

## Effect of gamma-irradiation on the chemical composition and antioxidant activity of dried black chokeberry (*Aronia melanocarpa*) fruits

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Gamma-irradiation is very fast, efficient, inexpensive, secure and safe method for sterilization of food products. The aim of the present study was to investigate the influence of gamma-irradiation with different intensity on the chemical composition and antioxidant activity of dried black chokeberry fruits (*Aronia melanocarpa*). Dried berries were irradiated at a <sup>60</sup>Co source with 8200 Ci activity with doses of 10 kGy and 25 kGy. Irradiation with  $\gamma$ -rays increased the total sugar content, as well as the amount of fructose and sorbitol in dried aronia samples as differences were the most distinct in the 25 kGy sample. Gamma-irradiation caused reduction of organic acids content being significant ( $p < 0.05$ ) for shikimic, ascorbic and succinic acids. Irradiation had profound effect on the total amount of anthocyanins in the fruits. The anthocyanin content of the untreated control sample was  $1144 \pm 46$  mg/100 g dried weight (DW) and increased up to  $1192 \pm 73$  mg/100 g DW and  $1291 \pm 9$  mg/100 g DW after irradiation with 10 kGy and 25 kGy, respectively. However, irradiation did not affect significantly the total polyphenol content of the fruits, neither their ORAC antioxidant activity.

**Keywords:** black chokeberry (*Aronia melanocarpa*), gamma-irradiation, polyphenols, anthocyanins, antioxidant activity.

### INTRODUCTION

Food irradiation was developed as a technology during the second half of the 20<sup>th</sup> century and nowadays it is among the most widely used methods for microbial decontamination of foods [1]. The extension of shelf life of foods is the main purpose of gamma-irradiation [2]. This treatment is considered to be safe, efficient, environmentally clean and energy efficient process and is particularly valuable as an end product decontamination procedure [3]. Radiation decontamination of dry ingredients such as dried fruits, herbs, spices and nuts with doses of 3–10 kGy proved to be a viable alternative to fumigation with microbicidal gases [4], but doses up to 20 kGy have been used for reduction of microbial population in dry foods [5]. However, along with the benefits of such treatment, negative effects related to changes in the chemical composition of irradiated foods could be expected. For example, irradiation caused negative effects on the fatty acid composition of different nuts [6] and significant decrease in the total anthocyanins and phenolic content on fresh pomegranates [7]. The rich variety of foods, every specific with its chemical composition, requires systematic studies on the effect of gamma-irradiation on their chemical composition. Black chokeberry (*Aronia melanocarpa*) fruits are among the richest sources of

polyphenol compounds and particularly anthocyanins [8]. In the last decade, these intensively colored berries have been thoroughly studied for their chemical composition [9, 10] and health benefits [11–17]. Black chokeberries are suitable raw material for the production of functional foods and nutraceuticals, but dried berries are also available on the market and sold as a rich source of phenolic antioxidants. Although EPR spectroscopy has been used to identify gamma-irradiated dried chokeberries [18], to the best of our knowledge, there are not studies on the effect of gamma-rays on the chemical composition change of dried black chokeberry fruits. Therefore, the aim of the current study was to investigate the effect of gamma-irradiation with different intensities (10 kGy and 25 kGy) on sugar, organic acid, total polyphenol and total anthocyanin contents, and ORAC antioxidant activity of dried chokeberry fruits.

### MATERIALS AND METHODS

#### Chemicals

All solvents (HPLC grade) and reagents were purchased from Sigma-Aldrich (Steinheim, Germany).

#### Black chokeberry fruits

Black chokeberry fruits were supplied from Vitanea Ltd. (Plovdiv, Bulgaria) in the stage of full maturity, in August 2017. Fresh fruits were put in polyethylen e bags and frozen at  $-18^{\circ}\text{C}$ . After that, the frozen fruits were lyophilized (Christ Alpha 1-4

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LDplus, Martin Christ GmbH, Germany) and stored in a desiccator until use.

### ***Gamma-irradiation of dried black chokeberry (*Aronia melanocarpa*) fruits***

Freeze dried berries were irradiated at a  $^{60}\text{Co}$  source with 8200 Ci activity. The gamma-rays facility has a mobile irradiation chamber with 4.0 L volume and dimensions: 13.5 cm diameter and 22 cm height. During the irradiation, the chamber rotates on its vertical axe. For the study of the absorbed dose, distribution Alanine dosimeters (Kodak BioMax) were used, measured by ESR spectrometer E-scan Bruker and calibrated in units of absorbed dose in water. In each point, three dosimeters were placed. The chosen absorbed doses were 10 kGy and 25 kGy. Controls and irradiated samples were kept in polyethylene bags.

### ***Extraction of polyphenols***

Before extraction and analysis, samples were milled in a laboratory mill to fine powder. Approximately 0.5 g of the fruit powder were weighted accurately, transferred to an extraction tube and mixed with 40 mL of the extragent (60% ethanol acidified with 0.5% formic acid). The samples were extracted for 1h at room temperature on a magnetic stirrer. After that, the samples were centrifuged ( $6000\times g$ , 20 min) and the supernatants were further used for antioxidant activity determination and analyses of total anthocyanins and total polyphenols.

### ***Extraction of sugars and organic acids***

One gram of the fruit powder was weighted accurately and extracted for 1 hour, at 30°C with 30 mL 3% meta-phosphoric acid solution in distilled water and shaking on a thermostatic water bath (NUVE, Turkey). After that, the samples were centrifuged ( $6000\times g$ , 20 min) and the supernatants were used for HPLC analysis of sugars and organic acids.

### ***High Performance Liquid Chromatography (HPLC) analysis of sugars***

HPLC determination of sugars was performed on HPLC system Agilent 1220 (Agilent Technology, USA), with a binary pump and Refractive Index Detector (Agilent Technology, USA). The column was Zorbax Carbohydrate (150 x 4.6 mm, 5  $\mu\text{m}$ , Agilent), connected to a guard column Zorbax Reliance Cartridge (Agilent), and as eluent was used 80% acetonitrile in water at a flow rate of 1.0 mL/min and temperature 25°C. Results were expressed as g/100 g dry weight (DW).

### ***HPLC determination of organic acids***

HPLC determination of organic acids was performed on HPLC system Agilent 1220 (Agilent

Technology, USA), with binary pump and UV-Vis detector (Agilent Technology, USA). Organic acid separation was performed on Agilent TC-C18 column (250 x 4.6 mm, 5  $\mu\text{m}$ ) at 25°C and the eluate was monitored at 210 nm. The mobile phase was 25 mM phosphate ( $\text{K}_2\text{HPO}_4/\text{H}_3\text{PO}_4$ ) buffer (pH 2.4), flowing at 1.0 mL/min. Results were expressed as mg/100 g DW.

### ***Total polyphenol compound analysis***

The total polyphenols were determined according to the method of Singleton & Rossi, with Folin-Ciocalteu's reagent [19]. Gallic acid was used for calibration curve and results were expressed as gallic acid equivalents (GAE) per 100 g DW.

### ***Total anthocyanin content determination***

Total anthocyanin content was determined by the pH-differential method [20] and the results were expressed as mg of cyanidin-3-galactoside equivalents per 100 g DW.

### ***Oxygen Radical Absorbance Capacity (ORAC) assay***

ORAC was measured according to the method of Ou et al. [21] with some modifications described in details by Denev et al., [22]. Results were expressed as  $\mu\text{mol TE/g DW}$ . ORAC analyses were carried out on FLUOstar OPTIMA plate reader (BMG Labtech, Germany) with excitation wavelength of 485 nm and emission wavelength of 520 nm.

### ***Statistical analysis***

The processing was repeated two times and the analyses were performed in duplicates or triplicates. The results were expressed as mean values  $\pm$  standard deviations. One-way analysis of variance (ANOVA) and Student's t-test were used to evaluate the differences of the mean between groups. P values less than 0.05 were considered to be significant.

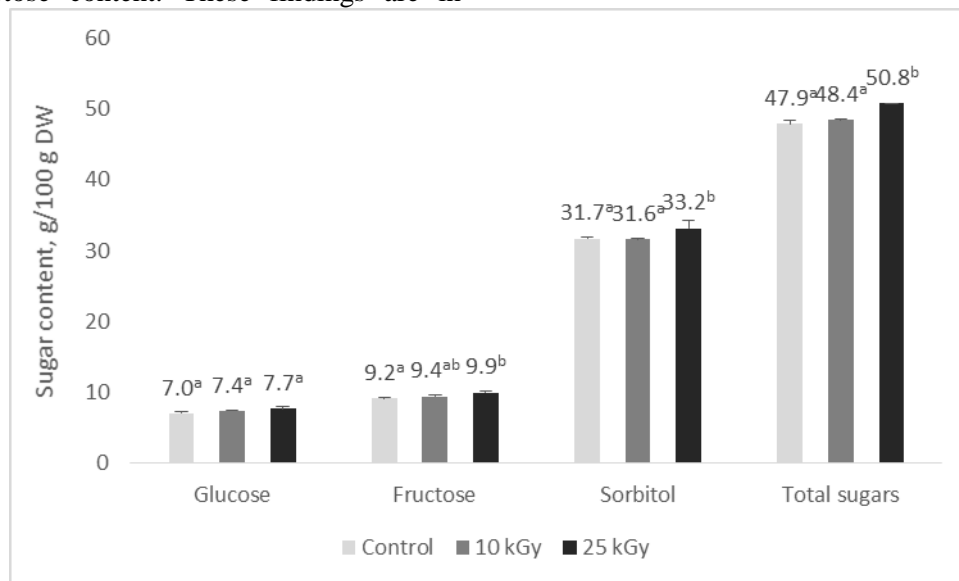
## **RESULTS AND DISCUSSION**

### ***Effect of gamma-irradiation on sugar and organic acid composition of dried chokeberries***

From the literature, it is known that gamma-irradiation could affect the content of sugars and organic acids in irradiated foods. **Figure 1** presents the content of sugars in irradiated samples in comparison to the untreated control. As it is evident from the results, sorbitol is the main carbohydrate of dried berries, nevertheless irradiated or not, which complies with our previous results [10]. Interestingly, irradiation with  $\gamma$ -rays increased the total sugar content, as well as the amount of fructose and sorbitol in dried aronia samples and differences were the most distinct in the 25 kGy sample. For example, the amount of fructose, sorbitol and total sugars were significantly different ( $p<0.05$ ) in that

sample in comparison to the untreated control. For 10 kGy sample, the increase was not so distinctive being significantly different from the control only for the fructose content. These findings are in

agreement with previous studies that report increase in total and reducing sugar contents in dates, onions, potatoes and sweet potatoes [23-25].



**Figure 1.** Sugar content (g/100 g) of untreated (control) and  $\gamma$ -irradiated (10 kGy and 25 kGy) dried black chokeberries. Results are presented as mean values  $\pm$  SD. There are no significant differences among values marked with the same letters in individual groups.

Organic acids are another important component of foods, since their content and composition strongly affect food palatability. The composition of organic acids in untreated and irradiated dried chokeberries was the same and embraced quinic acid, malic acid, ascorbic acid, shikimic acid, citric acid, oxalic acid and succinic acid. The irradiation with  $\gamma$ -rays affected the content of several organic acids in dried chokeberries and results are shown in **Table 1**. In contrast to sugars, gamma-irradiation caused reduction of organic acids content being significant ( $p < 0.05$ ) for shikimic, ascorbic and succinic acids.

**Table 1.** Content and composition of organic acids in untreated (control) and  $\gamma$ -irradiated (10 kGy and 25 kGy) dried black chokeberries.

	Control (untreated)	10 kGy	25 kGy
Quinic acid	1117 $\pm$ 16	1108 $\pm$ 59	1116 $\pm$ 16
Malic acid	836 $\pm$ 3	846 $\pm$ 5	847 $\pm$ 5
Ascorbic acid	241.6 $\pm$ 2.8	239.5 $\pm$ 7.7	214.7* $\pm$ 4.6
Shikimic acid	5.8 $\pm$ 0.1	5.8 $\pm$ 0.2	4.7* $\pm$ 0.3
Citric acid	139 $\pm$ 8	136 $\pm$ 4	134 $\pm$ 3
Oxalic acid	7.5 $\pm$ 0.9	7.8 $\pm$ 0.7	8.6 $\pm$ 0.1
Succinic acid	17.5 $\pm$ 2.3	11.4* $\pm$ 1.1	10.0* $\pm$ 1.0

Results are presented in mg/100g DW as mean values  $\pm$  SD. Values marked with an asterisk are significantly different from the control.

Previous studies have shown that ascorbic acid is one of the most sensitive vitamins to irradiation and the effect is dose-dependent. Irradiation causes oxidation of ascorbic acid to dehydroascorbic acid, which however is also biologically active. For example, Wen et al. observed that increasing the radiation dosage gradually decreased the vitamin C concentration in goji berries [26]. The gamma-rays dose with the most profound effect in that study was 14 kGy, which is close to the doses used in our work. Other studies with lower doses of  $\gamma$ -rays reveal less profound effect on ascorbic acid content. For example, Golding et al. found that there were few changes in the nutritional content of raspberry fruit following irradiation and storage [27]. Some components, such as sucrose, ascorbic, citric and malic acids were affected by irradiation, but the measured changes were inconsistent and minor. For ascorbic acid, the mean concentration for fruit irradiated at 0.15 kGy was similar to that for untreated fruit. The mean concentration of ascorbic acid for raspberries irradiated at 0.4 kGy and 1 kGy were lower than for the untreated and 0.15 kGy irradiated fruits. In the same study was found no effect of phytosanitary irradiation on the nutritional content of northern highbush blueberry (cv. Brigitta). In a similar study, Zegota et al. revealed that low doses of gamma-irradiation (2-2.5 kGy) had very mild effect on ascorbic acid content of strawberries [28]. In another work was detected that

irradiation affected vitamin C concentration in strawberries, but change was small in comparison with the large variations observed between varieties [29]. Application of the commercial practicing dose (10 kGy) of  $\gamma$ -radiation caused significant loss in vitamin C in black pepper, cinnamon, nutmeg, oregano, and sage, and decreased the content of carotenoids in cinnamon, oregano, parsley, rosemary, bird pepper, and sage when all compared with control samples [30].

***Effect of gamma-irradiation on total polyphenol and total anthocyanin content, and ORAC antioxidant activity of dried chokeberry fruits***

Irradiation can influence the levels of antioxidant phytochemicals and the capacity of a specific plant sample to produce them at different levels. Therefore, we investigated the effect of gamma-irradiation on total polyphenol and total anthocyanin contents, and ORAC antioxidant activity of dried chokeberry fruits. The results are shown in **Table 2**. Gamma-rays, at the tested doses did not have a significant effect on the total polyphenol content and ORAC antioxidant activity of the irradiated black chokeberry fruits. On the contrary,  $\gamma$ -rays increased the total anthocyanin content of the samples, being significant for 25 kGy-irradiated fruits.

**Table 2.** Effect of gamma-irradiation on total polyphenol and total anthocyanin content, and ORAC antioxidant activity of dried chokeberry fruits

	Total polyphenols, mg/100 g DW	Total anthocyanins, mg/100 g DW	ORAC $\mu$ mol TE/g DW
Control (untreated)	6715 $\pm$ 149	1144 $\pm$ 46	899 $\pm$ 75
10 kGy	6935 $\pm$ 79	1192 $\pm$ 73	1054 $\pm$ 110
25 kGy	6825 $\pm$ 79	1292 * $\pm$ 19	1081 $\pm$ 86

Results are presented as mean values  $\pm$  SD. Values marked with an asterisk are significantly different from the control.

Different studies report contradictory results on the effect of gamma-irradiation on the content of anthocyanins and other phenolics in irradiated foods. It seems that the effect of gamma-rays is very complex and the sensitivity of the antioxidants or phenolic components depends on the food matrix and its properties (water content, pH, etc.), their structure and the applied radiation dose. Usually, low and medium doses have insignificant effects on antioxidants, and the effect of irradiation itself on other food constituents might be responsible for the production and/or the accumulation of phytochemicals/antioxidants in the plant. Breitfellner et al. have reported that  $\gamma$ -irradiation (1–10 kGy) in strawberries leads to the degradation of cinnamic, p-coumaric, gallic and hydroxybenzoic

acids [31]. The hydroxylation (decomposition) of these phenolic acids has been attributed to the formation of free hydroxyl ( $\cdot$ OH) radicals during the treatment. Kong et al. showed that the anthocyanin contents of blueberries treated with different doses irradiation were similar to that found in the control blueberries and there was no significant effect of radiation on the anthocyanin content among blueberries stored at 4°C for 7 days and 15 days [32]. Erkan et al. revealed that UV-C treatment for different durations (1, 5 and 10 min) increased the antioxidant capacity and the concentrations of anthocyanins and phenolic compounds in strawberries [33]. Similarly, Perkins-Veazie et al., (2008) observed that 2 or 4 kJ/m<sup>2</sup> UV-C exposures did not change the total phenolic content, while it increased the total anthocyanin content and FRAP values of blueberries (Bluecrop cv.) [34]. In contrast, a massive decrease of anthocyanins content (81–84%) in blueberries was observed after thermal processing (80°C for 30 min), suggesting that anthocyanins were much less sensitive to irradiation at low doses than to temperature [35]. In another study, the anthocyanin content of fresh peaches was enhanced by gamma-irradiation at dose range of 1.2–1.4 kG [36]. Contrary, Alighourchi et al. revealed that gamma-irradiation (0–10 kGy) significantly reduced total and individual anthocyanins in pomegranate juice [37]. In another study,  $\gamma$ -irradiation slightly affected the quantitative profile of phenolic compounds of a wild thyme ethanolic extract. The content of p-coumaric and caffeic acids decreased and that of flavonoid aglycons increased after the  $\gamma$ -ray treatment [38].

Variyar et al. reported that gamma-irradiation was capable of breaking the glycosidic bonds of polyphenols, thereby releasing soluble phenols of low molecular weight, leading to an increase of antioxidant rich phenolics, responsible for higher antioxidant activities [39]. Many authors have also reported the enhancement in antioxidant activities through ionizing radiations. Alothman et al. summarized the effects of different radiation source and the dose delivered on various types of antioxidants and their activity, as obtained from different plant sources [40]. Verde et al. reported that irradiation dose of 1.5 kGy did not result in a major impact on raspberries sensory and quality attributes with a beneficial effect of reducing microbiota by 1 log (95% inactivation), and enhancement of phenolic compounds and antioxidant activity for 7 days of refrigerated storage [41]. Similarly, Pérezet et al. assessed the effects of  $\gamma$ -irradiation (30 kGy) on the dry rosemary leaves extracted in methanol, ethanol and water extracts [42]. Accordingly, they found that  $\gamma$ -radiation improved antioxidant activity and

polyphenol content in ethanol and water extracts, but had no significant effect on the methanol extracts. A more recent study revealed that the radical scavenging activity of irradiated tea samples was either maintained or increased after gamma-irradiation [43]. However, our results indicate that although there was a trend towards increase in ORAC values of irradiated samples, results did not reach significant values, nevertheless the increase in anthocyanin content. Black chokeberry fruits contain several classes of phenolic compounds, such as proanthocyanidins, anthocyanins, hydroxyl-cinnamic acids, flavanols and flavan-3-ols [8, 10]. Probably because of that, the increase of anthocyanin content did not result in elevated total polyphenol content and ORAC antioxidant activity. Moreover, we could assume that phenolic components other than anthocyanin could be affected in different way by gamma-irradiation. Therefore, we plan to perform a further detailed study on the influence of gamma-irradiation on the individual polyphenol constituents of aronia berries, in order to explain better the observed effects.

## CONCLUSIONS

Our study demonstrated for the first time that irradiation with  $\gamma$ -rays affects the chemical composition of dried fruits from black chokeberry (*Aronia melanocarpa*). Irradiation with  $\gamma$ -rays increased the total sugar content, as well as the amount of fructose and sorbitol in dried aronia samples and differences were the most distinct in the sample, irradiated at dose of 25 kGy. In addition, gamma-irradiation caused reduction of organic acid content, being significant for shikimic, ascorbic and succinic acids. Irradiation had profound effect on the total amount of anthocyanins in aronia berries, but did not affect significantly their total polyphenol content and ORAC antioxidant activity. Probably this effect is associated with the natural protective role of anthocyanins against UV-irradiation in plants. However, further studies on the influence of gamma-irradiation on the individual polyphenol constituents of aronia berries are required, in order to explain the observed effect.

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