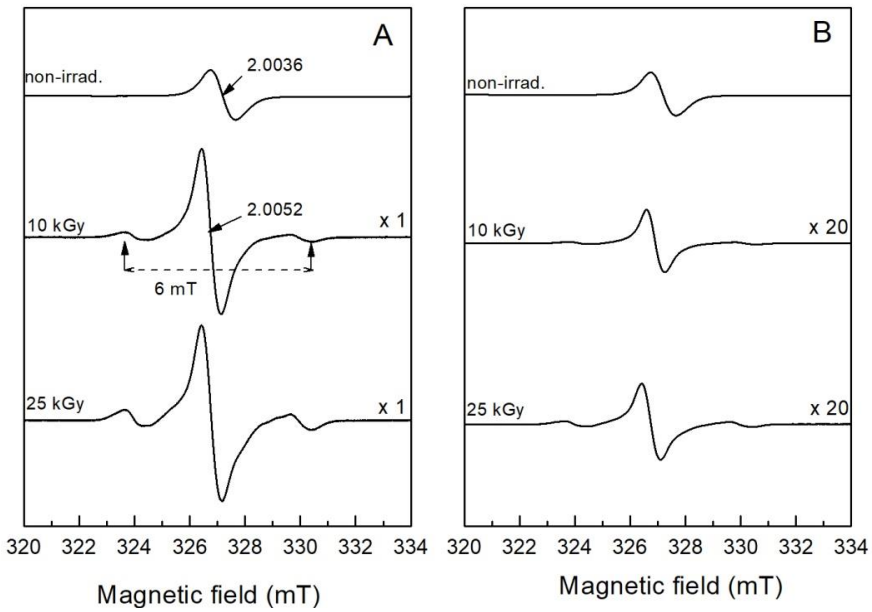

Fig. 1. EPR spectra of hazelnuts recorded: (**A**) immediately after irradiation; (**B**) 230 days after irradiation.

The fading kinetics of the radiation induced EPR signal is important characteristic of the materials because after irradiation it limits the time interval in which identification of radiation processing is possible. In order to find the time stability of radiation-induced EPR signals of irradiated hazelnut samples their decay kinetics was studied for a period of 230 days after irradiation. The results showed (Fig. 2) that all studied signals decayed exponentially with time. The central line decreased with ca. 90 % from its initial intensity of first day after irradiation whereas satellite lines with ca. 83 %. This behavior of central line and satellite peaks proved the case for at least two radiation-induced signals. No significant difference in the kinetic behavior of the samples irradiated at 10 kGy and 25 kGy was observed. From the other side the central line had the same intensity for the two doses whereas the satellite lines were more intensive in the spectra of 25 kGy irradiated samples.

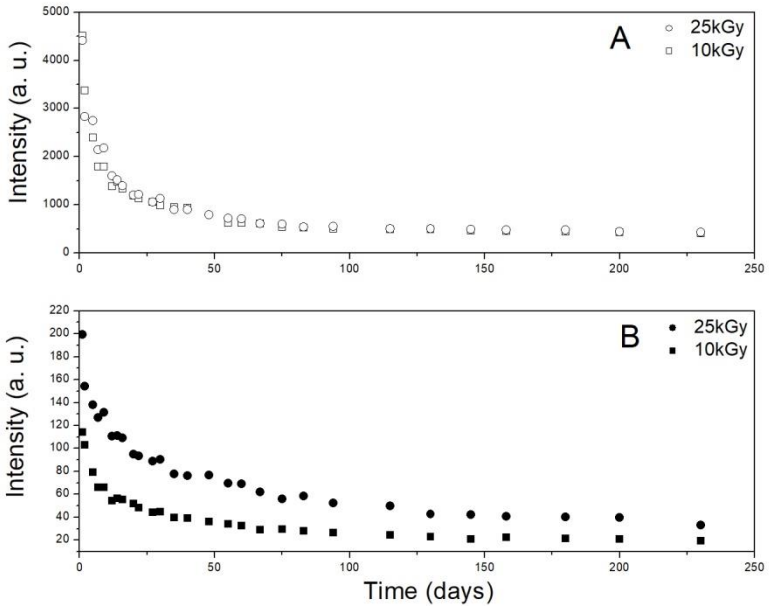

Fig. 2. Fading kinetics of 10 kGy and 25 kGy radiation induced signals in hazelnut samples: (**A**) central line; (**B**) satellite lines.

The effect of time after irradiation on antiradical activity was studied (Fig. 3). For the purpose irradiated hazelnut samples were measured six months after irradiation. The results revealed no change in free radical scavenging activity of hazelnuts treated with 10 kGy gamma-rays. However, in samples irradiated with 25 kGy free radical scavenging activity decreased six months after irradiation. Comparing to the non-irradiated samples the free radical scavenging activity decreased with 12.5 % for the samples irradiated with 10 kGy (independently of the time after irradiation), 31 % for the samples irradiated with 25 kGy few hours after irradiation and 43 % for the samples irradiated with 25 kGy six months after irradiation. The kinetic curves showed linear dependence of the signal up to 55 minute and then gradually fading.

Fat content and fatty acids composition

Only three papers were found in literature about the effect of gamma-irradiation on hazelnuts fat content. Güler et al. [15] tested hazelnuts treated with up to 1.5 kGy gamma-rays. Increasing of 4-5% in fat content was determined but the authors explained that as a result of heterogeneity of the samples. Other researchers [16] detected by Fourier transform infrared spectroscopy (FTIR) slight increasing of total lipid content at 1.5 kGy gamma-irradiation but its decreasing at 10 kGy dose. On the other hand, Gecgel et al. [17] had investigated nuts with 66% fat after 1 kGy, 3 kGy, 5 kGy and 7 kGy gamma-rays treatment and they had not observed significant differences between samples. Similar to that, our results (Table 1) revealed no effect of gamma-irradiation on the fat content of treated hazelnuts even at 25 kGy.

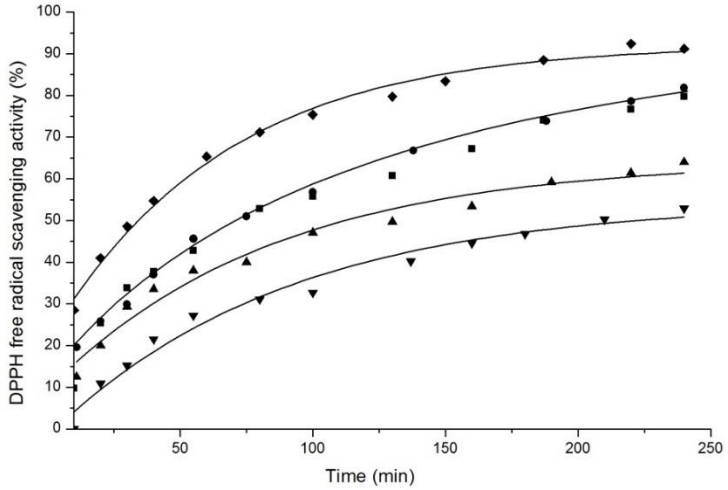

Fig. 3. Determination of DPPH free radical scavenging activity of non-irradiated (♦) hazelnuts and irradiated samples with: 10 kGy in the first hours (●) and six months (■) after irradiation; 25 kGy in the first hours (▲) and six months (▼) after irradiation.

Data in literature about fatty acid composition of irradiated nuts are occasional and contradictory. Thus, Dogan et al. [16] found by FTIR that, on one side, unsaturated lipids slightly increased in hazelnuts treated with 1.5 kGy but, on the other side, at 10 kGy they decreased (no data about individual fatty acids). Mexis and Kontominas [18] and Gecgel et al. [17] observed slight decreasing of total mono- and poly-unsaturated fatty acids with increasing of saturated acids at gamma-rays doses from 0 to 7 kGy. However, no changes in unsaturated fatty acids content (presented as iodine value) was detected by Ozyardimci et al. [19] in their investigation with up to 3 kGy. Our results show also no significant changes in fatty acids composition after 10 kGy and 25 kGy gamma-rays treatment (Table 1).

Table 1. Fat content (wt. %) and fatty acids composition (rel. %) of oil from gamma-irradiated hazelnuts

	0 kGy	10 kGy	25 kGy
Fat content [wt.%]	66.9 ± 2.2*	67.9 ± 1.3	67.8 ± 1.1
Fatty acids [rel.%]			
16:0	6.8 ± 0.1**	6.9 ± 0.1	6.9 ± 0.1
16:1	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01
18:0	3.3 ± 0.1	3.4 ±0.1	3.4 ± 0.1
18:1	83.1 ± 0.8	83.3 ± 0.9	83.1 ± 0.6
18:2	6.2 ± 0.5	5.8 ± 0.6	6.0 ± 0.4
20:0	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
20:1	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01

* mean value ± standard deviation

** within each row, no statistically significant difference between values was found (at P=0.95)

Oxidative stability

Hazelnut oil has rather high oxidative stability because of above 80% content of monounsaturated fatty acid. In order to study the effect of gamma-rays on oil quality peroxide values and kinetics of peroxides accumulation were evaluated at 80°C, 100°C and 120°C autoxidation of oil from non-treated and gamma-irradiated hazelnuts. The results are presented in Figure 4 and the corresponding induction periods are given in Table 2. As can be seen the values of IP at 100°C and 120°C are practically the same whereas slight difference between non-irradiated and gamma-treated oils can be noted at 80°C but that is statistically insignificant.

Table 2. Induction period (IP, hours) at 80°C, 100°C and 120°C autoxidation of oil from gamma-irradiated hazelnuts.

	0 kGy	10 kGy	25 kGy
IP 80°C	226 ± 11	206 ± 11	205 ± 11
IP 100°C	49 ± 3	48 ± 3	48 ± 3
IP 120°C	10.1 ± 0.5	9.8 ± 0.5	9.8 ± 0.5

* mean value ± standard deviation. Within each row no statistically significant difference was found (P=0.95).

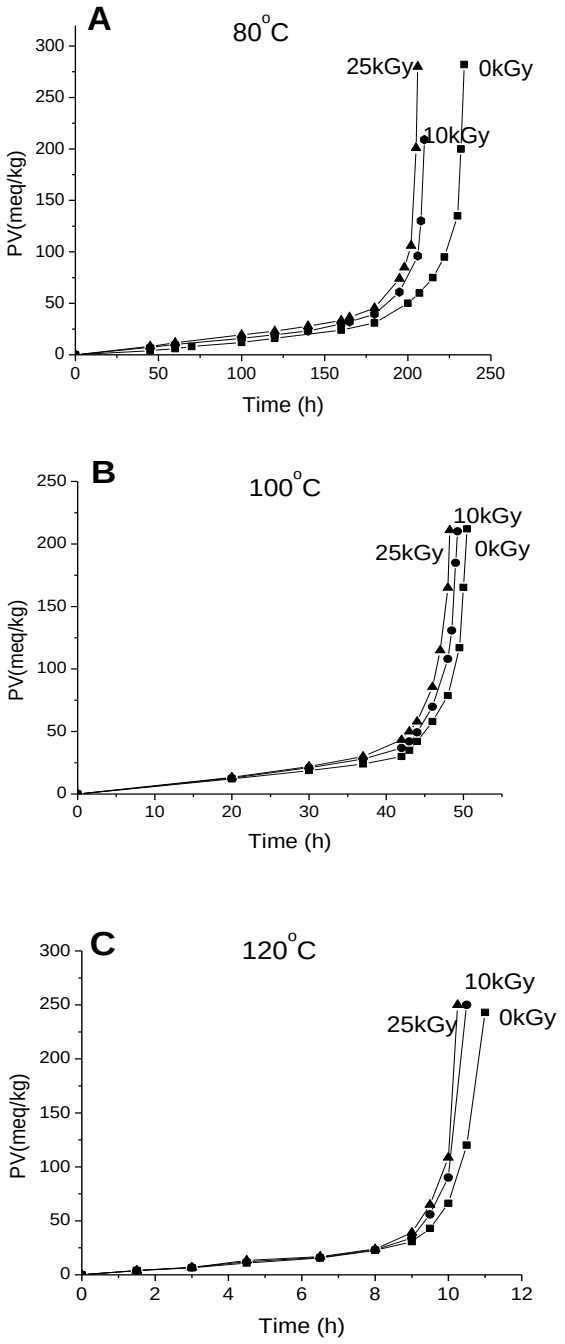

Fig. 4. Kinetics of peroxide accumulation at 80°C (**A**), 100°C (**B**) and 120°C (**C**) during the oil autoxidation from gamma-irradiated (■-0 kGy; ●-10 kGy; ▲-25 kGy) hazelnuts.

Table 3. Acid value and conjugated dienes and trienes in oil from gamma-irradiated hazelnuts.

	0 kGy	**10 kGy**	**25 kGy**
Acid value [mg KOH/g]	0.70 ± 0.08[a]	0.90 ± 0.09[b]	1.8 ± 0.2[c]
conj. Dienes A_{232} [1%]	1.1 ± 0.1[a]	1.6 ± 0.1[b]	2.3 ± 0.1[c]
conj. Trienes A_{268} [1%]	0.05 ± 0.01[a]	0.09 ± 0.02[b]	0.13 ± 0.02[c]

* mean value ± standard deviation. Different letters within each row indicate statistically significant difference (P=0.95).

In addition, the dependence between conjugated dienes (A_{232}) and peroxide values at 80°C autoxidation of hazelnut oils was estimated. The results (Figure 5) respond to identical linear relationship.

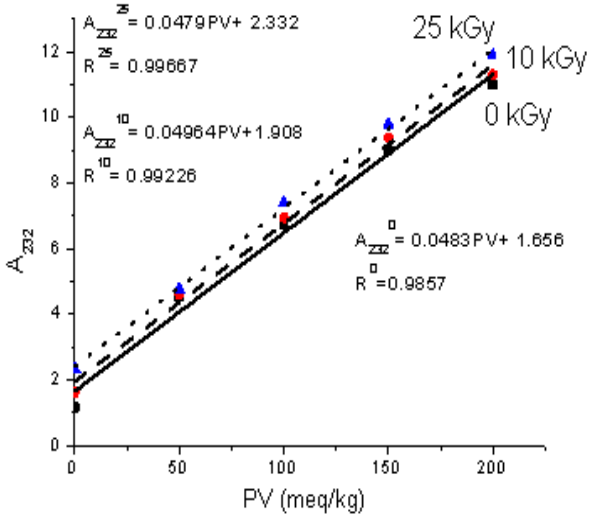

Fig. 5. Relationship between PV and D_{conj} at 80°C of oil from gamma-irradiated (■-0 kGy; ●-10 kGy; ▲-25 kGy) hazelnuts.

No data in literature about induction periods (i.e. oxidative stability) have been found to compare with our results. Only initial peroxide values of irradiated hazelnut oils have been published in three papers at that the results are various, i.e. with incresing of radiation dose to 7 kGy PV has increased to 1.6 meq/kg [17] and in other case to 6.8 meq/kg [18], whereas according to other authors gamma-rays dose of 1.5 kGy has not changed the PV [15].

Other characteristics of oxidized oils are the acid value (AV) and the presence of conjugated dienes and trienes. As is seen from the results in Table 3 their values slightly increase with increasing of radiation dose to 25 kGy. Some increasing of acid value (expressed as % free fatty acids) has been reported by Gecgel et al. [17] in contrast to other two papers [15, 19] where no changes in AV have been announced. Concerning conjugated dienes and trienes no data from other authors were found in literature.

CONCLUSIONS

EPR spectroscopy enables to identify gamma-irradiation treatment of hazelnuts even after 230 days. Doses of 10 kGy and 25 kGy do not affect practically their fat content, fatty acids composition and oxidative stability of oils from irradiated samples. These new results are of importance for the practice, because they prove that even at much higher dose of gamma-irradiation (25 kGy), the hazelnut oil saves its important lipid characteristics.

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REFERENCES

1. Codex Alimentarius, CAC / CEP 19-1979, Rev. 1-2003.
2. Radiation Technologies for Food Processing, National Center for Radiobiology and Radiation Protection, Sofia, Bulgaria (2013).
3. R. Jiang, D.R. Jacobs Jr., E. Mayer-Davis, M. Szklo, D. Herrington, N.S. Jenny, R. Kronmal, R.G. Barr, *Am. J. Epidem*., **163**, 222 (2006).
4. ISO 659:2009, Oilseeds – Determination of oil content (Reference method), p. 12 (2009).
5. W.W. Christie, Lipid Analysis, The Oily Press, Bridgwater, England (2003).
6. AOCS Official method Cd 3d-63.
7. AOCS Official method Ch 5-91.
8. N. Yanishlieva, A. Popov, E. Marinova, *Compt. Rend. Acad. Bulg. Sci.*, **31**, 869 (1978).
9. B. Le Tutour, D. Guedon, *Phytochem.*, **31**, 1173 (1992).
10. K. I. Aleksieva, N. D. Yordanov, *Food Res. Intern.*, **105**, 1019 (2018).
11. EN 1787, 2000. Foodstuffs -Detection of irradiated food containing cellulose - Method by ESR spectroscopy, European Committee for Standardization, Brussels, Belgium.
12. M. Polovka, V. Brezova, A. Stasko, *Biophys. Chem.*, **106**, 39 (2003).
13. H. Kameya, M. Ukai, Y Shimoyama, *Rad. Phys. Chem.*, **84**, 232 (2013).
14. J. Raffi, N. D. Yordanov, S. Chabane, L. Douifi, V. Gancheva, S. Ivanova, *Spectrochim. Acta Part A: Mol. Biomol. Spectr*., **56**, 409 (2000).
15. S. K. Güler, S. Z. Bostan, A. H. Çon, *Postharv. Biol. Technol.*, **123**, 12 (2017).
16. A. Dogan, G. Siyakus, F. Severcan, *Food Chem.*, **100**, 1106 (2007).
17. U. Gecgel, T. Gumus, M. Tasan, O. Daglioglu, M. Arici, *Rad. Phys. Chem.*, **80**, 578 (2011).
18. S. F. Mexis, M. G. Kontominas, *Rad. Phys. Chem.*, **78**, 407 (2009).
19. B. Ozyardimci, N. Cetinkaya, E. Denli, E. Ic, M. Alabay, *J. St. Prod. Res.*, **42**, 183 (2006).