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# EFFECTS OF *GAMMA*-IRRADIATION ON FATTY ACIDS AND OXIDATIVE STABILITY OF OIL FROM TREATED WALNUTS. IMPACT OF ANTIOXIDANT COMPOSITIONS ON OIL AUTOXIDATION

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**Abstract**

*Gamma*-irradiation at doses of 10 kGy and 25 kGy was applied on walnuts in order to study changes in the fat yield, fatty acids composition, conjugated dienes and trienes, tocopherols and oxidative stability of the extracted glyceride oils. The results revealed no significant changes in fat yield and fatty acids composition but an increase of dienes and trienes, and a decrease in tocopherols amount. Decrease of Induction periods of oils autoxidated at 80 °C, 100 °C, and 120 °C was observed as well. In order to reduce the negative effects of radiation-related lipid oxidation walnut oils were supplemented with binary antioxidant mixtures. Thus, the composition of ascorbyl palmitate and $\alpha$-tocopherol in 2:1 ratio slightly prolonged the induction periods while halving of the initial oxidation rate of oils extracted from irradiated walnuts at the same time.

**Key words:** walnuts, *gamma*-irradiation, glyceride oil, fatty acids, oxidative stability, induction period, antioxidants, kinetics

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**Introduction.** Food irradiation technology is approved and practiced in more than 50 countries around the world, and mostly in USA, Canada, Japan, China, Russian Federation, France, South Africa, Belgium, etc. Since 1970 *gamma*-treating has been used in Bulgaria, too, for allowed processing of herbs, spices, teas, dried fruits, food supplements, as well as nuts [1, 2]. Although the irradiation doses applied for food treatment usually do not cause immediate deterioration in quality, especially of nuts, some damages could occur during storage [3]. There are still many questions and ambiguities about the ongoing processes of secondary ionization, which are the subject of discussions in the international scientific community, but it is already known that free radicals generated by *gamma*-radiation attack lipid molecules first.

Walnut oil contains the exceptionally important alpha-linolenic acid (18:3 n-3) which is the first member in the omega-3 family of polyunsaturated fatty acids (PUFA). They reduce plasma low density lipoprotein-cholesterol (LDL-C, the so-called "bad" cholesterol), prevent diabetes and other ailments, and improve blood pressure levels and symptoms of inflammatory diseases [4, 5]. A walnut-enriched diet versus a control diet has caused a significant decrease in LDL-C, triglycerides and apolipoprotein in healthy mature adults [6]. In addition, nuts are extremely rich in antioxidants.

Only isolated studies about the effects of *gamma*-treatment on walnuts can be found in the literature. In addition to being unsystematic, these reports are also controversial. For that reason, the aim of the present study was to evaluate the effect of *gamma*-irradiation of walnuts at 10 kGy and 25 kGy on the glyceride oil yield, its fatty acids composition, tocopherols amount as well as its oxidative stability. Decreasing of the negative effects of radiation-related lipid oxidation, or at least minimizing them, by further enriching of walnut oil with antioxidant mixtures, was the other goal of the study. Radiation doses of 10 kGy and 25 kGy, respectively, were chosen for experiments, the former as the maximum allowable for food irradiation [1], and the latter to ensure some comparison of results.

**Materials and methods.** ***Samples and reagents.*** Shelled walnuts were purchased from the local market in the year 2021 and subsequently tested by electron paramagnetic resonance (EPR) spectroscopy if they had previously been treated with *gamma*-rays [7]. Then two portions of 200 g nuts were irradiated at doses of 10 kGy and 25 kGy ($^{60}$Co-source) at the National Centre of Radiobiology and Radiation protection, Sofia, Bulgaria [7]. Another portion of 200 g non-irradiated walnuts was used as a control sample.

All reagents and solvents were of analytical grade. They and the standards were from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA).

***Extraction of glyceride oil and determination of fat content.*** Portions of 30 g walnuts of the control and irradiated samples, respectively, were unshelled, ground and extracted with *n*-hexane in Soxhlet apparatus for 8 h. After distillation of the solvent in a rotary evaporator the fat content was calculated

by the equation:

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

where $m$ was the mass [g], of the residue (glyceride oil) and the initial sample (nuts), respectively.

Then 10% stock solutions of oils in hexane were prepared and stored at $-20\,^{\circ}\text{C}$ until analyses.

***Analysis of fatty acids composition.*** Fatty acids composition was determined by gas chromatography (GC) with a flame ionization detector after acid-catalized transesterification of oil to fatty acids methyl esters [8] at conditions explained in [7]. The results are presented as rel.% fatty acid.

***Analysis of tocopherols.*** Tocopherols were measured directly by high-performance liquid chromatopgraphy (HPLC) [7, 9]. The results are presented as [mg tocopherol/kg oil].

***Determination of the conjugated dienes and trienes.*** The content of conjugated dienes and trienes ($K_{232}$ and $K_{268}$) was estimated by their absorbance at 232 nm and 268 nm, respectively [10]. The results are presented as $K_{232}$ and $K_{268}$.

***Determination of oxidative stability.*** The acid value (AV) was determined by titration of oil with ethanolic KOH [11]. Peroxide value (PV), expressed as meq$O_2$/kg oil, was analysed according to the method of Yanishlieva et al. [12]. The induction period (IP, hours) at $80\,^{\circ}\text{C}$, $100\,^{\circ}\text{C}$, and $120\,^{\circ}\text{C}$, respectively, was fixated on the graph of increasing PV during time of 2 g oil oxidized by a stream of air [7, 12]. In addition, oxidative stability index (OSI) was determined by Rancimat equipment (Metrohm, Switzerland) with 20 L/h air flow at $80\,^{\circ}\text{C}$, $100\,^{\circ}\text{C}$, and $120\,^{\circ}\text{C}$, too.

***Statistics.*** Measurements were carried out in triplicate. Results are presented as a mean value of 3 parallel determinations ± standard deviation and have been compared by Students' $t$-test (MS Excel 2010 software).

**Results and discussion.** ***Effect of gamma-irradiation on the characteristics of walnut oil and its composition.*** Due to the high degree of unsaturation of walnut oil (more than 70% PUFA, Table 1), there is a risk of changes in its characteristics and composition after irradiation of nuts. Results in the literature about fatty acids composition of oils from *gamma*-treated walnuts are controversial: some authors report a decrease of PUFA content [13–16] while others do not observed changes [17]. Regarding amounts of monounsaturated fatty acids Sinanoglou et al. [15] and Mexis and Kontominas [17] found some decrease but other authors [16] established increase of these fatty acids after irradiation. Our results (Table 1) show no significant changes in fatty acids composition of oils after *gamma*-ray treatment of walnuts with doses of 10 kGy and 25 kGy.

Concerning the free fatty acids (measured as Acid value) in walnut oil, an

Table 1

Fat content, fatty acids composition, tocopherols content, acid value, conjugated dienes and trienes of oils from non-irradiated (0 kGy) and irradiated (10 kGy, 25 kGy) walnuts

| Walnut oil | 0 kGy | 10 kGy | 25 kGy |
|---|---|---|---|
| **Fat yield** (w/w %) | $50.1 \pm 2.4$ | $49.5 \pm 3.6$ | $49.9 \pm 3.8$ |
| **Fatty acids** (rel.%) | | | |
| Palmitic acid (16:0) | $6.1^{a*} \pm 0.1$ | $6.2^{a} \pm 0.1$ | $6.1^{a} \pm 0.1$ |
| Palmitoleic acid (16:1) | $0.1^{a} \pm 0.01$ | $0.1^{a} \pm 0.01$ | $0.1^{a} \pm 0.01$ |
| Stearic acid (18:0) | $2.1^{a} \pm 0.1$ | $2.2^{a} \pm 0.1$ | $2.2^{a} \pm 0.1$ |
| Oleic acid (18:1 n-9) | $19.1^{a} \pm 0.5$ | $18.7^{a} \pm 0.5$ | $18.9^{a} \pm 0.7$ |
| cis-Vaccenic acid (18:1 n-7) | $0.7^{a} \pm 0.1$ | $0.7^{a} \pm 0.1$ | $0.7^{a} \pm 0.1$ |
| Linoleic acid (18:2 n-6) | $62.7^{a} \pm 0.9$ | $63.6^{a} \pm 1.0$ | $62.8^{a} \pm 0.9$ |
| Linolenic acid (18:3 n-3) | $9.1^{a} \pm 0.4$ | $8.5^{a} \pm 0.5$ | $9.1^{a} \pm 0.6$ |
| Arachidic acid (20:0) | $0.1^{a} \pm 0.01$ | $0.1^{a} \pm 0.01$ | $0.1^{a} \pm 0.01$ |
| **Tocopherols** (mg/kg) | $340^{a} \pm 23$ | $220^{b} \pm 6$ | $211^{c} \pm 4$ |
| **Acid value** (mg KOH/g) | $3.7^{a} \pm 0.1$ | $3.9^{a} \pm 0.2$ | $3.8^{a} \pm 0.1$ |
| **conj. Dienes** ($A_{232,\ 1\%}$) | $1.3^{a} \pm 0.02$ | $2.4^{b} \pm 0.02$ | $3.1^{c} \pm 0.02$ |
| **conj. Trienes** ($A_{268,\ 1\%}$) | $0.13^{a} \pm 0.01$ | $0.20^{b} \pm 0.02$ | $0.18^{b} \pm 0.01$ |

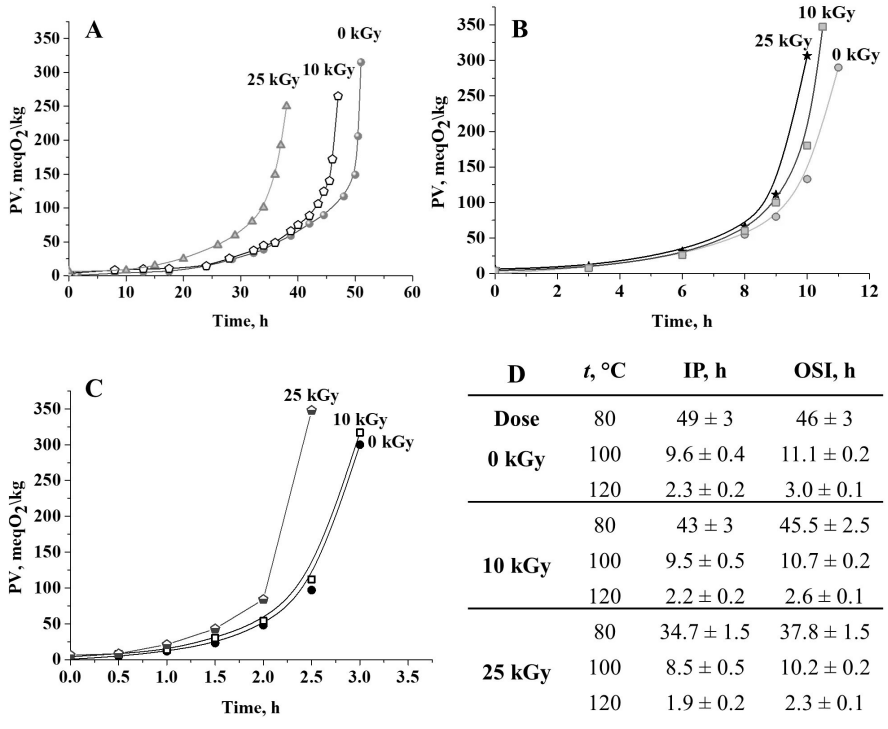
*Different letters within each row indicate statistically significant difference ($p < 0.05$)

increasing trend as a result of irradiation with doses higher than 6 kGy, with a maximum value recorded at 10 kGy, was reported by Iqbal et al. [16]. According to these authors, the effect on conjugated dienes and trienes was negligible when the sample was irradiated up to 4 kGy, but above this dose the increase was significant. Our results (Table 1) confirmed the increasing trend of conjugated dienes and trienes but the acid value showed no significant change. Fat yield was not affected by irradiation, too, as observed also by Iqbal et al. [16].

***Impact of gamma-irradiation on the oxidative stability of walnut oils.*** To investigate the effect of *gamma*-irradiation, the concentration of the lipid hydroperoxides (LOOHs) was measured (as the Peroxide value, PV) and the kinetics of their accumulation at 80 °C, 100 °C, and 120 °C under lipid autoxidation conditions was studied. The oxidation stability indexes (OSI) at the same conditions were determined as well. All these results are presented in Fig. 1.

In fact, the induction period (IP) is the time required to reach an end point of the oxidation process, after which the rate of formation and accumulation of LOOH (primary oxidation products) increases exponentially. During IP, the concentration of antioxidants (tocopherols, TOHs) is completely consumed. It should be noted that while PV and IP are parameters measured during the initial stage(s) of the autoxidation process (Fig. 1), OSI is a measure of the volatile degradation products when the process is underway.

Réblová [18] has reported that the activity of tocopherols (as the main




| D | *t*, °C | IP, h | OSI, h |
|---|---|---|---|
| **Dose** | 80 | 49 ± 3 | 46 ± 3 |
| **0 kGy** | 100 | 9.6 ± 0.4 | 11.1 ± 0.2 |
| | 120 | 2.3 ± 0.2 | 3.0 ± 0.1 |
| | 80 | 43 ± 3 | 45.5 ± 2.5 |
| **10 kGy** | 100 | 9.5 ± 0.5 | 10.7 ± 0.2 |
| | 120 | 2.2 ± 0.2 | 2.6 ± 0.1 |
| | 80 | 34.7 ± 1.5 | 37.8 ± 1.5 |
| **25 kGy** | 100 | 8.5 ± 0.5 | 10.2 ± 0.2 |
| | 120 | 1.9 ± 0.2 | 2.3 ± 0.1 |

Fig. 1. Kinetics of LOOH accumulation during the autoxidation of oils from non-irradiated (0 kGy) and irradiated (10 kGy and 25 kGy) walnuts: (A) 80 °C; (B) 100 °C; (C) 120 °C; (D) the induction period (IP, obtained after processing the curves) and oxidative stability index (OSI, measured by Rancimat)

antioxidants) is practically constant in the temperature range between 80 °C and 110 °C but decreases at temperatures higher than 110 °C. TOHs' concentration drops significantly (Table 1) as a result of their participation in the free radical scavenging processes initiated after *gamma*-irradiation. This affects the values of the parameters IP and/or OSI (Fig. 1D) as a measure of the oxidation stability after extraction of oils and is most evident comparing the results obtained at 80 °C (Fig. 1). The difference between IP and OSI values of oils obtained from nuts treated with *gamma*-rays at doses of 10 kGy and 25 kGy is about 10 h. The results are also explained by the rather higher degree of unsaturation of the walnut oil, a prerequisite for the higher rate of the accumulation of LOOHs in the initial oxidation stages.

***Impact of addition of antioxidants and synergist(s) on the oxidative stability of walnut oils.*** Antioxidant mixtures based on ascorbyl palmitate (AscPH) and *alpha*-tocopherol ($\alpha$-TOH), added to the oils, were studied considering the concentration of the native TOHs (Table 1). Experiments in the presence of

the compositions were performed at 80 °C. No statistically significant difference in oxidative stability at temperatures above 100 °C was observed for walnut oil from non-irradiated (0 kGy) and *gamma*-treated nuts at 10 kGy (Fig. 1) which is the maximum permitted level for food *gamma*-irradiation [1].

Figure 2 presents the kinetic curves constructed as a result of monitoring the autoxidation process at 80 °C of oil obtained from non-irradiated walnuts after the addition of double mixtures of AscPH and $\alpha$-TOH (at 0.5 mM) in a ratios of 1:1 and 2:1. These curves were compared with the kinetic curves obtained in the presence of the same mixtures and under the same oxidation conditions of oils from *gamma*-irradiated (10 kGy) walnuts (Fig. 2). The concentration of $\alpha$-TOH is 0.5 mM in both ratios.

There is an optimum concentration (concentration interval) for maximum antioxidant efficacy of $\alpha$- and $\gamma$-TOH depending on the unsaturation degree of the vegetable oils. At concentrations higher than the optimum one, TOHs participate in side reactions leading to decrease of their activity and even to prooxidant effect [19]. For this reason, it is not advisable to stabilize the oils by supplementing them only with TOHs, increasing their concentration, and the addition of a synergist is strongly recommended. Our previous studies proved synergism between $\alpha$-TOH and AscPH, a lipid-soluble analogue of vitamin C (E 304), in lipid substrates with a lower degree of unsaturation than walnut oil [19, 20].

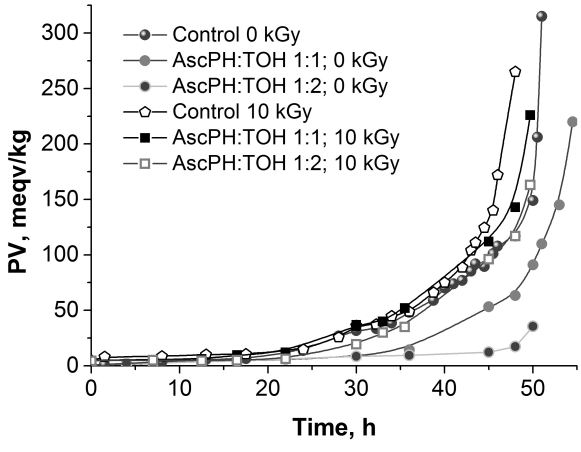



Fig. 2. Kinetic curves of LOOH accumulation during autoxidation of oil extracted from *gamma*-irradiated walnuts before and after addition of double mixtures of ascorbyl palmitate (AscPH) and *alpha*-tocopherol ($\alpha$-TOH), 0.5 mM, in a ratio of 1:1 and 2:1

Kinetic parameters obtained after processing the curves are presented in Table 2. Irradiation of walnuts with dose of 10 kGy resulted in a decrease of IP of the oils and increased initial rate of oxidation R at the same time (Fig. 2, Table

Table 2

Kinetic parameters characterizing autoxidation at 80 °C of glyceride oil from non-irradiated (control sample) and irradiated (10 kGy) walnuts before and after addition of antioxidants

| Walnut oils (oxidation at 80 °C) | *Kinetic parameters | | | |
|---|---|---|---|---|
| | $IP$, h | R, $10^{-6}$, $Ms^{-1}$ | ID, – | Effect |
| Control sample C (0 kGy) | $50 \pm 3$ | $0.055 \pm 0.005$ | – | – |
| + 0.5 mM AscPH: $\alpha$-TOH (1:1) | $51 \pm 3$ | $0.040 \pm 0.005$ | 1.4 | week |
| + 1 mM AscPH: $\alpha$-TOH (2:1) | $60 \pm 3$ | $0.038 \pm 0.005$ | 1.4 | moderate |
| Irradiated sample (10 kGy) | $43 \pm 3$ | $0.082 \pm 0.004$ | – | – |
| + 0.5 mM AscPH: $\alpha$-TOH (1:1) | $47 \pm 3$ | $0.078 \pm 0.005$ | 1.1 | week |
| + 1 mM AscPH: $\alpha$-TOH (2:1) | $48 \pm 3$ | $0.041 \pm 0.005$ | 2.0 | moderate |

*IP – Induction period, R – initial oxidation rate, ID – Inhibition degree as a ratio between the initial rates of lipid autoxidation in the absence ($R_0$ and $R_{10}$, respectively) and in the presence of antioxidants

2). Enrichment of the oils with binary compositions has a higher efficiency for those extracted from non-treated walnuts (Fig. 2) whose initial PV is lower and conjugated dienes and trienes are ∼ two-fold lower in comparison with the oils from irradiated nuts (Table 1). In all cases, the two-fold increase of AscPH concentration (1 mM), added to 0.5 mM $\alpha$-TOH (2:1), has a higher efficacy. The 2:1 mixture, in the case of irradiated samples, resulted in a halving of the R value (0.041 $Ms^{-1}$) relative to the oxidation rate of the irradiated control sample (0.082 $Ms^{-1}$).

**Conclusions.** Doses of 10 kGy and 25 kGy *gamma*-rays do not affect significantly the yield and fatty acids composition of the oils from irradiated walnuts. An increase of conjugated dienes and trienes, and decrease in tocopherols amount have been observed with an impact on the oil oxidative stability. Enrichment of the oils with binary compositions of ascorbyl palmitate and $\alpha$-tocopherol has a higher efficiency for those extracted from non-irradiated walnuts whose concentration of primary lipid oxidation products is lower in comparison with that in oils from irradiated walnuts. The antioxidant composition in which the concentration of ascorbyl palmitate is twice as high as that of $\alpha$-tocopherol is more effective, probably because the latter can be regenerated by the palmitate.

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