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TOCOPHEROL CONTENT IN PEANUTS AFTER *GAMMA*-IRRADIATION: EFFECT OF ASCORBYL PALMITATE ADDITION ON OIL STABILITY

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Abstract

Food irradiation has a number of advantages over the other conventional preservation technologies like canning, freezing, pasteurization and addition of chemical preservatives. It is a fast and inexpensive treatment which can be performed in the original packaging eliminating the risk of re-pollution. Unfortunately, changes as a result of irradiation may occur in biologically active compounds, especially lipids and antioxidants, thus decreasing the oil oxidative stability. So, this study is focused on the evaluation of changes in the content of tocopherols in oil, extracted from peanuts subjected to *gamma*-irradiation with a dose of 10 kGy. The effect of ascorbyl palmitate added to the oil in different proportions has been studied as well. The results showed decreasing of tocopherols in the oil from irradiated peanuts. On the other hand, addition of ascorbyl palmitate in 1:1 ratio to the native γ-tocopherol indicated synergistic effect which not only compensated the negative effect of irradiation but decreased twice the oxidation rate compared to that of non-irradiated oil.

Key words: peanut oil, *gamma*-irradiation, tocopherols, ascorbyl palmitate, synergism, effective protectors, food analysis

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1. Introduction. Nuts are important source of unsaturated fatty acids, proteins, some vitamins and minerals [1,2]. The structure of the soil and nutrients are of particular relevance for Peanut plant (*Arachis hypogaea*) which blooms above the ground but forms its kernels below the ground. Tropical and subtropical climate countries have favourable environmental conditions for development of the main types of genotoxicant fungi [3] while Bulgaria has a temperate-continental climate with moderate features. Bulgarian peanut production as a potential supply route to the European Union has the advantage of delivering aflatoxin free nuts, compared to other parts of the world, and may be able to produce *organic nuts* [4,5] justifying the higher market price. Before 1998, the areas sown with peanuts in the country were over 15 000 ha. Unfortunately, these areas in 2018 and 2019 were below 1000 ha [6]. The demand is about 50 times higher than the achieved production in Bulgaria which relies on imports.

One of the most effective ways to overcome the problem with *Aspergillus flavus* or/and *A. parasiticus* (pathogenic microorganisms responsible for the production of aflatoxins), is applying of γ-irradiation [7]. Although radiation is one of the conservation techniques that cause fewer damages to food nutrients, vitamin losses resulting from the food irradiation can be substantial [8,9]. To the best of our knowledge, no data on the ability of various antioxidants and their compositions to increase the oxidative stability of peanut oils, derived from irradiated nuts, have been published yet. On the other hand, peanut oil is susceptible to deterioration since its linoleic acid content reaches 35% [9,10]. So, the main goal of the current study was to find possibility to neutralize or at least minimize the negative effects of radiation-related lipid oxidation by additional enrichment of the peanut oil with co-antioxidants.

2. Materials and methods. ***2.1. Chemicals and plant samples.*** Peanuts were purchased from the local market and were tested by Electron paramagnetic resonance (EPR) spectroscopy [10] that they had not been previously treated by gamma-rays. All solvents were of HPLC grade (Merck, Darmstadt, Germany). Ascorbyl palmitate (AscPH) and tocopherol isomers (DL-α-, DL-β-, DL-γ- and DL-δ-TOH) were purchased from Sigma Adrich.

2.2. Gamma-irradiation of nuts under study. The peanuts were irradiated at a 60-Co source with 8 200 Ci activity according to the method previously described by us [10]. For the study of the absorbed dose distribution Alanine dosimeters (Kodak Bio Max) were used, measured by an EPR spectrometer E-scan Bruker and calibrated in units of absorbed dose in water. Three dosimeters were placed in each point.

2.3. Extraction procedure. About 20 g peanut kernels were milled and a precisely weighed portion of them was subjected to Soxhlet extraction with *n*-hexane [11]. The fat content was calculated towards the initial weight.

2.4. HPLC analysis of the content of tocopherols. A Shimadzu system, consisting of a DGV-20A 5R degassing unit, LC-30AD pump, SIL-30AC autosam-

pler, CTO-20AC column oven, RF-20AXS fluorescence detector and CBM-20A controlling unit was employed. The separation was carried out in 25 °C using LUNA SILICA (2) column (5 µm, 250 × 4.6 mm; Phenomenex, USA) and hexane:dioxane (95:5; v/v) as a mobile phase with 1 ml/min flow rate. An injection volume of 10 µl was used. Detector was set at excitation of 292 nm and emission of 325 nm.

Molar concentration of each one of tocopherols in the control (0 kGy) and in the irradiated (10 kGy) samples is calculated by the equation: $C_{M^{\alpha\text{-TOH}}} = \frac{m}{M_W V}$, where m is the mass of the related compound [g]; M_W is its molar mass [g/mol]; V is volume of the oil sample [l].

2.5. Determination of the oxidative stability of oil samples. Autoxidation was carried out in a thermostatic bath at 100 ± 0.2 °C by blowing air through the oil samples (2.0 g) at a rate of 100 ml/min in glass vessels. Samples containing different concentrations of ascorbyl palmitate (AscPH) were prepared by adding aliquots of its acetone solution according to KANCHEVA et al. [12]. The process was monitored by withdrawing samples at measured time intervals and subjecting them to determination of the peroxide value (PV) [13].

Induction period (IP) was determined by the method of tangents to the two parts of kinetic curves of lipid peroxide accumulation during the whole oxidation course [10,12].

Protection factor (PF) of the irradiated sample was calculated as a ratio between its induction period IPγ and that of the control non-irradiated sample, i.e. PF = IPγ/IP$_C$. PF of irradiated samples enriched with AscPH was calculated as a ratio between their induction periods (IP$_A$) and IPγ (22±2 h), i.e. PF = IP$_A$/IPγ. Inhibition degree (ID) of the irradiated samples was calculated as a ratio between initial rate of oxidation of the control non-irradiated sample (Rc) and that of the irradiated sample, i.e. ID = R$_C$/Rγ. ID of the irradiated samples enriched with AscPH was calculated as a ratio between Rγ, i.e. in absence and in presence of AscPH (ID = Rγ/R$_A$).

Three measurements of two parallel samples were done and the results were presented as mean value ± standard deviation.

3. Results and discussion. Results obtained from the HPLC analysis, summarized in Table 1 confirm that changes in tocopherol (TOH) content definitely occur after irradiation process. It can also be seen in the chromatograms (Fig. 1).

Gamma-TOH is present in the highest amount in the oil, being reduced by a

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Tocopherols quantified (mg/100 g oil) in peanut oil of irradiated and non-irradiated peanuts

Irradiation dose	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol
0 kGy	7.421 ± 0.202	1.024 ± 0.026	18.25 ± 0.538	1.072 ± 0.037
10 kGy	2.776 ± 0.008	0.649 ± 0.003	10.01 ± 0.003	0.745 ± 0.017

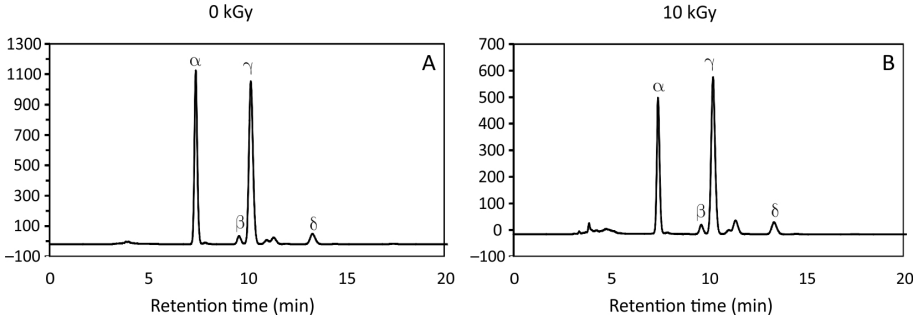

Fig. 1. HPLC chromatograms of α-, β-, γ- and δ-tocopherols in peanut oil obtained from (A) non-irradiated (0 kGy) and (B) irradiated (10 kGy) peanuts

half after irradiation process. In case of α-TOH (Table 1), almost 3-fold decrease after the irradiation is observed.

The results of HPLC analysis have shown that α-TOH content in the control (0 kGy) sample is 7.421 mg/100 g. Since the density of the peanut oil is 0.91 g/ml at 25 °C then α-TOH content is 7.421 mg/110 ml oil (M_W of α-TOH is 430.71 g/mol) which means its molar concentration in the oil is 0.16 mM. For the oil from irradiated nuts (10 kGy) it decreased to 0.06 mM. The molar concentrations of β- and δ-TOH in irradiated samples were 0.014 mM and 0.017 mM, respectively. In the oil of non-irradiated nuts it was 0.02 mM for both tocopherols. *Gamma*-TOH was present in the highest concentration in the peanut oil (0.4 mM) and was reduced by a half after irradiation (0.22 mM).

Figure 2 (A and B) presents kinetics of lipid peroxides (LOOH) accumulation during bulk phase autoxidation of peanut oil extracted from irradiated peanuts (10 kGy) in absence and in presence of 0.1 mM and 0.2 mM AscPH.

Irradiated sample has reduced oxidation stability due to the higher level of LOOH on the one hand, and to the involvement of its antioxidants (TOHs) in capturing of the free radicals generated by γ-rays, on the other. The molar concentrations of α- and γ-TOH in the oil, further enriched with AscPH, influence

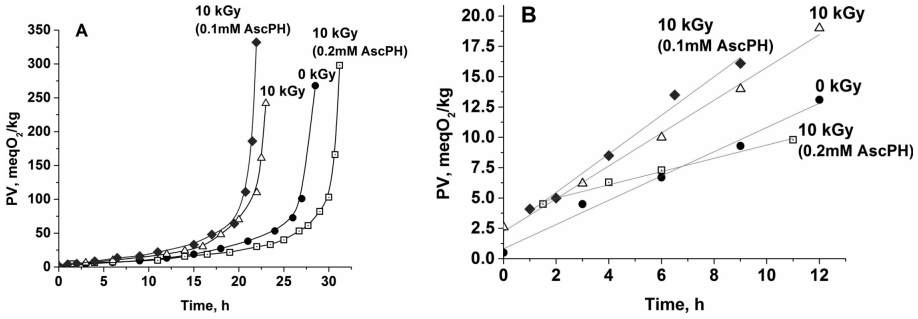

Fig. 2. Kinetic curves of LOOH accumulation during autoxidation of oil extracted from gamma-irradiated peanuts before and after addition of 0.1 mM and 0.2 mM ascorbyl palmitate (AscPH) for (A) the whole oxidation process and (B) for the initial stage of oxidation

significantly the effect between the antioxidants. TOHs' molecules can be regenerated in H-atom transfer reaction (HAT) from the molecules of AscPH, which is a good prerequisite for a synergism. This reaction of H-atom transfer is reversible. However, in case of synergism between AscPH and TOH, the reaction is shifted to regeneration of TOH, which is the stronger antioxidant:

$$\mathrm{AscPH} + \mathrm{TO}^{\bullet} \leftrightarrow \mathrm{TOH} + \mathrm{AscP}^{\bullet}.$$

Cross-disproportionation between ascorbyl palmitate radical ($\mathrm{AscP}^{\bullet}$) and tocopheryl radical ($\mathrm{TO}^{\bullet}$) with regeneration of TOH and dehydroascorbyl palmitate (DHAsc) formation, is also possible:

$$\mathrm{AscP}^{\bullet} + \mathrm{TO}^{\bullet} \rightarrow \mathrm{TOH} + \mathrm{DHAsc}.$$

Kinetic parameters, namely induction period (IP), protection factor (PF), initial rate of oxidation (R) and inhibition degree (ID), obtained after processing the kinetic curves, are given in Table 2.

T a b l e 2

Kinetic parameters characterizing peanut oil before and after addition of ascorbyl palmitate (AscPH)

Sample	IP, h	PF (IPγ/IPc)	R, 10^{-7} Ms^{-1}	ID (Rc/Rγ)
Control (0 kGy)	27 ± 2	—	1.6 ± 0.1	—
Irradiated (10 kGy)	22 ± 2	0.8	2.1 ± 0.2	0.8
Irradiated (10 kGy) enriched with 0.1 mM AscPH (0.5:1)*	21 ± 2	0.8	2.2 ± 0.1	1.0
Irradiated (10 kGy) enriched with 0.2 mM AscPH (1:1)*	30 ± 2	1.1	0.75 ± 0.05	2.8

*Ratio between AscPH and the native γ-tocopherol present in oil

As can be seen in Table 2 higher protective effect is observed at a ratio of 1:1 between AscPH and native γ-TOH while at ratio of 0.5:1 (addition of 0.1 mM AscPH) such effect is absent. The induction period (Table 2) and the stability in case of equimolar ratio, i.e. in presence of 0.2 mM AscPH, are even higher than those of the non-irradiated control sample (Fig. 2A). The initial rate of oxidation in this case (0.75×10^{-7} Ms^{-1}) is two-fold lower in comparison with the oxidation rate of the control (Fig. 2B, Table 2).

Conclusions. The results obtained in the current study show that ascorbyl palmitate (AscPH) reduces the negative effects of irradiation-related lipid oxidation of peanut oil in a concentration dependent manner and thus could be used as an oil additive to prevent oil deterioration.

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