Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 263-269) 2019

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

Sv. M. Momchilova[1*], S.P. Taneva[1], I.R. Totseva[1], Y.I. Nikolova[1], Y.G. Karakirova[2], K.I. Aleksieva[2], R.B. Mladenova[2], V.D. Kancheva[1]

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria*
[2]*Institute of Catalysis, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 11, 1113 Sofia, Bulgaria*

Received March 21, 2019; Accepted April 17, 2019

Gamma-irradiation is very suitable method for treatment of peanuts which often tend to get contaminated with pests. However, along with all positive issues, gamma-rays provoke reactive oxygen species and other radicals which cause changes firstly in the lipid molecules. So, in this study 10 kGy and 25 kGy gamma-irradiated peanuts were characterized by EPR, as well as changes in their fat content, fatty acid composition and oxidative stability were evaluated. EPR experiments revealed that the higher dose of radiation induced free radicals not only in the cellulose but also in the peanut starch. Non-significant difference in the kinetics behavior of the samples irradiated at 10 kGy and 25 kGy was observed, and EPR spectroscopy enabled to identify gamma-irradiation even 230 days after treatment. The free radical scavenging activity decreased with increasing of irradiation dose. On the other hand, in 25 kGy irradiated sample it was time dependent and decreased with 16 % six months after irradiation comparing to several hours after treatment, whereas in 10 kGy irradiated sample it was not changed during the time. Doses of 10 kGy and 25 kGy did not affect significantly the fat content and fatty acids composition. Although slight increasing in acid value (from 1.26 mg KOH/g oil of non-irradiated oil to 1.35 mg KOH/g oil of treated samples) and in specific absorption of conjugated dienes and trienes (from 1.82 to 7.26 and from 0.23 to 0.69, respectively) was observed at 0 kGy and 25 kGy gamma-rays, the irradiated samples still fulfilled the requirements for edible oil. The induction periods of autoxidation as a measure of oxidative stability at different temperatures (80°C–120°C) of the oils from treated peanuts slightly decreased.

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

INTRODUCTION

Peanut (*Arachis hypogaea*) is a legume crop species of global importance as a valuable source of oil and other biologically active components as proteins, dietary fibres, vitamins, antioxidants, microelements [1]. These nuts are favorite part of human diet because of their excellent taste and nutritional value due to the high content of healthy unsaturated essential fatty acids. However, peanuts often tend to get contaminated with pests such as molds, insects, surface microorganisms, microbial populations, etc. Fortunately, this problem can be easily overcome by application of gamma-irradiation which is a fast, very efficient, inexpensive, secure and safe method for sterilization of food. The maximum permissible dose for the purpose is 10 kGy [2], but China, USA and Canada carry out quarantine radiation processing up to 30 kGy [3]. Along with all positive issues of gamma-irradiation, some negative effects are possible too as a result of reactive oxygen species and other radicals which cause changes firstly in the lipid molecules. Although these processes have been investigated for many years the results published so far are sometimes contradictory. For that reason the aim of our study was to evaluate the effect of gamma-irradiation at 10 kGy and 25 kGy doses on the fat content, fatty acids composition and oxidative stability of oil from gamma-treated peanuts. 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 peanuts as well as their DPPH free radical scavenging activity.

MATERIALS AND METHODS

Samples and reagents

Peanuts (crop 2018) were purchased from the local market and were tested by Electron paramagnetic resonance (EPR) spectroscopy (see below) that they had not been previously treated by gamma-rays. 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).

* To whom all correspondence should be sent:
E-mail: svetlana@orgchm.bas.bg

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 peanuts shells were cut into small pieces 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 peanuts

Two parallel samples (about 100 g each) of shelled peanuts were gamma-irradiated at 10 kGy and at 25 kGy in a mobile irradiation chamber (4.0 L volume) using Co-60 source with 8200 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) peanuts – non-irradiated and gamma-rays treated at 10 kGy and 25 k Gy, respectively, were ground and extracted with hexane in Soxhlet apparatus for 8 h [4]. The solvent was distilled under vacuum 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 ethanol and 2.5 mL distilled water were added to 0.5 g dry residue peanuts (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 peanut extract and 1 mL 0.002 M ethanolic solution of DPPH were mixed. Then EPR spectroscopy was applied for monitoring 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 was determined by gas chromatography (GC) on methyl esters (FAME). For the purpose, peanut oils from the respective non-irradiated and gamma-rays treated nuts were transmethylated using 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 equipped 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 that 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, and 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

Two parallel samples from a representative portion of peanuts were analysed and each measurement was done in triplicate. 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 causes formation of free radicals in food which are relatively stable and thus can be detected easily by EPR spectroscopy. Advantages of the EPR as method for investigation of irradiated foods are speed of analysis, lack of continuous sample preparation and determination without the need of non-irradiated (control) sample [10]. The European Standard EN 1787 [11] is applied in such analyses.

EPR spectra of irradiated and non-treated peanuts are presented in Figure 1A. The weak singlet line at g = 2.0032 appears in every plant food which has not been exposed to radiation. It is accepted that this weak signal originates from stable semiquinone structures produced by oxidation of polyphenolic compounds present in samples of plant origin or lignin [12]. In EPR spectrum of peanuts upon irradiation can be seen a pair of extra lines separated *ca*. 3 mT left and right to the central line with g = 2.0052. The presence of two satellite peaks (marked with arrows in Fig. 1A) is considered in the Protocol EN 1787 [11] as unambiguous evidence for previous radiation treatment of plant origin foodstuffs. Radiation induced spectrum, called "cellulose-like", is attributed to a C(5) carbon-centered cellulose free radicals [13]. The second radiation induced signal which was a strong singlet with g-factor of 2.0052 overlapping the "cellulose-like" EPR spectrum. On the other hand, additional doublet of lines (marked with asterisks) spaced about 2 mT was recorded in the EPR spectra of 25 kGy irradiated samples. This doublet might be attributed to free radicals of starch known as "carbohydrate" spectrum [14]. The starch free radicals were not observed in the EPR spectra of 10 kGy irradiated peanuts samples. Figure 1B shows the EPR spectra of the same samples but 230 days after irradiation where the spectra reveal the same features. In spite of their reduced intensity (note different spectrometer gain) the satellite lines are still visible.

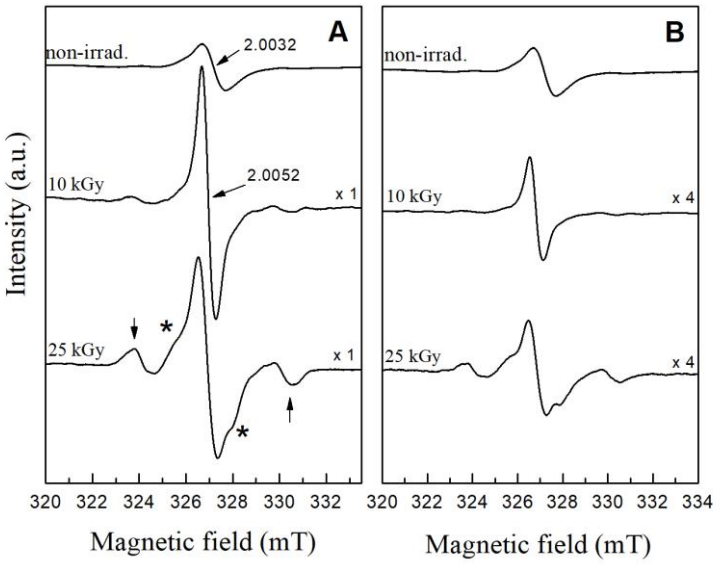

Fig. 1. EPR spectra of peanuts recorded: (**A**) immediately after irradiation; (**B**) 230 days after irradiation.

The fading kinetics of the EPR signal induced by radiation is important characteristic of materials since after irradiation it limits the time interval in which identification of radiation processing is possible. The results (Fig. 2) show a decay over time of the central line and two satellite peaks with the two different doses of gamma-irradiation. The time stability of radiation-induced EPR signals of irradiated peanuts samples was studied for a period of 230 days after irradiation. As can be seen all studied signals decay exponentially with time. Kinetic studies show that for 230 days the central

line decreases with ca. 90 % (10 kGy) and 87 % (25 kGy) from its initial intensity of first day after irradiation, whereas satellite lines with ca. 92 % and 85 % for 10 kGy and 25 kGy, respectively. No significant difference in the kinetic behavior of the samples irradiated at the two doses is observed. However, the intensity of central and satellite lines in the spectra of 25 kGy irradiated peanuts is higher than that of 10 kGy gamma-rays treated samples.

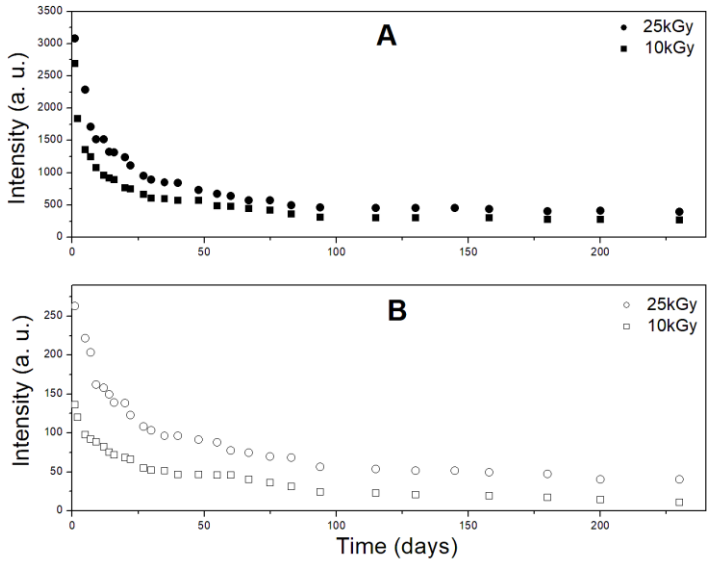

Fig. 2. Fading kinetics of 10 kGy and 25 kGy radiation induced signals in peanuts samples: (**A**) central line; (**B**) satellite lines.

The effect of time after irradiation on the antiradical activity is presented in Figure 3. The investigation shows decreasing of the free radical scavenging activity with increasing of irradiation dose. It has been found also that the antiradical activity of 25 kGy irradiated sample is time dependent and it decreases with 16 % six months after irradiation in respect to the sample few hours after irradiation. On the other hand there is no difference in free radical scavenging activity of the 10 kGy irradiated peanuts few hours and six months after irradiation. Comparing to non-treated peanuts the free radical scavenging activity decreases with 21 % for the samples irradiated with 10 kGy (irrespective of time after gamma-rays treatment), 31 % for the samples irradiated with 25 kGy few hours after irradiation and 47 % for the peanuts irradiated with 25 kGy six months after irradiation. The study on free radical scavenging activity of non-irradiated and irradiated peanuts shows increasing of percent scavenged DPPH radicals as a time function. In the first 45 minutes the kinetic curves exhibit a linear dependence and then gradually fading, excepting the 25 kGy treated sample six months after irradiation. The scavenging reaction between DPPH radicals and 25 kGy irradiated peanuts extract six months after irradiation is achieved slower - approximately 55 minutes after mixing of reactants.

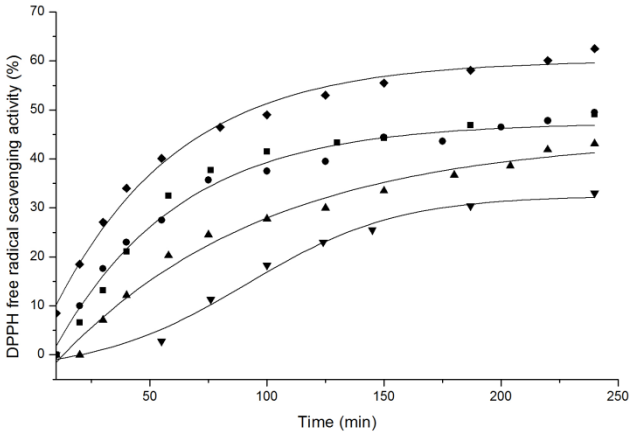

Fig. 3. Determination of DPPH free radical scavenging activity of non-irradiated (♦) peanuts and irradiated samples with: 10 kGy in the first hours (●) and six months (■) after irradiation; 25 kGy in the first hours (▲) and six months (▼) after irradiation.

Fat content and fatty acids composition

The peanuts studied here contained 45.2 % fat and that amount did not changed significantly after irradiation with 10 kGy and 25 kGy (Table 1). The same result was obtained by other authors for peanuts with similar fat content, i.e. 46 % [15] and 47 % [16] treated with 9 kGy and 8 kGy gamma-rays, respectively. On the other hand, only one paper was found in literature reporting a decrease of peanuts fat from 43 % to 40 % after irradiation with 10 kGy [17].

Results published yet about fatty acids of gamma-irradiated peanuts are contradictory, too. In general, peanuts contain above 70% unsaturated fatty acids among which oleic (9-18:1) and linoleic (9,12-18:2) acids are the main components, in similar proportions, and that is why it is important and interesting to evaluate the effect of irradiation on them. Thus, two papers [18, 19] report decreasing of unsaturated and increasing of saturated fatty acids after 7–7.5 kGy gamma-rays treatment of peanuts. Liu et al. [17] have obtained some peculiar result, i.e. decreasing of oleic and linolenic (9,12,15-18:3) acids but increasing of linoleic and saturated fatty acids at dose of 10 kGy. On the contrary, de Camargo et al. [20], investigating two peanut cultivars at the same dose of irradiation, have found decreasing of saturated and increasing of unsaturated fatty acids. However, other four papers [16, 21–23] demonstrate no change in fatty acids proportions after irradiation with, respectively, 3 kGy, 8 kGy, 10 kGy or 15 kGy gamma-rays. Our results (Table 1) are the same and reveal no effect of irradiation even at 25 kGy dose on the fatty acids composition of peanuts.

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

	0 kGy	**10 kGy**	**25 kGy**
Fat content [wt.%]	45.2 ± 0.3*	45.0 ± 0.4	45.2 ± 0.2
Fatty acids [rel.%]			
16:0	10.7 ± 0.1**	10.8 ± 0.1	10.8 ± 0.1
18:0	2.8 ± 0.1	2.9 ± 0.2	2.8 ± 0.1
18:1	44.6 ± 0.1	44.6 ±0.3	44.8 ± 0.2
18:2	35.4 ± 0.6	35.3 ± 0.6	34.9 ± 0.5
20:0	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
20:1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
22:0	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1
24:0	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1

* mean value ± standard deviation

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

Oxidative stability

Since peanut oil contains about 35 % polyunsaturated (linoleic) acid it is expected to be accessible to oxidation especially after some radiation treatment. In our study, oxidative stability of oil from gamma-irradiated peanuts was estimated by its acid value, the presence of conjugated dienes and trienes, and particularly, by the induction periods during autoxidation of oil at three different temperatures. All these parameters indicate the quality of oil and its edibility.

The results about acid value and conjugated dienes and trienes in peanut oil from irradiated nuts are given in Table 2. As can be seen, the acid value slightly increased from 1.26 mg KOH/g oil for non-irradiated oil to 1.35 mg KOH/g oil for 10 kGy treated sample but without further significant change at 25 kGy dose. Even that increasing, the acid values are below the limit of 10 mg KOH/g oil for edible fats and oils [2].

Table 2. Acid value (mg KOH/g oil) and conjugated dienes (A_{232} [1%]) and trienes (A_{268} [1%]) in oil from gamma-irradiated peanuts

	0 kGy	**10 kGy**	**25 kGy**
Acid value	1.26^{a} ±0.04*	1.35^{b} ±0.03	1.39^{b} ±0.04
conj. Dienes	1.82^{a} ±0.04	3.79^{b} ±0.05	7.26^{c} ±0.09
conj. Trienes	0.23^{a} ±0.05	0.41^{b} ±0.07	0.69^{c} ±0.04

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

Although two papers [15, 21] report no significant effect of irradiation (at 9 and 3 kGy, respectively) on the acid value of peanut oils, other

authors [16, 17] confirm our observation for its slight increasing after gamma-rays treatment (at 8 and 10 kGy, respectively).

The absorption of conjugated dienes and trienes also increases (Table 2), respectively from 1.82 to 7.26 and from 0.23 to 0.69 at 0 kGy and 25 kGy doses, similarly to the observations of other authors [16, 23, 24].

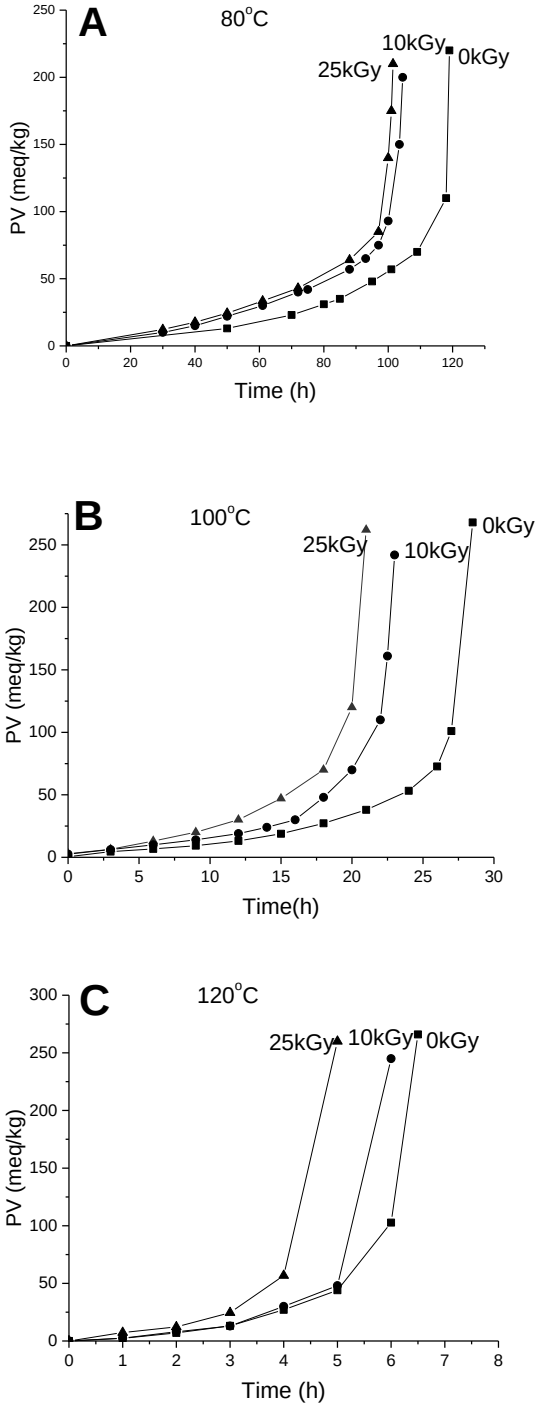

Fig. 4. Kinetics of peroxide accumulation at 80°C (**A**), 100°C (**B**) and 120°C (**C**) during the oil autoxidation from gamma-irradiated (■-0 kGy; ●-10 kGy; ▲-25 kGy) peanuts.

Table 3. Induction period (IP, hours) at 80°C, 100°C and 120°C autoxidation of oil from gamma-irradiated peanuts

Temperature	0 kGy	10 kGy	25 kGy
IP 80°C	$117^{a} \pm 7$*	$101^{b} \pm 6$	$99^{b} \pm 6$
IP 100°C	$27^{a} \pm 2$	$22^{b} \pm 2$	$19^{b} \pm 2$
IP 120°C	$5.7^{a} \pm 0.3$	$4.8^{b} \pm 0.2$	$8^{c} \pm 0.2$

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

As for the peroxide value of peanut oil, two papers report no effect on it of gamma-irradiation up to 5 kGy [17, 21] but above that dose, i.e. 5 kGy and 10 kGy [17], 7 kGy [18], 8 kGy [16] or 15 kGy [23], the peroxide value has increased.

In our investigation the kinetics of peroxides accumulation was evaluated at 80°C, 100°C and 120°C autoxidation of oils from non-treated and gamma-irradiated peanuts and thus the corresponding induction periods were determined. The results are given in Table 3 and are presented graphically on Figure 4.

As can be seen (Table 3) some decrease of IP with increasing of the radiation doses is observed at the three tested temperatures of autoxidation. At 80°C and 100°C the difference in IP is significant only between non-irradiated and radiated samples, whereas at 120°C significant difference is observed also between 10 kGy and 25 kGy treated peanuts (Table 3, Figure 4). Only one paper was found in literature with an IP value for oil from gamma-irradiated peanuts [24]. The applied radiation doses there were 0 kGy, 5 kGy, 7.5 kGy and 10 kGy, and the IP decreased respectively from 10.3 h to 8.3 h. Since the autoxidation was carried out at 110°C these results [24] could not be compared directly with ours (Table 3) but a parity could be revealed by analogy for the other temperatures.

CONCLUSIONS

The higher dose of radiation induces free radicals not only in the cellulose but also in the starch in the peanuts detected by EPR. Not significant difference in the kinetics behavior of the samples irradiated at 10 kGy and 25 kGy has been observed, and EPR spectroscopy enables to identify gamma-irradiation even 230 days after peanuts treatment. Doses of 10 kGy and 25 kGy do not affect practically their fat content and fatty acids composition. Although some increasing in acid value and of conjugated dienes and trienes has been detected in treated samples they still fulfill the requirements for edible oil. The induction periods of autoxidation as a measure of oxidative stability

of oils at different temperatures (80°C–120°C) slightly decrease for irradiated peanuts.

Acknowledgements: *Financial support from the National Science Fund of Bulgaria, Grant № DN 19/14 from 12.12.2017, is gratefully acknowledged.*

REFERENCES

1. F. Shahidi, C. Alasalvar, Ch. M. Liyana-Pathirana, *J. Agric. Food Chem.*, **55**, 1212 (2007).
2. Codex Alimentarius Stan 210 - Codex standards for named vegetable oils (Amended 2003, 2005).
3. Radiation Technologies for Food Processing, National Center for Radiobiology and Radiation Protection, Sofia, Bulgaria (2013).
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. R. W. A. Franco, L. Martin-Neto, M. S. A. Kato, G. R. Furlan, J. M. M. Walder, L. A. Colnago, *Intern. J. Food Sci. Technol.*, **39**, 395 (2004).
15. M. Al-Bachir, *Acta Sci. Pol. Technol. Aliment*., **15 (2)**, 171 (2016).
16. I. A. Bhatti, S. Ashraf, M. Shahid, M. R. Asi, S. Mehboob, *Appl. Rad. Isot.*, **68**, 2197 (2010).
17. K. Liu, Y. Liu, F. Chen, *LWT – Food Sci. Technol.*, **96**, 535 (2018).
18. S. F. Mexis, M. G. Kontominas, *J. Sci. Food Agric.*, **89**, 867 (2009).
19. A. M. R. Afify, M. M. Rashed, A. M. Ebtesam, H. S. El-Beltagi, *Grasas Aceit.*, **64 (4)**, 356 (2013).
20. A. C. de Camargo, S. G. Canniatti-Brazaca, D. N. Mansi, M. A. Calori Domingues, V. Arthur, *Cienc. Tecnol. Aliment., Campinas*, **31 (1)**, 11 (2011).
21. M. Al-Bachir, *Rad. Phys. Chem.*, **106**, 56 (2015).
22. A. C. de Camargo, T. M. F. de Souza Vieira, M. A. B. Regitano-d`Arce, S. M. de Alencar, M. A. Calori-Domingues, M. H. F. Spoto, S. G. Canniatti-Brazaca, *Int. J. Mol. Sci.*, **13**, 10935 (2012).
23. R. Y.-Y. Chiou, C. M. Lin, S.-L. Shyu, *J. Food Sci.*, **55 (1)**, 210 (1990).
24. A. C. de Camargo, T. M. F. de Souza Vieira, M. A. B. Regitano-d`Arce, S. M. de Alencar, M. A. Calori-Domingues, S. G. Canniatti-Brazaca, *Int. J. Mol. Sci.*, **13**, 2827 (2012).