

SUPPLEMENTARY MATERIAL

Gamma Irradiation Affects the Total Phenol, Anthocyanin and Antioxidant Properties in Three Different Persian Pistachio nuts

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Abstract:

The effects of gamma irradiation (GR) on total phenol, anthocyanin, and antioxidant activity were investigated in three different Persian pistachio nuts at doses of 0, 1, 2, and 4 kGy. The antioxidant activity, as determined by FRAP and DPPH methods, revealed a significant increase in the 1-2 kGy dose range. Total phenol content (TPC) revealed a similar pattern or increase in this range. However, when radiation was increased to 4 kGy, TPC in all genotypes decreased. A radiation dose of 1 kGy had no significant effect on anthocyanin content of Kale-Ghuchi (K) and Akbari (A) genotypes, while it significantly increased the anthocyanin content in the Ghazvini (G) genotype. In addition, increasing the radiation to 4 kGy significantly increased the anthocyanin content of K and G genotypes. To conclude, irradiation could increase the phenolic content, anthocyanin and antioxidant activity of pistachio nuts.

Keywords: Gamma irradiation, Pistachio, total phenol, anthocyanin, antioxidant activity

1. Experimental

1.1. Plant material and irradiation condition

Samples of three different pistachio nut (*Pistacia vera* L.) genotypes were collected at commercial maturity after harvest from different pistachio production areas of Iran (Table S1) and their green hulls were removed and exposed to gamma rays at doses of 0, 1, 2 and 4 kGy. Samples of approximately 300 g of pistachio nuts of each genotype (gathered from one tree) were packed into several polyethylene bags in a single layer for irradiation. In this design, each bag was used as a separate trial. For each treatment, three replicates were independently conducted. The irradiation treatments were performed in a Cobalt 60 irradiator with a dose rate of 7.5 kGy/h at room temperature (22 ± 2 °C) and a relative humidity 40% to 60%. The absorbed dose was determined by an alcoholic chlorobenzene dosimeter (Al-Bachir, 2004). The total uncertainty of the measured dose was 3.44% at the 95% confidence level. Subsequently, after the irradiation process; total phenol, anthocyanin, and antioxidant properties of the samples were measured.

1.2. Determination of total phenol content

The Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) was used to determine the total amount of phenolic compounds in the pistachio nut extracts. The samples were milled into a fine powder (particle size ≤ 0.5 mm). Twenty ml of methanol was added to each one g milled pistachio nuts sample before being thoroughly shaken for 20 min and kept for four hours at room temperature (25 ± 1 °C). The mixture was filtered using filter paper (Whatman, Grade 1 Chr, UK) and kept in a freezer at -20°C until assayed. (Singh et al., 2002). Forty microliters of the sample along with standard concentrations of Gallic acid were added to 3160 microliters distilled water, following 200 microliters of Folin indicator and 600 microliters saturated sodium carbonate. After keeping the mixtures in the dark for three hours, the absorption of the samples at the wavelength of 765 nanometers was measured. The total phenol content in the extracts was expressed as mg gallic acid equivalent (GAE) per g of the dried sample.

1.3. Antioxidant activity

1.3.1. Diphenylpicrylhydrazyl (DPPH) radical

Oil antioxidant capacity from irradiated genotypes was assessed via stable DPPH radicals following the method introduced by Oliveira *et al* (Oliveira et al., 2008). Extracts (0.2 milliliter) at different concentrations were added to 4 milliliters of a 6×10^{-5} molar methanol solution of

the free radical DPPH. The mixtures were then kept at room temperature for four hours. Following this, the intensities of absorption were read at a wavelength of 517 nanometers using a spectrophotometer. One sample containing 0.2 milliliter of methanol with 4 milliliters of the DPPH solution was used as the control sample; the solvent methanol was employed to set the spectrometer at zero. The experiment was conducted in three replications. The radical scavenging activity (%) of the extract was determined using the following formula:

$$\% \text{ RSA} = [1 - (A_c - A_s) / A_c] \times 100$$

Where, A_c and A_s were the absorbance of control and test samples, respectively. RSA is the radical scavenging activity of the extract.

1.3.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP was assessed following the method of Benzie and Strain (Benzie and Strain, 1996). Briefly, 0.1 mL of each sample was mixed with 3 mL of FRAP reagent then incubated for 15 min at 37 °C, and the absorbance at 593 nm was observed. A standard curve with different concentrations of FeSO₄ 3 7H₂O was prepared so that the test results could be compared with it. FRAP values were calculated as follows:

$$\frac{\text{sample OD at 593 nm}}{\text{standard sample OD at 593 nm}} \times \text{FRAP value standard } (\mu\text{M})$$

1.4. Determination of Anthocyanin Content

Anthocyanin content was quantified by the pH differential method introduced by Giusti and Wrosted (Giusti and Wrosted, 2001). An amount of 100 mg pistachio seeds along with 10ml acidified methanol solution (99% Methanol / 1% HCL) was completely milled and kept for 24h in dark at room temperature (25 ± 1 °C) and then centrifuged at 4000g for 15 min; supernatant was used for analysis. Two dilutions of the above extracts were prepared for each sample. The first sample was diluted with 0.025 mol/L potassium chloride buffer at pH 1 and the second by the same volume with 0.4 mol/L sodium acetate buffer at pH 4.5. Both samples were incubated for 15 min at room temperature and the absorbance of each dilution was observed at 513 and 700 nm. The absorbance difference between pH values and wavelengths was calculated as follows:

$$A = (A_{513} - A_{700})_{\text{pH}1.0} - (A_{513} - A_{700})_{\text{pH}4.5}$$

The concentration of monomeric anthocyanin pigment was calculated as follows:

$$(\text{mg/mL}) = (A \times MW \times DF \times 1000) / (\epsilon \times l)$$

Where, MW is the molecular weight, DF is the dilution factor, ϵ is the molar absorptivity, and 1 defines liter. Because of the unknown sample composition, the molar absorptivity of 26900 L/mol cm was used for cyanidin 3-glucoside (molecular weight = 449.2).

1.5. Statistical Analysis

The experiment was conducted in completely randomized design (CRD) with three replications, values are means with standard deviations, and Duncan's multiple range test was used to evaluate differences between means. All analyses were performed using statistical analysis system computer software (SAS Institute, Cary, NC, USA, 1988). P values of <0.05 were considered significant. Coefficient of correlation was determined by the Karl Pearson method.

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Table S1

Three pistachio genotypes with their collection area (region) included in the study

No.	Genotype	Collection area	Latitude (N)	Longitude (E)	Altitude (M)
1	Akbari	Kerman	30°16'59"	57°04'43"	1760
2	Kale Ghuchi	Rafsanjan	30°24'24"	55°59'38"	1514
3	Ghazvini	Qazvin	36°16'07"	50°00'14"	1300