



Effect of gamma irradiation on the extraction yield, antioxidant, and antityrosinase activities of pistachio green hull extract

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ABSTRACT

In this study, the antioxidant activity and tyrosinase inhibitory of non-irradiated and irradiated pistachio green hull (PGH) extracts were investigated. After irradiation of PGH by different doses of gamma ray (0, 10, 20, 30 and 40 kGy), their phenolic compounds were extracted by water. Antioxidant activities of extracts were examined by DPPH[•] and FRAP methods. The results showed that irradiation not only do not have negative effects on antioxidant activity but also it can increase the amount of total phenolic compounds of water extract in comparison with non-irradiated sample. Water extract of irradiated PGH at the dose of 30 kGy, showed the highest antioxidant activity in the DPPH[•] test with EC₅₀ equal to 289.0 ± 1.2 µg/ml. Irradiated (30 kGy) and non-irradiated water extracts had the highest antityrosinase activities with IC₅₀ of 10.8 ± 1.1 and 11.9 ± 1.2 µg phenolic/ml, respectively. In addition, it was found that the water extract of irradiated PGH can prevent enzymatic browning in sliced raw potatoes. According to the antityrosinase potential of PGH extract, it may be suggested as an antibrowning agent in some foodstuffs or cosmetic products.

1. Introduction

The color is an important factor when buying a food product to be understood by the customers. Browning can be started due to processing, handling, and storage (Weijn et al., 2011) and causes the destruction of color and sensory characteristics such as aroma and loss of nutritional quality in beverages and plant-derived foods (Maisuthisakul and Gordon, 2009). Enzymatic browning of fresh fruits, beverages, and vegetables is undesirable. Browning after harvest and/or processing are a common phenomenon in different crops which decreases the commercial value of the products (Chang, 2009).

Tyrosinase (polyphenol oxidase) is a multifunctional copper containing enzyme which causes oxidation of phenolic substrates into quinones and these products undergo pursuant reactions and transformed into the dark melanin pigments (Weijn et al., 2011). Tyrosinase is responsible for enzymatic browning in plants and melanin biosynthesis in human skin (Maisuthisakul and Gordon, 2009). In addition to the standard tyrosinase inhibitors, a lots of new inhibitors were introduced and they classified into five major classes: polyphenols, benzaldehyde and benzoate derivatives, long-chain lipids and steroids, other natural or synthetic inhibitors, and irreversible inactivators based on either the chemical structures or the inhibitory mechanism. Polyphenols represent a diverse group of compounds containing multiple phenolic functionalities and are widely distributed in nature.

Polyphenols are also the largest groups in tyrosinase inhibitors until now. Gallic acid has been identified as a tyrosinase inhibitor from many plants, and its inhibitory mechanism together with those of its ester derivatives has been well studied by Kubo et al. (2003). Lately, the tyrosinase inhibitors derived from natural resources are used not only in food but also in the cosmetic industry as skin-whitening agents (Miyazawa et al., 2003). There are a limited number of tyrosinase inhibitors in the food industry due to off flavor, food safety and economic efficiency (Loizzo et al., 2012). According to the challenges, phenolic compounds with high antioxidant activity can be considered as tyrosinase inhibitor (Mazlan et al., 2013).

Iran is a leading country in the production and exports of pistachio in the world (Rajaei et al., 2010a). Pistachio green hull (PGH) is less valuable byproduct of the pistachio production that is accumulated in large volumes and this can cause serious environmental problems (Grace et al., 2016). So, there is special attention to the reuse and processing of agricultural waste as a source of low cost bioactive compounds with high antioxidant activity (Maisuthisakul and Gordon, 2009). PGH is a rich source of phenolic compounds with high antioxidant activity (Goli et al., 2005). Gallic acid is a predominate compound of PGH (Rajaei et al., 2010a) and can scavenge free radicals, chelating metals and reducing tocopherol radicals (Harrison and Were, 2007). These phenolic compounds have several medical effects such as anti-allergenic, antiatherogenic, anti-inflammatory, antimicrobial,

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antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Rajaei et al., 2010a). Also, recently anti-microbial and antimutagenic activities of PGH are studied by Rajaei et al. (2010b). Various antioxidant compounds have been detected in PGH including gallic acid, 4-hydroxybenzoic acid, protocatechuic acid, naringin, eriodictyol-7-O-glucoside, isorhamnetin-7-O-glucoside, quercetin-3-O-rutinoside, isorhamnetin-3-O-glucoside and catechin (Barreca et al., 2016). In our previous published work, we found that PGH has higher phenolic content compared to many of phenolic sources and can effectively prolong the oxidation of soybean oil at 60 °C compared to synthetic antioxidants (Goli et al., 2005).

Application of gamma irradiation as a phytosanitary treatment is increasing in the food industry and plant materials. Irradiation is taken into consideration as an effective tool for reducing microbial contamination and insect damage (Khattak et al., 2008). Irradiation can be used to release low molecular weight phenolic compounds with antioxidant activity in agricultural wastes. Many studies have shown that gamma radiation can maintain or increase the antioxidant properties of studied samples (Harrison and Were, 2007). However, some researchers have observed a reduction in total phenolic content after gamma irradiation and its effects are varied according to irradiation dose (de Toledo et al., 2007). Zare et al. (1993) harvested freshly pistachio nuts inoculated with *A. Flavus* spores and exposed them to radiation treatment (1–5 kGy) and stored at 15–20 °C at 75–80% relative humidity for six months. They found that the 3.0 kGy was the suitable dose for reducing the microbial contamination. The effect of gamma irradiation (10, 20, 30, 40, 50 and 60 kGy) on tannin, total phenolics, antioxidants activity and in vitro digestion of pistachio hulls has been investigated by Behgar et al. (2011). The possibility of using the radial diffusion method based on software measurement of the rings area has also been investigated in this study. They reported that irradiation reduced the tannin content ($p < 0.01$) and activity of antioxidants of pistachio hull extracts (by acetone 70%) but increased the total phenolic content. There was no effect of gamma irradiation on the in vitro digestion of the pistachio hull. Also, Gecgel et al. (2011) irradiated different nuts with 1, 3, 5, and 7 kGy of gamma ray. Oil content, free fatty acid, peroxide value, and fatty acid composition of the nuts were studied after irradiation. The results showed that gamma irradiation did not cause any significant change in the oil content of nuts. In contrast, free fatty acid and peroxide value of the samples increased proportionally to the dose. In another study, Moradi et al. (2015) evaluated the effects of treating pistachio by-products (PBP) with electron irradiation (ER, 30 kGy), sodium hydroxide and polyethylene glycol nutrient digestion, growth performance and blood metabolites of Zandi lambs. Treating PBP with ER decreased (– 5.21%) neutral detergent fiber (NDF). The concentrations of phenolic compounds were lower in NaOH-PBP than in control or ER-PBP. Feeding ER-PBP improved feed intake, weight gain and average daily gain compared to other experimental diets ($p < 0.05$). Akbari et al. (2017) studied the effects of gamma irradiation on total phenol, anthocyanin and antioxidant activity of three different Persian pistachio nuts at doses of 0, 1, 2 and 4 kGy. The antioxidant activity of samples (FRAP and DPPH methods) significantly increased in the 1–2 kGy dose range. In addition, increasing the radiation to 4 kGy significantly increased the anthocyanin content of *Kale-Ghouchi* and *Ghazvini* genotypes. They concluded that irradiation could increase the phenolic content, anthocyanin and antioxidant activity of pistachio nuts.

Based on our knowledge, the effect of irradiated PGH extract on inhibition of tyrosinase enzyme and browning of potato has not been studied. The objectives of this study were i) to study effect of irradiation on the extraction yield and antioxidant activity of PGH extract ii) to apply the irradiated extract at the optimal dose as an inhibitor of the tyrosinase enzyme iii) to investigate the effect of irradiated PGH extract on inhibition of browning in real food systems (potato slices).

2. Materials and methods

2.1. Materials

Pistachio (*Ahmadaghaei* variety) green hull was obtained from the Yazd Agricultural Research Center of Iran. Hulls were dried and ground. Then, a fraction between 10 and 40-mesh sieve was collected. Methanol, ethyl acetate and acetone were purchased from LOBA chemie (Mumbai, India). 2, 2'-diphenyl-1-picrylhydrazyl (DPPH[•]), sodium dihydrogen phosphate and disodium hydrogen phosphate, mushroom tyrosinase EC (1.14.18.1) with activity 1000 units/mg and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), 3-(3, 4-dihydroxyphenyl)-L-alanine (L-DOPA) substrate were purchased from Acros Organics Company (Geel, Belgium). Folin-Ciocalteu reagent, ferric chloride, and sodium carbonate were purchased from Merck Chemical Co. (Darmstadt, Germany). Other solvents and chemicals were of analytical grade and used without any further purification.

2.2. Gamma irradiation

The dry powdered sample (1000 g) were placed in polyethylene bags (20 × 15 cm). Irradiation was carried out at several doses (0, 10, 20, 30 and 40 kGy) under the same conditions (temperature and humidity) using a Gamma cell-220 irradiator (Nordion, Canada). The source strength was approximately 12,470 Ci with the dose rate of 3.05 Gy/s. To minimize variations in radiation-dose absorption, the bags were rotated 180° halfway during the irradiation process. Dosimetry was performed using Red-Perspex dosimeter (Harwell Dosimeters, UK) at the outer side of bags. The dosimetry team calculated the dose map via Monte Carlo N-particle transport code (mcnp) calculations and evaluate it by practical results. The actual doses were within ± 2% of the target dose. All treatments were carried out triplicates.

2.3. Preparation of plant extracts

Irradiated and nonirradiated (control) samples were separately extracted by water, methanol, ethyl acetate and acetone (Rajaei et al., 2010a). Subsequently, all extracts filtered by using Whatman filter paper No.1 (Sigma-Aldrich, USA). Then, extracts concentrated under vacuum at 40 °C. Water extract was dried by a CoolSafe CS55-4 freeze drier (Libogen, Lyngø, Denmark).

2.4. Determination of total phenolic content (TPC)

The total phenolic content of extracts was determined using the Folin-Ciocalteu reagent (Waterhouse, 2002), and results were expressed as mg of gallic acid equivalent (GAE) per gram of dry weight (gdw).

2.5. DPPH free radical scavenging assay

The radical scavenging activities of PGH extracts were measured according to Oliveira et al. (2009) method with some modifications. The scavenging ability was calculated using the following equation:

$$\% \text{ Scavenging activity} = [(A_{517 \text{ control}} - A_{517 \text{ sample}}) / A_{517 \text{ control}}] \times 100$$

For better comparison, the DPPH[•]-scavenging activity of PGH extracts were also determined and expressed as EC₅₀. The EC₅₀ value (µg/ml) is the effective concentration of extract which reduces DPPH free radicals by 50%.

2.6. Ferric reducing antioxidant power (FRAP)

The reducing powers of PGH extracts were determined by the method of Benzie and Strain (1996) with minor modifications. The

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